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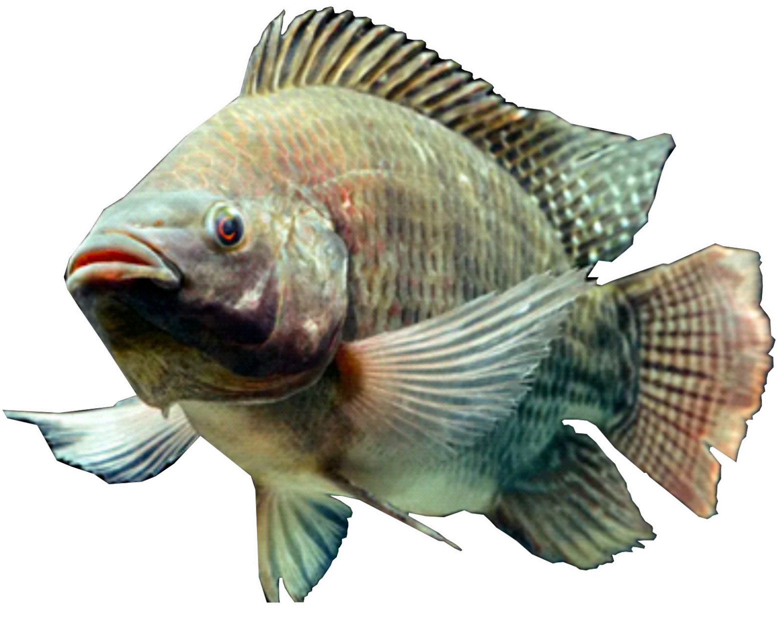
# Evaluation of locally available feed resources for Nile tilapia (*Oreochromis niloticus*) in Rwanda

Leon Niyibizi



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Cover: Nile tilapia *Oreochromis niloticus*
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## Abstract

Aquaculture was introduced in Rwanda in the 1940s as an extensive pond-based system but the sector has gained in popularity during the past two decades, resulting in greater numbers of fish ponds and a corresponding increase in demand for quality fish feeds for sustainable aquaculture production. The aim of this thesis was to identify, sample and evaluate the nutritive value of some locally available feed ingredients that could be used by fish farmers producing Nile tilapia.

An initial countrywide survey revealed that a semi-intensive farming system prevails (81% of total production) in Rwanda, three main fish species are cultured (Nile tilapia (*Oreochromis niloticus*) (most common), common carp (*Cyprinus carpio*) and North African catfish (*Clarias gariepinus*)) and around 31 feed ingredients are used, either individually or in mixtures in supplementary tilapia feeds. The nutrient content of local feed resources was evaluated.

Digestibility trials in which fishmeal protein (reference diet, RD) was partly replaced with protein from spent brewer's grain (SBG), spent brewer's yeast (SBY), sweet potato leaf meal (SPLM), kidney bean leaf meal (KBLM) or wheat middlings (WM) showed that apparent digestibility (AD) of crude protein was highest for diets with SPLM and SBG (83%), followed by RD and SBY (78-82%) and then KBLM and WM (69-73%). Mean AD of indispensable amino acids ($AD_{IAA}$) in the experimental diets was high (range 73-87%), and was above 81% for SPLM, SBG, RD and SBY.

Weight gain, final body weight and specific growth rate were high and comparable to the control in fish fed SPLM, SBY, and SBG, but low in fish fed WM and KBLM. Hepato-somatic index and viscero-somatic index did not differ between diets, but red and white blood cell counts indicated a tendency for possible negative effects of KBLM on blood physiology in tilapia.

These results suggest that SPLM, SBY, and SBG protein can replace fishmeal in Nile tilapia diets without compromising growth, feed utilisation or body indices, thus acting as a valuable local protein source for sustainable tilapia production.



*Keywords*: Pond fish farming, fishmeal, food processing by-products, vegetable ingredients, nutrient digestibility, amino acids.

# Preface

*Alone you can go quickly, together we can go far!*

# Dedication

*To my dear family*

# 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. Leon Niyibizi, Aleksandar Vidakovic, Anna Norman Haldén, Simon Rukera Tabaro & Torbjörn Lundh (2022). Aquaculture and aquafeed in Rwanda: current status and perspectives. *Journal of Applied Aquaculture* 1- 22. DOI: 10.1080/10454438.2021.2024315.

II. Leon Niyibizi, Anna Norman Haldén, Simon Rukera Tabaro, Torbjörn Lundh & Aleksandar Vidakovic (2023). Digestibility and haematological indices in Nile tilapia (*Oreochromis niloticus*) fed diets based on food processing by-products and plant-derived ingredients. (Manuscript)

III. Leon Niyibizi, Simon Rukera Tabaro & Aleksandar Vidakovic (2023). Growth performance, nutrient utilization, and body indices of Nile tilapia (*Oreochromis niloticus*) fingerlings fed local feed ingredients. *Livestock Research for Rural Development* 35 (2), 1-12.

Papers I and III are reproduced with the permission of the publisher.

The contribution of Leon Niyibizi to Papers I-III was as follows:

I. Performed the fieldwork (at the selected study sites countrywide) with support from the co-authors. Collected the ingredients and performed chemical analysis with the support of laboratory technicians. Worked on data analysis and interpretation with the support of the co-authors. Drafted the manuscript with inputs from the co-authors and main supervisor, and corresponded with the journal.

II. Performed the digestibility experiment and chemical analysis of feed ingredients and diets with the support of laboratory technicians. Collected faeces samples and conducted analyses on these with support from laboratory technicians and the co-authors. Performed data analysis and interpretation with support from the co-authors.

III. Performed the growth performance experiment and chemical analysis of diets 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.

# List of tables

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# Abbreviations

| | |
|---|---|
| AD | Apparent digestibility |
| ADC | Apparent digestibility coefficient |
| ADiCP | Apparent digestibility of crude protein |
| ADiDM | Apparent digestibility of dry matter |
| ADiE | Apparent digestibility of energy |
| ADiOM | Apparent digestibility of organic matter |
| AOAC | Association of Official Analytical Chemists |
| BW | Body weight |
| CF | Crude fibre |
| C:N | Carbon: nitrogen ratio |
| COD | Chemical oxygen demand |
| CP | Crude protein |
| CW-RAS | Clear water-recirculating aquaculture system |
| DAA | Dispensable amino acids |
| DHA | Docosahexaenoic acid |
| DM | Dry matter |
| DO | Dissolved oxygen |
| DWG | Weight gain |
| EAA | Essential amino acids |
| EE | Ether extract |
| EFA | Essential fatty acids |
| EPA | Eicosapentaenoic acid |
| FCR | Feed conversion ratio |
| FI | Feed intake |
| IAA | Indispensable amino acids |
| IFFO | International fishmeal and fish oil organizations |

| | |
|---|---|
| GIFT | Genetically improved farmed tilapia |
| Hb | Haemoglobin |
| Hct | Haematocrit |
| HPLC | High performance liquid chromatography |
| HSI | Hepato-somatic index |
| HUFA | Highly unsaturated fatty acid |
| KBLM | Kidney bean leaf meal |
| LC-PUFA | Long-chain polyunsaturated fatty acid |
| MCH | Mean corpuscular haemoglobin |
| MCHC | Mean corpuscular haemoglobin concentration |
| MCV | Mean corpuscular volume |
| MINAGRI | Ministry of Agriculture and Animal Resources |
| NDF | Neutral detergent fiber |
| NFE | Nitrogen free extract |
| PER | Protein efficiency ratio |
| PI | Protein intake |
| RAS | Recirculating aquaculture system |
| RAB | Rwanda Agriculture Board |
| RBC | Red blood cell |
| SBG | Spent brewer's grain |
| SBM | Soybean meal |
| SBY | Spent brewer's yeast |
| SGR | Specific growth rate |
| SR | Survival rate |
| SPLM | Sweet potato leaf meal |
| TAN | Total ammonia nitrogen |
| UR | University of Rwanda |
| VSI | Viscero-somatic index |
| WAPI | World aquaculture performance indicators |
| WG | Weight gain |
| WM | Wheat middlings |

# 1. Introduction

Aquaculture is the fastest-growing animal food production sector in the world. It contributed around 17% of human total animal protein consumed in 2016 and currently represents around 50% of global fish consumption. Since 2010, aquaculture increased at an annual rate of 5.8% and has potential to meet the increasing global demand for aquatic foods created by worldwide population growth and stagnation of global capture fisheries caused by over-exploitation of wild-capture fisheries (FAO, 2018; Stevens *et al*., 2018).

Aquaculture (farming of aquatic organisms, including fish, molluscs, crustaceans and aquatic plants) is an important agricultural activity capable of reducing nutritional deficiencies in developing countries and contributing to poverty reduction. Aquatic food or seafood is a natural part of a balanced and nutritious diet, and over 3 billion people worldwide consume fish protein as an essential part of their diet (FAO, 2020). Fish are an excellent source of protein and lipids, especially unsaturated fatty acids, which have merits for human health (Nakagawa *et al*., 2007). Fish protein accounts for approximately 20% of the global population's animal protein intake and is an outstandingly rich source of omega-3 (n-3) long-chain polyunsaturated fatty acids (LC-PUFA), particularly EPA and DHA, with beneficial impacts on a range of human pathologies such as cardiovascular disease, improvement of visual acuity and strengthening of mental health (Lu *et al*., 2022). However, fish consumption and fish production differ widely between countries worldwide. Production of the main groups of farmed species also differs significantly across regions and countries. Most fish production today is in freshwater systems (99%), where carp, tilapia and catfish are the major fish species (Adeleke *et al*., 2021). These three freshwater species are predicted to comprise around 60% of total aquaculture production by 2025 and have accounted for most of the increase in aquaculture production in recent decades (FAO, 2016). Nile tilapia (*Oreochromis niloticus*), one of the most important tilapia species, is widely cultured in tropical, subtropical and temperate regions of the world, with annual growth in production of about 12.2% at present (El-Sayed, 2020).

In the past two decades, there has been increasing interest in tilapia and catfish farming in Rwanda. Previous studies in Rwanda identified several local feed ingredients with potential in African catfish and tilapia aquaculture, including soybean meal, cotton seed cake, sunflower oil cake and groundnut oil cake (Munguti *et a*l., 2012; Nyina-wamwiza *et al*., 2007). Other potential local ingredients yet to be assessed include various plant leaves, agro-industrial by-products such as cereal residues, spent brewer's grain and novel feeds such as spent brewer's yeast (*Saccharomyces cerevisiae*).

Information is scarce on currently farmed species, pond-farm practices and management, locally available fish feeds and the potential nutritive value of local feed ingredients that can be used in fish farming in Rwanda. Knowledge on other key inputs, including fingerling availability countrywide, is also scarce or lacking. In parallel, demand for good-quality fish diets to support the nascent fish farming industry in Rwanda has increased. Therefore, there is an urgent need for research on the availability and proximate chemical composition of potential local and novel ingredients that could be used as alternatives to fishmeal, fish oil and soybean. In order to support long-term development of sustainable fish production and productivity in Rwanda, investigations are also needed on the digestibility of novel ingredients and on effects on tilapia fish growth performance of diets formulated with local feed ingredients.

# 2. Background

## 2.1 Global aquaculture production

Since the 1950s, the worldwide aquaculture industry has increased by approximately 10% per annum, making it the fastest-growing animal food production sector in the world. In 2020, global aquaculture production reached a record 122.6 million tonnes, with 87.5 million tonnes of aquatic animals (49.2% total aquatic animals production) and 35.1 million tonnes of algae, worth USD 264.8 billion and USD 16.5 billion, respectively (FAO, 2022) (Figure 1). In the same year, the amount of aquatic animals destined for human consumption was 20.2 kg per capita, more than double the average of 9.9 kg per capita in the 1960s. Overall, aquaculture production retained its growth trend in 2020 despite the worldwide COVID-19 pandemic (FAO, 2022).

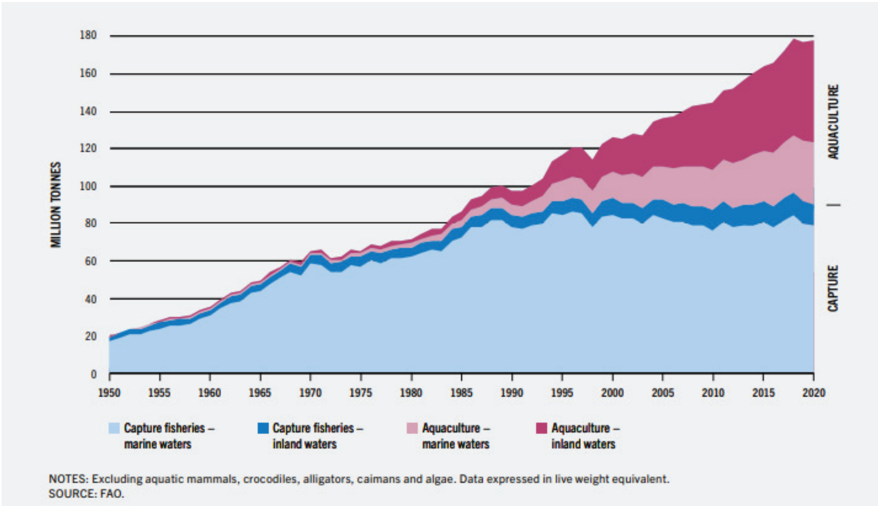


*Figure 1.* World capture fisheries and aquaculture production, 1950-2020. (Source: FAO, 2022).

Asia continues to dominate world aquaculture, producing 91.6% of global aquatic animals and algae in 2020. China is the world's leading aquaculture producer, accounting for 58% of global production in 2018 (FAO, 2020; FAO, 2018). Since 1991, China (mainland) has produced more farmed aquatic animals than the rest of the world, with 56.7% in 2020. China is a key player in the global seafood trade and is one of the largest producers, consumers, importers and exporters of seafood in the world (Crona *et al*., 2020).

Africa contributes only 2.6% of the world's aquatic animal production. Apart from Egypt and Nigeria, which recently experienced a decrease, African countries have displayed 14.5% growth in aquaculture since 2019 (FAO, 2022). Within Africa, Egypt and Nigeria are the first- and second-largest producers of fish, respectively (Kaleem & Bio Singou Sabi, 2021). Over the past decade, sub-Saharan countries led by Nigeria, Uganda and Ghana have reported a significant increase in aquaculture production, from 106,000 tonnes in 2000 to 709,000 tonnes in 2018, with a farm-gate value of about USD 1.68 billion. Since 2000, aquaculture production in sub-Saharan Africa has grown by 11% annually on average, almost twice as fast as in the rest of the world, with a few countries showing growth of 12-23% per year. Tilapia and African catfish (*Clarias gariepinus*) are the two dominant species in SSA and account for 70% of its aquaculture production volume

(Ragasa *et al*., 2022; Chan *et al*., 2019). However, sub-Saharan Africa still accounts for less than 1% of global aquaculture production, despite abundant potential for aquaculture development, existing financial and technical support and past government efforts to assist fish farmers (Ragasa *et al*., 2022).

Globally, aquatic foods provide about 17% of animal protein, reaching over 50% in several countries in Asia and Africa (FAO, 2022) (Figure 2). In recent years, there has been a significant increase in worldwide consumption of aquatic products (FAO, 2022; FAO, 2016), which is expected to continue and over the next 30 years. For instance, a remarkable dietary transition is occurring in sub-Saharan Africa, where demand has grown faster than supply (Naylor *et al*., 2021). Asia had the highest consumption of aquatic foods in 2019 (24.5 kg capita$^{-1}$), while Africa had a low level (10.1 kg capita$^{-1}$). Asia, Europe and Oceania show a high level of edible fish consumption per capita in relation to the global average (14.6 kg capita$^{-1}$ year$^{-1}$). Fish consumption in North America is close to the global average, whereas Africa and South America are significantly below the global per-capita average (FAO, 2020).

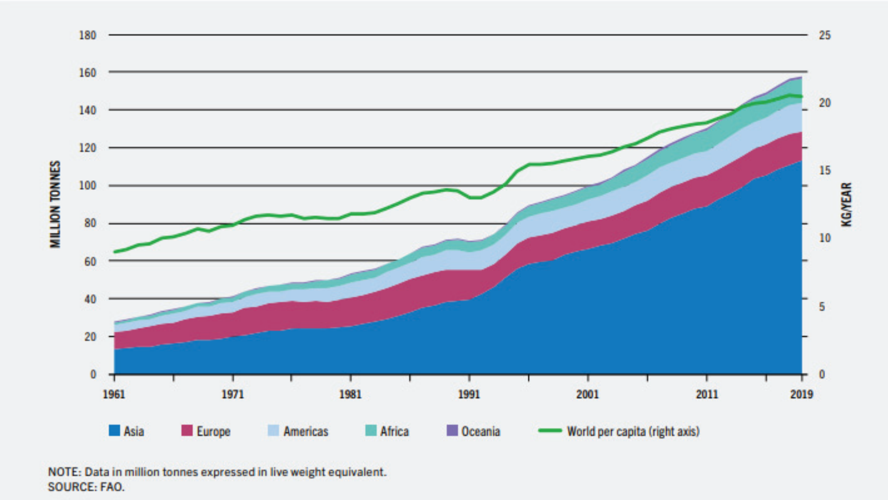


*Figure 2*. Aquatic food consumption by continent, 1961-2019 (Source: FAO, 2022).

In Africa, consumption ranges from a maximum of about 12 kg capita$^{-1}$ in West Africa to 5 kg capita$^{-1}$ in East Africa (FAO, 2022). Rwanda has the

lowest per-capita consumption of protein in the East Africa Community (EAC), far below the FAO-recommended world average of 32 kg capita$^{-1}$ day$^{-1}$ at population level (FAO, 2018).
In Rwanda, aquaculture started in the 1940s as small-scale extensive tilapia pond farming. From 1948 it was promoted mainly as a government-sponsored activity and the nascent fish farming sector was fostered by the Belgian colonial administration until the early 1960s (Dadzie, 1992; Schmidt *et al*., 1981).

By 2030, global aquatic food production is forecast to increase by a further 15% and it is projected to expand and intensify further by 2050, almost doubling its current production (FAO, 2022) (Figure 3). The main factors behind the increase in global consumption of aquatic food include high demand resulting from worldwide population growth and per-capita income growth, urbanisation and improvements in aquatic production, post-harvest methods and distribution channels. Demand is also being stimulated by changes in dietary trends with the focus on healthy and nutritious aquatic diets (FAO, 2022; FAO, 2018 ; Stevens *et al*., 2018).

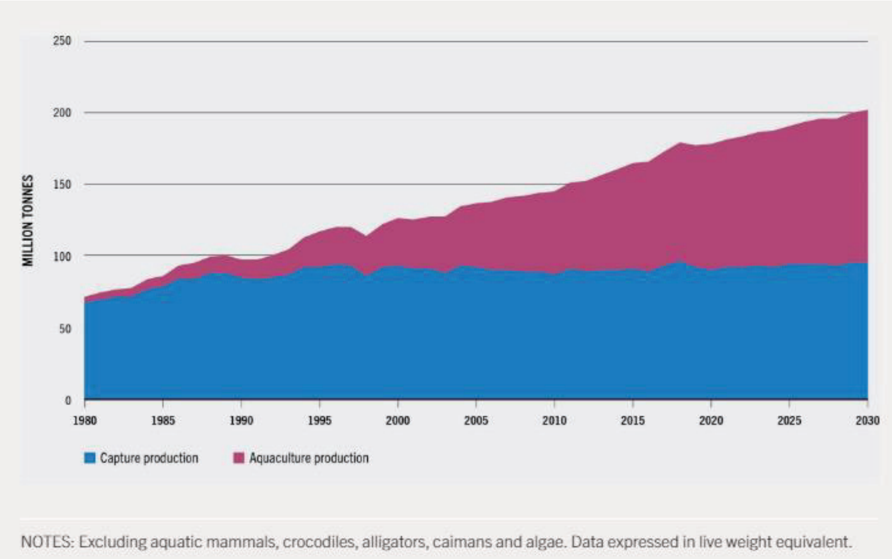


*Figure 3*. World capture fisheries and aquaculture production, 1980-2050 (Source: (FAO, 2022).

The future fish supply will depend on aquaculture production, which has to nearly double to supply a global population (estimated to reach 9.7 billion by 2050) with appropriate amounts of nutritious and safe aquatic food (Naylor *et al*., 2021), and will continue to depend more on land than sea (Costello *et al.*, 2020). Production of aquatic products (excluding algae) is expected to expand to reach 202 million tonnes by 2030. Aquaculture is expected to break the 100 million tonnes threshold in 2027, and to reach 106 million tonnes by 2030 (FAO, 2022). Advances in modern technology, innovations and farming methods, such novel and disruptive technologies to increase fish production includes genome editing, artificial intelligence, recirculating aquaculture systems and Internet of things (K. Yue & Shen, 2022)). This will allow adequate and efficient fingerling production, culture systems, culture methods and high-quality fish feed production needed to ensure expected aquaculture expansion (FAO, 2020).

To sustain such production levels, large volumes of feed will be needed in terms of affordable protein, essential amino acid, additives, omega-3 fatty acids, key minerals, vitamins and energy sources. This will require the sourcing of additional raw materials that are currently either not available or otherwise used (FAO, 2020). Demand could be partly met by use of locally available feed ingredients in the diets fed to farmed fish (Munguti *et al*., 2012). Studies show that in well-fertilised semi-intensive ponds, bran and other by-products of maize (*Zea mays*), wheat (*Tritium aestivum*) and rice (*Oryza sativa*) may be utilised, when available, to supplement natural pond food in African aquaculture (FAO, 2021; Satia, 2017).

## 2.2 Fish feed

### 2.2.1 Fish feeds and feeding systems in Rwanda

In Rwanda, fish are farmed mainly in extensive and semi-intensive ponds systems, using animal manure and dry grasses collected around the ponds to support autotrophic and heterotrophic primary productivity in fishponds (Green, 2015 ;Tabaro et *al.,* 2013). Extensive culture systems, for instance for tilapia, depend solely on natural food, through fertilisation of the ponds, while both natural food and supplementary feeds are generally used in semi-intensive farming systems (El-Sayed, 2020). To improve commercial returns for farmers, appropriate feeding strategies should consider *e.g*. holding conditions, fish size at stocking, grading, and adjusting stock density, feeding rate and pellet size (Ng & Romano, 2013; Saoud *et al*., 2005). For optimal performance, fish fry and spawning females usually have a higher protein requirement than fingerlings and the grow-out stages (Siddiqui *et al.*, 1988). The objective of feeding fish is to meet their nutritional requirements for good health, optimum growth, optimum yield and minimum waste, at a reasonable cost so as to optimise profits (El-Sayed, 2006).

For aquaculture to continue its current high growth rate, equivalent growth in feed supply is essential, providing a balanced diet for proper growth and healthy fish. High-yielding, efficient aquaculture production requires high-quality feeds with a balanced protein content and amino acid profile (ideal amino acids) that cover the indispensable amino acid (IAA) requirement of the fish (El-Sayed, 2006).

Feed and feeding represent the largest operating expense (50-70%) in all types of intensive aquaculture (Tacon & Metian, 2008; Rumsey, 1993). This high cost mainly derives from the cost of the protein included in aquafeed, chiefly fishmeal and soybean meal. Recent estimates show that 68.2% of total fishmeal worldwide and 88.5% of fish oil are utilised for aquafeed production (Ghamkhar & Hicks, 2020). Approximately 70% of aquatic-based production of animals (around 68% of which involves commercial fish species) consists of fed aquaculture, which uses high-protein aquafeeds (Tacon, 2020). Fishmeal, produced mainly from wild-caught small pelagic anchovies and sardines, remains the major protein source in aquafeed, due to its high nutritional value (Tacon & Metian, 2008; Gatlin *et al*., 2007).

Although fishmeal and fish oil have traditionally been the major protein and lipid source, respectively, in aquafeed, their consumption has declined in the past few years due to increased use of plant oils in alternative applications Tacon & Metian, 2008). Globally, fishmeal and fish oil consumption in aquaculture declined after 1996, but increased slightly over the past decade (Tacon *et al*., 2011; Naylor *et al*., 2009)). Between 2018 and 2030, the proportion of total fish oil obtained from fish waste is projected to increase from 40 to 45 %, while for fishmeal the projected increase is from 22 to 28 % (FAO, 2020). World fish production (1990-2030) from whole fish and from fish by-products is shown in Figure 4.

In general, fishmeal is not environmentally and economically sustainable. To achieve successful and sustainable production of fish, one key challenge for the expanding aquaculture industry is to utilise alternative sustainable feed sources and formulate cheaper diets that result in high growth rate, good health and low environmental footprint, through a proper feeding regime. Since 2006, many advances have been made in replacing part of the fishmeal in aquafeeds with alternative protein sources (NRC, 2011). However, more research and development in that area are required to help farmers replace fishmeal and fish oil with more sustainable alternative sources.

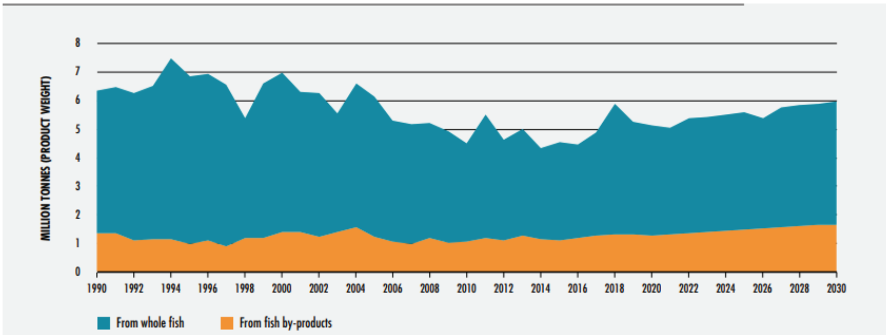


*Figure 4.* World fishmeal production of whole fish (dark blue, mainly caught fish) and fishmeal from by-products (orange), 1990-2030. (Source: FAO, 2022).

### 2.2.2 Fish feed resources

*Animal-based feed sources*

Globally, fishmeal has become an expensive feed ingredient due to its limited availability and high competition amongst diverse animal production sectors (IFFO, 2017). Fishmeal is still the major dietary protein source, comprising between 20 and 60 % of fish feed (De Silva & Anderson, 1995); (Watanabe, 2002) (Figure 5). Replacing even a portion of the fishmeal in aquafeeds is crucial for expansion of aquaculture beyond the level at which fishmeal supply restricts further growth (Stickney, 1997). Around 300 amino acids in proteins are reported in natural sources, but only 20 amino acids make up most proteins, each with different physical and chemical properties (Molina-Poveda, 2016). Consequently, the use of alternative sources to formulate economic and sustainable aquafeed is constantly renewed and includes local ingredients from animal wastes and plants, either terrestrial or aquatic (Mmanda *et al*., 2020; Wassef *et al*., 2013).

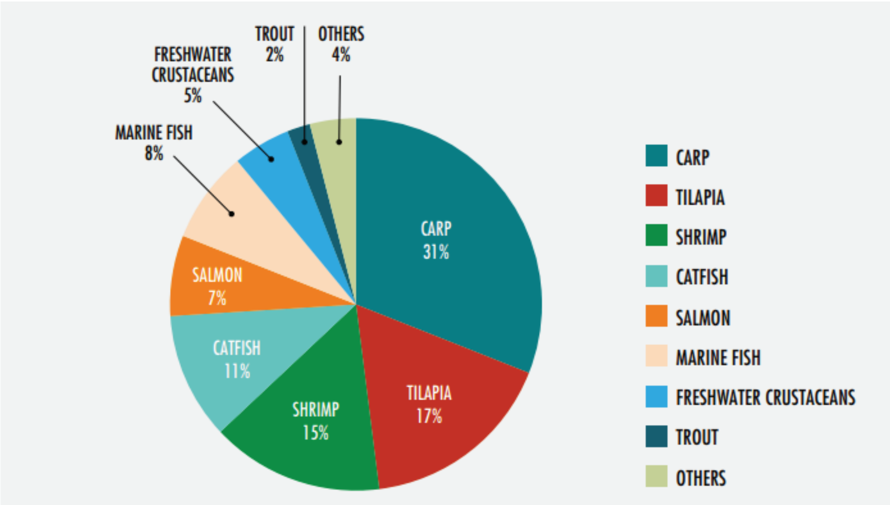


*Figure 5.* Worldwide share (%) of consumption of total aquaculture feed by species group, 1955-2015. (Source: FAO, 2018).

Several fishmeal alternatives of animal origin, mainly animal by-products, have been assessed for digestibility and the growth performance of cultured species has been extensively studied over decades. Animal by-products most commonly used as protein feed ingredients in fish feed are high in crude protein and are therefore able to meet the protein requirements of *e.g.* tilapia for growth, reproduction and development (Abdel-Tawwab *et al*., 2010). Furthermore, the amino acid profile of animal-origin ingredients meets the essential amino acid requirements for fish growth and reproduction, particularly lysine and methionine plus cysteine and tryptophan (NRC, 2011). Animal-origin ingredients most investigated locally include cattle blood (*Bos taurus*), bone and meat meal (Suloma *et al*., 2013) , poultry by-products (Amm & Aa, 2015; Soltan et *al*., 2017) maggot fly, freshwater shrimp (*Caridina nilotica*) and shrimp waste (Leal *et al*., 2010). Aquatic animal protein used in aquaculture today is mainly fishmeal derived from wild-harvested whole fish (see Figure 4). However, the focus has recently shifted to fish by-products, insect and other protein sources as a partial or even total substitute for fishmeal (Gasco *et al*., 2018 ; NRC, 2011).

*Plant-based feed sources*

Plant ingredients are considered cheap protein sources that could replace fishmeal in aquafeed without compromising feed quality (Dorothy *et al*., 2018 ;El-Sayed, 1999). Plant resources range from roots or tubers to grains and leaves, but also various important agricultural or industrial by-products such as cakes, meals, brans and middlings. Plant ingredients are commonly grouped into distinct categories, such as agricultural by-products, agro-industrial by-products, terrestrial plant leaves and aquatic plants. In aquaculture, plant sources provide nutrients such as protein, carbohydrates as energy, fibre and oils (lipid or fat). In addition, plant sources contain minerals and vitamins, but also are known to contain more or less undesirable anti-nutritional compounds (Gatlin *et al*., 2007). Soybean (*Glycine max*) and sunflower (*Helianthus annus*) seeds are used as an oil source, in the form of meal or cake. Cereals such as rice, wheat, and maize are used in the form of by-products, namely crushed bran and middlings. In addition, pulses and protein concentrate meals (*e.g*. peas, lupin) are used (NRC, 2011; Tacon *et al*., 2011; Gatlin *et al*., 2007).

The most commonly used terrestrial leaf ingredients (dried) as alternatives to fishmeal include leaves of cassava (*Manihot esculenta*) (Madalla *et al*., 2016), kidney bean (*Phaseolus vulgaris*) and sweet potato (*Ipomoea batatas*) (Adewolu, 2008) and moringa (*Moringa oleifera*) (Tabassum *et al.*, 2023; Puycha et al., 2017). However, plant leaves have a high fibre content and contain various anti-nutritional factors that could affect fish health and growth (Naylor *et al*., 2009; Francis *et al*., 2001). Plant sources, such as legumes, are used as protein sources in commercial aquaculture diets. Despite the good nutrient profile of many plant leaves with respect to protein, vitamins and minerals, aquatic leaves are not commonly used in commercial fish feed production, as they are only available seasonally (Das *et al*., 2018). Thus plant leaves available year-round need to be assessed as alternatives. For instance, sweet potato leaf meal and kidney bean leaf meal could be used more widely as feed ingredients for animal and fish diets (Adewolu, 2008). Such ingredients have relatively lower cost and constant availability for fish diets (Bergamin *et al*., 2013).

The most studied aquatic plants as replacements to fishmeal in fish diets include aquatic ferns (*Azolla* spp.), water lettuce (*Pistia stratiotes*) and duckweed (*Lemnoideae* spp.) (Das *et al*., 2018; Thy *et al*., 2008; *Nandi et al*., 2023). However, high replacement rate of fishmeal with aquatic plants results in poor fish growth performance (Das *et al*., 2018). A high level of crude fibre and low level of lysine in plant ingredients are the most limiting factors that decrease their nutritional value in fish diets (Bomfim *et al.*, 2010) (Furuya *et al*., 2000). Generally, plant ingredients/feedstuffs contain bioactive compounds that may positively or negatively affect fish (Gatlin *et al*., 2007), in addition to indigestible organic matter in the form of insoluble carbohydrate and fibre (Naylor *et al*., 2009). Some plant ingredients are possible alternatives that can be used in fish feed without compromising the nutritional quality of the feed (El-Sayed, 1999). Between 1990 and 2020, the composition of fish feed shifted towards the use of plant resources in fish feed, as these resources are considered more efficient, sustainable and economically viable (Zlaugotne *et al*., 2022). However, few studies have investigated the effects of plant leaf and agro-industrial by-product feed ingredients on digestibility and growth performance in tilapia produced in Rwanda, where there is an emerging need for suitable and sustainable (plant leaves and agriculture by-products) and novel feed ingredients for aquaculture.

*Agro-sector by-product resources*

Agro-sector by-products that are interesting for aquaculture include by-products from the food industry, breweries, the wood and paper industry and biogas production. In Rwanda, the main agro-industrial by-product feed ingredients used by fish farmers and aquafeed producers are cereals (Niyibizi *et al*., 2022). These cereals are particularly important as staple food crops in many areas of the world, providing carbohydrates, proteins, dietary fibres and vitamins in different by-products (Guimarães *et al*., 2008). Wheat is the most widely cultivated and used cereal for human consumption and a large quantity of wheat is milled into flour, providing by-products such as wheat middlings (Reynolds & Braun, 2022; FAO, 2009).

Wheat middlings consist of fragments of the outer skin and particles of grain containing variable amounts of endosperm, and are often mixtures of different types of milling residues. They are typically richer in starch and less coarse than wheat bran, and poorer in starch and coarser than wheat feed flour. Wheat contains all basic nutrients, but is deficient in essential amino acids, *i.e.* leucine, lysine and phenylalanine. The bran fraction is very rich in protein, vitamins, minerals and dietary fibre, but during milling of wheat water-soluble vitamins, proteins and dietary fibre are lost. Cereals and legumes are still rich in minerals, although the bioavailability of these is hindered by the presence of anti-nutritional factors such as phytate, trypsin inhibitor and polyphenols (Ram *et al*., 2020). In wheat and rice, starch is distributed in larger proportions in the external cover of the pericarp and in the aleurone layer (Cheryan & Rackis, 1980), and this constitutes wheat middlings portion (Cangussu *et al*., 2018). The availability of wheat fractions could increase the flexibility of feed formulation for aquaculture, principally for aquaculture areas proximal to grain fractionation facilities (Gatlin *et al*., 2007).

Other ingredients used as low-cost alternative energy and protein sources that contribute to the lipid content in fish diets include soybean meal and soybean full fat, cotton seed cake, sunflower seed cake, rice polish and maize bran (Ogello *et al*., 2017; Toghyani *et al*., 2015; NRC, 2011). Most agricultural by-products are often low in limiting amino acids such as lysine, methionine and tryptophan (Maina *et al*., 2002; Gorissen *et al*., 2018), and contain higher levels of indigestible organic matter in the form of insoluble plant fibres than animal-origin ingredients (Naylor *et al*., 2009; Maina *et al*., 2002).

Baker's yeast (*Saccharomyces cerevisiae*), a single-cell eukaryotic fungus, is the most commonly used yeast species in aquaculture (Agboola *et al*., 2021; Øverland *et al*., 2013). It is a by-product of brewing consisting of yeast remaining in the fermentation vats of malt wort after removal of the fermented liquid and it is generally sold in dried form (Chauhan & Kanwar, 2020). Yeast cells contain considerable amounts of crude protein (about 40-55%) and have a favourable amino acid profile. However, when yeast is used as major protein ingredient in fish feeds, dietary concentrations of sulphur-containing methionine and cysteine are typically low (Agboola *et al*., 2021; Nasseri *et al.*, 2011) . Yeasts have rather low lipid content, high ash content and moderate levels of carbohydrates (Halász & Lásztity, 2017; Øverland et al., 2013). Yeast contains other bioactive components beneficial for fish growth and development (Hansen *et al*., 2019; Rawling *et al*., 2019; Vidakovic *et al*., 2020). For example, when used as a nutritional supplement in fish feed, yeast is known to have beneficial effects on the immune response and gut health (Yilmaz *et al*., 2007; Torrecillas *et al*., 2012; Eryalçin *et al*., 2017). Spent brewer's yeast can also be used as a raw material for production of β-glucan, and is used in the food industry as a thickener and as an emulsifier and stabiliser due to its good viscosity and water retention properties (Thammakiti *et al*., 2004). In particular, spent brewer's yeast contains various immunostimulatory compounds such as β-glucan, nucleic acids and mannan oligosaccharides, known for their health-stimulating effects in various fish species, and also chitin, among other compounds (Li & Gatlin, 2005; Tukmechi *et al*., 2011), It is thus a sustainable alternative to fishmeal in aquafeeds.

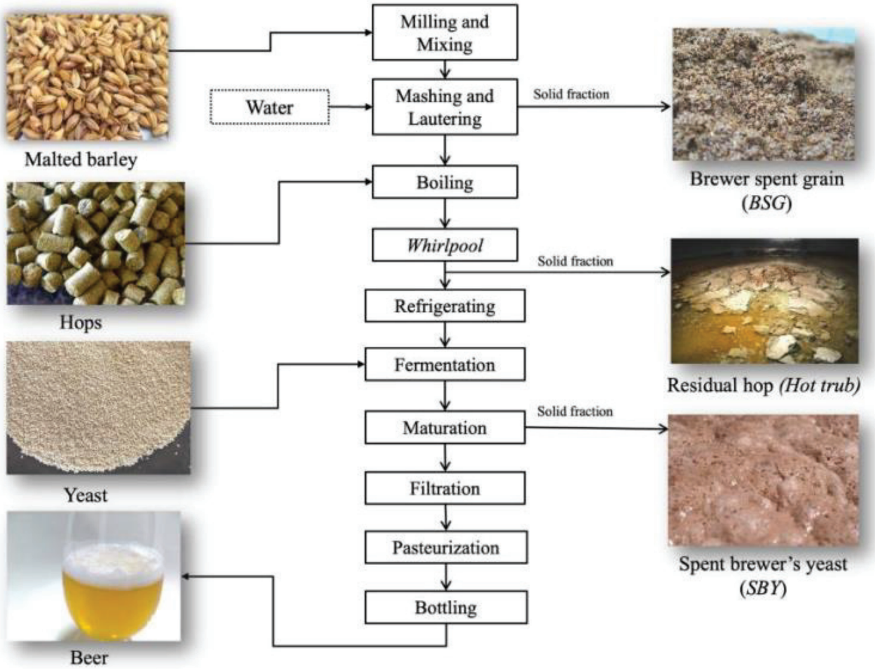


*Figure 6.* Schematic representation of beer processing and main by-products generated. (Adapted from (Mussatto, 2009).

Beer is the fifth most consumed beverage in the world and in 2021 global beer production amounted to about 1.86 billion hectolitres, up from 1.3 billion hectolitres in 1998 (Statista, 2022). In the manufacture of beer, various residues and by-products are generated. The most common of these are waste water, spent grains, spent hops and surplus yeast, which are generated from the main raw materials (Karlović *et al*., 2020; Mussatto, 2009) (Figure 6). Spent brewer's grain is the main by-product generated during beer production based on barley, wheat, maize, rice or oats and is available in large quantities throughout the year. Around 15-20 kg of spent brewer's grain is obtained per hectolitre of beer produced. It represents ~85% of all by-products generated (Mussatto, 2014) and approximately 30% (w/w) of the starting malted grain (Arranz *et al*., 2018). Fresh spent brewer's grain has around 75-80% moisture content (Robertson *et al*., 2010a). It is essentially composed of a lignocellulosic material containing protein (∼30% on a dry weight basis), lignin (∼28%), hemicelluloses (∼25%) and cellulose (∼17%) (Zerai *et al*., 2008; Celus *et al*., 2006). The main minerals found in spent brewer's grain are calcium, cobalt, copper, iron, magnesium, manganese, phosphorus, potassium, selenium, sodium and sulphur (Biswas & Naveen, 2011). It is also a rich source of good-quality protein that contains essential amino acids (Ikram *et al*., 2017). To date it has been almost exclusively as an animal feed (Mussatto *et al*., 2006), but it can be used to feed fish (Jayant *et al*., 2018) and is a potentially valuable resource for industrial exploitation (Robertson *et al*., 2010b). It is also a by-product of great interest for the biotechnology, food, and pharmaceutical sectors, given its nutritional and functional characteristics (Lynch *et al*., 2016; *Mussatto et al.*, 2006). Active investigations are underway in many countries on highly sustainable 'circular' feeds that incorporate waste of various types, including 'circular' ingredients from waste and from nature (Zerai *et al*., 2008).

## 2.3 Tilapia

Tilapia, a group of species in the family Cichlidae, order Perciformes, is the second major species cultured in world aquaculture after carp species (El-Sayed, 2020; FAO, 2018). More than 125 countries performed tilapia farming in 2017 (FAO, 2019). In Africa, tilapia is by far (50.7%) the most commonly cultured fish species, with Egypt and Nigeria the largest producers (WAPI/FAO, 2022). In Rwanda, tilapia was the major species raised in small-scale extensive pond systems in the 1960s (FAO/UNDP, 1981).

Nile tilapia (*Oreochromis niloticus*) is one of the most important tilapia species in tropical, subtropical and temperate regions. It is popular due to its distinctive characteristics such as ease of reproduction, high growth rate, significant ability to withstand a wide range of environmental stresses, acceptance of artificial diets in all stages of production and high nutritional value, carcass taste and of high market demand, and is proposed to be the next-generation aquaculture species (G. H. Yue *et al*., 2016).

## 2.4 Nutrient requirements of tilapia fish

In general, nutrients or dietary constituents are critically important for physiological body metabolism and for optimum growth, reproduction and health (Lall & Dumas, 2015). It is widely believed that fish require more dietary protein than other vertebrates, and protein is considered the most important constituent in fish diets (Teles *et al*., 2019; Ogunji & Wirth, 2002). Lovell (1980) suggested 25-50% crude protein in fish feed to reach maximum growth rate. Mammals and birds typically achieve maximum growth rate on diets containing 12-25% protein. However, efficiency of protein utilisation by fish is lower than in other animals (Fry *et al*., 2018).

### 2.4.1 Protein requirement

Proteins are complex biomolecules linked into chains by peptide bonds and cross-links between chains with sulfhydryl and hydrogen bonds (Lall & Dumas, 2015; Molina-Poveda, 2016). Protein plays the most important role in fish growth, development and reproduction (Volkoff & London, 2018);Cho & Kaushik, 1990), by supplying amino acids, and is generally the most expensive ingredient in fish diets (Leal *et al*., 2010; El-Sayed, 1999). In fish and shrimp, protein represents 65-85% of body weight (Jauncey, 1982). In general, total protein requirement in tilapia can be influenced by digestibility, fish life stage and amino acid profile of the protein source. For instance, Nile tilapia fingerlings must be fed a diet with high levels of protein, lipids, vitamins and minerals, but low in carbohydrates, whereas sub-adult and adult tilapia effectively need lower levels of protein and relatively high level of lipids and carbohydrates for acceptable growth rate (Lovell, 1989). However, the protein requirements of tilapia can vary and recommendations are sometimes inconsistent. Purified or semi-purified protein sources are not recommended under the conditions prevailing in commercial tilapia farming.

The quality of commercial feeds currently used for tilapia production in Rwanda is greatly variable, with crude protein content ranging from 25 to 32 % of dry matter, while the quality of farm-made feeds is unknown (Niyibizi *et al*., 2022). In general, tilapia larval stages or fry have the highest protein requirement (45-50%) (El-Sayed & Teshima, 1992) and 33% crude protein is optimal requirement in the diet of fingerlings Nile Tilapia (Nasr Sayed, 2018). In the adult stage, the protein requirement is around 30% (Al Hafedh *et al*., 1999). Protein deficiency in fish results in severe growth retardation, depletion of body protein and amino acids, and low survival rate, and may be reflected in selected haematological indices (Ogunji & Wirth, 2002).

### 2.4.2 Essential amino acid requirement

Fish, like other animals, do not have a precise protein requirement, but rather a requirement for a well-balanced composition of essential and non-essential amino acids (Wilson, 2003). Formulating a cost-effective diet that meets the essential amino acid (EAA) requirement of fish and shrimp can be a challenge (Kaushik & Seiliez, 2010), as this will depend on exact data on both EAA requirements of the species and the EAAs supplied by the prospective feed ingredients.

Fish, shrimp and most monogastric animals require the same 10 EAA (arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine) (Table 1). The quantitative requirement for each of these 10 EAAs is known only for a limited number of fish species (NRC, 2011; Kaushik & Seiliez, 2010) (Table 1). The EAA required in fish are most important for growth and maintenance, as they are involved in a wide variety of other metabolic reactions beside protein synthesis and are subjected to significant endogenous losses (Wilson & Halver, 1986). Further, EAAs are required as precursors for various neurotransmitters, hormones and cofactors (NRC, 2011).

Table 1. *Estimated requirement (g 16 g$^{-1}$ N) of essential amino acids (arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine) in some commonly farmed fish and shrimp species*

| | Arg | His | Iso | Leu | Lys | Met | Phe | Thr | Trp | Val |
|---|---|---|---|---|---|---|---|---|---|---|
| Channel catfish *(Ictalurus punctatus)* | 4.3 | 1.5 | 2.6 | 3.5 | 5.1 | 2.3 | 2.1 | 2.2 | 0.5 | 3.0 |
| Common carp *(Cyprinus carpio)* | 4.3 | 2.1 | 2.5 | 3.3 | 5.7 | 2.0 | 6.5 | 3.9 | 0.8 | 3.6 |
| Nile tilapia *(Oreochromis niloticus)* | 4.2 | 1.7 | 3.1 | 3.4 | 5.1 | 2.7 | 3.8 | 3.8 | 1.0 | 2.8 |
| Mrigal carp *(Cirrhimus mrigala)* | 4.6 | 2.1 | 3.2 | 3.9 | 5.8 | 3.0 | 3.3 | 4.5 | 1.0 | 3.8 |
| Japaneese eel *(Anguila japonica)* | 4.2 | 2.0 | 3.8 | 4.7 | 5.1 | 4.8 | 5.8 | 3.8 | 1.1 | 3.8 |
| Rainbow trout *(Oncorhynchus mykiss)* | 4.2 | 1.2 | 2.8 | 2.9 | 5.3 | 1.9 | 2.0 | 2.6 | 0.4 | 3.4 |
| Black tiger shrimp *(Penaeus monodon)* | 5.3 | 2.2 | 2.7 | 4.3 | 5.8 | 2.9 | 3.7 | 3.5 | 0.5 | 2.8 |

Source: (NRC, 2011).

Lysine is the amino acid found in the highest concentrations in the carcass of several fish species (NRC, 2011; Wilson & Cowey, 1985) and it is the first limiting EAA in most protein sources used for commercial feed production (Hauler & Carter, 2001; NRC, 2011). In addition, fish generally have a high requirement for dietary arginine because it is one of the most versatile amino acids, serving as the precursor for the synthesis of nitric oxide, urea, polyamines, proline, glutamate and creatine in fish (Zhao *et al*., 2011 ; Wu & Morris, 1998). Arginine is also abundant in protein and tissue fluid (Li *et al*., 2009).

When dietary lysine requirement is known, it is possible to predict the requirements of other EAAs using the 'ideal protein concept', defined as the exact amino acid profile that meets the requirements of a given species with no excess or deficit. The ideal amino acid pattern is usually stated as the ratio of each EAA to lysine, which is given an arbitrary value of 100 (NRC, 2011). This method has been used to estimate the amino acid requirements for several fish species, based on the amino acid profile of whole-body tissue of the species (Furuya *et al*., 2004; NRC, 2011; Green & Hardy, 2002). Other EAA requirements in fish maybe assessed through dose-response trials, where an amino acid is added or removed from the experimental diet and the survival, growth or condition of the animal are assessed *(do Nascimento et al*., 2020). Examining the amino acid profile of the whole fish may also help to predict their amino acid requirements (Rodehutscord & Pack, 1999). Discrepancies can arise in the given EAA requirement for a specific fish species, such as tilapia (Table 2), due to differences in the methods used to estimate amino acid requirements (Cowey, 1995).

Table 2. *Essential amino acid requirement of Nile tilapia as a percentage of dietary protein, according to different literature sources*

| Amino acid | Santiago (1985) | Santiago & Lovell (1988) | Fagbenro (200) | NRC (2011) |
|---|---|---|---|---|
| Arginine | 4.3 | 4.1 | 4.1 | 1.2 |
| Histidine | 1.5 | 1.7 | 1.5 | 1.0 |
| Isoleucine | 2.6 | 3.1 | 2.6 | 1.0 |
| Leucine | 3.5 | 3.4 | 4.3 | 1.9 |
| Lysine | 5.1 | 5.1 | _ | 1.6 |
| Methionine | 2.2 | 2.7 | 1.3 | 0.7 |
| Phenylalanine | 5.0 | 3.8 | 3.2 | 1.1 |
| Threonine | 5.0 | 3.8 | 3.3 | 1.1 |
| Tryptophan | 0.5 | 1.0 | 0.6 | 0.3 |
| Valine | 3.0 | 5.1 | 3.0 | 1.5 |

### 2.4.3 Lipid requirement

Lipids comprise five classes, waxes, sterols, triacylglycerides, sphingolipids and phosphoglycerides (or phospholipids), and are classified as either polar or nonpolar lipids depending on their water solubility (NRC, 2011). Lipids and their constituents, fatty acids, are vital organic components of fish, with the latter being an essential source of metabolic energy for growth, reproduction and movement in fish (Tocher, 2003). Dietary lipids are also important sources of highly digestible energy and the only source of the essential fatty acids needed by fish for normal growth and development and reproductive performance. They are also carriers and assist in the absorption of fat-soluble nutrients such as sterols and vitamins A, D, E and K (Lim *et al*., 2011). Fat-soluble vitamins are important in various physiological processes, such as bone health, immune function, blood coagulation and vision (Reddy & Jialal, 2022). Lipids, especially phospholipids, are major constituents of cell membranes and are key to the normal function of every cell and organ. They are thus vital for maintenance of membrane flexibility and permeability (Tocher, 2003). Lipids are also precursors of steroid hormones and prostaglandins, improving the flavour of feeds and affecting feed texture (Lim *et al*., 2011). Inclusion of phospholipids in aquafeeds ensures increased growth, better survival and stress resistance, and prevention of skeletal deformities in larval and juvenile stages of fish and shellfish species.

Appropriate dietary lipid inclusion level is an important consideration, since deficiencies can reduce growth and excesses can produce an excessively fatty fish. The minimum requirement in feeds for hybrid tilapia has been shown to be 5% and the optimal level is 12% dietary lipid (Chou & Shiau, 1996; De Silva & Anderson, 1995). Increasing dietary lipid content above the minimum level will support higher growth rates, partly due to a protein-sparing effect in the utilisation of dietary protein. The level of protein in diets for Nile tilapia can be reduced from 33.2 to 25.7 % by increasing the dietary lipid content from 5.7 to 9.4 % (NRC, 2011; Lim *et al*., 2011).

Fatty acids are a type of lipid composed of carbon, hydrogen and oxygen arranged as a variable-length linear carbon chain skeleton with an even number of atoms at one end. Fatty acids are classified as essential fatty acids (EFA) or as not essential, based on their ability or inability to be synthesised by animals, and where deficiency can be reversed by dietary addition. In addition, 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). The polyunsaturated, linolenic and linoleic fatty acids in lipids are dietary essentials for tilapia because the fish cannot biosynthesise them. Like other fish species and vertebrates, tilapia cannot biosynthesise C18 polyunsaturated fatty acids (PUFAs), linoleic acid (18:2 omega-6) or linolenic acid (18:3 omega-3) (Lim *et al*., 2011). The amount and quality of EFA required vary among fish species. For instance, the optimum dietary level of the n-6 acids has been estimated to be about 1% for redbelly tilapia (*Tilapia zillii*) and 0.5% for Nile tilapia. Lipid levels ranging from 5 to 12 % are considered the optimum in diets for tilapia (Lim *et al*., 2011).

Freshwater species normally possess the ability to desaturate and chain-elongate C18 EFAs to longer-chain highly unsaturated fatty acids (HUFAs) (18:2 omega-6 to arachidonic acid, 20:4 omega-6; and 18:3 omega-3 to eicosapentaenoic acid, 20:5 omega-3 and docosahexaenoic acid, 22:6 omega-3). Thus they only have a requirement for linoleic acid or linolenic acid, or both. In contrast, marine species that cannot perform this conversion at a sufficient rate have a dietary requirement for long-chain HUFAs. Research on fatty acid requirements has shown that linoleic series fatty acids are dietary essentials for tilapia.

### 2.4.4 Carbohydrate and fibre requirements

Carbohydrates are abundant, low-cost, excellent sources of energy and carbon in feed formulations, improving growth and protein utilisation, and are efficiently utilised in several fish species (Zhao *et al.*, 2011; Hung *et al.*, 2003). Carbohydrates provide protein and have lipid-sparing effects for growth and their inclusion can improve the quality of pelleted feeds (FAO, 2018). Fish do not have a specific requirement for carbohydrates, because amino acid and fatty acid precursors can supply the required glucose via gluconeogenesis (Lall & Dumas, 2015; NRC, 1993). However, adequate dietary carbohydrate supply is important for fish growth (Han *et al*., 2021), increased retention of protein and lipid in farmed fish and reduced nitrogen discharge via farm effluents (Kamalam *et al*., 2017).

In omnivorous and warmwater fish such as Nile tilapia, carp and channel catfish (*Ictalurus punctatus*), dietary carbohydrates are more important than lipids (Hung *et al*., 2003; Wilson, 1994). In a number of fish species, provision of appropriate levels of dietary carbohydrates appears to produce positive effects on growth and digestibility, metabolism and health (Li *et al*., 2013; Hung *et al*., 2003; Watanabe, 2002). Tilapia can efficiently use high levels (30-70%) of dietary carbohydrates as a primary energy source, whereas the maximum recommended level of dietary carbohydrate is 15-25% for salmonids and marine fish (Kamalam *et al*., 2017; NRC, 2011). For instance, an inclusion level of starch at 10-40% of dry matter has been found to improve growth rate in tilapia (Maas *et al*., 2020 ; Amirkolaie *et al*., 2006).

Factors that affect carbohydrate utilisation efficiency include carbohydrate origin, dietary level, physical state, technological treatments and molecular complexity. These factors may adversely affect fish health through metabolic disorders with physio-clinical signs such as hyperglycaemia, increased glycogen deposition, liver hypertrophy and histopathological development (Azaza *et al*., 2020). Additionally, excess carbohydrates may cause high visceral fat accumulation in fish at harvest

(Hung *et al*., 2003). Carbohydrate-rich ingredients obtained from major cereal grains (maize, wheat and rice), such as rice bran, rice polishings and broken rice, and from vegetables can make up 60-80% of the total feed ration (Cacot, 1994).

### 2.4.5 Energy requirement

Like all living organisms, fish need energy for maintenance. The energy requirement of fish depends on the species, water temperature and physiological stage of the animal itself (Chabot *et al.*, 2016). Hence, a typical diet must be well-defined for each fish species and should be based on at least the appropriate dietary protein and protein to energy ratio, or simply based on the protein and energy needs of the species (Li *et al*., 2013; Sanz *et al*., 2000). Fish preferentially use energy sourced from protein than from lipids or carbohydrates (Walton & Cowey, 1982). Hence, energy expenditure is largely dependent on protein catabolism (Peres & Oliva-Teles, 2001). In general, the diet should provide at least 15-18 MJ digestible energy per kg dry matter. For instance, for freshwater fish (10-250 g body weight), average daily energy expenditure is 25-45 KJ $kg^{-1}$ (NRC, 1993). Average gross energy content ranging from 3.83 to 11.49 MJ $kg^{-1}$ has been reported for freshwater fish samples collected in the wild and farmed species (Schreckenbach *et al*., 2008).

### 2.4.6 Mineral requirement

All aquatic organisms require minerals (nutritionally essential major and minor inorganic elements) for their normal life processes (Halver & Hardy, 2014). Fish are also unique among vertebrates in their ability to absorb minerals not only from their diets but also from water through their gills and skin (Lall & Tibbetts, 2009). These include skeletal formation, colloidal system maintenance, acid-base equilibrium regulation, enzyme activation and hormone production (Chanda *et al*., 2015). Tilapia, like other finfish, obtain the mineral(s) they require either from the diet offered or from the surrounding water through the gills (Chanda *et al*., 2015; Watanabe *et al*., 1997). Dietary sources of minerals include ingredients made from animals, plants and related by-products, plant leaves, aquatic plants and weeds.

## 2.5 Nutrient digestibility in fish

Before promoting any local feedstuffs as potential ingredients in fish feeds, assessment of their digestibility in cultured fish species is vital (Allan *et al*., 2000). Knowledge on the nutritional value of some local ingredients is available in proximate composition tables based on chemical analyses, but data on nutrient digestibility in different fish species are generally scarce. Digestibility is a measure of the degree of absorption or disappearance of a nutrient from a meal as it passes through the digestive system and is egested in faeces, whereby dietary proteins, lipids and carbohydrates are degraded into absorbable units in the form of amino acids, fatty acids and monosaccharides (Lall & Dumas, 2015). Digested nutrients can be presumed to be available to the organism for growth and metabolism (NRC, 2011).

Evaluation of the apparent digestibility (AD) of feedstuffs utilised in fish diets is one of the most important steps in formulating properly balanced diets to satisfy the nutrient requirements and energy of fish cultured in modern aquaculture (Bureau *et al*., 1999; Cho *et al*., 1982).

### 2.5.1 Nutrient and amino acid digestibility

The potential nutritive value of any feed ingredient can be measured based on the digestibility of its energy and nutrient components (NRC, 2011; Allan *et al*., 2000). Proteins and amino acids are expensive but essential dietary components for body composition and metabolism in all species. Fish cannot synthesise all amino acids and must acquire some protein or mixtures of amino acids through the diet. Proteins are hydrolysed into amino acids prior to their absorption, and hence dietary protein is the principal source of amino acids necessary for protein synthesis for growth, tissue repair and metabolic functions (NRC, 2011). Protein quality is measured by the digestible EAA score (Wolfe *et al*., 2016) or determined by the amino acid profile (Jauncey, 1982). Balanced and high availability of EAA can be expected to enhance growth performance, while EAA imbalance and poor EAA availability will reduce growth performance (Ogello *et al*., 2017).

### 2.5.2 Methods used in digestibility assessment

Determining the digestibility of feedstuffs and diets in animals requires collection of faecal material. Two important methodological approaches used in assessing feed digestibility are direct and indirect assessment methods. In direct digestibility assessment, a complete account of all feed inputs and faecal outputs is required, and then digestibility is measured directly from the difference between intake and faecal output of a nutrient or energy source (NRC, 2011). The direct method's main advantage is that faecal excretion is qualitatively evaluated, making it possible to determine digestibility with high accuracy. However, quantitative total collection of faeces in water is not possible for fish (Lall & Dumas, 2015). Another challenge is that this method can easily stress the fish, which may affect digestive and metabolic processes and result in digestibility values that are less credible (NRC, 2011). The indirect method for determination of digestibility relies on collection of a representative sample of faeces, free of uneaten feed particles, and use of an indigestible marker for calculation of total digestibility (Lall & Dumas, 2015; NRC, 2011). The marker can either be added to the feed or can be a component in the feed. Acid-insoluble ash (AIA) is a common and reliable feed-associated indigestible marker used to assess digestibility in pigs (McCarthy *et al*., 1974) and fish (Montaño-Vargas *et al*., 2002). The added marker should be non-toxic and inert and possible to include at low concentrations. Indigestible markers commonly used are titanium (IV) dioxide, chromic oxide ($Cr_2O_3$) and yttrium oxide ($Y_2O_3$) (NRC, 2011).

Both evaluation methods involve feeding test feed ingredients singly or, more commonly, as a component of a diet (NRC, 2011). Diet design, feeding strategy, faecal collection method and method of calculation are key in determining the digestible value of nutrients from any ingredient ( Glencross *et al*., 2007). The amount of the marker in the feed and faeces is assumed to be constant throughout the experiment and ingested entire marker will appear in the faeces. The ratio of the marker in the feed and faeces determines the digestibility of dietary components and energy (Glencross *et al*., 2007). The apparent digestibility coefficient (ADC) of nutrients and energy in the diets can be determined according to (Cho *et al.*, 1982), while that of ingredients can be calculated according to (Bureau *et al*., 1999).

## 2.6 Growth performance and nutrient utilisation in fish

In development of sustainable aquaculture production, improving feed efficiency in fish at the economic, social and environmental level is crucial (de Verdal *et al*., 2018). Efficient use of diets by farmed fish enhances fish growth rate and thus shortens the rearing time to market-sized fish. This lowers operating costs and reduces environmental pollution due to lower waste output. Feed intake in fish can be indirectly estimated from measurement of the growth performance, which reflects net nutrient deposition in the tissues of the fish body. However, direct feed intake assessment is difficult (Glencross *et al*., 2007), and the growth response is influenced by factors such as life stage, fish size and species, physiological conditions, genotype and environmental factors (NRC, 2011). Daily weight gain (DWG), final weight gain (FWG) and specific growth rate (SGR) remain the most commonly used growth performance indices (Abdel-Warith *et al*., 2019; Hassaan *et al*., 2018; Vidakovic *et al.*, 2016). The feed industry uses feed utilisation as another important index to evaluate fish growth. The most extensively considered measurement of fish production efficiency is feed conversion ratio (FCR), calculated as the weight of feed administered over the lifetime of an animal divided by weight gain (de Verdal *et al*., 2018). Other commonly used feed utilisation indices include protein efficiency ratio (PER), protein intake and feed intake (Qi *et al*., 2012).

# 3. Aim of the thesis

The overall aim of this thesis was to identify, collect and evaluate the nutritive value of locally available feed ingredients fed to Nile tilapia (*Oreochromis niloticus*), as a step towards sustainable development of fish farming in Rwanda. Specific objectives were to:

- Provide baseline data on the potential for supplying future high-quality fish feed to support increased fish farming in Rwanda, and identify and assess the chemical composition of locally available feed ingredients used by tilapia fish farmers across all five provinces of Rwanda (Paper I)

- Determine the nutritional quality of local feed sources in the diet of Nile tilapia, based on assessment of apparent digestibility of dietary components, energy and amino acids and effects on fish haematology (Paper II)

- Assess growth performance and feed utilisation in Nile tilapia fed diets with fishmeal replaced to different levels by vegetable ingredients and agro-industrial by-products (Paper III).

# 4. Materials and methods

## 4.1 Brief outline of the studies performed

The research started with desk work (contextual or literature), prior to a countrywide field survey. A list of fish farms was obtained from the Ministry of Agriculture and Animal Resources, Rwanda Agriculture Board, and complementary information was gathered from the University of Rwanda. Based on this initial list, active farms were selected for survey and verified in collaboration with district directors of agriculture under the Ministry of Local Government, resulting in a final list of 212 fish farms. This preliminary work was followed by a field survey which provided data on the status of aquaculture and aquafeed in Rwanda (Paper I), samples of which were collected for proximate chemical composition analysis (Paper I). Two experiments were then performed, using a complete random block design, with six dietary treatments for a digestibility assessment (Paper II) and six dietary treatments for a growth performance analysis (Paper III).

## 4.2 Field survey (Paper I)

In Paper I, a structured survey questionnaire was designed and tested in a pilot study prior to its use in the field in the main survey. The questionnaire contained a total of 102 questions, including closed (n=36) and open-ended (n=66) questions, grouped under the following headings: general information on the respondent and farm manager, farm practices and management, and feed and fertilisation of fish ponds. The field survey was conducted in all five provinces of Rwanda (Northern, Southern, Eastern, Western, Kigali City), subdivided into 30 districts, and was carried out from

November 2017 to February 2018. In total, 67 pond farms were randomly selected from a study population of 212 pond fish farms, applying 95% confidence interval and 10% margin of error. The number of pond farms differed across the five provinces. For the sample to be representative, randomisation was performed at province level considering existing pond farms in each province, and respondents were the fish farm owners or representatives. All field data and feed ingredient samples in Paper I were collected by the same team through interviews, from farm records, and through on-site observation. Farm visits and interviews were scheduled and agreed in advance with the respondents.

## 4.3 Experimental facilities (Papers II & III)

The experiments described in Papers II and III were carried out at the fish farming and research station hatchery at Rwasave, part of the University of Rwanda (UR), Huye campus, located in Southern Province, Rwanda (2°40´S, 29°45E). The experiments in both Paper II and Paper III were conducted in a recirculating aquaculture system consisting of 18 fibreglass tanks, each 100 L in volume, installed above 4480 L concrete tanks equipped with a mechanical and biological water filtration system (Photo). Mixed-sex tilapia fingerlings collected from the University of Rwanda's Fish Farming Research Station were used. Prior to acclimatisation, fish in Paper II received a five-minute bath treatment with saline solution (5 NaCl g $L^{-1}$) to prevent potential ectoparasites, bacteria or fungi. All the tanks had a common water supply and were handled in the same way throughout. All tanks had a plastic mesh top cover to prevent fish from escaping and the fish were kept at a natural photoperiod of 12 h light: 12 h dark.

Recirculating aquaculture system at Rwasave. (Photo by Leon)

## 4.4 Test feed ingredients (Papers II & III)

The reference diet contained fishmeal (*Rastrineobola argentea*) as the main protein source. Ingredients used in Papers II and III were purchased from local markets, obtained from food and beverage industries or freshly harvested in local fields. Some collected ingredients were pre-treated (rinsed, cooked or autoclaved, sundried and milled prior to mechanical mixing and diet production). The local feed ingredients included cotton seed meal, soybean meal, spent brewer's yeast, spent brewer's grain, sunflower oil cake, rice bran, wheat middlings, sweet potato leaves and kidney bean leaves. Cattle blood was collected from cattle abattoirs, spent brewer's yeast and spent brewer's grain were obtained from a brewery, and fish for fishmeal were purchased at a local market.

## 4.5 Experimental diets (Papers II & III)

The experimental diets compared in Paper II comprised one reference diet with fishmeal as the main protein source, and five test diets containing 70% basic ingredients (fishmeal and other same ingredients) and 30% test feed ingredient (*i.e.* 70:30 ratio) on a dry matter basis, according to Cho and Slinger (1979). The five test feed ingredients were spent brewer's yeast, spent brewer's grain, kidney bean leaf meal, sweet potato leaf meal and wheat middlings. Other feed ingredients included to balance the nutrient content in the diets were soybean meal, cotton seed meal, rice bran, sunflower seed cake, broken maize, molasses, sunflower oil and vitamin and mineral premix. Titanium (IV) dioxide, a non-toxic inert marker, was added at a rate of 0.5% (dry weight) to all experimental diets for indirect assessment of digestibility.

In Paper III, six experimental diets were formulated; one reference diet and five test diets. Considering the proximate composition of ingredients, the reference diet was fishmeal-based, while the five test diets were made with the maximum possible fishmeal replacement without affecting dietary crude protein and energy content. All ingredients used and their inclusion rates in Paper II and III are presented in Table 3. All the experimental diets used in Papers II and III were pelleted using a meat grinder and the pellets produced (2 mm in diameter) were sun-dried for 2-3 days and stored at -20 °C until use. Table 3. *Formulation (g $kg^{-1}$dry matter, DM) of the reference diet (RD)/control diet (CD) and test diets based on spent brewer's yeast (SBY), spent brewer′s grain (SBG) sweet potato leaf meal (SPLM), wheat middlings (WM) and kidney bean leaf meal (KBLM)*

Table 3. *Formulation (g kg$^{-1}$dry matter, DM) of the reference diet (RD)/control diet (CD) and test diets based on spent brewer's yeast (SBY), spent brewer's grain (SBG) sweet potato leaf meal (SPLM), wheat middlings (WM) and kidney bean leaf meal (KBLM)*

Paper II

| Ingredients | RD | SBY | SBG | SPLM | WM | KBLM |
|---|---|---|---|---|---|---|
| Fishmeal * | 269 | 188 | 188 | 188 | 188 | 188 |
| Spent brewer's yeast | - | 299 | - | - | - | - |
| Spent brewer's grain | - | - | 299 | - | - | - |
| Sweet potato leaf meal | - | - | - | 299 | - | - |
| Wheat middlings | - | - | - | - | 299 | - |
| Kidney bean leaf meal | - | - | - | - | - | 299 |
| Soybean meal | 149 | 105 | 105 | 105 | 105 | 105 |
| Cotton seed meal | 100 | 70 | 70 | 70 | 70 | 70 |
| Rice bran | 199 | 139 | 139 | 139 | 139 | 139 |
| Sunflower seed cake | 60 | 42 | 42 | 42 | 42 | 42 |
| Broken maize | 149 | 105 | 105 | 105 | 105 | 105 |
| Molasses | 40 | 28 | 28 | 28 | 28 | 28 |
| Vitamin and mineral premix** | 20 | 14 | 14 | 14 | 14 | 14 |
| Sunflower oil | 10 | 7 | 7 | 7 | 7 | 7 |
| Titanium (IV) dioxide | 5 | 5 | 5 | 5 | 5 | 5 |

Paper III

| Ingredients | CD | SBG | KBLM | WM | SPLM | SBY |
|---|---|---|---|---|---|---|
| Fishmeal | 220 | 150 | 170 | 150 | 150 | 110 |
| Soybean meal | 150 | 150 | 150 | 150 | 150 | 150 |
| Cottonseed meal | 100 | 100 | 100 | 100 | 100 | 100 |
| Rice bran | 200 | 200 | 200 | 200 | 200 | 200 |
| Sunflower seedcake | 60 | 60 | 60 | 60 | 60 | 60 |
| Maize middlings | 190 | 190 | 190 | 190 | 190 | 190 |
| Blood meal | 50 | 50 | 50 | 50 | 50 | 50 |
| Sunflower oil | 20 | 20 | 20 | 20 | 20 | 20 |
| Premix* | 10 | 10 | 10 | 10 | 10 | 10 |
| Spent brewer's grain meal | - | 70 | - | - | - | - |
| Kidney bean leaf meal | - | - | 60 | - | - | - |
| Wheat middlings meal | - | - | - | 70 | - | - |
| Sweet potato leaf meal | - | - | - | - | 70 | - |
| Spent brewer's yeast meal | - | - | - | - | - | 110 |
| Replacement rate for fishmeal (%) | 0 | 32 | 27 | 32 | 32 | 50 |

*Vitamin and mineral content in premix: Vitamin A 4,000,000 I.U, Vitamin D3 750,000 I.U, Vitamin E 3,500 I.U, Vitamin K 500mg, Vitamin B1 200mg, Vitamin B2 600mg, Vitamin B6 600mg, Vitamin B12 5,000mg, folic acid 250mg, biotin 0.75mg, nicotinic acid 5,000mg, pantothenic acid 2,000mg, choline 40,000mg, Fe 8,750mg, Mg 12,500mg, Cu 1,500mg, Zn 12,500mg, Co 270mg, I 250mg, Se 50mg, P 1,050mg, Ca 750,000mg, lysine 1200mg, methionine 8,000mg, phytase 20,000U.

## 4.6 Experimental conditions (Papers II & III)

In the digestibility study (Paper II) and the growth study (Paper III), a total of 360 mixed-sex tilapia , with an average weight of 31.2±1.9 g and 30.2 ± 1.54 g, respectively, were used. In each experiment, the fish were acclimatised for one week, then weighed and randomly allocated to 18 fibreglass tanks (100 L), with 20 fish per tank. The experiment was conducted in a recirculating aquaculture system. The rearing tanks were divided into six groups or treatments, with three tanks per group. One group was assigned to the reference diet (control) and five groups received test diets. The tanks were filled with 60 L, had a common well water supply and were cleaned regularly to improve visibility within the water column. The water in the tanks was continuously aerated using an electric air pump connected to stone diffusers, to ensure adequate oxygen supply (Table 4). The fish were weighed again at the end of the experiment

## 4.7 Feeding (Papers II & III)

The Nile tilapia used in the digestibility study (Paper II) and the growth study (Paper III) were hand-fed a ration to satiation at 4.5% of body weight per day, on three occasions per day (9.00 h, 13.00 h and 15.00-17.00 h). In Paper III, the feed ration was adjusted every two weeks as the fish grew, to ensure maximum growth throughout the 70-day experimental period.

## 4.8 Sample collection (Papers I-III)

In Paper I, representative samples of 1-2 kg of feed ingredients commonly used by farmers and local fish feed makers were collected. Each feed ingredient was placed separately in an appropriate container and stored at 4°C until analysis.

In Paper II, faeces samples were collected through siphoning and uneaten feed were siphoned out within 30 minutes post-feeding. Faecal matter was collected from each experimental tank twice daily (11.00 h and 15.00 h) within two hours post-feeding, using a 2-cm pipe. The siphoned faeces were collected on a 100 µm nylon filter mesh. All faeces collected (average 40 g) were transferred to an appropriate plastic container, placed on ice and stored at -20 °C and kept frozen until further analysis. During faecal matter sampling, caution was taken to ensure maximum retrieval of a relatively intact string of faeces. The collection process was repeated daily throughout the experiment.

In Paper II, haematological parameters were analysed. Three fish were randomly collected from each tank (n=54 fish) and anesthetised with a solution (50 mg $L^{-1}$) of tricaine methane-sulphonate (MS-222; Topical Anesthetic Chemicals Inc., USA). Blood samples (1.0 mL) were collected from the caudal vein of the fish using heparinised syringes (2 mL), and immediately transferred into heparinised vials and placed on ice until further analyses.

In Paper III, all 20 fish stocked in each tank were weighed, providing initial biomass. Then, throughout the growth experimental period, every 14 days a sample of six fish were randomly netted from each experimental tank and weighed to monitor intermediate body weight growth. At the end of the experiment, all fish were anesthetised with 100 mg $L^{-1}$ of MS-222, counted and weighed (final biomass). Three fish from each tank were randomly collected and dissected for determination of hepato-somatic index (HSI %) and viscero-somatic index (VSI %).

## 4.9 Water quality monitoring (Papers II & III)

In Papers II and III, water parameters such as pH, temperature (°C) and dissolved oxygen (mg $L^{-1}$) were recorded twice daily (at 08.00 h and 15.00 h) in each experimental tank, using a portable multiparameter probe. Water temperature was kept around 28 °C using aquarium heaters. Concentrations of nitrite (mg $L^{-1}$) and ammonia (mg $L^{-1}$) were monitored on a bi-weekly basis, using a HACH water analysis kit (DR/890.

## 4.10 Chemical analysis (Papers I-III)

All feed ingredients used in Papers I-III were evaluated for their proximate chemical composition. Proximate analyses of feed ingredients and experimental diets (Papers II and III) were performed according to commonly used standards. Dry matter (DM) content was determined by oven-drying at 105 °C for 24 h. Ash content was determined by incineration of samples at 550 °C for 4 h. Total nitrogen (N) content was determined using the Kjeldahl method, and crude protein (CP) was calculated as N x 6.25. Crude lipid content (EE) was measured using the Soxhlet method, after acid hydrolysis of the sample, and crude fibre (CF) content was analysed using standard methods according to Official Methods of Analysis (AOC, 2000). Nitrogen-free extract (NFE) was calculated as NFE (%) = 100 - (CP+CL+CF+Ash), according to Castell and Tiews (1980) and gross energy (GE) as GE = 5.72 x CP + 9.50 x EE + 4.79*CF + 4.17 x NFE (g $kg^{-1}$ DM) according to Schiemann *et al*. (1966). Further proximate analysis of the final test diets, amino acids and faeces samples in Paper II was performed. Amino acid content of the diets was analysed by high-performance liquid chromatography (HPLC) according to Vázquez-Ortiz *et al*. (1995). Titanium oxide concentration was measured according to Short *et al*. (1996).

In Paper II, haematological parameters measured were red blood cell count (RBC), white blood cell count (WBC), haematocrit (Hct), haemoglobin (Hb), mean cell volume (MCV), mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC). RBC and WBC were determined using an improved Neubauer haemocytometer (Reichert, Inc., Depew, NY, USA) after blood dilution with phosphate-buffered saline (pH 7.2), according to the haemocytometer manufacturer's instructions, as described by Rusia and Sood (1992). Haematocrit values were determined after centrifuging blood in capillary tubes for 5 min at 12,000 rpm (Nelson & Morris, 1989). Haemoglobin concentration was determined colorimetrically by measuring formation of cyanomethaemoglobin using a spectrophotometer at wavelength 540 nm according to Van Kampen and Zijlstra (1961). Erythrocyte indices (mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC)) were calculated using standard formulae according to Lavanya *et al.* (2011), Bain *et al*. (2017) and Stoskopf (1993) (MCV = Hct/ RBC x10), MCH = Hb/RBC x 10, (MCHC = Hb/Hct x 100).

## 4.11 Calculations (Papers II & III)

In Paper II, the following calculations were performed:

Apparent digestibility coefficient (ADC) of the diets was calculated according to Cho and Slinger (1982) as:

$ADC_{diet}$ (%)= [1- (F/D x Di/Fi)]x100 where F is % nutrient (or kJ $g^{-1}$ gross energy) of faeces, D is % nutrient (or kJ $g^{-1}$ gross energy) of diet, Di is % digestion indicator of diet and Fi is % digestion indicator of faeces.

ADC of the test ingredients was calculated as (Bureau *et al*., 1999):

$ADC_{test\ ingr.} = ADC_{test\ diet.} + [(ADC_{test\ diet} - ADC_{ref.\ diet}) \times (0.7 \times D_{ref}/0.3 \times D_{test\ ingr})]$

where $D_{ref}$ is % nutrient (or kJ $g^{-1}$ gross energy) of reference diet (as-is) and $D_{test\ ingr}$ is % nutrient (or kJ $g^{-1}$ gross energy) of the test ingredient (as-is).

In Paper III, growth performance and biological indices were calculated using the following equations:

Specific growth rate (SGR %/day) = [(ln Wf−ln Wi)/T] ×100, where Wf is final weight, Wi is initial weight and T is time (days)

Protein intake (g) = Feed intake (g) × Protein in the diet (%)

Total feed intake per fish (FI) = [Total feed intake (g)/Number of fish]

Survival rate (SR %) = (TFf/TFi) ×100, where tTFf is total number of fish at harvest and TFi is total number of fish at start

Feed conversion ratio (FCR) = [Total feed intake (g)/Total wet weight gain (g)]

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

Hepato-somatic index (HSI %) = [100 × (Liver weight (g)/Body weight (g))]

Viscero-somatic index (VSI %) = [100× (Viscera weight (g)/Body weight (g))].

## 4.12 Statistical analysis (Papers II & III)

In Paper II, values obtained in the apparent digestibility assessment for DM, OM, CP and GE were encoded into Microsoft Excel worksheets and imported into IBM SPSS STATISTIC (2021) program version 27 software for statistical analysis. The data were subjected to one-way analysis of variance (ANOVA). When appropriate, Duncan's multiple range test was applied to evaluate differences ($p<0.05$) between means. All means were recorded, with standard error of the mean (SEM). Tank was considered a fixed effect, while diet was considered a random effect.

In Paper III, data obtained on growth performance, feed utilisation and body composition were encoded into Microsoft Excel worksheets, imported into IBM SPSS STATISTIC (2011) program version 19 software for statistical analysis. The data were subjected to one-way analysis of variance (ANOVA), and then Duncan's multiple range test was used for comparison of means ($p<0.05$ level of significance). Rearing tank was considered to be the experimental unit and the same method was used for testing all parameters. All means were recorded, ± SEM.

# 5. Main results

## 5.1 Field survey: Aquaculture and aquafeed in Rwanda (Paper I)

### 5.1.1 Pond fish farming description and ownership structure

The results from the field survey are summarised in Table 4. These results were obtained with 212 active fish farmers who participated in the countrywide survey, of whom 79.1% were male and only 20.9% female. Around 75% of respondents were aged between 31 and 55 years, almost 15% were 56 years and above and only 10.4% were young (18-30 years). Approximately 63% of participating fish farms were owned by cooperatives and 27% were privately owned. All respondents were engaged in other side employment as extra source of income, including mining, brick-making, teaching, commerce and other business. More than 70% of the respondents had 4-9 years of fish farming experience (Table 4).

Photo: cooperative ponds

Table 4. *General description of the survey respondents (N = 67) on fish farms in Rwanda*

| Characteristic of respondent* | Category | % of total |
|---|---|---|
| Sex | Male | 79.1 |
| | Female | 20.9 |
| Age | From 16 to 30 years | 10.4 |
| | From 31 to 55 years | 74.6 |
| | From 56 & above | 14.9 |
| Education (level) | Primary | 23.4 |
| | Secondary | 21.9 |
| | Tertiary** | 54.7 |
| Farming experience | Up to 4 years | 12.3 |
| | Between 4 & 9 years | 70.8 |
| | 10 years and more | 16.9 |
| Farmer ownership | Cooperative member | 62.7 |
| | Private owners | 26.9 |
| | Other | 10.4 |

*Respondent was farm owner and/or farm manager/representative. **Ranging from one-year college course to university.

### 5.1.2 Farm practices and management (Paper I)

Most of the fish ponds represented in the survey were located in Northern and Southern Province, followed by Eastern and Western Province and Kigali City. Approximately 98% of fishponds countrywide were earthen ponds, while the remaining 2% were concrete ponds (concrete or plastic-lined). A typical earthen pond was approximately 300 $m^2$ in size.

Three fish species, Nile tilapia (*Oreochromis niloticus*), North African catfish (*Clarias gariepinus*) and common carp (*Cyprinus carpio*), were the only cultured fish species reported countrywide (Table 5). On average, the most common production approach (73.6%) was a tilapia monoculture system. The frequency of this system ranged between the different areas of Rwanda, from 89% of farms in Kigali to 56% in Southern Province (Table 5). Polyculture of tilapia with catfish was most prevalent in Southern Province. A total of 11 active hatcheries were identified countrywide, with the number per province varying between one and three. The majority of the hatcheries were privately owned, run by individuals, had only been in operation for less than 10 years, and produced only Nile tilapia. The maximum production capacity varied between hatcheries, ranging from 160,000 to 480,000 fingerlings annually. Two larger hatcheries (Rwasave and Kigembe), owned by the government, had been operating since the 1950s and produced both tilapia and catfish fingerlings.

No hatchery was producing carp fingerlings at the time of the survey and the few farms culturing carp confirmed that fingerlings were captured from rivers and inland lakes. The majority of tilapia farmers produced their own mixed-sex fingerlings in their monoculture ponds.

The average tilapia stocking density applied was 2-3 fingerlings per $m^2$, regardless of fish size or sex (Table 5). Fingerling feed was reported to be lacking (84.6%) or irregularly available and expensive (1.30-1.60 US\$ $kg^{-1}$, as of September 2020).

Table 5. *Distribution of fish species cultured and tilapia stocking density in different provinces of Rwanda (% of fish farms per province)*

| *Species cultured* | Farm location (province) | | | | |
|---|---|---|---|---|---|
| | Kigali City | Eastern | Southern | Western | Northern |
| Tilapia (%) | 89 | 69 | 56 | 75 | 79 |
| Tilapia and catfish (%) | 11 | 23 | 33 | 25 | 11 |
| Tilapia and carp (%) | | 8 | | | 5 |
| Tilapia, catfish, and carp (%) | | | 11 | | 5 |
| Tilapia stocking density (fish $m^{-2}$) | | | | | |
| 2 | 22 | 50 | 36 | 63 | 79 |
| 3 | 45 | 40 | 31 | 25 | 21 |
| 4 | 22 | 10 | 31 | 13 | |
| 5 | 11 | | | | |

Approximately 81% of farms practised a combination of agro-livestock and fish farming activities in so-called integrated agriculture aquaculture. All farms used organic fertiliser in their ponds to stimulate growth of the natural food web. Supplementary feeding with dry feed was practised by 81% of respondents, with 67% using commercial feeds and 14% using feeds produced on-farm. Feeding frequency varied from 1 to 4 times daily for the grow-out phase and up to eight times daily for fry.

### 5.1.3 Feed ingredient availability and proximate composition

The ingredients most commonly used by fish farmers and local fish feed producers were locally produced maize (*Zea mays*), wheat (*Triticum aestivum*) and rice (*Oryza sativa*), usually in the form of broken grains, bran and middling polishes (Table 6). Other feed resources, such as dried leaf meal from kidney beans (*Phaseolus vulgaris*), cassava (*Manihot esculenta*) and sweet potatoes (*Ipomea batatus*) were used occasionally. Soybean (*Glycine max*) and sunflower (*Helianthus annus*) seeds were an oil source provider, and were also used in the form of meal or cake. Other ingredients available and used to different extents in different provinces included spent brewer's grain, cattle blood, poultry by-products and fishmeal made from local small cichlids (*Haplochromis* spp.) and from small cyprinid sardine species (*Rastrineobola argentea*) and the clupeid *Stolothrissa tanganicae*, mostly imported from Tanzania and Uganda. Fish oil was not used and the farmers surveyed instead used vegetable oils.

During the survey (Paper I), a sample of feed ingredients was collected and analysed in terms of proximate composition. The results showed that the crude protein (CP) content of the ingredients varied between 67 and 701 g $kg^{-1}$ DM. In locally available fishmeal (three fish species), the CP content ranged between 549 and 614 g $kg^{-1}$ DM. Fish oil was exclusively imported and seldom used locally, and was therefore not analysed. In general agricultural by-products, industrial by products and plant leaves had low to medium CP content (<400 g $kg^{-1}$ DM). The content in spent brewer's yeast and spent brewer's grain was 380 and 235 g CP $kg^{1}$ DM, respectively, while in kidney bean and sweet potato leaf meal it was 242 and 318 g CP $kg^{-1}$ DM, respectively.

Table 6. *Feed ingredients used by fish farmers and local fish feed producers in the five provinces of Rwanda*

| Ingredients | Northern | Southern | Eastern | Western | Kigali city |
|---|---|---|---|---|---|
| Plant-origin feed ingredients | | | | | |
| Rice bran | * | * | * | * | * |
| Maize bran | * | * | * | * | * |
| Soybean meal | | * | * | * | * |
| Broken maize | | * | | | * |
| Rice polishes | * | | * | * | * |
| Wheat bran | * | | | * | * |
| Wheat middlings | * | | | * | * |
| Sweet potato leaf meal | * | | * | * | |
| Sunflower cake | | * | * | | * |
| Cotton seed cake | * | * | * | | * |
| Soybean crude oil | | | * | * | * |
| Brewer's grain (or by-products) | * | | | * | * |
| Kidney bean leaf meal | * | | | * | |
| Sweet potato root meal | | | * | * | |
| Sugar cane molasses | * | | | * | * |
| Ripe banana and peels | | | | | * |
| Coffee cherry husks, pulps | | | | * | |
| Groundnut (or peanut) cake | | * | | | |
| Sunflower oil | | * | | | |
| Animal-origin feed ingredients | | | | | |
| Bone meal (cattle) | * | * | * | * | * |
| Blood meal (cattle) | * | * | * | * | * |
| Sea shells | | * | * | * | * |
| Fish meal, *Haplochromis* spp. | * | | * | * | |
| Fish meal, *Stolothrissa tanganicae* | | * | | * | * |
| Fish meal, *Rastrineobola argentea* | * | | * | | * |
| Slaughter waste | * | | * | | |
| Freshwater shrimp meal | * | | | | * |
| Poultry by-product meal | | * | | | |
| Tilapia fish by-products | | | | * | * |
| Snail shells | | | | * | |
| Fish oil | | | * | | * |

*The ingredients are presented in descending order based on abundance for each category

### 5.1.4 Proximate composition of test diets (Papers II & III)

The proximate composition of the diets used in Papers II and III is summarised in Table 7. The results obtained in Paper II showed that DM content ranged from 861 g kg$^{-1}$ in fishmeal (FM) to 925 g kg$^{-1}$ sweet potato leaf meal (SPLM). The highest CP (548 g kg$^{1}$ DM) was found in fishmeal, the lowest in Kidney bean leaf meal (167 kg$^{1}$ DM). The crude lipid content ranged from 170 g kg$^{-1}$ observed in fishmeal to 33 g kg$^{-1}$ found in spent brewer's yeast.

Table 7. *Proximate composition (g kg$^{-1}$ dry matter, DM) of feed ingredients used in the control diet and in test diets for Nile tilapia fingerlings in Papers II and III*

Paper II

| Ingredient | DM | CP | CL | CF | Ash | NFE | OM |
|---|---|---|---|---|---|---|---|
| Fishmeal* | 861 | 548 | 170 | 123 | 17 | 141 | 844 |
| Spent brewer's yeast | 920 | 380 | 33 | 21 | 91 | 516 | 829 |
| Spent brewer's grain | 917 | 245 | 106 | 153 | 76 | 395 | 841 |
| Sweet potato leaves | 925 | 318 | 40 | 130 | 145 | 366 | 780 |
| Kidney bean leaves | 909 | 167 | 35 | 116 | 164 | 618 | 745 |
| Wheat middlings | 878 | 178 | 59 | 84 | 67 | 614 | 811 |
| Cotton seed meal | 904 | 371 | 115 | 169 | 62 | 283 | 842 |
| Maize middlings | 896 | 127 | 165 | 121 | 96 | 491 | 800 |
| Soybean meal | 897 | 382 | 115 | 175 | 82 | 245 | 815 |
| Sunflower seedcake | 916 | 273 | 73 | 158 | 54 | 441 | 862 |
| Rice bran | 903 | 126 | 71 | 159 | 235 | 408 | 668 |
| Blood meal | 914 | 701 | 16 | 12 | 31 | 240 | 883 |

Paper III

| | DM | CP | CL | CF | Ash | NFE | OM |
|---|---|---|---|---|---|---|---|
| Fishmeal* | 861 | 548 | 170 | 123 | 51 | 108 | 810 |
| Spent brewer's yeast | 920 | 380 | 48 | 21 | 91 | 475 | 829 |
| Spent brewer's grain | 917 | 235 | 163 | 130 | 76 | 396 | 841 |
| Sweet potato leaves | 925 | 318 | 40 | 130 | 145 | 367 | 780 |
| Kidney bean leaves | 909 | 242 | 73 | 116 | 164 | 443 | 745 |
| Wheat middlings | 878 | 178 | 59 | 84 | 67 | 612 | 811 |

*CP = crude protein, CL = crude lipid, CF = crude fibre, OM= organic matter.*

## 5.2 Composition and digestibility in Nile tilapia of the diets (Paper II)

The reference diet and the test diets formulated in Paper II were analysed for their proximate composition and amino acids content prior to use in the experiments. Total amino acid content was highest (274.5 g $kg^{-1}$) in the reference diet and diet SBG, and lowest (233.4 g $kg^{-1}$) in diet SPLM. Sum of IAA was highest (131-129 g $kg^{-1}$) in the reference diet and diet SBG, moderate (118-118 g $kg^{-1}$) in diets SBY and KBLM, and lowest (113-110 g $kg^{-1}$) in diets SPLM and WM.

Apparent digestibility coefficient (ADC) for CP in the five test diets showed different patterns (Table 8). The highest apparent digestibility of CP (83.1%) was found in the sweet potato leaf meal diet and the lowest wheat middlings diet (67.7%). The highest GE content was recorded in the reference diet, and the lowest in middlings diet. In general, three of the test ingredients (SPLM, SBG, and SBY) performed better or almost as well as the reference diet. KBLM and WM consistently showed the lowest AD.

Table 8. A*pparent digestibility (% DM) of dry matter, crude protein, crude lipid, organic matter and gross energy, and indispensable and dispensable amino acid (AA, %) content, in the reference diet RD, with fishmeal) and in test diets for Nile tilapia*

| | RD | SBY | SBG | WM | SPLM | KBLM | SEM | p_value |
|---|---|---|---|---|---|---|---|---|
| Dry matter | 87.7 | 87.6 | 87.7 | 89.0 | 86.6 | 90.0 | 0.277 | 0.940 |
| Crude protein | 81.6 $^{b}$ | 77.7 $^{c}$ | 83.0 $^{a}$ | 69.7 $^{e}$ | 83.1 $^{a}$ | 73.1 $^{d}$ | 1.317 | <.0001 |
| Crude lipid | 81.7 $^{a}$ | 78.4 $^{c}$ | 81.4 $^{ab}$ | 76.8 $^{c}$ | 82.6 $^{a}$ | 63.1 $^{d}$ | 1.047 | <.0001 |
| Organic matter | 68.9 $^{a}$ | 69.7 $^{a}$ | 63.7 $^{c}$ | 67.4 $^{b}$ | 69.2 $^{a}$ | 69.4 $^{a}$ | 0.272 | <.0001 |
| Gross energy | 60.0 $^{a}$ | 56.7 $^{a}$ | 57.6 $^{a}$ | 47.7 $^{b}$ | 56.7 $^{a}$ | 48.8 $^{b}$ | 0.268 | <.0001 |
| Indispensable amino acids | | | | | | | | |
| Arginine | 88.6 $^{b}$ | 83.0 $^{c}$ | 87.9 $^{b}$ | 77.3 $^{d}$ | 91.1 $^{a}$ | 81.3 $^{c}$ | 2.299 | <.0001 |
| Histidine | 83.8 $^{b}$ | 81.0 $^{c}$ | 83.9 $^{b}$ | 71.6 $^{e}$ | 86.7 $^{a}$ | 77.1 $^{d}$ | 0.852 | <.0001 |
| Isoleucine | 85.3 $^{b}$ | 82.6 $^{c}$ | 87.8 $^{a}$ | 71.0 $^{e}$ | 87.5 $^{a}$ | 76.7 $^{d}$ | 0.817 | <.0001 |
| Leucine | 82.9 $^{c}$ | 81.2 $^{d}$ | 84.8 $^{b}$ | 73.1 $^{f}$ | 86.4 $^{a}$ | 76.5 $^{e}$ | 0.782 | <.0001 |
| Lysine | 87.5 $^{b}$ | 84.8 $^{c}$ | 85.4 $^{bc}$ | 77.3 $^{e}$ | 89.7 $^{a}$ | 79.7 $^{d}$ | 0.817 | <.0001 |
| Methionine | 84.7 $^{b}$ | 81.7 $^{c}$ | 86.9 $^{ab}$ | 75.0 $^{e}$ | 88.8 $^{a}$ | 77.4 $^{d}$ | 1.627 | <.0001 |
| Phenylalanine | 82.4 $^{b}$ | 80.6 $^{c}$ | 84.3 $^{a}$ | 72.1 $^{e}$ | 85.7 $^{a}$ | 76.0 $^{d}$ | 0.852 | <.0001 |
| Threonine | 78.9 $^{b}$ | 75.9 $^{c}$ | 82.7 $^{a}$ | 68.9 $^{e}$ | 83.8 $^{a}$ | 71.8 $^{d}$ | 1.627 | <.0001 |
| Valine | 81.0 $^{b}$ | 78.6 $^{c}$ | 84.0 $^{a}$ | 70.1 $^{e}$ | 84.5 $^{a}$ | 74.3 $^{d}$ | 1.177 | <.0001 |
| Average | 83.9 | 81.0 | 85.3 | 72.9 | 87.1 | 76.8 | | |
| Dispensable amino acids | | | | | | | | |
| Alanine | 78.1 $^{b}$ | 72.7 $^{c}$ | 83.3 $^{a}$ | 70.4 $^{c}$ | 83.6 $^{a}$ | 72.9 $^{c}$ | 4.232 | <.0001 |
| Aspartic acid | 83.8 $^{b}$ | 80.4 $^{c}$ | 86.4 $^{ab}$ | 75.0 $^{d}$ | 87.0 $^{a}$ | 78.0 $^{c}$ | 2.039 | <.0001 |
| Cysteine +Cystine | 79.9 $^{a}$ | 74.9 $^{b}$ | 79.0 $^{a}$ | 61.1 $^{d}$ | 79.9 $^{a}$ | 70.1 $^{c}$ | 3.602 | <.0001 |
| Glutamic acid | 88.4 $^{b}$ | 86.6 $^{c}$ | 89.8 $^{ab}$ | 78.9 $^{e}$ | 91.4 $^{a}$ | 82.1 $^{d}$ | 0.852 | <.0001 |
| Glycine | 64.2 $^{b}$ | 59.0 $^{c}$ | 77.0 $^{a}$ | 60.9 $^{c}$ | 79.1 $^{a}$ | 64.5 $^{bc}$ | 3.872 | <.0001 |
| Proline | 74.7 $^{c}$ | 71.3 $^{d}$ | 78.8 $^{b}$ | 63.7 $^{f}$ | 81.9 $^{a}$ | 66.8 $^{e}$ | 1.977 | <.0001 |
| Serine | 81.8 $^{b}$ | 78.6 $^{c}$ | 85.3 $^{a}$ | 71.2 $^{e}$ | 86.3 $^{a}$ | 74.9 $^{d}$ | 1.797 | <.0001 |
| Average | 78.7 | 74.8 | 82.8 | 68.7 | 84.2 | 72.4 | | |

Spent brewer's yeast (SBY), spent brewer's grain (SBG) sweet potato leaf meal (SPLM), wheat middlings (WM) and kidney bean leaf meal (KBLM). SEM: standard error of the mean. *Values within columns with different superscript letters are significantly different as determined by Duncan's multiple range test at $p<0.05$.

## 5.3 Haematological indices (Paper II).

In Paper II, the blood collected from fish in each dietary treatment was analysed for white blood cell count (WBC), red blood cell count (RBC), haemoglobin (Hb), haematocrit (Hct), mean corpuscular volume (MVC), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC). The results showed significantly different levels for WBC, Hb and Hct between the experimental diets. The RBC level was highest in diet SPLM and lowest in diet KBLM (2.053 and 1.334 x$10^6$ $mL^{-1}$, respectively), while the WBC level was highest (107.6 x$10^3$ $mL^{-1}$) in KBLM. The Hb level ranged from 5.26 to 7.54 g $dL^{-1}$, in RD and SBY, respectively. The other haematological indices (MCV, MCH and MCHC) did not differ significantly between fish fed the different diets.

## 5.4 Growth performance in Nile tilapia (Paper III)

Body weight gain changes was consistent throughout the 70 days of feeding fish the test diets. From an initial average weight of 28.9±1.88 g $fish^{-1}$, the fish grew to a final weight of 60.2±2.81g $fish^{-1}$ (Figure 7). However, fish fed the control diet showed a higher growth rate than those in other treatments from day 14 to the end of the feeding experiment. Fish fed the diets containing kidney bean leaf meal and wheat middlings (diets KBLM and WM) typically showed lower growth performance throughout the experiment.

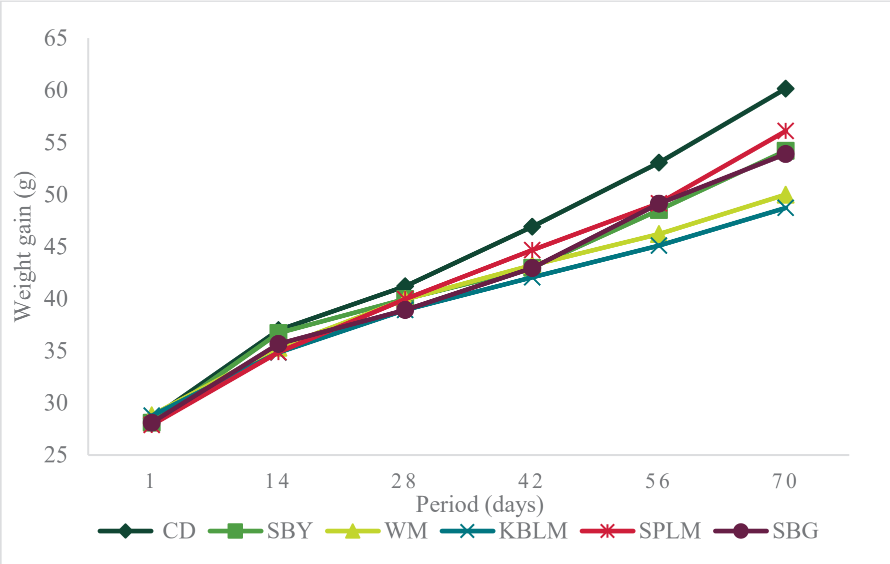


*Figure 7*. Growth performance recorded over the 70-day rearing period of Nile tilapia (*Oreochromis niloticus*) fed the control diet (CD) and test diets based on spent brewer's yeast (SBY), wheat middlings (WM), kidney bean leaf meal (KBLM), sweet potato leaf meal (SPLM) and spent brewer's grain (SBG).

In addition to final and daily weight gain (FWG and DWG), specific growth rate (SGR) was significantly highest in the control diet and SPLM fish, followed by SBY and SBG fish, and lowest in fish fed diets WM and KBLM. Feed conversion ratio (FCR) was highest in KBLM fish. Feed utilisation and body indices (feed intake, protein efficiency ratio, hepato-somatic index), and survival rate showed no significant differences between fish fed the control diet and the test diets (Table 9).

Table 9. *Growth performance, feed utilisation and somatic indices of Nile tilapia (Oreochromis niloticus) fingerlings fed the control diet (CD) and test diets based on spent brewer's grain (SBY), spent brewer's grain (SBG), wheat middlings (WM), kidney bean leaf meal (KBLM), and sweet potato leaf meal (SPLM) for 70 days*

| | CD | SBY | SBG | WM | KBL | SPLM | SE | P-value |
|---|---|---|---|---|---|---|---|---|
| IBW (g) | 27.3 | 28.1 | 29.7 | 29.0 | 29.3 | 27.8 | 1.33 | 0.32 |
| FBW (g) | $60.2^{a}$ | $54.2^{bc}$ | $53.9^{bc}$ | $50.0^{cd}$ | $48.7^{d}$ | $56.1^{ab}$ | 1.17 | 0.01 |
| DWG (g) | $30.7^{a}$ | $25.1^{bc}$ | $24.8^{bc}$ | $21.2^{c}$ | $19.9^{c}$ | $28.3^{ab}$ | 8.51 | 0.01 |
| SGR (%) | $1.10^{a}$ | $0.90^{ab}$ | $0.90^{ab}$ | $0.80^{c}$ | $0.80^{c}$ | $1.00^{a}$ | 0.07 | 0.03 |
| FCR | $1.40^{b}$ | $1.60^{ab}$ | $1.70^{ab}$ | $1.80^{ab}$ | $2.10^{a}$ | $1.40^{b}$ | 0.78 | 0.02 |
| PER | 0.50 | 0.40 | $0.40^{c}$ | 0.40 | 0.30 | 0.50 | 0.08 | 0.36 |
| FI (g) | 41.1 | 39.5 | 40.3 | 36.5 | 40.6 | 39.6 | 0.16 | 0.98 |
| PI | 70.5 | 64.3 | 69.1 | 61.2 | 68.9 | 67.5 | 0.19 | 0.97 |
| VSI (%) | 10.1 | 9.60 | 9.20 | 8.60 | 9.80 | 9.60 | 0.29 | 0.77 |
| HSI (%) | 1.40 | 1.50 | 1.20 | 1.40 | 1.30 | 1.10 | 0.08 | 0.68 |
| SR (%) | 85. 0 | 78.4 | 83.4 | 75.0 | 86.7 | 80.0 | 1.88 | 0.53 |

IBW = initial body weight (g), FBW = final body weight, DWG (g) = daily weight gain, SGR = specific growth rate, FCR = feed conversion ratio, PER = protein efficiency ratio, FI = total feed intake per fish, PI = protein intake, HSI = hepato-somatic, VSI = viscero-somatic (VSI) index, SR = survival rate, SE = standard error of difference of means. Means within rows with different superscript letters are significantly different ($p \leq 0.05$), as determined by Duncan's multiple range test. For all growth and feed utilisation parameters, n = 18.

## 5.5 Water quality

In Papers II and III, water quality parameters recorded during both experiments remained stable and showed no differences between treatments ($p > 0.05$). Mean water temperature (°C) range was 27.1-27.3, pH range was 7.60-7.40, and dissolved oxygen content range was 4.81-5.50 mg $L^{-1}$. The concentration range for total ammonia-nitrogen was 0.23-0.30 mg $L^{-1}$ and that for nitrite was 0.11-0.10 mg $L^{-1}$.

# 6. General discussion

This thesis examined the status of land-based aquaculture in Rwanda, with emphasis on the problem of unsustainable use of fishmeal as a major feed ingredient for Nile tilapia. Rwanda has the lowest fish consumption in Africa, of around 3 kg capita$^{-1}$, compared with an average for the continent of 10.1 kg capita$^{-1}$. People in Rwanda also consume small sardines used in the animal feed industry, as the fish produced by fish farmers are far too expensive for many. By comparing the results obtained in this thesis with earlier reports, it was possible to obtain up-to-date insights on the status of pond aquaculture and aquafeed and the possibility of using food processing by-products and plant-derived ingredients in Rwanda.

## 6.1 Current status and perspectives on aquaculture in Rwanda (Paper I)

The survey in Paper I revealed the status of pond-fish farming across all five provinces of Rwanda and identified a range of currently available local feed ingredients, which were later evaluated for their nutritive value. Over 50% of these fish farms were located in Northern and Southern provinces. More than 60% of the fish farms surveyed were owned by cooperatives and this seems to be the case in many other countries in Africa, *e.g*. aquaculture value chain development in Nigerian Egypt, Uganda and Ghana has been markedly driven by private sector initiatives (Adeleke *et al*., 2021). The majority (79%) of Rwandan fish farm managers surveyed in Paper I were male, as reported previously for Rwanda and Tanzania (Mmanda *et al*., 2020; FAO, 2017), and for sub-Saharan Africa in general (Satia, 2017)). Around 69% of households of Rwanda's current population of 13.2 Million are engaged in agriculture activities (Rphc, 2022), A more equal gender balance in aquaculture could contribute to poverty reduction, improve household decision-making and consequently allow better management of ponds, land and capital (Galiè *et al*., 2015; Johnson *et al*., 2016). Most women working in fish farming in Rwanda are employed downstream, in post-harvest and marketing activities in the aquaculture value chain. Furthermore, young (16-30 years old) fish farmers are not common in Rwanda (only 10%), but this level may increase considering the proportion of the labour force made up of young people aged 16-24 years (females 43.1% and males 50.6% in 2020). This age group is increasing faster than the adult population aged 25 years and above, and over 50% of the population in Africa is below 25 years of age while in Rwanda the proportion of young people (below 30 years) were 65.3% in 2022 (Rphc, 2022;FAO, 2014; NISR, 2011).

In terms of fish species diversity, tilapia was found to be by far the most farmed species in Rwanda (89%) (Paper I). Compared with earlier findings (Hishamunda *et al*., 1996; MINAGRI, 2011), the survey revealed an increased number of hatcheries in Rwanda, and thus a substantial improvement in the fingerling supply countrywide. Tilapia was mainly produced in monoculture systems, which agrees with previous findings in East African countries (Charo-Karisa *et al*., 2006; Mbugua, 2002; Vincke, 1987). African catfish, the second most farmed species, was produced in both monoculture and polyculture systems.

Most of the farms surveyed (80%) practised a combination of agro-livestock and fish farming activities, using only organic fertilisers in their ponds. The main fertilisers used were dried grasses, maize and rice stalks, and rabbit and poultry faeces. These fertilisers undergo decomposition to release mainly the three primary plant nutrients (NPK) to stimulate phytoplankton photosynthesis that is the base of the food web culminating in fish production (Boyd, 2018). According to Bhujel (2013), the daily recommended fertilisation rate for tilapia fish ponds is 0.4 g nitrogen and 0.1-0.2 g phosphorus per $m^2$. Rwandan farmers need recommendations on fertilisation and on how to assess satiation or fertiliser dose and thus avoid over- or under-fertilising their ponds. Fertilization remains an important practice for smallholder farmers in Rwanda, but also in other developing countries, and usually results in two to fivefold increase in aquaculture production (Boyd, 2018). Tilapia cultured in water with high primary production of natural feed can be supplemented with simple feed with imbalanced nutrient content (Nguyen *et al*., 2018).

Lack of fry and fingerling feed is a major constraint faced by hatchery operators in Rwanda. Fish feed has been a bottleneck for many African countries (Moyo & Rapatsa, 2021), even in the most developed aquaculture countries (Egypt and Nigeria) (Adeleke *et al.*, 2021; Dickson *et al.*, 2016). The low level of aquaculture in sub-Saharan countries, including Rwanda, may be due to lack of quality feedstuffs and poor feed supply chains, lack of skilled manpower, poor management and use of outdated technology and methods (MINAGRI, 2020; FAO, 2020). Commercial fingerling feeds are rare and expensive, and thus the price of fingerlings is high for many smallholder farmers. To cope with this situation and lower the feed costs in fingerling production, most hatchery owners use homemade feed, instead of imported good quality feed. Others mix a portion of commercial feed with available low-cost single ingredients, such as rice bran or wheat bran (Paper I). According to Edwards and Allan (2004), comparable strategies are applied by farmers in the Mekong region of Vietnam. Based on the results obtained in this thesis, there is potential to increase production and quality of catfish and tilapia fingerlings, but also to diversify and supply the carp fingerlings needed by farmers.

The survey results also showed also that, in the prevailing semi-intensive earthen pond system in Rwanda, the ponds are predominantly stocked with mixed-sex tilapia fingerlings. Male monosex tilapia culture is preferred, due to faster growth in males than females. In males, metabolic energy is channelled toward growth and anabolic growth-enhancing androgens are produced (Angienda *et al*., 2010; Tran-Duy *et al*., 2008). Monosex male tilapia suppliers are still scarce in Rwanda, and therefore it is important that hatchery operators develop the capacity to progressively provide them to farmers, to improve tilapia production in Rwanda.

Fish stocking density is a key factor in the optimal management of farmed fish. The survey found that prevailing stocking density was 2-3 fingerlings per $m^2$. Increasing the stocking density to about 3-4 fingerlings per $m^2$ in semi-intensive systems would have no adverse effect on yield (Hishamunda *et al*., 1996). The stocking density can be optimised to match the overall cultivation strategy, and is influenced by the desired final fish size at harvest (Shumway & Parsons, 2016). Increasing the stocking density 4-5 fish $m^{-2}$ in semi-intensive ponds farms in Rwanda would increase the yield, provided that adequate supplementary feeding is provided. Early-adopter farmers in the survey reported a marked increase in fish yield.

## 6.2 Feed resources and aquafeeds (Papers I-III)

To obtain information about the feed ingredients used by farmers, as identified in the survey, 31 ingredients were collected and five ingredients were evaluated against a fishmeal control for their suitability and effect on fish growth. In general, the nutritive value of these local feed ingredients was within the range previously reported for feedstuffs in the East Africa region (Mmanda *et al*., 2020; Munguti *et al*., 2012; Nyina-wamwiza *et al*., 2007). This shows that a range of ingredients widely available in Rwanda can potentially be used in fish feed formulation. Many of the feed ingredients found in Rwanda are also found elsewhere in the world and have been tested in a mixture, as partial or total replacers for fishmeal, for different fish species (El-Saidy & Gaber, 2003; Khan *et al*., 2013; Liti *et al*., 2006). Based on analysis of proximate composition, all grain by-products had a low content of CP (<178 g $kg^{-1}$ DM), but a high content of NFE (408-684 g $kg^{-1}$ DM). However, the fat content (EE) in sunflower oil cake (73 g $kg^{-1}$ DM) differed considerably from that (244 $kg^{-1}$ DM) reported by Mmanda *et al*. (2020). The CP content in sweet potato leaf (318 g $kg^{-1}$) was comparable to that reported in previous studies (Munguti *et al*., 2012). Nutritive value may differ depend on the method used for analysis, but probably also depends on natural variations in *e.g*. the growing environment and leaf maturity stage at harvest (McDonald *et al*., 2002; Church, 1980).

Fishmeal used in Rwanda was from relatively low-grade fish species, mainly sun-dried sardines (*Rastrineobola argentea* and *Stolothrissa tanganicae*, both known locally as '*ndagaa*'). The proximate composition of fishmeal used in Papers II and III was consistent with most previous findings for East Africa (Munguti *et al*., 2012; Mmanda *et al*., 2020). These fish are also used directly as human food and their use in fish feeds is thus unsustainable.

Spent brewer's yeast, the second major by-product from the brewing industry in Rwanda, was found to be readily available and had proximate composition featuring high CP content (380 g $kg^{-1}$ DM). Thus, as in many other countries, it is a potential high-volume alternative to fishmeal protein in the diet of Nile tilapia in Rwanda (Agboola *et al*., 2021; Nhi *et al*., 2018).

Spent brewer's grain is also widely available but displayed moderately low CP content (235g $kg^{-1}$ DM), and a high content of NFE (408-684 g $kg^{-1}$ DM). Efficient use of the alternative ingredients identified, considering their proximate composition, could decrease fishmeal use and allow production of low-cost fish feeds, contributing to sustainable aquaculture production and improved food security in Rwanda.

The most commonly used ingredients in semi-intensive pond systems according to the survey in Paper I included rice, wheat and maize bran. Feeding frequency applied by farmers differed across farms. Feeding twice per day was the most common for grow-out ponds. Tilapia have a relatively small stomach and display continuous foraging behaviour, so multiple feeding can improve growth and feed efficiency (NRC, 2011; Shiau, 2002). Feeding frequency is key for cultured fish, as it can affect overall growth, survival and yield (Macintosh & Little, 1995; Sanches & Hayashi, 2001). For profitable fish farming, farmers should apply an appropriate feeding strategy that considers pellet size and feeding rate, but should also grade the fish prior to their stocking (Creswell, 2005; Saoud *et al*., 2008). The survey showed that the grow-out period ranged from six to nine months, and in most cases harvested fish were sold at the farm gate as fresh whole (Paper I). However, most tilapia grow-out ponds in Rwanda comprise mixed sexes and ages, so it can be challenging to determine the optimum pellet size and the precise amount to utilise for adequate feeding, which could also influence the harvesting period. Quantifying the contribution of naturally available food organisms in fertilised pond systems is difficult, and hence adequate feeding remains a challenge for cultured fish species (Rahman *et al*., 2006; Spataru *et al*., 1983; Veverica *et al*., 2000).

## 6.3 Digestibility of diets (Paper II)

The five ingredients analysed for digestibility were spent brewer's grain (SBY), spent brewer's grain (SBG), wheat middlings (WM), kidney bean leaf meal (KBLM), sweet potato leaf meal (SPLM) and the fishmeal used in the reference diet (RD). Digestibility values are crucial for obtaining accurate matrix values for different ingredients in feed formulation, as diets are formulated based on digestible nutrients rather than the chemical composition of ingredients (Glencross *et al*., 2021). The nutritive value of a formulated diet depends on the digestibility of each ingredient, but also on interactions between ingredients (Abro, 2014; Sørensen, 2012). The nutritional value of feed and the effect of diet composition on absorption are reflected in digestibility values (Koushik Roy & Mraz, 2021). Dietary treatment formulations in Papers II and III generally followed the concept that most aquafeeds used today are made with multiple ingredients instead of a single protein source, which allows the creation of complementary nutritional profiles from multiple protein sources (Tacon *et al*., 2011).

Apparent digestibility of crude protein ($AD_{CP}$, %) was high for diets SPLM, SBG, RD and SBY (83.1%, 83.0%, 81.6% and 77.7%, respectively) but relatively low for diets KBLM and WM (73.1% and 69.7%, respectively). In general, the test diets showed acceptable $AD_{CP}$ (range 69.7-83.1%), with equivalent or higher levels than most $AD_{CP}$ values reported previously for Nile tilapia (Mmanda *et al*., 2020; El shafai *et al.,* 2004; Hanley, 1987). Of the different diets tested, diet SPLM had the highest digestibility of amino acids ($AD_{AA}$), even better than the reference diet. Sweet potato leaf meal has been successfully used previously as feed for Nile tilapia and hybrid catfish (Tram *et al*., 2011), and in livestock feed (Nguyen *et al*., 2012; Hue *et al*., 2010; Phuc & Lindberg, 2000). Differences observed in the amino acid content of test ingredients and in diet digestibility could be due to various factors, including feeding and the individual fish (*e.g*. species, age, gut health and physiological status) (Lall & Dumas, 2015; NRC, 2011). However, the lower digestibility values observed for kidney bean leaf meal and wheat middlings (Paper II) may impose limitations on their use in feed formulations for possible replacement of fishmeal in the diet of Nile tilapia.

## 6.4 Growth performance and feed utilisation.

The growth performance observed in this thesis, *e.g*. FCR range 1.4-2.4, was similar to that commonly reported for tilapia (Fry *et al*., 2018). Growth performance may vary due to differences in nutritional quality or properties between feed ingredients used, size and age of fish, and culture systems, but also due to environmental conditions, feeding duration and other unknown factors (Nhi *et al*., 2018; Liti *et al*., 2006). Tilapia fed SPLM (up to 32% replacement) showed equally good growth performance as fish fed the control diet (CD), which indicates that SPLM is a suitable feed ingredient for tilapia fingerlings. In addition to rather good crude protein content (up to 310 g CP $kg^{-1}$ DM) identified in this thesis, SPLM is known to have high nutritive value (Ishida, 2000; Woolfe, 1992). Sweet potato leaf contains various bioactive compounds (Nguyen *et al*., 2021) and several essential minerals (iron, calcium, magnesium) and trace elements (chromium, cobalt, nickel, copper, zinc) (Taira *et al*., 2013). Brewer's by-products (SBG, SBY) showed satisfactory growth performance relative to the control diet. For instance, up to 50% fishmeal, replacement with SBY did not affect fish performance, as evidenced by high SGR and FCR, and also WG and FWG similar to that in fish fed the control diet. These results corroborate earlier findings on tilapia reported by (Islam et al., 2021, Abdel-Tawwab et al., 2020), and (Nhi et al., 2018). Furthermore, SBY has a high protein content and favourable amino acid profile, and contains important bioactive compounds such as β-glucans, nucleic acids, mannans oligosaccharides *etc*., which can substantially improve fish growth and health (Vidakovic *et al*., 2020; Øvrum Hansen *et al*., 2019; Ferreira *et al*., 2010). The results obtained in this thesis are consistent with previous findings showing that tilapia, which is an omnivorous fish, can efficiently utilise feed from different sources, such as plant, animal and microbial origin (Felix *et al*., 2020; Adewolu, 2008); El-Sayed, 1999). The slight variation in some growth and feed utilisation indices observed for fish fed diets containing brewery by-products compared with fish fed the control diet could be due to factors including the type of barley, malting and mashing conditions, and additives used during beer processing, in different breweries (Santos *et al*., 2003), or even between batches within the same brewery (Gallone *et al*., 2018).

In contrast, fish fed diets KBLM and WM showed consistently decreasing growth (final weight, weight gain, specific growth rate), possibly due to the presence of anti-nutritional factors such as phytate, trypsin inhibitor, phytohaemagglutinin and other compounds commonly found in cereals and legumes, which reduce the bioavailability of nutrients and minerals and thus affect growth and fish health (Ram *et al.*, 2020; Vasconcelos & Oliveira, 2004; Francis *et al*., 2001). Tannin, oxalate, and phytate have been detected in bean leaves (Alalade *et al*., 2016). The results obtained in this thesis indicated that KBLM and WM should not be included as feed ingredients or should be kept at low levels in diets so as not to affect growth of tilapia.

Blood indices have been used previously as biological indicators reflecting animal health status, physiological status, signs of stress originating from disease feed and environmental conditions (Blaxhall, 1972; Taira *et al*., 2013; Harikrishnan *et al.*, 2011). The results obtained in this thesis showed no significant differences between diets in terms of MCV, MCH or MCHC, which are valuable in morphological classification of anaemia (Grant, 2015). Although the interpretation of blood information requires caution, since particular physiological perturbations do not necessarily depend on a given experimental protocol, the highest RBC, Hct and Hb values found in SPLM seemingly confirm its potential as a suitable ingredient in tilapia feed. Red blood cells contain Hb, which supplies oxygen to all body tissues, so Hb level determines fish endurance (Qiang *et al*., 2013). In the feed ingredients tested in this thesis, RBC level was highest in SPLM and lowest in KBLM (2.053 and 1.334 $x10^6$ $mL^{-1}$, respectively, while WBC level were highest in KBLM (107.6 $x10^3$ $mL^{-1}$). The low values for RBC, Hct and Hb recorded in KBLM may indicate possible negative effects of kidney bean leaf meal on blood physiology in tilapia. Phytohaemagglutinin (PHA), a lectin or plant protein found mostly in red kidney bean and whole grains like wheat, may be the cause (Nagae *et al*., 2014). Thus, it can be concluded that despite being potentially low-cost and abundant ingredients, kidney bean leaves and wheat middlings are not good candidates as tilapia feedstuffs in Rwanda, at least at the levels evaluated here.

## 6.5 Water quality parameters

Optimum water quality plays a significant role in the biology and physiology of fish and requires continuous oversight in aquaculture systems (Abdel-Tawwab *et al*., 2019). The temperature and dissolved oxygen content used in experiments in this thesis were set at the optimum level for tilapia. Water temperature was thermostatically controlled and stabilised at around 28 °C, the optimal temperature for tilapia growth (Azaza *et al*., 2008). The concentrations of nitrogenous compounds remained at minimum levels and thus did not affect the performance of fish (Papers II and III). Deviations in water quality in the culture environment of tilapia can affect fish health, for instance it can result in retardation in growth, mortality or some harmfully physiological responses like osmo-regulatory disturbances and kidney damage (Zeitoun *et al*., 2016; Yanbo *et al*., 2006). However, the tolerance of cultured fish to water quality deviations depends on different factors, including species, size and health status (Mustapha & Atolagbe, 2018). In general, water quality parameters remained stable through the experiments in Papers II and III, and were acceptable for good performance of the tilapia fingerlings. Thus, any difference arising between treatments were not due to rearing water quality parameters.

# 7. General conclusions

- Semi-intensive earthen pond farming of mixed-sex tilapia is the predominant culture system in Rwandan aquaculture.

- Most Rwandan fish farms combine agro-livestock and fish farming activities and use only organic fertilisers in their ponds.

- Tilapia is by far the most common farmed species in Rwanda.

- Most Rwandan fish farms are located in Northern and Southern province.

- Fishmeal used in Rwanda is made from relatively low-grade sun-dried sardines (*Rastrineobola argentea* and *Stolothrissa tanganicae*, both known locally as '*ndagaa'*) that are also used directly as human food, and are thus unsustainable as fish feeds.

- Compared with a fishmeal-based reference diet, apparent digestibility of indispensable amino acids was higher in a diet where fishmeal was replaced with sweet potato leaf meal and lower in diets where fishmeal was replaced with wheat middlings and kidney bean leaf meal.

- Apparent digestibility of crude protein, crude lipid and gross energy was higher in diets where fishmeal was replaced with sweet potato leaf meal and spent brewer's by-products, which appear to be suitable ingredients for tilapia diets.

- Three of the test ingredients (sweet potato leaf meal, spent brewer's yeast, spent brewer's grain) performed better or almost as well as the reference diet.

- Analysis of blood samples showed the lowest red blood cell count, haematocrit and haemoglobin concentrations, and highest white blood cell count, for kidney bean leaf meal, a possible indication of negative effects of this feedstuff on blood physiology in tilapia.

- Efficient use of identified local ingredients in respect of their nutritive values could decrease fishmeal use and reduce fish feed costs, contributing to sustainable aquaculture production and improved food security in Rwanda.

# 8. Future perspectives

The number of fish farming operations in Rwanda is continually increasing, leading to high demand for quality fish feed at affordable prices. This thesis identified and analysed local ingredient sources in Rwanda that had not been investigated previously. Analyses showed that these feed ingredients could partly replace fishmeal in tilapia diets to up to 50% of dry matter without affecting fish growth performance. In order to build upon these findings, future studies should address the following issues relating to tilapia farming in Rwanda:

- Perform a feed cost analysis of replacing fishmeal and soybean meal with local ingredients in feed for tilapia in Rwanda. This information is needed to compare imported tilapia feeds with locally produced feeds.

- Investigate the effects of novel, non-conventional feed ingredients available locally or with scope to be produced locally, including earthworm meal and black soldier fly larvae as sustainable fish feeds; and conduct an economic evaluation and comparative analysis to identify pros and cons of their mass production and use.

- Provide baseline data on floating cage farming in Rwanda. This sector is developing and mapping its food contribution and environmental implications is important.

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# Popular science summary

The growing global human population is projected to reach approximately 10 billion by 2050, resulting in an estimated 40-75% increase in total demand for food protein (rising to 72% in developing countries), of which protein from fish is predicted to make up a significant part. Wild-capture fishery harvests have remained basically flat since the late 1980s and have been unable to satisfy growing demand. However, global seafood consumption has almost doubled in recent decades, due largely to increasing aquatic production, mainly of farmed fish. This has increased demand for fish protein in the diets fed to farmed fish, and the sector will continue to require more fish protein in future as it expands further. However, the fish protein currently used in aquaculture mainly consists of fishmeal made from either small fish or from discarded products from wild stocks, but the supply of these fish-based resources has decreased in recent years. This practice has also been criticised as unsustainable and finite, since it increases pressure on wild stocks and reduces biodiversity. In addition to its use in aquaculture, fishmeal is also widely used as feed for poultry and other animals, but most could be used as human food. This competition for fish-based resources makes fish feed expensive, especially in land-locked countries such as Rwanda.

In Rwanda, as in many other developing countries, substantial future development of the aquaculture sector will require increased availability of quality fish feed at affordable prices. This can best be achieved if locally available feed ingredients are identified and used in fish feed formulation in these countries.

Prior to the work in this thesis, there was only outdated and incomplete information about various locally available feed ingredients in Rwanda. The aim of the thesis was thus to identify, sample and evaluate the nutritive value

of some locally available feed ingredients that could be used by fish farmers producing Nile tilapia, a common farmed fish species in Rwanda. As a first step, the current status of fish farming in Rwanda was surveyed, since such information is of great importance for the development of future fish farming in Rwanda. An initial countrywide survey revealed that semi-intensive fish farming was the most common system in Rwanda (81% of the total), that over 60% of pond fish farms were owned by cooperatives, and that 79% of fish farm managers were male. Only three fish types were cultured, of which Nile tilapia was by far the most common, followed by African catfish and common carps. Lack of quality feed, mainly for fingerlings, was reported by Rwandan fish farmers to be a constraint on production. More than 30 local ingredients were identified in the survey, including cattle blood meal, agro-industrial wastes such as spent brewer's grain and spent brewer's yeast, agricultural by-products such as wheat middlings, maize bran and rice bran, and different types of leaves (sweet potato, cassava, kidney bean) used to make meals. All these appeared to be widely available and very cheap to use. Moreover, by-products from the brewing industry are high in protein, while cereal by-products and sweet potato and kidney bean leaves are not used as human foodstuffs and can be a possible replacement for fishmeal in farmed fish diets.

However, before these local ingredients can be used in confidence in fish feed, they need to be evaluated in terms of their nutrient content, cost, feed acceptability and digestibility, and growth performance of fish fed diets containing the ingredients. Some previous studies have revealed that feeding high amounts of agro-industrial by-products and leaves may reduce fish growth. Among the locally available ingredients with relatively high protein content identified in the countrywide survey in Rwanda, spent brewers' yeast, spent brewer's grain, wheat middlings, sweet potato leaf meal and kidney bean leaf meal were selected for further analysis in this thesis. Experiments were performed to measure the digestibility and growth performance of tilapia fed these ingredients, in order to determine their nutritive value and suitability for use in aquaculture.

The results showed that spent brewer's yeast can replace up to 50% of fishmeal in the diet of Nile tilapia without impairing fish growth and yield, while spent brewer's grain and sweet potato leaf meal can replace up to 32% of fishmeal. However, kidney bean leaf meal and wheat middlings resulted in poor performance outcomes and are not recommended as suitable replacers.

Overall, the results presented in this thesis show the current status of aquaculture in Rwanda and available feed ingredients and their chemical composition. They also show that inexpensive quality tilapia feed can be produced locally through a change in feeding strategy, by replacing fishmeal with non-conventional local ingredients such as sweet potato leaf meal, brewer’s yeast and grain by-products. This novel information should be used in future development of sustainable fish farming in Rwanda and for reducing the current use of fishmeal in farmed fish feed.

# Populärvetenskaplig sammanfattning

Världens ökande befolkning beräknas uppgå till cirka 10 miljarder år 2050, vilket resulterar i en uppskattad ökning på 40-75 % av den totala efterfrågan på matprotein (upp till 72 % i utvecklingsländer), varav protein från fisk förutspås utgöra en betydande del. Fångster av vilt fisk har varit i stort sett oförändrade sedan slutet av 1980-talet och har inte kunnat tillgodose den växande efterfrågan. Den globala konsumtionen av sjömat har nästan fördubblats under de senaste decennierna, till stor del beroende på ökad akvakultur främst av odlad fisk. Detta har ökat efterfrågan på fiskprotein i foder till odlad fisk och sektorn kommer att fortsätta att kräva mer fiskprotein i framtiden när den expanderar ytterligare. Det fiskprotein som idag används inom vattenbruket består dock huvudsakligen av fiskmjöl tillverkat av antingen småfisk eller från kasserade produkter från vilda fiskbestånd, men tillgången på dessa fiskbaserade resurser har minskat de senaste åren. Denna praxis har också kritiserats som ohållbar och ändlig, eftersom det ökar trycket på vilda bestånd och minskar den biologiska mångfalden. Förutom att det används i vattenbruk används fiskmjöl också i stor utsträckning som foder för fjäderfä och andra djur, men det mesta skulle kunna användas som mat för människor. Denna konkurrens om fiskbaserade resurser gör fiskfoder dyrt, särskilt i inlandsstater som Rwanda.

I Rwanda, liksom i många andra utvecklingsländer, kommer en betydande framtida utveckling av vattenbrukssektorn att kräva ökad tillgång på kvalitetsfiskfoder till överkomliga priser. Detta kan bäst uppnås om lokalt tillgängliga foderingredienser identifieras och används i fiskfoderformulering i dessa länder.

Före arbetet med denna avhandling fanns det endast föråldrad och ofullständig information om lokalt tillgängliga foderingredienser i Rwanda. Syftet med avhandlingen var därför att identifiera och utvärdera näringsvärdet av några lokalt tillgängliga foderingredienser som kan användas av fiskodlare som producerar Niltilapia, en vanligt odlad fiskart i Rwanda. Som ett första steg kartlades fiskodlingens nuvarande status i Rwanda, eftersom sådan information är av stor betydelse för utvecklingen av framtida fiskodling i Rwanda. En första landsomfattande undersökning visade att semi-intensiv fiskodling var det vanligaste odlingssystemet i Rwanda (81 % av det totala), att över 60 % av dammfiskodlingarna ägdes av kooperativ och att 79 % av fiskodlingarna var män. Endast tre fisksorter odlades, varav Niltilapia var den absolut vanligaste, följt av afrikansk ålmal och vanlig karp. Brist på kvalitetsfoder, främst till fiskyngel, rapporterades av Rwandiska fiskodlare vara ett hinder för produktionen. Mer än 30 lokala ingredienser identifierades i undersökningen, inklusive nötkreatursblodmjöl, agro-industriellt avfall som drav (förbrukad spannmål från bryggerier) och förbrukad bryggerijäst, jordbruksbiprodukter som vetekli, majskli och riskli och olika typer av blad (sötpotatis, kassava, kidneyböna) används för att göra fiskfoder. Alla dessa verkade vara allmänt tillgängliga och mycket billiga att använda. Dessutom har biprodukter från bryggeriindustrin höga halter av protein, medan spannmålsbiprodukter och sötpotatis- och kidneybönsblad inte används som livsmedel till människor och kan vara en möjlig ersättning för fiskmjöl i fiskfoder till odlad fisk.

Men innan dessa lokala ingredienser kan användas med säkerhet i fiskfoder, måste de utvärderas med avseende på näringsinnehåll, kostnad, foderacceptans, smältbarhet och tillväxtprestanda för fiskfonder u som innehåller ingredienserna. Vissa tidigare studier har visat att utfodring av stora mängder agro-industriella biprodukter och blad kan minska fiskens tillväxt.

Bland de lokalt tillgängliga ingredienser med relativt högt proteininnehåll som identifierats i den landsomfattande undersökningen i Rwanda, valdes förbrukad bryggerijäst, förbrukad bryggerisäd, veteklil, sötpotatisbladsmjöl och mjöl av njurbönor för vidare analys i denna avhandling. Experiment utfördes för att mäta smältbarheten och tillväxtprestandan för tilapia som matats med dessa ingredienser, för att fastställa deras näringsvärde och lämplighet för användning i vattenbruk.

Resultaten visade att förbrukad bryggerijäst kan ersätta upp till 50 % av fiskmjölet i kosten till Niltilapia utan att försämra fiskens tillväxt och avkastning, medan förbrukat bryggerimjöl och sötpotatisbladmjöl kan ersätta upp till 32 % av fiskmjölet. Emellertid resulterade njurbönbladsmjöl och vetemjöl i dåliga resultat och rekommenderas inte som lämpliga ersättningsmedel.

Sammantaget visar resultaten som presenteras i denna avhandling den aktuella statusen för vattenbruket i Rwanda och tillgängliga foderingredienser och deras kemiska sammansättning. Avhandlingen visade också att billigt tilapia-foder av hög kvalitet kan produceras lokalt genom en ändrad utfodringsstrategi, genom att ersätta fiskmjöl med icke-konventionella lokala ingredienser som sötpotatisbladmjöl, bryggerijäst och spannmålsbiprodukter. Denna nya information bör användas i framtida utveckling av hållbar fiskodling i Rwanda och för att minska den nuvarande användningen av fiskmjöl i foder till odlad fisk.

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God bless you All!

# Appendix

Tilapia blood sampling


Rwasave fish farm and research station /University of Rwanda

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# Aquaculture and aquafeed in Rwanda: current status and perspectives

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**ABSTRACT**

A survey was conducted to obtain up-to-date information and create a knowledge base on pond fish farming, local feed ingredients, and their nutritive properties in Rwanda. Sixty-seven pond-farms were randomly sampled from a population of 112 countrywide. Semi-intensive was the dominant (81%) farming-system and three fish species were cultured: Nile tilapia (*Oreochromis niloticus*), common carp (*Cyprinus carpio*), and North African catfish (*Clarias gariepinus*). Tilapia was the most commonly farmed species, and >50% of pond-farms were located in Northern and Southern provinces. There were 1–3 hatcheries per province, all producing tilapia. In total, 31 feed ingredients were identified, with rice, wheat, and maize bran being most commonly used. Feed analysis revealed high protein content (>350 g $kg^{-1}$ CP) in local fishmeal, chicken viscera, and spent brewer's yeast. Local ingredients and potential novel-feeds need further investigation prior to their confident use in fish diets to improve aquaculture at a low-cost in Rwanda.

**KEYWORDS**

Fish farming; pond-aquaculture; local feed ingredients; semi-intensive farming system; survey; Rwanda

## Introduction

Globally, aquaculture is the fastest-growing animal food production sector which now provides over 50% of fish for human consumption and expected to continue to increase in the long term. Aquaculture has high potential to help meet the increasing global demand for aquatic foods created by worldwide population growth (FAO 2021; Stevens et al. 2018). In Africa, fish farming production is about 2.7% of the world fish farming production (Halwart 2020), led by Egypt, the largest producer (with 8.4% growth rate in the period 2009–2018) (FAO 2020). Additionally, SSA aquaculture has been led by countries like Nigeria, Uganda and Ghana, and has grown significantly over the last decade, from 106,000 tonnes in 2000 to 709,000 tonnes in 2018, with a farm-gate value of about USD

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1.68 billion (FAO 2021). Most of the fish production in Africa comes from fresh water systems (99%) where tilapia and African catfish are the major cultured fish species (Adeleke et al. 2021).

Rwanda has the lowest per-capita consumption of protein in East Africa, far below the FAO recommendation for the world's average of 32 g/capita/day at population level (FAO 2018b). Aquaculture is one way to increase the supply of high-value animal protein to the Rwandan population.

In Rwanda, aquaculture started in the 1940s as small-scale extensive pond farming. Since 1948, the nascent fish farming sector was fostered by the Belgian colonial administration (Dadzie 1992; FAO/UNDP 1981). In the 1960s-1970s, the main species raised in small-scale extensive pond systems in Rwanda were redbreast tilapia *(Coptodon rendalli)*, longfin tilapia (*Oreochromis macrochir*), Nile tilapia (*Oreochromis niloticus*), smoothhead catfish (*Clarias liocephalus*), and common carp (*Cyprinus carpio*) (FAO/UNDP 1981). Common carp (*Cyprinus carpio Linnaeus, 1758*) was introduced to Rwanda from Israel in 1960 for aquaculture, while other carp strains were introduced from Uganda, and thus there are established carp populations in Rwandan rivers and lakes (De Vos, Snoeks, and Van Den Audenaerde 2001; Welcomme 1988). Data on aquaculture in Rwanda are scarce and outdated. Previous investigations date back to the 1980s-1990s, focus on socio-economic aspects, and consider only some parts of the country (Engle, Brewster, and Hitayezu 1993; Hishamunda, Curtis, and Upton 1998; Molnar, Rubagumya, and Adjavon 1991).

To date, Rwandan fish farmers practice extensive and semi-intensive farming by fertilizing their ponds with animal manure and dry grasses collected around the ponds, to support primary productivity in fishponds and enhance autotrophic and heterotrophic fish food production (El-Sayed 2006). In semi-intensive tilapia farming system supplemental feeding is required for tilapia farmed at densities >3 fish per $m^{-2}$, as fish stocking density is an important factor that can potentially affect the amount of natural food available per fish in fertilized ponds (Bhujel 2013). Suitable timing for supplemental feeding is to grow the fish up to 100–150 g with fertilizers alone, followed by provision of supplemental feeds to 50% satiation (Diana, Lin, and Yi 1996). In intensive systems, fish feed is the single largest operating expense (50–70%) (Rumsey 1993), and has been identified as a significant limiting factor (FAO 2006). Protein is the most restricted nutrient in fish feed and, compared with adult fish, fry, and fingerlings have a higher protein requirement (30–40%) for optimal performance (Abdel-Tawwab et al. 2010; Siddiqui, Howlader, and Adam 1998). Fry and fingerlings are mostly fed with floating extruded pellets to obtain higher growth. An appropriate feeding management strategy should consider pellet size, feeding rate, and size grading before stocking, to improve commercial returns for farmers (Creswell 2005; Saoud et al. 2008). Use of fish oil in aquafeeds has raised sustainability concerns, prices have increased 3- to

4-fold in the past two decades alone and there is no foreseeable return to lower levels, and the supply from wild marine forage fish is being exceeded by growing demand, constituting an obstacle to aquaculture expansion (FAO 2016, 2018; Pauly and Zeller 2016). Potential fish oil substitutes include plant oils, stearidonic acid, and algae oils (Lenihan-Geels, Karen, and Ferguson 2013). Novel feeds such as spent brewer's yeast (*Saccharomyces cerevisiae*), earthworm species such as *Eisenia foetida*, and various fly larvae have high nutritive value, high digestibility, and good essential amino acid (EAA) content (Bondari and Sheppard 1981; NRC, 2011; Sogbesan and Ugwumba 2007; St-Hilaire et al. 2007). These materials could thus be used as a sustainable protein source in fish feed. Previous studies in Rwanda identified several local feed ingredients with potential in African catfish and tilapia aquaculture, including soybean meal, cotton seed cake, sunflower oil cake, and groundnut oil cake (Munguti et al. 2012; Nyina-wamwiza, Wathelet, and Kestemont 2007).

Information on currently farmed species, pond-farm practices and management, locally available fish feeds, the nutritional value of feed ingredients, and other key inputs such as fingerling availability for aquaculture in different provinces of Rwanda is scarce or lacking. In the present study, a survey on aquaculture status was conducted to fill information gaps and identify currently available local feed ingredients. Laboratory analyses were performed to determine the nutritive value of these feed ingredients. The aim was to contribute baseline data on supplying future high-quality fish feed to support increased fish farming in Rwanda.

## Material and methods

### *Study sites*

The study was conducted in all five provinces of Rwanda (Northern, Southern, Eastern, Western, Kigali City), subdivided into 30 districts (Figure 1). Temperatures in Rwanda vary little throughout the year but there are some variations between regions, with the highest mean annual values found in Bugarama Valley in Western province (23–24°C) and in Eastern province (20–21°C). Northern province and parts of Western province, considered the country's highlands, are the coldest agro-ecological zone (17–19°C) (Bonfils 2012).

Prior to the survey, a list of fish farms were obtained from the Ministry of Agriculture and Animal Resources (MINAGRI), Rwanda Agriculture Board (RAB), and complemented by existing recent information at the University of Rwanda (UR). From this initial list, active farms were selected and verified in collaboration with district directors of agriculture under the Ministry of Local Government (MINALOC), resulting in a final list of 212 fish farms.

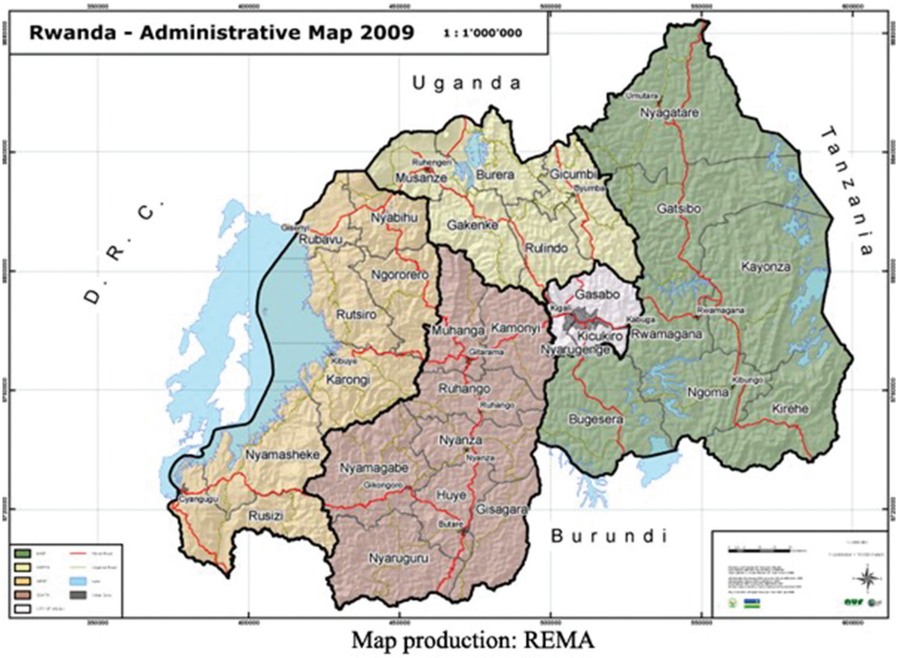


Figure 1. Map of Rwanda showing its five provinces (Northern, Southern, Eastern, Western, Kigali City), all of which were included in this survey of pond fish farming activities.

Inactive farms were either under rehabilitation, not yet stocked or abandoned due to other issues such as ponds filled with sands brought in by upstream erosion.

## *Field survey design*

The survey was carried out from November 2017 to February 2018. A total of 67 farms were selected randomly from a study population of 212 pond fish farms, applying 95% confidence interval and 10% margin of error. The number of pond-farms differed across the five provinces. For the sample to be representative, randomization was performed at province level considering existing pond-farms in each province.

The survey respondents were the fish farm owners or representatives. They were asked to complete a structured survey questionnaire (see Supplementary Material), which was designed and pre-tested in a pilot study before use for gathering field data in the main survey. The survey contained 102 questions, both closed (36) and open-ended (66), grouped under the following headings: general information on the respondent and farm manager, farm practices and management, and feed and fertilization of the ponds. Data were collected through interviews, from farm records, and through on-site observations and sampling of feed ingredients. The interview team, consisted of three people, collected all data per farm. Farm visits and interviews were scheduled and agreed in advance with the respondents.

### *Feed ingredient sampling and proximate composition*

Representative samples of 1–2 kg of feed ingredients commonly used by farmers and local fish feed makers were collected. Each feed ingredient sampled was placed separately in an appropriate container, labeled, transported to the laboratory, and stored at 4°C until analysis.

Proximate analyses of feed ingredients were performed at the food science laboratory of the College of Agriculture, Animal Sciences and Veterinary Medicine, University of Rwanda (Busogo campus), Northern Province, Rwanda. Moisture content was determined by oven-drying at 100–105°C to constant weight. Ash content was determined by incineration at 550°C for 4 h. Total nitrogen (N) content was determined by the Kjeldahl method (AOAC, 2000), and crude protein (CP) was calculated as N content × 6.25. Ether extract (EE) was measured using the Soxhlet method and crude fiber (CF) content was analyzed using standard methods (AOAC (Association of Official Analytical Chemists) 2000). Nitrogen-free extract (NFE) in dry matter (DM) was estimated by difference as: NFE (%) = 100-(CP(%)+EE(%)+CF(%)+Ash(%).

## Data analysis

Data obtained from the survey and feed ingredient evaluation were recorded and analyzed using Microsoft Excel®. Results are presented as means and percentages using descriptive statistics.

## Results

### *General description of survey respondents and farm ownership structure*

Fish farm representatives were predominantly male (79%) and 75% of respondents were aged between 31 and 55 years (Table 1). Most fish farms were owned by cooperatives (63%), and the number of members per cooperative varied from 7 to 158. Approximately one-quarter (27%) of the farms were privately owned, while public institutions like secondary schools, universities, prisons, and religious institutions owned the remaining 10%. More than 70% of respondents had 4–9 years of fish farming experience. All respondents were engaged in other employment, such as mining, brick-making, teaching, a shop or other business, and have fish farming as side employment.

**Table 1.** General description of the survey respondents (N = 67) on fish farms in Rwanda.

| Characteristics of respondent* | Category | % of total |
|---|---|---|
| Sex | Male | 79.1 |
| | Female | 20.9 |
| Age | 16–30 years | 10.4 |
| | 31–55 years | 74.6 |
| | >56 years | 14.9 |
| Education (level) | Primary school | 23.4 |
| | Secondary school | 21.9 |
| | Tertiary education** | 54.7 |
| Farming experience | 2–4 years | 12.3 |
| | 4–9 years | 70.8 |
| | >9 years | 16.9 |
| Farmer ownership | Co-operative | 62.7 |
| | Privately owned | 26.9 |
| | Other | 10.4 |

*Respondent was farm owner and/or farm manager/representative **One-year college course to university.

**Table 2.** Total number of fish farms surveyed in Kigali City, Eastern, Southern, Western, and Northern province, Rwanda.

| Province & districts | No. of fish farms surveyed | Province area* ($km^2$) | Total number of fish farms | % of total |
|---|---|---|---|---|
| *Kigali City* | 9 | 730 | 28 | 14 |
| Gasabo | 7 | | | |
| Kicukiro | 2 | | | |
| *Eastern* | 14 | 9,813 | 41 | 20 |
| Gatsibo | 5 | | | |
| Kayonza | 2 | | | |
| Nyagatare | 2 | | | |
| Rwamagana | 3 | | | |
| Bugesera | 2 | | | |
| *Southern* | 18 | 5,963 | 57 | 27 |
| Gisagara | 1 | | | |
| Huye | 3 | | | |
| Kamonyi | 4 | | | |
| Muhanga | 2 | | | |
| Nyanza | 2 | | | |
| Nyamagabe | 3 | | | |
| Ruhango | 3 | | | |
| *Western* | 7 | 5,883 | 25 | 12 |
| Karongi | 3 | | | |
| Nyamasheke | 1 | | | |
| Ngororero | 2 | | | |
| Rusizi | 1 | | | |
| *Northern* | 19 | 3,276 | 60 | 28 |
| Burera | 2 | | | |
| Nyabihu | 2 | | | |
| Gakenke | 2 | | | |
| Gicumbi | 4 | | | |
| Musanze | 2 | | | |
| Rulindo | 7 | | | |

*Source: Ministry of Local Government (MINALOC), Provinces and Districts of Rwanda, 2011.

### Farm practices and management

Northern and Southern province had highest numbers of fish farms, followed by Eastern and Western province and Kigali City province (Table 2). The majority of the fish farms owned by respondents were earthen fishpond farms (98%). Two farms, located in Northern and Eastern province, used concrete ponds, and concrete and plastic-lined ponds, respectively. The oldest fish farm, established in 1943, was located in Western province. The number and size of farms in each province varied (Table 2).

Nile tilapia, North African catfish, and common carp were the only cultured fish species reported country wide (Table 3). Respondents generally cultured tilapia in monoculture, with some differences in degree between provinces. This was especially evident in Kigali City province, where 89% of the farms practiced tilapia monoculture. Polyculture of tilapia with catfish was most prevalent in Southern province (Table 3). Previously introduced fish species, such as longfin tilapia, redbreast tilapia, and smoothhead catfish, were not reported on any farm.

A total of 11 active hatcheries were recorded in the country, while the number per province varied between one and three (Table 4). Most of the hatcheries were privately owned, run by individuals, had been in operation for less than 10 years, and produced only Nile tilapia. The maximum production capacity varied between hatcheries, ranging from 160,000 to 480,000 fingerlings annually (Table 4). Rwasave and Kigembe government farms, located in Southern province, had large hatcheries since 1950s, which produced both

**Table 3.** Distribution of fish species cultured in different provinces of Rwanda (% of fish farms per province).

| | Farm location (province) | | | | |
|---|---|---|---|---|---|
| Species cultured | Kigali City | Eastern | Southern | Western | Northern |
| Tilapia (%) | 89 | 69 | 56 | 75 | 79 |
| Tilapia and catfish (%) | 11 | 23 | 33 | 25 | 11 |
| Tilapia and carp (%) | | 8 | | | 5 |
| Tilapia, catfish, and carp (%) | | | 11 | | 5 |

**Table 4.** Number, location and capacity of surveyed fish hatcheries in Rwanda.

| | Province | | | | |
|---|---|---|---|---|---|
| Parameters | Kigali City | Western | Eastern | Northern | Southern |
| Number of hatcheries per province | 1 | 2 | 3 | 2 | 3 |
| Species produced | Nile tilapia | Nile tilapia | Nile tilapia | Nile tilapia | Africa catfish & Nile tilapia |
| Size of fingerlings (gram) | 1– 2 | 1– 2 | 2– 5 | 2 | 2– 5 |
| Minimum fingerlings (2 g) per hatchery per year* | 160,000 | 320,000 | 480,000 | 320,000 | 480,000 |
| Average price per fingerling (Rwandan francs)* | 20–30 | 20–30 | 30–45 | 30 | 30– 50 |

*Production cycle at hatchery level is 3 months. **850 Rwanda francs =1 US Dollar.

tilapia and catfish fingerlings. No hatchery produced carp fingerlings during the present study, but a few farms culture carp fingerlings captured from rivers and inland lakes.

Feed for juveniles were reported to be expensive (1.30–1.60 US$/kg, by September 30, 2020) and rarely available, and most hatcheries (84.6%) reported a lack of fingerling feed. Average stocking density applied on all farms in all provinces was 2–3 fingerlings per $m^2$, regardless of fish size. High stocking density of 5 fingerlings per $m^2$ was reported in Kigali province only (Table 5). Typical earthen pond size was approximately 300 $m^2$, and most farmers produced their own fingerlings in monoculture ponds. The majority of farmers (61%) reported constraints such as size differences; mainly small (<2 g), mixed sex, and high mortality of obtained fingerlings.

The majority of fish farms (81%) represented by respondents practiced a combination of agro-livestock and fish farming activities. All farms used organic fertilizers in their ponds to stimulate growth of the natural food web. The main fertilizers used were dried grasses, crop wastes like maize and rice stalks, and rabbit and poultry feces. In addition, 81% of respondents reported using supplemental feeding with dry feed, of which 67% used commercial feeds and 14% used feeds produced on-farm (Table 6).

Feeding frequency was 1–4 times daily for the grow-out phase and up to eight times daily for fry. For the grow-out phase, 55% of respondents reported feeding twice daily, at around 09–10 h and 15–16 h, and all performed hand-feeding (Table 6). The majority of farms that regularly practiced supplementary feeding were in Kigali City province (89%) use of

**Table 5.** Tilapia stocking density applied in different provinces in Rwanda (% of farms per province).

| Stocking density (fish $m^{-2}$) | Farm location (province) | | | | |
|---|---|---|---|---|---|
| | Kigali City | Eastern | Southern | Western | Northern |
| 2 | 22 | 50 | 36 | 63 | 79 |
| 3 | 45 | 40 | 31 | 25 | 21 |
| 4 | 22 | 10 | 31 | 13 | |
| 5 | 11 | | | | |

**Table 6.** Fish-feeding strategy applied in different provinces in Rwanda (% of farms per province).

| | Province | | | | | |
|---|---|---|---|---|---|---|
| *Feed used* | Kigali City | Eastern | Southern | Western | Northern | Mean values of all regions |
| Commercial | 89 | 67 | 76 | 37 | 63 | 67 |
| Home-made | 11 | 33 | 0 | 13 | 16 | 14 |
| Do not feed | | | 24 | 50 | 21 | 19 |
| *Feeding rate (times $day^{-1}$)* | | | | | | |
| 1 | 23 | 45 | 17 | 60 | 46 | 38 |
| 2 | 64 | 36 | 83 | 40 | 54 | 55 |
| 3 | | 19 | | | | 4 |
| 4 | 13 | | | | | 3 |

supplemental feeding showed a positive correlation with stocking density. Whereas, 50% of the farms located in Western province did not practise supplementary feeding.

### *Feed ingredient availability and proximate composition*

The main feed ingredients used by fish farmers and local fish feed producers in different provinces were reported to be locally produced maize (*Zea mays*), wheat (*Triticum aestivum*), and rice (*Oryza sativa*), which were commonly used as broken, bran, and middling polishes (Table 7). Dried leaves from kidney beans (*Phaseolus vulgaris*), cassava (*Manihot esculenta*), and sweet potatoes (*Ipomea batatus*) were milled and used as leaf meal. Cassava and sweet potato tubers were utilized as starch sources. Soybean (*Glycine max*) and sunflower (*Helianthus annus*) seeds were used as an oil source, as meal, or as cake. Rice, wheat, maize, beans, and potatoes were cultivated to different

**Table 7.** Feed ingredients used by fish farmers and local fish feed producers in all five provinces of Rwanda. The ingredients are presented in descending order based on abundance for each category.

| | | Province | | | | |
|---|---|---|---|---|---|---|
| No. | Ingredients | Kigali City | Northern | Southern | Eastern | Western |
| | *Plant-origin feed ingredients* | | | | | |
| 1 | Rice bran | * | * | * | * | * |
| 2 | Maize bran | * | * | * | * | * |
| 3 | Soybean meal | * | | * | * | * |
| 4 | Broken maize | * | | * | | |
| 5 | Rice polishes | * | * | | * | * |
| 6 | Wheat bran | * | * | | | * |
| 7 | Wheat middlings | * | * | | | * |
| 8 | Sweet potato leaf meal | | * | | * | * |
| 9 | Sunflower cake | * | | * | * | |
| 10 | Cotton seed cake | * | * | * | * | |
| 11 | Soybean crude oil | * | | | * | * |
| 12 | Brewer's grain (or by-products) | * | * | | | * |
| 13 | Kidney bean leaf meal | | * | | | * |
| 14 | Sweet potato root meal | | | | * | * |
| 15 | Sugar cane molasses | * | * | | | * |
| 16 | Ripe banana and peels | * | | | | |
| 17 | Coffee cherry husks, pulps | | | | | * |
| 18 | Groundnut (or peanut) cake | | | * | | |
| 19 | Sunflower oil | | | * | | |
| | *Animal-origin feed ingredients* | | | | | |
| 1 | Bone meal (cattle) | * | * | * | * | * |
| 2 | Blood meal (cattle) | * | * | * | * | * |
| 3 | Sea shells | * | | * | * | * |
| 4 | Fish meal, *Haplochromis* spp. | | * | | * | * |
| 5 | Fish meal, *Stolothrissa tanganicae* | * | | * | | * |
| 6 | Fish meal, *Rastrineobola argentea* | * | * | | * | |
| 7 | Slaughter waste | | * | | * | |
| 8 | Freshwater shrimp meal | * | * | | | |
| 9 | Poultry by-product meal | | | * | | |
| 10 | Tilapia fish by-products | * | | | | * |
| 11 | Snail shells | | | | | * |
| 12 | Fish oil | * | | | * | |

extents in all five provinces. Brewer's spent grains were available and used by farmers in Kigali City and Western province. Farmers in some cooperatives reported collecting cattle (*Bos taurus*) blood and poultry by-products, which they cooked and dried before use, and grinding bones and seashells for use in their fish feed production. Fish oil was rare and used only in small quantities. Fishmeal came from local small cichlids (*Haplochromis* spp.) and from two small cyprinid sardine species: *Rastrineobola argentea*, found in Lake Ruhondo in northern Rwanda or imported from Lake Victoria, Uganda, and clupeid *Stolothrissa tanganicae* imported from Lake Tanganyika, Tanzania (Table 7).

Feed ingredients from all five provinces were collected and analyzed for their proximate composition. The CP content of the ingredients varied between 67 and 701 g kg$^{-1}$ DM, with the lowest content in kidney bean leaves and the highest in cattle blood meal (Table 8). Locally available fishmeal had a protein content of 549- to 614 g kg$^{-1}$, while chicken viscera and spent brewer's yeast had a protein content >350 g kg$^{-1}$ CP (Table 8). Most agricultural by-products, industrial by-products, and plant leaves had low to medium CP content (<400 g kg$^{-1}$ CP) and CF content for most commonly used grains in bran form, including rice and wheat bran, ranged between 126 and 159 g kg$^{-1}$. The highest CF was found in soya bean meal (175 g kg$^{-1}$) followed by agricultural by-products such as cottonseed meal (168.5 g kg$^{-1}$) and rice bran (159.6 g kg$^{-1}$). The highest lipid content (EE) was found in groundnut cake (268 g kg$^{-1}$) and the lowest (16 g kg$^{-1}$) in blood meal (Table 8).

**Table 8.** Proximate composition and energy content of local feed ingredients (g kg$^{-1}$ DM).

| Ingredient | DM | CP | EE | CF | Ash | NFE |
|---|---|---|---|---|---|---|
| Fish meal (*Rastrineobola argentea*) | 861 | 548 | 170 | 123 | 17 | 141 |
| Fish meal (*Stolothrissa tanganicae*) | 908 | 614 | 150 | 118 | 12 | 107 |
| Fish meal (*Haplochromis* spp.) | 875 | 586 | 110 | 145 | 21 | 138 |
| Chicken/poultry viscera | 911 | 348 | 143 | 13 | 61 | 435 |
| Blood (*Bos taurus*) meal | 914 | 701 | 16 | 12 | 71 | 199 |
| Spent brewer's yeast | 920 | 380 | 73 | 21 | 1 | 516 |
| Soybean meal | 897 | 382 | 115 | 175 | 82 | 245 |
| Sunflower oil cake | 916 | 273 | 73 | 158 | 54 | 441 |
| Groundnut cake | 907 | 397 | 268 | 83 | 48 | 204 |
| Cotton seed meal | 904 | 371 | 115 | 169 | 62 | 283 |
| Spent brewer's grain | 917 | 235 | 163 | 130 | 76 | 395 |
| Rice bran | 903 | 126 | 71 | 159 | 235 | 408 |
| Rice middlings | 889 | 116 | 152 | 64 | 73 | 595 |
| Maize middlings | 896 | 127 | 165 | 121 | 96 | 491 |
| Wheat bran | 896 | 144 | 43 | 133 | 69 | 610 |
| Wheat middlings | 878 | 178 | 59 | 84 | 67 | 614 |
| Broken maize | 886 | 70 | 45 | 99 | 102 | 684 |
| Sweet potato leaves | 925 | 318 | 40 | 130 | 145 | 366 |
| Kidney bean leaves | 909 | 67 | 35 | 116 | 164 | 618 |
| Sugar cane molasses | 821 | 250 | | | 58 | 708 |

CP = crude protein, CF = crude fiber, EE = ether extract (lipid content), NFE = nitrogen-free extract (%, = 100 – CP).

Sugar molasses and broken maize had the highest content of NFE (708 and 684 g $kg^{-1}$, respectively), while the lowest value was found in fishmeal (range 107–142 g $kg^{-1}$) depending on species among the four used in fish meal Rwanda. Wheat displayed slightly higher protein content, and lower EE and ash content, than rice bran (Table 8).

## Discussion

The present study surveyed the status of pond-fish farming across all five provinces of Rwanda, it also investigated currently available local feed ingredients, and evaluated their nutritive value. Tilapia was the most farmed species, more than half of pond-farms located in Northern and Southern provinces. One to three hatcheries existed in each province, all produced tilapia fingerlings. We identified 31 feed ingredients, rice, wheat, and maize bran were the most commonly used ingredients in the prevailing extensive and semi-intensive pond farming system in Rwanda.

### *General description of survey respondents and farm owner structure*

More than 60% of the fish farms surveyed belonged to cooperatives, whereas, e.g.,, the aquaculture value chain development in Nigerian Egypt, Uganda, and Ghana is markedly driven by the private sector initiatives (Adeleke et al. 2021). Gender distribution among fish farm managers in Rwanda was predominantly male (79%), as reported previously for Rwanda and Tanzania (FAO 2017; Mmanda et al. 2020). The study by FAO (2017) showed an equally skewed gender distribution of fish farmers in Sub-Saharan Africa (SSA) in general. A more equitable gender balance in aquaculture could help reduce household poverty, improve household decision-making, and result in better management of ponds, land, and capitals (Galiè et al. 2015; Johnson et al. 2016; Ndanga, Quagrainie, and Dennis 2013). However, achieving this under Rwandan conditions could be challenging, since most women in Rwandan aquaculture are typically engaged in most of the downstream, post-harvest and marketing activities mainly as fishmongers.

Similarly, young (16–30 years old) fish farmers are still scarce in Rwanda. Only 10% of the respondents belonged to this age category, which is even less than the 17% reported in 1991 by Hishamunda, Curtis, and Upton (1998). In Rwanda, young people comprise 44.2% of the population and over 50% of the population in Africa is below 25 years of age (FAO 2014; NISR 2019). Young men are often employed as casual part-time workers, e.g., in pond construction and fish harvesting, while young women play a larger role in post-harvest steps but are often limited to sales and marketing (Cai, Leung, and Hishamunda 2009). Efforts are needed to achieve a more equal gender distribution in aquaculture and better integration of young people early on. This could contribute positively to aquaculture development and popularization, and also to economic growth.

### *Farm practices and management*

Three fish species namely Nile tilapia, African catfish, and common carp, are currently the main farmed species in Rwanda, with tilapia being the dominant species (56–89%). The present findings are supported by those previously reported for countries in East Africa (Charo-Karisa et al. 2006; Gatachew 1987; Mbugua 2002). Moreover, Nile tilapia was mostly produced in monoculture and was the main species in polyculture, which is in line with findings by Vincke (1987). African catfish was the second most farmed species in both monoculture and polyculture. It is mainly reared to control high tilapia populations in mixed ponds in Rwanda. In a previous study, Hishamunda, Curtis, and Upton (1998) found that two more tilapia species were farmed in monoculture, namely longfin tilapia (2% of farms) and redbreast tilapia (0.1% of farms). In the present survey, these earlier introduced species, as well as Smoothhead catfish, were reported to be no longer farmed on any of the farms represented by respondents. It can be assumed that farming of these species in Rwanda has ceased, based on reported low growth rates (El-Sayed 2006; FAO/UNDP 1981).

Our results revealed that lack of fry and fingerling feed is a major constraint faced by hatchery operators in Rwanda. Commercial fingerling feeds are rare and expensive, and thus the price of fingerlings is high for many smallholder farmers. For tilapia, the most widely farmed species in Rwanda, early stage feeding is a general challenge for most hatcheries/farmers, due to lack of proper feed. We found that most hatchery owners use homemade feed, instead of imported feed, in order to lower the feed costs in fingerling production. Other hatchery owners mix a portion of commercial feed with available low-cost single ingredients, such as rice or wheat bran. Similar strategies are applied by farmers in the Mekong region of Vietnam, who mix manufactured fish feed with formulated poultry feed and plant by-products such as rice bran and sweet potato roots (Edwards and Allan 2004).

The present study finds an increased number of hatcheries countrywide in Rwanda compared with earlier reports by Hishamunda, Curtis, and Engle (1996) and MINAGRI (2011) shows an improvement in the fingerling supply situation.

Northern and Southern provinces have slightly higher numbers of pond-fish farms than the remaining provinces. Northern province is topographically diverse, featuring many rivers and high, steep slopes. Thus, a number of government and NGO projects in this province focus on environmental protection and flood and landslide erosion control. These projects have resulted in construction of bench and progressive terraces, fishponds, and buffer zone protections around water bodies, in order to improve land productivity and reduce soil erosion (MINAGRI 2014). This has created favorable conditions for fish farming, resulting in a higher number of fish farms in Northern province.

Southern province is best suited for cultivation of fish, due to relative high daily temperature and flat valley bottoms with very gentle slopes, which lend themselves to easy construction of ponds. Southern province also hosts two major and active public aquaculture stations, Rwasave and Kigembe, which were founded in 1952 and 1954, respectively. These continue to provide direct support to fish farmers in the form of extension services, seed, and other inputs, albeit with no reported or tangible increments (MINAGRI 2011). Based on our results, there is potential to increase the production and quality of catfish and tilapia fingerlings, but also to diversify and avail carp's fingerlings needed by farmers. Lower numbers of fish ponds in Western province can be explained by presence of Lake Kivu, since both fishery activities and cage culture occur in the lake itself.

Our survey showed that, in the prevailing semi-intensive earthen pond farms in Rwanda, stocking is predominantly done with mixed-sex fingerlings. A small number of farmers currently produce their own mixed-sex fingerlings in their hatcheries. Male monosex tilapia culture is preferred, due to faster growth in males than females. In males, metabolic energy is channeled toward growth and anabolic growth-enhancing androgens are produced (Angienda, Aketch, and Waindi 2010; Tran-Duy et al. 2008). Monosex male tilapia in Rwanda are scarce, therefore it is important that hatchery operators have the capacity to progressively provide monosex male fingerlings to farmers to improve tilapia production in Rwanda.

On average, the most common stocking density reported by survey respondents was 2–3 fingerlings per $m^2$, regardless of fish size, although a small proportion of farms (17.3%) stocked 4–5 fingerlings per $m^2$. There are large discrepancies in recommended pond stocking densities. In Kenya, a stocking rate of 3 fish $m^{-2}$ is commonly used in ponds to achieve yields of 1 kg per $m^{-2}$ (Opiyo et al. 2018). According to Hishamunda, Curtis, and Engle (1996), increasing the stocking density to about 3–4 fingerlings per $m^2$ in semi-intensive systems increases yield. The optimal density is related to the overall cultivation strategy, and is influenced by the desired final fish size at harvest (Shumway and Parsons 2016). Fish stocking density is a key factor in the optimal management of fish culture. It affects the amounts of natural food available (in fertilized ponds) per fish, and the level of supplemental feeding required (Bhujel 2013). Increasing the stocking density on pond-farms in Rwanda to 4–5 fish $m^{-2}$ could provide higher yield at harvest, provided that adequate supplemental feeding is practiced.

The majority of the farms surveyed (81%) practiced a combination of agro-livestock and fish farming activities. Respondents reported using only organic fertilizers in their ponds, such as animal manures, different dried grasses, and crop residues. The main fertilizers used were dried grasses, maize and rice stalks, and rabbit and poultry feces. Organic fertilizers include different plant-derived materials ranging from fresh or dried plant material to animal manures and litters to agricultural by-products (Das and Jana 2003). Most of commonly used fertilizers must undergo decomposition to release mainly the three primary plant nutrients nitrogen, phosphorus, and potassium for phytoplankton growth. The daily recommended application rate of these elements is 0.4 g N $m^{-2}$ and 0.1–0.2 g P $m^{-2}$ (Bhujel 2013). The farmers surveyed do not have experience or know the recommendations for fertilization, and rarely measure satiation or fertilizer dose, so it is likely that they end up over- or under-fertilizing their ponds. This is a challenge that needs to be addressed, as previous results show that tilapia reared in fertilized ponds and fed supplemental diets at 50%, 75%, and 100% satiation produce comparable yields, but the 50% level represents a considerable reduction in production costs and in nutrient loading (Lin and Yi 2003). Additionally, most tilapia grow-out ponds in Rwanda contain mixed sexes and ages, so it is difficult to know precisely the pellet size to utilize for adequate feeding. Little is known about the dietary nutrient requirements and supply for species cultured in fertilized pond systems, due to the difficulty in quantifying the contribution of naturally available food organisms (Rahman et al. 2006; Spataru, Wohlfarth, and Hulata 1983; Veverica et al. 2000). Nguyen et al. (2018) demonstrated that simple feed with imbalanced nutrient content can be given to tilapia cultured in water with high primary production of natural feed. In our survey, feeding twice per day was the most common supplementary feeding regime used for grow-out ponds. Feeding frequency is an important factor for cultured fish and can affect overall growth, survival, and production of the fish (Macintosh and Little 1995; Phillips, Summerfelt, and Clayton 1998; Sanches and Hayashi 2001; Tung and Shiau 1991). In species such as tilapia with relatively small stomachs and continuous foraging behavior, multiple feeding can improve growth and feed efficiency (NRC (National Research Council) 2011; Shiau 2002).

We found that the average grow-out period on the surveyed farms ranged from six to nine months, and in most cases harvested fish were sold at the farm gate as fresh whole. This is in accordance with earlier findings that in semi-intensive aquaculture, where ponds are fertilized with manure and inorganic fertilizers, the production cycle is 6–9 months (Suresh, 2003).

### *Feed ingredients and their proximate composition*

Our analyses showed that a range of ingredients widely available in Rwanda can potentially be used in fish feed formulation. All grain by-products tested had a low content of CP (<178 g $kg^{-1}$ DM), but a high content of NFE (408–684 g $kg^{-1}$ DM) (Table 8). These ranges are similar to those reported for other countries in East Africa, such as Tanzania and Kenya (Mmanda et al. 2020; Munguti et al. 2012). The fat content (EE) in sunflower oil cake (73 g $kg^{-1}$ DM) differed considerably from that (244 $kg^{-1}$ DM) reported by Mmanda et al. (2020). Most of the ingredients identified in Rwanda have been studied previously in a mixture, as partial or total replacers for fishmeal for different fish species (El-Saidy and Gaber 2003; Khan, Siddique, and Zamal 2013; Liti et al. 2006). Nyina-wamwiza, Wathelet, and Kestemont (2007) found that groundnut oil cake could replace 50% of fishmeal in the diet of North African catfish, without amino acid supplementation. However, anti-nutritional factors commonly found in a number of plant-based ingredients, and their effect on fish, should be considered during ingredient processing (Francis, Makkar, and Becker 2001). Most anti-nutrient and toxic effects of these compounds can be destroyed by processing methods such as soaking, germination, heat processing (boiling and autoclaving), fermentation, or by genetic manipulation, without altering the nutritional value (Hamid, Thakur, and Kumar 2017). Additionally, plant by-products contribute high levels of indigestible organic matter in the form of insoluble plant fibers and often contain low levels of limiting amino acids (lysine, methionine, tryptophan) (Gorissen et al. 2018; Naylor et al. 2009). In cases of full substitution of animal protein with plant protein in formulated fish feeds, supplementation with (synthetic) lysine and methionine is necessary. Sweet potato leaves meal had slightly lower CP content (318 g $kg^{-1}$) and could be comparable with 359 g $kg^{-1}$ DM reported in previous studies (Munguti et al. 2012). Such differences maybe a natural variation related to the growing environment, leaf maturity stage at harvest, or analytical method used (Church 1980; McDonald et al. 2002). Sweet potato is cultivated on 89,427 ha across Rwanda (NISR, 2020) and its leaves are an important feed ingredient available country-wide.

Fishmeal available and used in Rwanda is made from relatively low-grade fish species, predominantly sun-dried sardines (*Rastrineobola argentea* and *Stolothrissa tanganicae*, both known locally as "*ndagaa*"). These indigenous pelagic fish species from Lake Victoria and Lake Tanganyika, respectively, have been introduced in Lake Ruhondo and Kivu in Rwanda (De Vos, Snoeks, and Van Den Audenaerde 2001). *R. argentea* is the most used, and its proximate composition was consistent with most previous findings in East Africa (Mmanda et al. 2020; Munguti et al. 2012). Despite its high cost compared with other ingredients used for feed formulation, fishmeal is an ideal source of protein in feeds for most fish species. However, high demand for these small sardines, both as fishmeal and for direct human consumption, makes their use in feed formulations expensive (1500–6000 Rwandan francs (equivalent to 2–7 US$, by July 7th 2018) per kg dry weight), but also non-sustainable, since they can be used directly as human food. Therefore, replacement of fishmeal with other ingredients from sustainable sources not used as human food would be beneficial in reducing feed costs and food fish prices, especially in landlocked developing countries such as Rwanda.

Other locally identified ingredients of animal origin include blood meal, poultry by-products, and fishery by-products. These ingredients are inexpensive, readily available, and suitable for aquaculture diets, and may be good alternative protein source for use in fish feed. Feed containing 10% blood meal has been found to be most efficient in terms of total tilapia fish production, average weight gain, and average final fish weight (Otubusin 1987). According to Sabbagha et al. (2019), total substitution of fishmeal with poultry by-product meal in the commercial diet of gilthead sea bream (*Sparus aurata*) is achievable without compromising fish growth performance, fish welfare, or fillet quality. Poultry by-product meal is palatable and has a high protein content and an EAA profile similar to fishmeal, but contains low levels of dietary methionine and lysine (Bureau, Harris, and Cho 1999; González-Rodríguez et al. 2016; NRC (National Research Council) 2011). However, combining the poultry by-product meal in tilapia feed recipes with other available lysine-rich ingredients such as fishery by-products or fish skeletons could be a solution (Ahmed and Khan 2004).

Brewer's yeast biomass is the second major by-product from the brewing industry in Rwanda. Our survey found that spent brewers' yeast is available for use as a major ingredient in fish diets in Rwanda. However, despite its proximate composition featuring high CP content (380 g $kg^{-1}$ DM), it is not currently used in fish feed formulation in Rwanda. Brewer's yeast has been used in aquaculture elsewhere since the 1990s and is a potential high-volume alternative to fishmeal protein in the diet of Nile tilapia (Agboola et al. 2020; Nguyen et al. 2018). Based on our survey results, fish oil is rarely used in Rwanda, most likely due to low availability and high market price but also because freshwater fish, including tilapia, have a low lipid requirement and can be satisfied with C18 PUFA at around 1% of diet dry weight (NRC (National Research Council) 2011; Tocher 2010). Alternatively, plant oil sources such as sunflower oil, crude palm oil, and soybean oil locally available in Rwanda can be used. Currently, sunflower oil is the most commonly used.

The proximate composition of local feed ingredients was generally within the range reported in previous studies, apart from some values for individual feed ingredients reported within East Africa (Mmanda et al. 2020; Munguti et al. 2012; Nyina-wamwiza, Wathelet, and Kestemont 2007). However, the proximate composition of feed ingredients may vary due to many factors, such as climate conditions, production season, geographical zone, soil type, stage of maturity at harvest, animal species, processing, handling, storage, and contamination by mycotoxin and other toxic compounds (Church 1980).

In summary, aquaculture in Rwanda is dominated by Nile tilapia, farmed mainly in semi-intensive systems. Fish farming practices generally indicate lack of training and management skills, reflected in relatively low production levels and inadequate use of resources. These are thus potential areas for improvement. Many of the potential feed ingredients analyzed in this study are available in all five provinces of Rwanda. Although relatively expensive, fishmeal is still used as the main protein source. Use of novel feeds can be a sustainable strategy in future aquaculture development. Efficient use of existing local ingredients, considering their proximate composition, could decrease fishmeal use and allow production of less expensive fish feed locally. This would contribute to more sustainable aquaculture production and improved food security in Rwanda. However, the suitability of local feed ingredients (and their EAA profile) and novel feeds for use in Nile tilapia and catfish feeds needs further assessment, preferably in vivo, before their use by Rwandan fish feed producers and farmers can be recommended.

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III

# Growth performance, nutrient utilization and body indices of Nile tilapia (*Oreochromis niloticus*) fingerlings fed local feed ingredients

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## Abstract

A 10-week trial was conducted to evaluate growth performance, feed utilization, and somatic indices of Nile tilapia (*Oreochromis niloticus*) fingerlings fed five diets based on local feed protein ingredients (kidney bean leaf meal (KBLM), spent brewer's grain (SBG), spent brewer's yeast (SBY), sweet potato leaf meal (SPLM), and wheat middlings (WM) and a fishmeal-based control diet (CD). The experimental diets were formulated to be iso-nitrogenous and iso-energetic, with 27-50% of fishmeal in CD replaced ('as is' basis) with test ingredient. Initial average body weight of individual fish was 28.9±1.88 g and final body weight (FBW) was 60.2±2.81 g. Weight gain (WG) and FBW were highest ($p<0.05$) for fish fed CD, followed by SPLM, SBY, SBG, WM, and KBLM in that order. Specific growth rate (SGR) was highest in fish fed CD and SPLM, followed by SBY and SBG, and lowest in fish fed WM and KBLM. Feed conversion ratio (FCR) was highest in fish fed KBLM and lowest in fish fed CD and SPLM. Survival was 75-87% and did not differ between the groups. Hepato-somatic index (HSI) and viscera-somatic index (VSI) also did not differ across dietary treatments. These results indicate that SPLM, SBY, and SBG protein can efficiently replace fishmeal in Nile tilapia diets without adverse effects on growth, feed utilization, or body indices, acting as a valuable protein source for sustainable tilapia production.

***Key words:*** *agro-industrial by-products, aquaculture, fishmeal, Rwanda, vegetable ingredients*

## Introduction

Aquaculture is the fastest growing animal food-producing sector. It currently produces over 50% of all fish consumed worldwide and has high potential to meet the increasing global demand for aquatic foods created by global population growth (FAO 2018; Stevens et al 2018). Seafood is a balanced and nutritious foodstuff, and over 3 billion people worldwide consume fish protein as an essential part of their diet (FAO 2020). Aquaculture in Africa currently represents only 2.7% of global aquaculture, but this proportion is expected to increase by 48% in the near future, driven by additional aquaculture capacity introduced in recent years (FAO 2020). Most fish production in Africa is based on freshwater systems (99%) with tilapia (*Oreochromis niloticus)* and African catfish ( *Clarias gariepinus*) being the major cultured fish species (Adeleke et al 2021).

In Rwanda, aquaculture started in the 1940s as small-scale extensive tilapia pond farming, while from 1948 the nascent fish farming sector was promoted by the Belgian colonial authorities (Schmidt and Vincke 1981; Dadzie 1992). According to the latest annual report by Rwanda's Ministry of Agriculture and Animal Resources (MINAGRI 2021) fish production reached an estimated 41,664 tonnes, with 7059 in 2020 tonnes from aquaculture.

Rwanda's burgeoning aquaculture sector is predicted to expand further and to become an important source of high-value animal protein for the population. The country is endowed with important untapped aquaculture potential, including sufficient water resources, but future expansion will require use of locally available ingredients to make aquafeed, and robust fish species suitable for culture (such as tilapia). Local fish farmers are already struggling to obtain fish feed of good quality, which constitutes a major challenge to successful growth and intensification of aquaculture production in Rwanda. Feed constraints are most critical for Nile tilapia, which is by far the most commonly cultured fish species (89%) in Rwandan aquaculture (Niyibizi et al 2022).

Tilapia is a warm-water omnivorous fish species capable of utilizing nutrients from animal and plant feedstuffs (Felix et al. 2020; El-Sayed 1999) and agro-industrial by-products (Agboola et al 2021; Nhi et al 2018). It is the second most cultured and economically important fish species worldwide (FAO 2020), mainly due to its adaptation to a wide range of environmental conditions, tolerance against stressful conditions, acceptance of artificial diets for all stages of production, and high nutritional value (El-Sayed 2006). The future supply of fish for human diets will largely depend on the availability of farmed fish (FAO 2020). Due to its robustness, tilapia is likely to be an important fish species for sustainable aquaculture (Yue et al 2016).

Feed input is the single largest operating cost in aquaculture, representing 40-75% of total production costs (FAO 2018; Rumsey et al 1990) Approximately 70% of all commercial fish species produced in aquaculture rely on fishmeal-based feeds (Tacon 2020). Fishmeal is a major protein source in commercial fish feeds and is predominantly used in aquafeeds for high-trophic finfish and crustaceans. Its use in diets for low-trophic finfish like tilapia is limited (incorporated at 3-10%), where it is valued mostly for its ability to enhance growth (Tacon et al 2011). High inclusion rates of fishmeal are not sustainable, particularly in landlocked countries such as Rwanda where it is rarely available and where the fish species used in fishmeal are also used as food for humans (Niyibizi et al 2022). Identification of accessible, highly digestible proteins to reduce reliance upon expensive and unsustainable marine ingredients is essential, and numerous studies have examined the effect of replacing marine ingredients (meals and oils) in tilapia feeds with alternative ingredients such as low-cost, indigenous plant and animal ingredients (Lim et al. 2011; El-Saidy and Gaber 2003). Animal raw materials as an alternative to fishmeal have been tested in tilapia diets, with varying results. Poultry waste products can be included at rates up to 40%, but only limited amounts of blood meal (10%) can be used without affecting average weight gain (Felix et al 2020; El-Sayed 1998).

A recent study identified 31 ingredients in Rwanda worth investigating as tilapia feed alternatives (Niyibizi et al 2022). These included agro-industrial by-products such as spent brewer's yeast (SBY), spent brewer's grain (SBG), and wheat middlings (WM), and plant materials such as sweet potato leaves (SPL) and kidney bean leaves (KBL), which are all readily available ingredients. These ingredients could be suitable feed resources to increase the protein and energy content in fish diets in general and in tilapia diets in particular. Tilapia is an omnivorous species known to efficiently utilize high levels (30-70%) of dietary carbohydrates as a primary energy source, which allows protein to be saved for growth (Kamalam et al 2017; FAO 2018).

Spent brewer's grain is the major by-product of the beer industry, representing ~ 85% of total by-products generated when producing beer (Mussatto 2014), and is thus an abundant feed resource. Brewing removes the soluble part of the grain, concentrating insoluble compounds as lignocellulosic residues (SBG) that are still rich in protein. This makes SBG potentially valuable as a high-volume and low-cost source of protein in the diet of farmed tilapia and e.g., striped catfish ( *Pangasianodon hypophthalmus*) (Jayant et al 2018; Zerai et al 2008; Mussatto et al 2006). Spent brewer's yeast biomass is the second major by-product from the brewing industry is another potentially interesting feed ingredient for use in tilapia feeds (Marson et al 2020). In Rwanda, SBY and SBG have never been used in fish feed formulation, although their good availability and high crude protein content (380 and 266 g $kg^{-1}$ dry matter (DM) in SBY and SBG, respectively) indicate good potential as a feed ingredient for use in aquaculture (Niyibizi et al 2022).

Plant-derived ingredients are a readily available alternative that can potentially be used in fish feed, provided that they do not compromise the nutritional quality of the feed (Dorothy et al 2018; El-Sayed 1999). Plant protein sources, including soy and other legumes, are currently key alternatives to fishmeal in most commercial fish diets (Gatlin et al 2007), while little use has been made of sweet potato leaves and kidney bean leaves as feed ingredients in animal and fish diets (Adewolu 2008). Such plant-derived ingredients are relatively low-cost and locally available, which are advantages for sustainable aquaculture (Bergamin et al 2013).

Fishmeal and fish oil (currently widely used in aquafeed) are expensive and the supply is gradually becoming limited as the world's fish stocks are either fully exploited or seriously depleted (FAO 2018; Tacon and Metian 2008). The sustainability of future commercial aquaculture will depend on reduced use of wild fish inputs in fish feed (Naylor et al 2000). A shift to alternative protein sources, especially by-products and other ingredients not used by humans, will be important and possibly cost-saving (Agboola et al 2021; Montoya-Camacho et al 2019; Gasco et al 2018). The challenge facing the aquaculture industry is to identify alternatives to fish oil that are economically viable and environmentally friendly, achieving least-cost and sustainable aquaculture production. The potential of alternative feed ingredients in fish diets can be established based on their proximate chemical composition (Mzengereza et al 2014). In Rwanda, there have been no attempts yet to replace fishmeal with plant and agro-industrial by-products in formulated tilapia feeds and there is a lack of empirical data on the feed potential of these materials. Thus the present study assessed growth performance of Nile tilapia fed experimental diets in which fishmeal was replaced by plant-derived ingredients and agro-industrial by-products.

## Materials and methods

### Study area and facilities

The study was carried out at the hatchery of University of Rwanda Fish Farming and Research Station (UR-FFRS), Rwasave, Huye campus, southern Rwanda (2°40´S; 29°45E), during the period August-December 2019. The experiment was conducted in a recirculating aquaculture system comprising 18 fiberglass tanks, each 100 L in volume, installed above 4480-L concrete tanks equipped with a mechanical and biological water filtration system. The recirculating system was continuously supplemented with fresh well water at a flow of 2 L min $^{-1}$. The water was constantly aerated using an electric air pump connected to stone diffusers supplied in each tank to ensure adequate oxygen supply.

### Fish and rearing conditions

A total of 360 mixed-sex Nile tilapia fingerlings bred at UR-FFRS were used in the feeding experiments. Before acclimatization, the fish were subjected a five-minute bathing treatment with NaCl (5 g $L^{-1}$) to remove potential ectoparasites, bacteria, or fungi (Barker et al 2002). All fish were weighed and measured (average weight 28.9 ±1.9 g, average length 11.8 ± 0.4 cm) and then 20 fish were randomly distributed to each of the 18 experimental tanks, with three replicate tanks per diet. The experimental tanks had a plastic mesh top cover to prevent fish from jumping out. The fish were acclimatized to the experimental conditions for one week, during which they were fed a commercial diet from Premier Animal Feed Industry (PAFI Ltd), Rwanda, and kept at a natural photoperiod of 12 hours light: 12 hours dark. All fish were weighed individually at the beginning and end of the experiment, using an electronic balance (Mettler PM4000, Hampton, NH 03842, USA). The three replicate tanks per dietary treatment were arranged in a completely randomized design.

### Experimental diets

Ingredients used in this study (see Table 1) were purchased from local markets, obtained from food and beverage industries, or freshly harvested in local fields. Dried soybean grain was first rinsed in clean cool water and then autoclaved at 110 °C for 30 minutes to reduce anti-nutritional factors. Fresh cattle blood was cooked for 30 min. Treated soybean grain and blood were then sundried for 2-3 days. All 12 dried feed ingredients (Table 1) were ground to flour using a Grain Hammer Mill Crusher (GMEC-280 Zhengzhou Runxiang Machinery Equipment Co., Ltd, Zhengzhou, Henan, China) and then mechanically mixed (Santos 10Ltr Dough Mixer, Lyon, France) with vitamin and mineral premix for 5 minutes. Sunflower oil was then added and mixed in for an additional 5 minutes. Finally, a small amount of clean water was added and mixing continued for 10 minutes to form a homogenous dough, which was pelleted using a meat grinder (FAMA FTS107, Brugnera, Italy). The pellets (2 mm diameter) were sun-dried for 2-3 days and stored at -20 °C until use. A small portion (enough to be offered within five days) was regularly taken from main diet batch and kept at 5 °C in sealed food-grade plastic bags.

Six different diets were produced by replacing fishmeal in the control diet with locally produced ingredients, and formulated to meet the nutritional needs of Nile tilapia (NRC 2011). The control diet (CD) was fishmeal-based and the five test diets were formulated with maximum possible fishmeal replacement rate on an 'as is' basis without affecting crude protein and energy content of the diet. Proximate composition of test ingredients used to formulate the diets is presented in Table 2. The fish were hand-fed to satiation (three portions, at 09.00 h, 12.00 h, and 15.00 h) over the 70-day study period, starting with a pre-determined ration (approximately 4.5% of body weight per day) that was adjusted according to measured growth.

**Table 1.** Formulation (g $kg^{-1}$ dry matter) of the control diet (CD) and of test diets based on spent brewer's grain (SBG), kidney bean leaf meal (KBLM), wheat middlings (WM), sweet potato leaf meal (SPLM), and spent brewer's yeast (SBY)

| Ingredient | Diet | | | | | |
|---|---|---|---|---|---|---|
| | CD | SBG | KBLM | WM | SPLM | SBY |
| Fishmeal | 220 | 150 | 170 | 150 | 150 | 110 |
| Soybean meal | 150 | 150 | 150 | 150 | 150 | 150 |
| Cottonseed meal | 100 | 100 | 100 | 100 | 100 | 100 |
| Rice bran | 200 | 200 | 200 | 200 | 200 | 200 |
| Sunflower seedcake | 60 | 60 | 60 | 60 | 60 | 60 |
| Maize middlings | 190 | 190 | 190 | 190 | 190 | 190 |
| Blood meal | 50 | 50 | 50 | 50 | 50 | 50 |
| Sunflower oil | 20 | 20 | 20 | 20 | 20 | 20 |
| Premix* | 10 | 10 | 10 | 10 | 10 | 10 |
| Spent brewer's grain meal | 0 | **70** | 0 | 0 | 0 | 0 |
| Kidney bean leaf meal | 0 | 0 | **60** | 0 | 0 | 0 |
| Wheat middlings meal | 0 | 0 | 0 | **70** | 0 | 0 |
| Sweet potato leaf meal | 0 | 0 | 0 | 0 | **70** | 0 |
| Spent brewer's yeast meal | 0 | 0 | 0 | 0 | 0 | 110 |
| Replacement rate for fishmeal (%) | 0 | 32 | 27 | 32 | 32 | 50 |

*Vitamin and mineral content in premix: Vitamin A 4,000,000 I.U, Vitamin D3 750,000 I.U, Vitamin E 3,500 I.U, Vitamin K 500mg, Vitamin B1 200mg, Vitamin B2 600mg, Vitamin B6 600mg, Vitamin B12 5,000mg, folic acid 250mg, biotin 0.75mg, nicotinic acid 5,000mg, pantothenic acid 2,000mg, choline 40,000mg, Fe 8,750mg, Mg 12,500mg, Cu 1,500mg, Zn 12,500mg, Co 270mg, I 250mg, Se 50mg, P 1,050mg, Ca 750,000mg, lysine 1200mg, methionine 8,000mg, phytase 20,000U*

## Sampling and measurements

On the day before the feeding experiment started, all 20 fish per tank were weighed, giving the initial weight per tank ($W_i$, sample zero). During the experiment, six fish were randomly netted from each tank every 14 days and weighed, to monitor intermediate body weight gain (BWG). Total number of fish remaining in each tank was also calculated on these occasions, for diet adjustment and mortality evaluation. At the end of the experiment, the remaining fish were counted and weighed ($W_f$, final biomass), after which they were anesthetized with 100 mg $L^{-1}$ of MS-222 and weighed again (BW, body weight) (Mettler PM4000, Hampton, NH 03842, USA). Three fish per tank were randomly collected and dissected for determination of hepato-somatic index (HSI, %) and viscero-somatic index (VSI, %).

## Chemical analyses

Prior to experimental diet production, all ingredients were analyzed for their proximate chemical content at the Food Science Laboratory, College of Agriculture, Animal Sciences and Veterinary Medicine, Busogo campus, University of Rwanda. Moisture content was determined by oven-drying at 105 °C to constant weight. Ash content was determined by incineration of samples at 550 °C for 4 h. Total nitrogen (N) content was determined by the Kjeldahl method (KEL PLUS, Pelican Equipment, Chennai, Tamil Nadu, India) and crude protein (CP) was calculated as N x 6.25. Crude lipid (CL) was determined by Soxhlet ether extraction (ALCON.51, Alcon Scientific Industries, Ambala Cantt, Haryana, India), and crude fiber (CF) content was analyzed using standard methods described in AOAC (2000). Nitrogen-free extract (NFE) was calculated by subtracting the sum of crude protein, crude lipid/ether extract (EE), ash, and crude fiber from the corresponding dry matter value: (NFE (%) = DM-(CP +CL +CF +Ash). Gross energy (GE) was calculated as (CP x 23.6+CF x 39.5+NFE x 17.2)/100, expressed as MJ $g^{-1}$.

At the end of the experiment, four fish per tank were used for whole body analysis of crude protein, crude lipid, crude fiber, moisture and ash.

Water parameters such as pH, temperature (°C) and dissolved oxygen (mg $L^{-1}$) were monitored twice daily (at 08.00 and 16.00 h) in each experimental tank, using a portable multi-parameter probe (Hanna HI 11310, Hanna Instruments Ltd., USA). Water temperature was kept at 27.0±0.1 °C using aquarium heaters (Aquazonic AZ-LED 100, Yi Hu Fish Farm Trading. Sungei Tengah, Singapore). Nitrite (mg $L^{-1}$) and ammonia (mg·$L^{-1}$) were monitored on a weekly basis using a Hach® water analysis kit (DR/890 Colorimeter, Hach Company, Colorado, USA).

### Growth performance and calculation of health indices

Growth performance and biological indices were calculated using the following equations:

Specific growth rate (SGR, %/day) = [(ln $W_f$–ln $W_i$)/T] ×100, where $W_f$ is final weight and $W_i$ is initial weight

Protein intake (g) = Feed intake (g) × Protein in the diet (%).

Total feed intake per fish (FI) = Total feed intake (g)/Number of fish

Survival rate (SR %) = ($TF_f$/$TF_i$) ×100, where $TF_f$ is total number of fish at harvest and $TF_i$ is total number of fish at start.

Feed conversion ratio (FCR) = Total feed intake (g)/Total wet weight gain (g)

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

Hepato-somatic index (HSI, %) = [100 × (Liver weight (g)/Body weight (g))].

Viscero-somatic index (VSI, %) = [100× (Viscera weight (g)/Body weight (g))].

### Statistical analysis

Data on growth performance, feed utilization, and body composition were encoded into Microsoft Excel worksheets, and then imported into *IBM SPSS STATISTIC (2011)* program version 19 software for statistical analysis. One-way analysis of variance (ANOVA), followed by Duncan's multiple range test, were used for comparisons of means ($p<0.05$ level of significance). Rearing tank was considered as experimental unit, and the same method was used for all parameter testing. All means were recorded, ± standard error of the mean (SEM).

## Results

### Proximate composition of feed ingredients and diets

The proximate composition of ingredients used in the experimental diets for Nile tilapia fingerlings is presented in Table 2. The CP content was highest in blood meal (701 g $kg^{-1}$ DM) and fish meal (547 g $kg^{-1}$ DM), followed by soybean meal, SBY, cottonseed meal, SPLM, sunflower oil cake, and SBG (382 to 245 g $kg^{-1}$ DM). It was low in WM and maize middlings, rice bran and KBLM (178 to 67 g $kg^{-1}$ DM). The CL content varied by up to 10-fold between the ingredients (16-170 g $kg^{-1}$ DM), with the highest values in fishmeal and the lowest in blood meal. The CF content in the experimental ingredients showed different patterns, with a high content (>153 g $kg^{-1}$ DM) in soybean meal, cottonseed meal, rice bran, sunflower seedcake and SBG. The ash content ranged from 17 to 235 g $kg^{-1}$ DM, with the highest values in rice bran and SPLM (235 and 145 g $kg^{-1}$ DM, respectively) and the lowest in fishmeal (17 g $kg^{-1}$ DM). The NFE content varied between 141 and 618 g kg$^{-1}$ DM, with the lowest content in fishmeal and the highest in KBLM and WM.

**Table 2.** Proximate composition (g kg$^{-1}$ dry matter, DM) of feed ingredients used in the control diet and in test diets for Nile tilapia (*Oreochromis niloticus*) fingerlings

| Ingredient | DM | CP | CL | CF | Ash | NFE |
|---|---|---|---|---|---|---|
| Blood meal | 914 | 701 | 16 | 12 | 31 | 240 |
| Cotton seed meal | 904 | 371 | 115 | 169 | 62 | 283 |
| Fishmeal* | 861 | 548 | 170 | 123 | 17 | 141 |
| Kidney bean leaves | 909 | 167 | 35 | 116 | 164 | 618 |
| Maize middlings | 896 | 127 | 165 | 121 | 96 | 491 |
| Rice bran | 903 | 126 | 71 | 159 | 235 | 408 |
| Soybean meal | 897 | 382 | 115 | 175 | 82 | 245 |
| Spent brewer's grain | 917 | 245 | 106 | 153 | 76 | 395 |
| Spent brewer's yeast | 920 | 380 | 33 | 21 | 91 | 516 |
| Sunflower seedcake | 916 | 273 | 73 | 158 | 54 | 441 |
| Sweet potato leaves | 925 | 318 | 40 | 130 | 145 | 366 |
| Wheat middlings | 878 | 178 | 59 | 84 | 67 | 614 |

*CP = crude protein, CF = crude fiber, CL = crude lipid, NFE = nitrogen-free extract (NFE (%) = 100-(CP (%) +EE (%) +CF (%) +Ash (%)). *Fishmeal made of Rastrineobola argentea*

The aim to produce iso-nitrogenous and iso-energetic diets was almost achieved, with CP content ranging between 282 and 300 g kg$^{-1}$ DM, and energy content between 16.5 and 17.3 MJ kg$^{-1}$ (Table 3).

**Table 3.** Proximate composition (g kg$^{-1}$ dry matter, DM) and gross energy (MJ kg$^{-1}$ DM) content of the control diet (CD) and of test diets based on spent brewer's grain (SBY), spent brewer's grain (SBG), wheat middlings (WM), kidney bean leaf meal (KBLM), and sweet potato leaf meal (SPLM)

| Nutritional component | CD | SBY | SBG | WM | KBLM | SPLM |
|---|---|---|---|---|---|---|
| Crude protein | 298 | 288 | 276 | 241 | 266 | 285 |
| Crude lipid | 51.0 | 53.0 | 61.0 | 53.0 | 53.0 | 52.0 |
| Crude fiber | 82.0 | 80.0 | 83.0 | 82.0 | 76.0 | 79.0 |
| Dry matter | 911 | 887 | 902 | 901 | 898 | 895 |
| Ash | 87.0 | 78.0 | 71.0 | 75.0 | 98.0 | 91.0 |
| NFE | 397 | 395 | 402 | 402 | 406 | 389 |
| *Gross energy (MJ kg$^{-1}$) | 17.1 | 16.8 | 17.3 | 17.0 | 16.6 | 16.5 |

**Gross energy was estimated using the following coefficients: 23.6 kJ g$^{-1}$ for crude protein, 39.5 kJ g$^{-1}$ for crude lipid and 17.2 kJ g$^{-1}$ for carbohydrates (National Research Council (U.S.), 1993). NFE = nitrogen-free extract (total dietary carbohydrates)*

### Growth performance, feed utilization and somatic indices

Measurements of body weight changes over the rearing period showed a consistent trend of daily weight gain for all treatments (Figure 1). From day 14 until the end of the experiment, fish fed CD displayed higher growth than those in other treatments. The WM and KBLM diets consistently displayed lower growth performance.

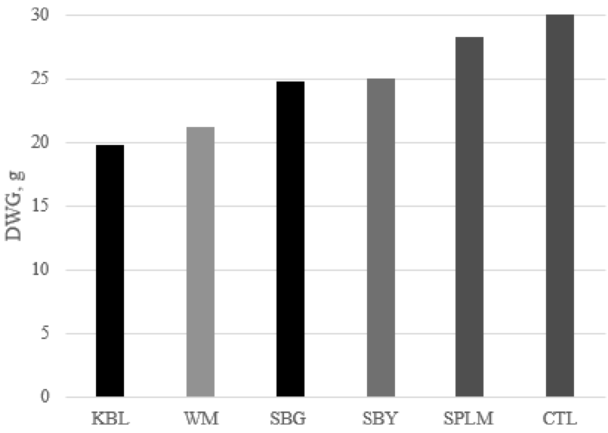


**Figure 1.** Growth performance the 70-day of Nile tilapia (*Oreochromis niloticus*) fed the control diet (CD) and test diets based on spent brewer's yeast (SBY), wheat middlings (WM), kidney bean leaf meal (KBLM), sweet potato leaf meal (SPLM), and spent brewer's grain (SBG)

During the 70-day experimental period, fish grew from an initial average weight of 28.9±1.88 g/fish to a high final weight 60.2±2.81g/fish, and there was no difference in fish initial body weight between the treatments (Table 4). Final body weight (FBW) and weight gain (WG) were significantly highest for fish fed CD, followed by fish fed diets SPLM, SBY, SBG, WM, and KBLM, in that order. Specific growth rate (SGR) was significantly highest in CD and SPLM fish, followed by SBY and SBG fish, and lowest in fish fed diets WM and KBLM. Feed conversion ratio (FCR) was highest in KBLM fish and lowest in CD and SPLM fish. Other feed utilization indices, including feed intake (FI) and protein efficiency ratio (PER), were not significantly affected by dietary treatment and showed only small numerical differences across treatment groups. In addition, survival rate (SR) was not significantly different between treatments (range 75.0-87%) and no differences were detected in HIS and VSI between fish on CD and the test diets.

**Table 4.** Growth performance, feed utilization and somatic indices of Nile tilapia (*Oreochromis niloticus*) fingerlings fed the control diet (CD) and test diets based on spent brewer's grain (SBY), spent brewer's grain (SBG), wheat middlings (WM), kidney bean leaf meal (KBLM), and sweet potato leaf meal (SPLM) for 70 days

| | CD | SBY | SBG | WM | KBL | SPLM | SE | *p-value* |
|---|---|---|---|---|---|---|---|---|
| IBW (g) | 27.3 | 28.1 | 29.7 | 29.0 | 29.3 | 27.8 | 1.33 | 0.32 |
| FBW (g) | 60.2 $^{a}$ | 54.2 $^{bc}$ | 53.9 $^{bc}$ | 50.0 $^{cd}$ | 48.7 $^{d}$ | 56.1 $^{ab}$ | 1.17 | 0.01 |
| DWG (g) | 30.7 $^{a}$ | 25.1 $^{bc}$ | 24.8 $^{bc}$ | 21.2 $^{c}$ | 19.9 $^{c}$ | 28.3 $^{ab}$ | 8.51 | 0.01 |
| SGR (%) | 1.10 $^{a}$ | 0.90 $^{ab}$ | 0.90 $^{ab}$ | 0.80 $^{c}$ | 0.80 $^{c}$ | 1.00 $^{a}$ | 0.07 | 0.03 |
| FCR | 1.40 $^{b}$ | 1.60 $^{ab}$ | 1.70 $^{ab}$ | 1.80 $^{ab}$ | 2.10$^{a}$ | 1.40$^{b}$ | 0.78 | 0.02 |
| PER | 0.50 | 0.40 | 0.40$^{c}$ | 0.40 | 0.30 | 0.50 | 0.08 | 0.36 |
| FI (g) | 41.1 | 39.5 | 40.3 | 36.5 | 40.6 | 39.6 | 0.16 | 0.98 |
| PI | 70.5 | 64.3 | 69.1 | 61.2 | 68.9 | 67.5 | 0.19 | 0.97 |
| VSI (%) | 10.1 | 9.60 | 9.20 | 8.60 | 9.80 | 9.60 | 0.29 | 0.77 |
| HSI (%) | 1.40 | 1.50 | 1.20 | 1.40 | 1.30 | 1.10 | 0.08 | 0.68 |
| SR (%) | 85. 0 | 78.4 | 83.4 | 75.0 | 86.7 | 80.0 | 1.88 | 0.53 |

*IBW = initial body weight (g), FBW = final body weight, DWG (g) = daily weight gain, SGR = speci fi c growth rate, FCR = feed conversion ratio, PER = protein ef ficiency ratio, FI = total feed intake per fi sh, PI = protein intake, HIS = hepato-somatic, VSI = viscero-somatic (VSI) index, SR = survival rate, SE = standard error of difference of means. Means within rows with different superscript letters are signi ficantly different (p≤ 0.05), determined by Duncan's multiple range test. For all growth and feed utilization parameters, n= 18*

## Water quality

Water quality parameters recorded during the experiment remained stable and showed no differences between treatments (p>0.05). Average temperature (°C) was 27.3±0.66, pH was 7.40±0.20, and dissolved oxygen content was 5.50±0.70 mg $L^{-1}$. The concentration of total ammonia-N and nitrite-N was 0.30±0.03 mg $L^{-1}$ and 0.10±0.02 mg $L^{-1}$, respectively.

## Discussion

Tilapia fish fed SPLM (up to 32% replacement) achieved as good growth performance as fish fed the fishmeal-based control (CD), indicating high suitability of SPLM as a feed ingredient for tilapia fingerlings. This agrees with previous findings that tilapia, which is typically an omnivorous species, is capable of using nutrients from animal and plant feedstuffs, including leaves of vegetable crops such as sweet potato (Felix et al 2020; Adewolu 2008; El-Sayed 1999). High nutritive value of SPLM has been reported previously (Ishida 2000; Woolfe 1992). Furthermore, sweet potato leaves contain various bioactive compounds (Nguyen et al 2021) and several essential minerals (Fe, Ca, Mg) and essential trace elements (Cr, Co, Ni, Cu, Zn) (Taira et al 2013).

The fish in the present study fed brewer's by-products (SBG, SBY) generally showed adequate growth performance relative to fish fed CD, indicating that tilapia can utilize high amounts of agro-industrial-by products in their diet, which agrees with findings by Felix et al (2020) and El-Sayed (1999). Up to 50% fishmeal replacement with SBY gave high growth performance, as evidenced by high SGR and FCR, and also WG and FWG similar to that in CD fish (Table 4). High growth of tilapia fed SBY is in accordance with findings in earlier studies on tilapia cultured in different systems (Islam et al 2021; Abdel-Tawwab et al 2020; Nhi et al 2018). Nhi et al (2018) reported good growth and protein efficiency for tilapia fed up to 30% SBY, but for fish cultured in a biofloc environment instead of a clear water recirculating system. The good performance observed in our study for SBY can be related to its good dietary qualities, making it suitable for use as an alternative to fishmeal protein in fish feed (Agboola et al 2021). For instance, it has a high protein content and favorable amino acid profile , in addition to containing important bioactive compounds such as β-glucan, nucleic acids, mannan oligosaccharides, etc. that can substantially improve fish growth and health (Vidakovic et al 2020; Øvrum Hansen et al 2019; Ferreira et al 2010). Thus, according to our results and those in other studies, SBY can partly replace fishmeal in commercial diets for Nile tilapia.

The diets with SBG (32% replacement) and SPLM (32% replacement) gave only minor differences in weight gain compared with the control. SBG was moderately rich in CP (23.4 ± 0.2), CL 9.4, and cellulose 51 ± 0.7 and also contains other nutrients (Yu et al 2020). The CL content in our study was higher than the 66 g $kg^{-1}$ DM reported by Nhi et al (2018), and much higher than the 39 g $kg^{-1}$ DM reported for oven-dried SBG by Santos et al. (2003). Elevated dietary lipid content above the minimum required level can support higher growth rates, partly due to protein-saving effects (NRC 2011). Our values were within the optimum range (5-12% lipid) reported for tilapia diets (Lim et al 2011). However, higher dietary lipid content may increase flesh lipid levels in freshwater fish (Guo et al 2019).

In general, growth performance indices may vary due to differences in nutritional quality or properties between feed ingredients used, size and age of fish, and culture systems, in addition to environmental conditions, feeding duration, and other unknown factors (Nhi et al 2018; Liti et al 2006). For ingredients such as brewer's by-products, nutritional quality or properties may also vary with factors including the type of barley, malting and mashing conditions, and additives used during beer processing (Robertson et al 2010). Our results indicated that the body indices evaluated (HSI, VSI) did not differ across treatments, i.e., there were no significant effects of the dietary treatments on the physiological condition of the fish, fat accumulation and adaptation to the environment, and thus on fish welfare (Robb 2008).

From our results, it can be concluded that SPLM, SBY, and SBG are nutritionally adequate as sources of protein, fiber, carbohydrate, and energy and can be beneficial for tilapia fish growth performance, so their use in commercial tilapia diets can be recommended. The experimental diets containing those ingredients also did not affect fish somatic indices, so there were no obvious negative health effects in any treatment. In contrast, the results showed that weight gain for fish fed the KBLM and WM diets only reached about 40% and 45% of that in the control (CD) group. Fish fed KBLM and WM showed consistently decreasing growth (FW, WG, SGR), even though the replacement rate of fishmeal was only 5-7 g/kg in those diets, indicating that KBLM and WM should not be included as feed ingredients or should be kept at low levels in diets so as not to affect growth of tilapia. Tilapia has the capability to efficiently utilize high levels (30-70%) of dietary carbohydrates as a primary energy source, giving protein-saving effects for growth (FAO 2018; Kamalam et al 2017). Fish fed WM and KBLM displayed the worst growth performance of all treatments, possibly due to the presence of anti-nutritional factors such as phytate, trypsin inhibitor, and polyphenols commonly found in cereals and legumes, which reduce the bioavailability of nutrients and minerals (Ram et al 2020). Cereal grain has low overall phytic acid content (1-2% by weight), but in wheat this compound is concentrated in the external cover of the pericarp and the aleurone layer (Brouns et al 2012), which make up the wheat middlings fraction. Previous studies have concluded that use of plant-derived materials, including legume seeds, leaf meals, and root tuber meals, as fish feed ingredients is limited by the presence of different anti-nutritional substances, particularly protease inhibitors, phytates, glucosinolates, saponins, tannins, lectins, oligosaccharides and non-starch polysaccharides, gossypols, cyanogens, mimosine, and antivitamins (Francis et al 2001;Vasconcelos and Oliveira 2004). Tannin, oxalate, and phytate have been detected in bean leaves (Alalade et al 2016). Based on results in the present study, WM and KBLM should only be used in limited amounts in the diet of tilapia.

## Conclusions

Inclusion of SPLM in the diet of tilapia resulted in no or minor differences in WG, FWG, SGR and FCR compared with a control diet based on fishmeal, while inclusion of SBY and SBG gave only minor differences. Therefore these alternative protein sources can replace fishmeal in the diet of Nile tilapia without adverse effects on growth, feed utilization, or body indices, and can be valuable for future sustainable tilapia production in Rwanda and other countries in Africa. However, inclusion of KBLM and WM gave consistently poor growth in fish, indicating that these ingredients should be excluded or kept at low levels in diets so as not to affect growth of tilapia. These findings have practical implications for optimized inclusion of local ingredients, allowing aquaculture nutritionists to tailor practical diet formulations.

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This thesis has shown current status of aquaculture in Rwanda, available feed ingredients and their chemical composition. Evaluated and found that sweet potato leaf meal, brewers grain and yeast protein can replace fishmeal (32-50%) in Nile tilapia diets without reducing growth and feed utilization or impairing blood indices and are thus valuable local protein source for sustainable tilapia production.

**Leon Niyibizi** received his doctoral education at the Department of Animal Nutrition and Management, Swedish University of Agricultural Sciences, Uppsala. He obtained his Master of Science degree in Aquatic resources management at the University of Liège in Belgium.

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