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Declaração de autoria

Declaro ser a autora deste trabalho, que é original e inédito. Autores e trabalhos consultados estão devidamente citados no texto e constam da listagem de referências incluída

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Ana Rebotim

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Abstract

The United Nations' Sustainable Development Goal (SDG) 2 was developed in an endeavour to end world hunger as well as to achieve food security and improved nutrition by 2030. In developing countries, especially in Sub-Saharan Africa, food insecurity is probably still the biggest threat that comes with poverty. Aquaculture has been proposed to undermine this problem, as it has the potential to provide animal protein to low-income communities, without the need to further explore natural resources. The concept of community-based aquaculture is especially interesting as it involves local communities in the animal production process, creating not only a source of food but also a source of income, improving the livelihoods of those involved. Tilapia (*Oreochromis* spp.), referred by the International Development Agencies as "aquatic chicken", is a great candidate species to be used in this context as it is an extremely robust fish, adaptable to a wide range of culture conditions. For community-based aquaculture to be sustainable, it is important that the ingredients used in diet formulation are readily accessible to farmers and have also low environmental impact. Therefore, the aim of this study was to formulate a diet mainly based on local ingredients commonly found in Mozambican household and agricultural waste. This diet was used in Nile tilapia juveniles and its effect on growth performance, feed utilization and nutrient balances was evaluated and compared to those obtained using a typical commercial formulation. The results show that inclusion of local ingredients does not significantly impair growth neither feed utilization in Nile tilapia juveniles. Moreover, higher retention values of phosphorous and nitrogen obtained from this diet, suggest that this formulation is more environmental-friendly than the commercial formulations.

Keywords: Small-scale aquaculture, Sustainability, Sustainable Development Goals, Nile tilapia, Community.

Resumo

Em 2015, a Organização das Nações Unidas definiu um conjunto de objetivos de forma a promover o desenvolvimento sustentável. De entre estes, destaca-se o objetivo 2, que pretende terminar com a fome, a insegurança alimentar e a malnutrição até 2030. Em muitas áreas do mundo, de entre as quais se salienta a África subsaariana, a insegurança alimentar é ainda uma das mais sérias ameaças que vem com a fome, e com o ano 2030 a aproximar-se a um ritmo galopante, é imperativo que se desenvolvam soluções que sejam viáveis a longo prazo e que perdurem mesmo após o término dos projetos de promoção ao desenvolvimento das comunidades menos afortunadas. Sendo a aquacultura a indústria de produção animal com um maior crescimento nos últimos anos, esta tem sido sistematicamente sugerida como uma possível fonte de proteína animal suplementar, de modo a garantir a subsistência destas populações.

Contudo, a sustentabilidade, quer económica quer ambiental, deverá ser priorizada aquando da implementação da aquacultura nos países menos desenvolvidos. Para tal, a formulação das dietas é de maior importância, de forma a garantir o crescimento dos indivíduos bem como minimizar as perdas de fósforo e compostos azotados para o ambiente, minorando assim o impacto ambiental. A aquacultura praticada em comunidade tem também o potencial de desenvolver a economia das mesmas, criando postos de trabalho e gerando rendimento suplementar que pode subsequentemente ser usado para adquirir outros géneros alimentares e não só, promovendo assim uma melhoria na qualidade de vida. A aquacultura desenvolvida em contexto comunitário recorre ao uso de tanques de terra e de recursos como restos alimentares e das atividades agrícola e pecuária, que são utilizados para alimentar os peixes e consequentemente como fertilizante, para promover a produtividade primária do tanque, reduzindo assim a necessidade de *inputs* alimentares externos.

A tilápia do Nilo (*Oreochromis niloticus*) tem alto potencial para ser utilizada neste contexto de produção, uma vez que é uma espécie muito resiliente e que se encontra num nível baixo da cadeia trófica. A sua fácil manutenção em cativeiro, valeu à espécie a designação de "galinha aquática", vulgarmente empregue pelas agências de desenvolvimento. Apesar do seu indubitável potencial, a aquacultura em comunidade tem ficado aquém das suas promessas, principalmente em zonas como a África subsaariana.

Isto deve-se, em grande parte, a iniciativas levadas a cabo por organizações, governamentais ou não, com o objetivo de promover e estabelecer a aquacultura como um setor económico próspero nas mesmas áreas. Contudo, estas iniciativas têm-se baseado maioritariamente em incentivos monetários, desacreditando a formação contínua dos aquacultores, que uma vez findas as campanhas de promoção ao desenvolvimento se sentem incapazes de fazer face às despesas e às dificuldades que encontram, acabando assim por abandonar o seu ofício em detrimento de outro mais lucrativo. Muitas das vezes os aquacultores dependem de rações comerciais que podem constituir mais de 60% do total de custos operacionais. A eliminação ou minimização dos conteúdos de farinha e óleo de peixe nas dietas são de maior importância para reduzir os custos de produção, mas também para assegurar uma maior sustentabilidade das dietas. Muitas alternativas têm sido testadas, com resultados promissores, desde fontes alternativas de proteína vegetal, resíduos de produção da pecuária e aquacultura e também ingredientes alternativos como a farinha de inseto, os subprodutos da produção de bebidas alcoólicas e da pecuária, entre outros. O objetivo deste estudo foi, desta forma, formular uma dieta à base de alimentos locais de Moçambique, como a mandioca, o milho, o amendoim, e feijões. Esta dieta experimental continha um teor mínimo de farinha de peixe, para garantir a aceitação do alimento. Esta dieta foi testada em juvenis de tilápia do Nilo, durante um período de 57 dias. Os parâmetros utilizados para avaliar o potencial da dieta foram a performance de crescimento, a utilização do alimento, bem como os balanços de fósforo e azoto. Para tal determinaram-se parâmetros como o aumento de peso, o índice de ingestão voluntária, o índice de conversão alimentar, o índice de eficiência proteica, os índices hepatossomático e viscerossomático, bem como o fator de condição. A composição corporal dos indivíduos foi determinada para obter os teores de matéria seca e cinzas, bem como os teores brutos de proteína, lípidos, energia e fósforo. A retenção de energia, proteína, lípidos e fósforo foram calculadas como a percentagem de ingestão de cada componente. Por último foram também calculados os balanços de fósforo e azoto com base na ingestão, ganho e perda dos mesmos.

Os resultados foram comparados com os obtidos em indivíduos mantidos durante o mesmo período de tempo e em condições análogas, mas alimentados com uma dieta seguindo uma típica formulação comercial. As diferenças estatísticas entre os resultados foram calculadas através de um teste *t*, utilizando o software SPSS. Os resultados

mostram um menor crescimento dos indivíduos alimentados com a dieta experimental, o que pode estar relacionado com o menor teor de proteína presente nesta dieta. Relativamente à utilização do alimento, não foram encontradas diferenças na taxa de alimentação voluntária, o que sugere que a palatabilidade da dieta é adequada. Já a taxa de conversão de alimento foi superior para os indivíduos do grupo experimental, revelando uma menor eficiência na utilização do mesmo. Contudo, os valores registados neste estudo estão dentro da faixa de valores referidos na literatura para a tilápia do Nilo. A dieta experimental promoveu uma maior deposição de gordura na carcaça, o que pode ser devido à presença de ingredientes com alto teor energético como o amendoim e o milho. Por último, a dieta experimental promoveu a retenção de fósforo e compostos azotados, minorando a perda dos mesmos. Num contexto de cultivo em tanques de terra, esta é uma característica muito importante, pois permite evitar a eutrofização dos corpos de água que para além de comportarem a produção de peixe irrigam também os solos agrícolas circundantes. O baixo impacto ambiental da dieta experimental aliado à sua relação custo-benefício, fazem desta uma alternativa ideal para promover a aquacultura de espécies de baixo nível na cadeia trófica, como a tilápia do Nilo.

Palavras-chave: Aquacultura extensiva; Sustentabilidade; Objetivos de Desenvolvimento Sustentável; Tilápia do Nilo; Comunidade.

Abbreviations, acronyms, and symbols

ABW: Average body weight

AFI: Apparent feed intake

AFNOR: French Standardization Association

CBA: Community-based aquaculture

CF: Condition factor

CI: Commercial Ingredients

DGI: Daily growth index

DM: Dry matter

DW: Dry weight

FBW: Final body weight

FCR: Feed conversion ratio

FM: Fishmeal

g: gram

ha: hectare

HSI: Hepatosomatic index

IAA: Indispensable amino acids

IBW: Initial body weight

kg: kilogram

LI: Local Ingredients

mg: milligram

MJ: Mega joule

ml: millilitre

nm: nanometre

PER: Protein efficiency ratio

RAS: Recirculating aquaculture system

SDGs: Sustainable Development Goals

SPSS: Statistical package for the social sciences

UV: Ultra-violet

VFI: Voluntary feed intake

VSI: Viscerosomatic index

ºC: centigrade degree (degree Celsius)

µm: micrometre

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1. Introduction

1.1. Aquaculture as a tool to alleviate hunger and food insecurity

The Sustainable Development Goals (SDGs) (UN, 2015) were created as a framework for developing and developed countries to achieve prosperity without threatening planetary boundaries (Steffen *et al*. 2015). These contemplate the economic, social, and environmental dimensions of social development (Ogisi & Begho, 2021) in a total of 17 sustainable development goals, each with specific targets and indicators (Moyer & Hedden, 2020). These goals constitute the 2030 Agenda for Sustainable Development. Some SDGs are related not only to society but also to economy and the environment, which makes them pivotal for the success of the whole SDG agenda (FAO, 2016). Such is the case of the SDG 2 pledging to "end hunger, achieve food security and improved nutrition and promote sustainable agriculture". The eradication of hunger must be linked with food security, which is evaluated according with four different parameters: availability, access, utilization, and stability (FAO, 2008).

As of 2020, one fifth of the African population – 256 million people – was undernourished, and out of those, 239 million lived in Sub-Saharan Africa (FAO *et al*., 2020). In 2016, even the majority (60%) of a strong middle class was deemed vulnerable to slip back into poverty and, consequently, food insecurity (Chicago Council, 2016). In fact, both the number of undernourished people and its prevalence have shown an increase in recent years, accounting for one out of nine people being undernourished, worldwide, in 2017 (FAO, 2018). The onset of the COVID-19 pandemic makes it even less likely to achieve food security by 2030 in least developed areas of the globe, as Sub-Saharan Africa. Some of the immediate impacts of the pandemic were the loss of income and the disruption of food systems, culminating on an additional 26 to 40 million Sub-Saharan Africans prone to fall into poverty and suffer from food insecurity due to the pandemic, according to a World Bank report (Lakner *et al*., 2019).

In turn, the lack of income turned in the inability to purchase staple foods. In a recent paper, Josephson *et al*. (2021) inquired a sample of households in four low-income countries: Ethiopia, Malawi, Nigeria, and Uganda. The authors estimated that 77% of the population lived in households that have lost income during the onset of the pandemic. Other important estimates made by the authors are those of moderate and severe food insecurity across all countries: moderate to severe food insecurity affected 60% of the adult population (98 million individuals) and severe food insecurity affected 22% of the adult population. The pandemic is still evolving and data from low-income countries tends to be scarce or not updated; regardless, the current situation only exalts the sense of urgency that needs to be instilled when talking about hunger.

Fish is a key element in the diet of billions of consumers, not only as a source of high-quality protein but also of vitamins and fatty acids (Thilsted *et al*., 2016). Its importance is even greater in the least developed countries, where apparent fish consumption has increased from 6.1 kg *per capita* in 1961 to 12.6 kg *per capita* in 2017 at an average annual rate of 2.4%. In fact, in countries such as Bangladesh, Cambodia, Gambia, Ghana, Indonesia, Sierra Leone, Sri Lanka and several other small island developing states, fish provided 50% or more of the *per capita* animal protein intake (FAO, 2020). Production of fish in countries like Bangladesh, Brazil, China, Egypt, India, Indonesia, the Philippines, Myanmar, Thailand, and Vietnam, is likely growing, making them increasingly more relevant actors in the global market (FAO, 2016). Coincidentally, these countries are also home to 52% of the world's undernourished population.

In a study from 2016, Rashid *et al.* stipulated that if aquaculture development had stopped in the 80's, global fish supply *per capita* as of 2013 would have been about half of the actual supply in that year. The consequences of such scenario are not hard to imagine - higher prices of fish products and overall lower fish consumption. In fact, the production of fish in aquaculture settings has helped to drive down the prices of farmed species, making them increasingly accessible to poorer consumers. It is certainly not surprising that food price stability plays a big role in ensuring food security to low-income consumers, which spend a large fraction of their livelihoods in food acquisition (Troell *et al*., 2014). The price of fish coming from aquaculture is way less volatile than that of fish coming from capture fisheries, for two reasons: 1) aquaculture is immune to the seasonality and unpredictability of the natural environment (Asche *et al*., 2015), and 2) unlike capture fisheries, aquaculture is also immune to anthropogenic pressures (i.e., fishing efforts) that would otherwise compromise supply (Belton *et al*., 2018). Although some may argue that fish intake may not always be a synonym of improved nutritional status (Kongsbak *et al*., 2008), it has been proved that in households that practice farming as an income generating activity there is a higher ability to purchase nutrient-rich foods (Aiga *et al*., 2009). This pattern, known as the "Bennett's Law", has been known for long and it inversely correlates income with consumption of starchy staples; since increasing income will translate in a more diversified diet (Timmer *et al*., 1983). This is the pattern we should aim to attain in all nations struggling with food deficiencies, as it truly is a badge of socio-economic growth. From all stated above, one would dare to say that aquaculture could really play a role on achieving that.

1.2. Community-based aquaculture

When tailored specifically to enhance local well-being, aquaculture can play an important role in improving the livelihoods of those involved, especially, less-intensive production systems that meet the nutritional needs of the poor, aiding for food security (Golden *et al*., 2016). The concept of community-based aquaculture (CBA) ties small-scale aquaculture to the development of low-income communities. Its purpose embodies the definition of rural aquaculture given by Espinosa (FAO, 2000), as it focuses on improving natural water productivity, the use of polyculture, as well as alternative feeds prepared from local waste supplies. This type of farming is non reliant on external feed inputs and focuses on using low trophic level fish species. Moreover, culture systems are often integrated with agriculture or even livestock production (Mulokozi *et al*., 2020).

By diversifying coastal livelihoods and providing new skills to those involved, CBA also contributes to the adaptative capacity of communities to climate change and other environmental threats (Gentry *et al*., 2017). Further, CBA reduces the dependence on natural resources, ultimately contributing to biodiversity conservation through the reduction of fishing efforts (Troell *et al*., 2014). As it puts little pressure on natural resources and allows for the use of waste products as feeds, community-based aquaculture is also perceived as ecologically effective (Edwards *et al*., 2002).

Concerning its contribution to food security, small-scale aquaculture is believed to meet the subsistence fish consumption needs of rural households and generate supplemental income through sales of small marketable surplus that may be spent on purchasing staple foods (Ahmed & Lorica, 2002; Beveridge *et al*., 2013).

In many Asian, African, and South American nations, farmed fish is extensively consumed domestically, and its availability and accessibility to low-income consumers are improving (Belton *et al*., 2018). In the ten largest of these producers - Bangladesh, Brazil, China, Egypt, India, Indonesia, the Philippines, Myanmar, Thailand, and Vietnam (FAO, 2016) - about 89% of the farmed fish is consumed within domestic markets. An example of that is the increasing availability of low value farmed species, like tilapia in Egypt (Belton *et al*., 2018).

Regardless, many attempts of using aquaculture to eradicate poverty have not been fully successful (Slater *et al*., 2013). The main cause for the unsuccess of such initiatives has often been the disregard for the local socio-economic context in which they are inserted (Philcox *et al*., 2010). Initiatives that only take in consideration economic and environmental standards fail in addressing the social drivers that can lead individuals to perceive and embrace an unfamiliar activity such as aquaculture (Bush *et al*., 2009). Moreover, social organizations within the communities empower and support their members, making them more likely to pursue novel income generating activities (Cinner & Pollnack, 2004; Sesabo & Tol, 2005). Appropriate governance and policy, that take in consideration the perceptions and expectations of locals, are also in need for the successful establishment of community-based aquaculture (Torell *et al*., 2010; Bostock, 2011; Carneiro, 2011). Other constraints, pointed by the farmers themselves, are the technological gaps residing in the shortage of fish fingerlings and feed, the lack of appropriate training, low fish yield and the lack of appropriate infrastructures, as well (Haji & Workagegn, 2021).

1.3. The case of Africa and Mozambique

Aquaculture has become the main provider of fish protein in many developing countries (Golden *et al*. 2017) and more than 80% of the world's aquaculture production comes from small-scale farms owned and managed by families (Mulokozi *et al*., 2020). Despite aquaculture often being considered to have failed in Sub-Saharan Africa, it has expanded greatly in countries such as Egypt, Ghana, Kenya, Nigeria, Uganda, and Zambia (Satia, 2017). In other countries, such as Mozambique, there is a clear potential to establish this activity, but that potential is yet to be met. Fish production in Mozambique focuses mainly on freshwater species of the genus *Oreochromis* (Companhia & Thorarensen, 2012), with special attention to *O. niloticus*, due to the advantages of several genetic improvements it has undergone (Elabd *et al*., 2019; Tan *et al*., 2019). Fish production is expected to reach 27, 921 tons in 2024 (Idepa, 2020).

The lack of support from the private sector or even from government investment companies, hinders the development of the industry and makes farmers anxious to join it, as the costs of all inputs required for production are oftentimes unbearable (Muhala *et al*., 2021). For instance, in Egypt, between 75 to 85% of the operational costs of fish production are attributable to fish feed (Dickson *et al*., 2016). Difficulty in accessing quality feed is not exclusive to Mozambique, rather it extends to almost all African countries (Hasimuna *et al*., 2019; Musinguzi *et al*., 2019; Oyebola & Olatunde, 2019). Plus, many of the feed manufacturers purchase their ingredients from neighbouring countries, increasing the costs of feed even further (Ndah *et al*., 2011; Hasimuna *et al*., 2019). This unavailability of standardized feed has led to equally unstandardized feeding practices, and sometimes the only solution is to use alternative feeds. Thus, farmers rely on farm-made feed prepared with cassava and lettuce leaves, leftover food, corn and rice brans and other alternative ingredients (Muhala *et al*., 2021). Another significant production cost comes from the obtention of quality fry and fingerlings. Tilapia species mature early and breed profusely, so monosex male populations are generally preferred as a means of not retarding growth (Fuentes-Silva *et al*., 2013; Basavaraja & Raghavendra, 2017). This way, the production of fry and fingerlings requires technology and knowledge that are uncommon between rural farmers. This of course, paired with transportation, increases the costs of fish (Manliclic *et al*., 2018).

In Mozambique, the Research Aquaculture Centre of Southern Africa is already focusing on the production and availability of sex-reversed fry and genetic improvement research of Mozambique tilapia (*Oreochromis mossambicus*) (Das, 2019). This will probably help reducing fingerling costs for Mozambican farmers. Regardless, these issues are the reflection of a lack of research, education, and constant training of the farmers (Muhala *et al*., 2021). Poor water quality is also a big contributor to the stagnation of aquaculture development, as it often leads to outbreaks of opportunistic diseases and jeopardizes production (Chirindza & Thorarensen, 2010; Maulu *et al*., 2019; Prema, 2020; Hasimuna *et al*., 2020). Water quality deteriorates even faster since farmers often use organic fertilizers, such as manure, as the first feeding mechanism, which can cause eutrophication or the creation of anoxic zones by deposition (Tidwell, 2012; Boyd, 2020).

Recently, the aquaculture development strategy 2020-2030 was approved in Mozambique by the Institute of Fisheries and Aquaculture Development (Muhala *et al*., 2021). Although this is surely a step in the right direction, there are many dimensions to the unsuccess of aquaculture in Africa and so, a thorough understanding of them is mandatory to shift the paradigm.

1.4. Tilapia – why is it a good candidate for community-based aquaculture?

The name 'tilapia' derives from the African native Bushman word 'thiape', that literally means fish (Trewavas, 1982; Chapman, 2000). This designation comprises a group of freshwater fish species from the *Cichlidae* family, autochthonous to Africa and Palestine (Philippart & Ruwet, 1982). Three genera of cichlids fall under the scope of the tilapia definition - *Oreochromis*, *Sarotherodon* and *Tilapia*. Among tilapia's natural distribution range, 112 different species and subspecies have been identified (Mc Andrew, 2000), inhabiting freshwater streams, ponds, rivers and less commonly, brackish waters (Prabu *et al*., 2019).

Its popularity in aquaculture, especially in development initiatives, rendered tilapia the title of "food fish of the 21st century" (Prabu *et al*., 2019). Their high growth rates, adaptability to a wide range of culture and environmental conditions, their ability to grow and reproduce in captivity and the ability to feed in low trophic levels gave tilapia the designation of "aquatic chicken" and all these factors contribute to its great potential to be used in aquaculture (El-Sayed, 2019). The farming of tilapia, namely Nile tilapia goes back to Egypt, more than 4000 years ago (Prabu *et al*., 2019). However, during the 20th century, tilapia was introduced into other countries through Pan-African transplants as a resource both to aquaculture and fisheries, pushing farming boundaries beyond those of the species natural distribution (Pillay, 1990; Pullin *et al*., 1991). Nowadays, tilapia represents an important source of animal protein and income throughout the world. The genera of tilapia used in aquaculture are *Oreochromis* and *Sarotherodon* (Amoussou *et al*., 2019), being that the species farmed on a significant scale are Nile tilapia (*O. niloticus*), blackchin tilapia (*S. melanotheron*), blue tilapia (*O. aureus*), Mozambique tilapia (*O. mossambicus*), and their hybrids (Ansah *et al*., 2014). Nile tilapia is the third most produced species in aquaculture worldwide, only surpassed by silver carp and grass carp (FAO, 2020).

There are many attributes that make tilapia the ideal candidate to be used in developing countries. One of them, and unarguably the most important, is their ability to feed on low trophic levels and to accept inert feeds immediately after yolk-sac absorption (Elkatatani *et al*., 2020). By feeding on lower trophic levels, feed costs are lower than for carnivorous species, while not jeopardizing their suitability to human consumption as a high-quality protein source (El-Sayed, 2019). Such characteristics are of extreme importance for the economics of tilapia culture (El-Sayed, 2019). Species form the genus *Oreochromis*, such as *O. niloticus*, *O. mossambicus* and *O. aureus*, are primarily microphagous, feeding on phytoplankton, periphyton and detritus (El-Sayed, 2019).

1.5. Alternative ingredients in tilapia nutrition

Aquaculture will only contribute effectively to economic development and food security when the production of fish bares minimal impact to the environment and maximum for the society (Luthada-Raswiwsi *et al*., 2021). In their work in 2015, Bene *et al*., argued that this positive impact would only be possible if some conditions were met, in between which was the impending reduction of fishmeal and fish oil content in aquafeeds. As it was discussed in previous sections, the operational costs attributable to fish feed can reach values as high as 85% (Dickson et al., 2016). Therefore, to attain sustainability in aquaculture production, the reduction of such costs must be prioritized. This can be done by using alternative ingredients which are readily available to farmers. These ingredients ought to present, apart from a high protein content, a balanced amino acid profile, high nutrient digestibility and low levels of fibres, starches, and non-soluble carbohydrates (Gatlin *et al*., 2007).

In fact, to reduce production costs, tilapia farmers have long been relying on locally available feed ingredients to supplement the diet of their cultured fish (Chenyambuga *et al*., 2014). This is possible when cultivating herbivorous and omnivorous fish species, which nutritional requirements can be met by using plant ingredients, household waste and agricultural by-products instead of conventional ingredients (Craig & Mclean, 2005). In a study conducted by Mmanda *et al*. (2020) 80% of Tanzanian tilapia farmers reported to follow this practice. These farmers often used plant ingredients such as maize bran, rice polish, banana and sweet potato leaves, as well as animal by-products from poultry production. Kitchen and garden leftovers were also used, and fishmeal was replaced by sardines, caught by the farmers in nearby water bodies (Onyango *et al*., 2019; Mmanda *et al*., 2020). Also in Tanzania, the potential of fly maggots (Hezron *et al*., 2019) and cassava (*Manihot esculenta*) and moringa (*Moringa oleifera*) leaves (Madalla *et al*., 2013; Madalla *et al*., 2016) to be used in aquafeeds has been evaluated. It is noteworthy, however, that these local ingredients are often dependable on regionality and seasonality. Moreover, their nutritional value is highly variable (Kaliba *et al*., 2006; Onyango *et al*., 2019).

Many agricultural by-products, for example, have been incorporated in fish feeds as sources of protein, energy, or lipids. Some of these protein sources are soybean meal, rapeseed meal, coconut seed cake and cottonseed cake (Cho & Slinger, 1979; El-Sayed, 1999; Storebakken *et al*., 1998). Sources of energy include ingredients such as maize bran, rice polish and bran, and wheat pollard (Kaliba *et al*., 2006; Liti *et al*., 2006). Sunflower and soybean oils are often used as sources of lipids, regardless of other ingredients listed above (e.g., cottonseed cake) having the potential to contribute to the overall lipid content of the feed (NCR, 2011; Azaza et al., 2015; Ogello *et al*., 2017). Plant leaves from moringa (Javid *et al*., 2018), cassava (Madalla *et al*., 2016) and sweet potato (*Ipomoea batatas*) (Lochmann *et al*., 2013) can also be used as protein sources for fish.

Soybean meal has been indubitably the mostly used protein source of vegetable origin. Its popularity is due to its protein content (40-51%), balanced amino acid profile and high digestibility (Zhou & Yue, 2012; Ng & Romano, 2013; Ozkan *et al*., 2015). On the other hand, soybean meal is deficient in indispensable amino acids (methionine and cysteine) and contains antinutritional factors (*e.g.*: phytic acid) that often inhibit or overall affect growth and feed efficiency (Ng & Romano, 2013; Hassaan *et al*. 2015). This conventional protein source is, nonetheless, becoming scarce and competitive, with a price range that is on the verge of becoming inaccessible to many farmers (Guo *et al*., 2011; Deng *et al*., 2015). Other commonly used plant ingredient is cottonseed meal – a cheap, palatable, and nutritious by-product of oil extraction (Ng & Romano 2013; Montoya-Camacho *et al*., 2019). High inclusion levels (more than 75%) of cotton seed meal have been shown to cause impairments in growth, feed efficiency and phosphorous retention in tilapia (*Oreochromis* sp.) (Mbahinzireki *et al*., 2001). This is linked to low availability of some indispensable amino acids (lysine and methionine), and high concentrations of fibres and antinutritional factors (Ayadi *et al*., 2012). Nevertheless, an inclusion of 25% of cotton seed meal, have been shown to not affect the growth of tilapia species such as Mozambique tilapia (Jackson *et al*., 1982) and Nile tilapia (Ochieng *et al*., 2017). Sunflower seed meal is another popular alternative, with a nutritional value equivalent to that of soybean meal (Gonzalez-Salas *et al*., 2014). However, its high levels of crude fibres (Ochieng *et al*., 2017) and endogenous antinutritional factors (Becker *et al*. 2001) require caution when calculating inclusion levels of this ingredient. Rapeseed meal is another common alternative which has adequate nutritional quality as well as high protein levels (González-Salas *et al*., 2014). However, rapeseed meal has amino acid deficiencies which paired with high levels of antinutritional factors, limit its use in the formulation of aquafeeds (Ayadi *et al*. 2012). Corn gluten meal (Hisano *et al*., 2003) and distiller grains (Herath et al., 2016; Webster *et al*., 2016; Khalifa *et al*., 2018), have also been tested as alternative protein sources in tilapia diets, with promising results.

Distiller's dried grains with solubles, are a by-product generated in alcohol production from cereal grains (Bothast & Schlicher, 2005). Since they are subjected to fermentation, their nutritional value is improved, making them an appealing alternative protein/ energy source for aquaculture species (Chevanan *et al*., 2009; Mostafizur Rahman *et al*., 2015). Another fact contributing to this is that they also lack antinutritional factors (Welker *et al*., 2014). In one study, Gabr *et al*. (2013) have noticed positive effects on tilapia growth performance with inclusion of corn-based distiller's grains up to 16%. At an inclusion level of 20% feed utilization enhancement has also been reported using this resource in grass carp (Kong *et al*., 2020). Brewery spent yeast and grains are also alcohol production by-products that can be used in aquafeeds, due to the considerable protein content (Muthusamy, 2014; Nhi, 2019). Brewer spent yeast is nonetheless more promising for aquafeed production as it has a low fibre content and high digestibility compared to other plant ingredients (Mmanda, 2020).

Rubber seed, a by-product from natural rubber (*Hevea brasiliensis*) production, is another non-conventional ingredient used in aquafeeds (Deng *et al*., 2015). Inclusion of rubber seed kernel requires caution, as it contains toxic factors (*i.e.*, cyanogenetic glycoside) which can lead to health dysfunctions (Francis *et al*., 2001; Sharma *et al*., 2014). However, if properly processed with heat treatment, these toxic factors can be eliminated, and the product used for its high lipid and protein contents (Sharma *et al*., 2014). In their work in 2015, Deng *et al*., determined that rubber seed kernel inclusion does not affect feed intake in tilapia, and hypothesized that it therefore does not affect overall diet palatability. Inclusion of rubber seed kernel at 30% has also been showed to yield high protein efficiency ratios (PER) and low feed conversion ratios (FCR) in *O. niloticus* (Alegbeleye *et al*., 2012; Deng *et al*., 2015). Toxic factors such as hydrogen cyanide that are present in rubber seed kernel, can also be found in other plant ingredients such as linseed meal and cassava leaves. Regardless, some studies indicate that their inclusion does not affect growth rates of Nile tilapia (Ng & Wee, 1989; Hanafy, 2006).

Peanut meal, a by-product from oil extraction is another interesting ingredient to be used in aquafeed formulation. Despite some amino acid imbalances, peanut meal has a high protein content and low cost per protein unit (Goes *et al*., 2004; Batal *et al*., 2005). Inclusion levels below 25% showed no negative effects on feed intake neither digestibility in Nile tilapia juveniles (Silva *et al*., 2017). Conversely, cashew nut meal has also been considered for animal nutrition, as its production generates great amounts of by-products (Akande *et al*., 2015, Pradhan *et al*., 2020). This resource is rich in amino acids and has an appropriate protein content (Aremu *et al*., 2007). In their study in 2020, Pradhan *et al*., recorded higher growth levels of Mozambican tilapia when cashew nut meal was used in addition to soybean meal, than in the diets that used solely the latter.

The production of livestock and poultry also generates a great number of by-products, accounting for 33–43% of live weight (Martinez-Alvarez *et al*., 2015). These by-products include porcine meal (Hernandez *et al*. 2010), poultry by-product meal (Rossi & Davis, 2012), blood meal (Davies, 2011), meat and bone meal (Ozkan *et al*. 2015) and feather meal (Zhang *et al*. 2014). In Nile tilapia diets, poultry and porcine by-product meal are effective replacers of fishmeal. High dietary inclusion levels of blood meal, on the other hand, have been shown to impair growth performance for most fish species, which can be due to amino acid imbalances (Ayadi *et al*., 2012, Kirimi *et al*., 2016). Feather meal has also been used in aquafeeds, but its poor digestibility (Poppi *et al*., 2011) accounts for high processing efforts, limiting its use (Munguti *et al*. 2014). In general, animal by-products have high contents of crude protein, which enables their use in tilapia diets without affecting development (Abdel-Tawwab *et al*., 2010). In Tanzania, animal by-products have been used as alternative local ingredients in fish farming. Examples include the use of cattle blood (Bekibele *et al*., 2013; Kirimi *et al*., 2017), poultry by-products (Soltan, 2009; El-Sayed & Abdellah, 2012; Yones & Metwalli, 2015) and bone and meat meal (Mabroke *et al*., 2013; Suloma *et al*., 2013). Fisheries also generate a great number of by-products (skin and fins, scales, heads and bones, viscera, and muscle trimmings) that can be used as feed ingredients (Rustad et al., 2011; Martínez-Alvarez *et al*., 2015). Many studies have described that inclusion of fisheries by-products in aquafeeds does not substantially affect growth rate, weight gain, or even feed conversion ratio (Llanes *et al*. 2012; Diop *et al*. 2013; Hernandez *et al*. 2013; Lee *et al*. 2015). Mugo-Bundi *et al*. (2015) used freshwater shrimp (*Cardinia nilotica*) for tilapia diets and reported no negative effects on growth performance. The use of shrimp waste as a local alternative ingredient is also described in the work of Leal *et al*., (2010).

Insects are a natural food source for many freshwater species, including Nile tilapia (Howe *et al*., 2014; Whitley *et al*., 2014). Being an omnivorous species, Nile tilapia has some advantages in chitin degradation, which is present in zooplankton – a natural feed for tilapia (Spataru, 1978; Cottrell *et al*., 1999). Freccia *et al*., (2016) reported no negative effects on growth of Nile tilapia fingerlings fed cinerea cockroach meal (*Nauphoeta cinerea*). The use of insect oil from silkworm pupa (*Bombyx mori*) as a sardine oil replacer in common carp (*Cyprinus carpio*) has also been described in Nandeesha et al. (1999), with promising results. Moreover, the use of maggot fly as a local, alternative resource for aquafeeds has also been described in the works of Devic *et al*., (2013) and Obeng *et al*., (2015).

The number of studies using different and novelty ingredients in fish nutrition is ever increasing. In this section we focused on ingredients that are accessible to small-scale producers. Since many of them are by-products from agriculture, fisheries, and livestock production, or can even be found in household waste, they do not represent further expenses for farmers. By exploring the potential of such ingredients, farmers would be given the possibility to farm a safe and nutritious source of animal protein, without the feed related expenses which are often overbearing.

1.6. Aim

The aim of this work was to test the effects of a diet formulated from household and agricultural waste on growth performance, feed utilization and nutrient balance in Nile tilapia juveniles. The ingredients used to formulate the experimental diet (LI) were sourced in Mozambique and the main goal would be to use this diet in the autochthonous tilapia species, *Oreochromis mossambicus*, in a context of community-based aquaculture.

2. Material and Methods

2.1. Rearing system and conditions

The experiment, with a duration of 8 weeks (March 3rd, 2021-April 29th, 2021), was conducted at the Centre of Marine Sciences (CCMAR) facilities, located in the Gambelas Campus of the University of Algarve, Faro, Portugal (37°02'34.9"N 7°58'15.6"W). Both the CCMAR facilities and staff are certified to house and conduct experiments with live animals (Group-C licenses by the Direção Geral de Alimentação e Veterinária, Ministério da Agricultura, Florestas e Desenvolvimento Rural (DGAV, Portugal). Nile tilapia juveniles (Silver Natural Male Tilapia™) were acquired from Til-Aqua International B.V (Netherlands) and were reared in compliance with the Guidelines of the European Union Council (Directive 2010/63/EU) and Portuguese legislation for the use of laboratory animals, specifically exotic species with invasive potential (Ordinance nº 92/2019, July 10th).

The fish were reared in 100 L cylindrical tanks, in a recirculating aquaculture system equipped with a mechanical filter, a submerged biological filter and an UV sterilizer. Prior to the beginning of the experiment fish with an approximate weight of 11g were distributed evenly between the tanks to a final density of 3 kg/m^3 (27 fish per tank). Water temperature in the tanks was kept at 26 ºC and dissolved oxygen concentration was kept above 80%. Photoperiod was not manipulated; therefore, animals were subjected to natural light variations. Water quality parameters as well as mortality and feed intake were monitored and recorded daily. Backwash of the system was performed every other day to maintain water quality.

Triplicate tanks were assigned randomly to each of the two dietary treatments CI: Commercial Ingredients and LI: Local Ingredients. During the experiment, fish were hand-fed three times a day until apparent satiation from Monday to Saturday and twice on Sunday morning. On Sundays, the afternoon feeding was done with the aid of fish feeders, that released 2g of feed in each tank at a programmed time. An effort was made to keep daily apparent feed ingestion under 4% of total body weight, to avoid overfeeding, since tilapia are voracious animals. In the day preceding each sampling campaign fish were fasted. When tissue collection was required, this fasting period was 24 h. For screenings, this period was reduced to half a day.

2.2. Composition of the diets

The diet LI (Local Ingredients) was tested against a control diet, CI (Commercial Ingredients) that followed a typical commercial formulation regularly used in tilapia farming. In the LI diet, almost all traditional ingredients were replaced by alternative ones, usually waste products or ingredients meant to mimic food sources that occur naturally in ponds. The protein sources used in the commercial diet: fish, poultry, and soybean meals, as well as plant proteins, were totally or almost fully replaced by the local ingredients. Fishmeal was included in the experimental diet at a very low percentage (2.5%) to enhance palatability and maximize feed acceptance. The fat source used in the LI diet was also shifted from soybean oil to palm oil, which is cheaper and native to Africa. The inclusion of insect meal in the experimental diet, was meant to mimic the natural occurring feed in the earthen ponds.

Both diets were supplemented with additives such as minerals and vitamins, and with yttrium oxide was added as an inert marker to measure diet digestibility (results not in the framework of this Thesis). The diets had similar lipid content, but diverged in protein content, with the control diet (CI) having 32.5% protein and the experimental diet (LI) only 22.8%. Table 1 provides a thorough profile of the diets regarding formulation, as well as proximate composition and amino acid profile.

Table 1. Reduced formulation, proximate composition, and amino acid profile of the experimental diets – CI and LI.

Ingredients (%)	CI	LI
Fishmeal	7.50	2.50
Poultry meal	7.50	-
Insect meal	-	2.50
Soybean meal	25.00	10.00
Plant meals	49.73	-
Local ingredients *	-	78.88
Soybean oil	6.50	-
Palm oil	-	3.60
Vitamins, minerals, and other additives	3.75	2.50
Yttrium oxide	0.02	0.02
Proximate Composition	**CI**	**LI**
Dry Matter (DM, %)	92.80	92.70
Crude protein (DM, %)	35.00	24.60
Crude fat (DM, %)	11.90	11.30
Ash (DM, %)	7.80	4.60
Phosphorus (DM, %)	0.70	0.30
Gross Energy (MJ/kg DM)	20.20	20.00
Amino acids (mg/g DM)	**CI**	**LI**
Arginine	32.60	19.90
Histidine	9.80	5.90
Lysine	20.90	13.20
Threonine	17.30	8.30
Isoleucine	15.10	10.10
Leucine	24.70	17.70

Valine	17.40	12.00
Methionine	12.90	3.00
Phenylalanine	19.90	15.60
Cystine	2.90	1.60
Tyrosine	18.00	15.10
Aspartic acid + Asparagine	25.90	19.20
Glutamic acid + Glutamine	47.20	32.80
Alanine	16.40	10.20
Glycine	22.30	11.30
Proline	17.60	10.20
Serine	16.50	11.20
Taurine	0.90	-

* Local ingredients are peanuts, corn, cassava and moringa leaves and a variety of beans.

Both diets were formulated, manufactured, and extruded at SPAROS, Lda. (Olhão, Portugal). Throughout the duration of the trial, feeds were stored in a refrigerator at 4ºC.

2.3. Sampling

At the beginning of the experiment a pooled sample of 12 individuals was collected to perform a posterior whole-body composition analysis. These individuals were euthanized by overdose with 2-phenoxyethanol (VWR, United Kingdom) and individually weighed and measured. After measurement, this sample was frozen and kept at -20ºC until use. Another sample of nine fish was collected, and the individuals were sacrificed using the same procedure. In addition to weighing and measuring, the individuals were also dissected to extract the liver and the gut (including perivisceral fat). Tissues were weighed using a precision scale and individually frozen in liquid nitrogen at -80ºC. Information regarding the weights of the liver and viscera were posteriorly used to calculate the hepatosomatic and viscerosomatic indexes. In the middle of the experiment fish were bulk weighed (per tank) to ensure an accurate calculation of growth indexes and feed conversion ratio.

By the end of the experiment, a third sampling campaign was performed following the model of the first. A sample of 9 individuals per treatment (3 fish/tank) was collected to extract liver and the gut (including perivisceral fat). These fish were previously measured and weighed individually. A sample of 15 fish per treatment (5 fish/tank) was collected to perform a proximate composition analysis. These individuals were weighed one by one and then frozen whole at -20 ºC until further use. The remaining individuals were used to collect samples for other projects or handed to partners to perform posterior experiments. Out of those, 30 individuals (5/tank) were sampled to collect plasma and tissue samples and another 12 individuals of each tank were sent live to CESAM (University of Aveiro) (results not in the framework of this Thesis).

2.4. Proximate composition analysis

Samples were processed by cutting the fish with an electrical knife and then using a food processor to form a paste. A fraction of this paste (15 ml) was stored at -20ºC and was later used to determine dry matter and ash content of the fish. The remaining sample was frozen at -80ºC for a period of 12h and then freeze-dried. The freeze-dried samples were reduced to a powder using a coffee grinder. This powder was sieved through a 1000 μm sieve to ensure homogenous grain size, and then frozen at -80ºC and freeze-dried again before analysis.

Proximate composition analysis of experimental diets and whole-fish samples from the beginning and end of the experiment was performed following the work by Teodósio *et al.* (2021) and were done in duplicates. Determination of the dry matter content was performed by drying the samples at 105ºC for a day (24h). After this period, the samples were weighted and combusted in a muffle furnace at 550ºC for 12h, to assess the ash content. Diets and whole-body fish samples were also analysed for crude protein (N x 6.25) using a Leco nitrogen analyser (Model FP-528; Leco Corporation, St. Joseph, USA); crude fat by petroleum ether extraction using a Soxtherm Multistat/SX PC (Gerhardt, Germany); gross energy by combustion in an adiabatic bomb calorimeter (Werke C2000; IKA, Staufen, Germany) calibrated with benzoic acid. These analyses were performed by an external laboratory.

Total phosphorus content was determined according to Teodósio *et al.* (2021) following an adaptation of the AFNOR V 04-406 norm. Freeze-dried fish samples were analysed in triplicate. The analysis was performed after digestion using an oxidant reagent made from sodium molybdate and perchloric acid at 230 °C in a Kjeldatherm block digestion unit followed by digestion at 75 °C in a water bath. After digestion and addition of the molybdenum reagent, the absorbance of the samples at 820 nm was measured and phosphorus percentage in each sample was determined using a calibration curve. Total amino acid content was determined by ultra-high-performance liquid chromatography in a Waters reversed-phase amino acid analysis system, using norvaline as an internal standard. Samples were pre-column derivatised with Waters AccQ Fluor Reagent (6-aminoquinolyl-N-hydroxysuccinimidyl carbamate) using AccQ Tag method (Waters, USA) after acid hydrolysis (HCl 6 M at 116 °C for 48 h in nitrogen-flushed glass vials). Amino acids were identified by retention times of standard mixtures (Waters) and pure standards (Sigma-Aldrich). Instrument control, data acquisition and processing were achieved by the use of Waters Empower software.

2.5. Calculations

Growth performance was evaluated according to different parameters calculated using the expressions depicted in Table 2.

Table 2. Growth performance parameters and corresponding equations.

Weight Gain (% IBW[a])	$\frac{\text{FBW} - \text{IBW}}{\text{IBW}} * 100$

Daily Growth Index (DGI)	$\frac{FBW^{\frac{1}{3}} - IBW^{\frac{1}{3}}}{Days} * 100$
Condition Factor (k)	$\frac{BW}{Length^{3}} * 100$
Survival (%)	$\frac{Final\ nr\ individuals}{27} * 100$

[a] IBW and FBW are the initial and final body weights, respectively.

Estimates of the overall condition of fish were based on parameters such as the Hepatosomatic Index (HSI) and the Viscerosomatic Index (VSI). The expressions used to calculate each parameter are provided in Table 3.

Table 3. Condition parameters and corresponding equations.

Hepatosomatic Index (HSI)	$\frac{Liver\ Weight}{Body\ Weight} * 100$
Viscerosomatic Index (VSI)	$\frac{Liver\ Weight + Gut\ Weight}{Body\ Weight} * 100$

Feed utilization was evaluated according to the apparent feed intake, voluntary feed intake, protein efficiency and retention and lipid retention (Table 4). Nitrogen and phosphorus balances were also calculated based on intake, retention, and loss. The equations used to calculate such parameters are provided in Table 5.

Table 4. Feed utilization parameters and corresponding equations.

Apparent Feed Intake (AFI)	SUM (Daily Feed Intake)

Voluntary Feed Intake (VFI) [b]	$\frac{\text{Apparent Feed Intake}}{\text{ABW} * \text{days}} * 100$
Protein Efficiency Ratio (PER)	$\frac{\text{Wet Weight Gain}}{\text{Crude Protein Intake}}$
Protein Retention (% intake)	$\frac{\text{Final Protein Content} - \text{Initial Protein Content}}{\text{Protein Intake}} * 100$
Lipid Retention (% intake)	$\frac{\text{Final Lipid Content} - \text{Initial Lipid Content}}{\text{Lipid Intake}} * 100$
Feed Conversion Ratio (FCR)[c]	$\frac{\text{Apparent feed intake}}{\text{Wet Weight Gain}}$

[b] **Average body weight, ABW = (Initial weight + Final weight)/2**

[c] **Wet weight gain= FBW-IBW**

Table 5. Nitrogen (N) and Phosphorus (P) balances based on intake, retention (gain), and loss and corresponding equations.

Nitrogen (N) balance	
Nitrogen Intake (mg N/ kg fish/ day)	$\frac{\text{N intake}}{\text{ABW} * \text{days}} * 1000$
Nitrogen Gain (mg N/ kg fish/ day)	$\frac{\text{Final N Content} - \text{Initial N Content}}{\text{ABW} * \text{days}} * 1000$

Nitrogen Loss (mg N/ kg fish/ day)	Nitrogen Intake - Nitrogen Gain
Phosphorus (P) balance	
Phosphorus Intake (mg P/ kg fish/ day)	$\frac{\text{P intake}}{\text{ABW}*\text{days}}*1000$
Phosphorus Gain (mg N/ kg fish/ day)	$\frac{\text{Final Phosphorus Content}-\text{Initial Phosphorus Content}}{\text{ABW}*\text{days}}*1000$
Phosphorus Loss (mg N/ kg fish/ day)	Phosphorus Intake - Phosphorus Gain

2.6. Data treatment and statistical analysis

Data are expressed as means ± standard deviation. All data expressed as a percentage were arcsine transformed previously to statistical analysis. Comparison between the results of the two dietary treatments were made using a t-test for independent samples, when homogeneity among variances was verified. Whenever the assumptions of the test were violated, a non-parametric test was used, namely de Mann-Whitney test. In such cases, as the number of observations was reduced, normality was assumed. For parameters with more than three observations, normality was tested using the Shapiro-Wilk test and equality of variances and means was tested following the procedure stated above. All statistical analysis were performed using the SPSS statistics for windows software, version 27.0 (IBM Corp., Armonk, N.Y., USA).

3. Results

The effect of each diet in the growth performance of Nile tilapia juveniles was evaluated over a period of 57 days, with data being collected at the beginning (0 days), middle (28 days) and end (57 days) of the experiment. The parameters used to evaluate growth performance were weight gain, daily growth index (DGI), and condition factor (CF). Other condition parameters used were the hepatosomatic and viscerosomatic indexes. The results are presented bellow from Figures 1 to 5 and Tables 6 to 9.

At the 28th day of the experiment, fish fed the control diet (CI) weighed 24.97± 0.89g, while the fish fed the experimental diet (LI) weighed only 17.93 ± 0.24g. The same pattern was observed at the end of the experiment, with the mean weigh for the CI diet being more than double than that of LI (CI = 48.99 ± 3.69g *vs*. LI = 24.47 ± 3.69g) (Table 6). At the end of the experiment, fish fed the control diet were significantly heavier, having gained approximately 5 times their initial weight (initial mean weight = 11 ± 0.01 g *vs*. final mean weight = 48.99 ± 3.69 g). As for the LI group, fish doubled their initial body weight (initial mean weight = 11.02 ± 0.01 g *vs*. final mean weight = 24.97 ± 3.69 g).

Table 6. Mean body weight at the 29th and 57th days of the experiment.

Mean Body Weight (g)	LI	CI
Initial	11.02 ± 1.69	11.00 ± 1.75
Intermediate	17.93 ± 0.24^{a}	24.97± 0.89^{b}
Final	24.47 ± 3.69^{a}	48.99 ± 3.69^{b}

Values are the mean ± standard deviation (*n* = 81 individuals/diet). The presence of different superscript letters within the same line indicates significant differences (p<0.05) between diets.

Weight gain between treatments was statistically different at the 29th day, but not on the 57th (Fig. 1A). Data for the daily growth indexes differed significantly between treatments, both in the middle and in the end of the experiment (Fig.1B).

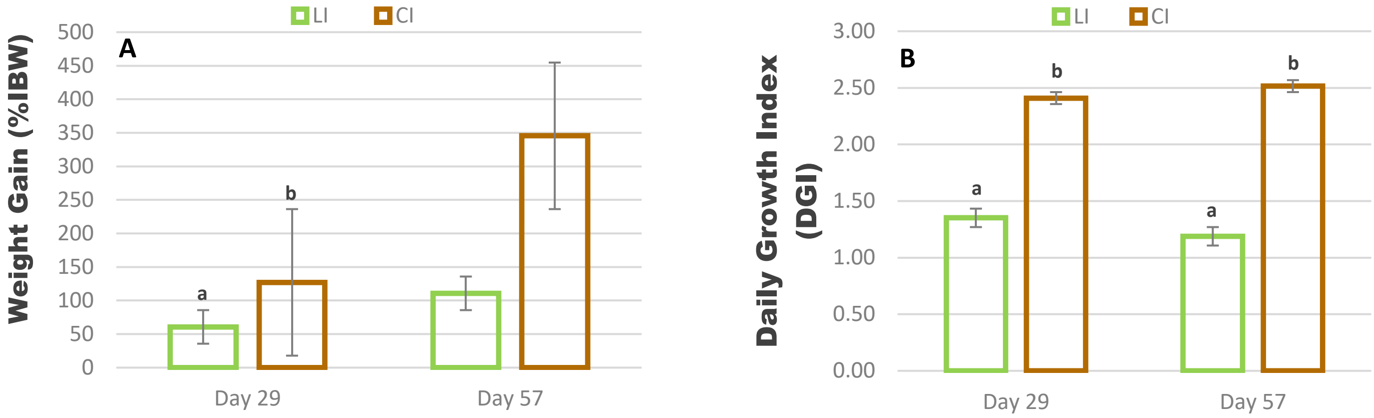

Fig.1. Growth performance parameters – weight gain as a percentage of the initial body weight (A) and daily growth index (B) - in the middle (day 29) and end (day 57) of the experiment. Values presented are the mean ± standard deviation (n = 3). Within the same sampling day, data represented in bars with different superscript letters are significantly different from each other (p<0.05).

The hepatosomatic index was significantly higher for the individuals fed the control diet (CI). The viscerosomatic index and condition factor were not affected by the dietary treatments. Values of HSI and VSI for both diets are depicted in Table 7.

Table 7. Hepatosomatic index (HSI), viscerosomatic index (VSI) and condition factor (k), for Nile tilapia individuals fed the experimental diets.

	HSI	**VSI**	**k**
CI	2.31 ± 0.83^{a}	9.44 ± 1.75	3.50 ± 0.50
LI	1.33 ± 0.35^{b}	8.54 ± 1.43	3.70 ± 0.30

The values represent the beginning and end of the experiment. Values are the mean ± standard deviation (n = 24). Different superscript letters within the same column indicate significant differences (p<0.05).

Note: Initial values: **HSI**: 1.55 ± 0.43; **VSI**: 8.13 ± 0.71; **k**: 3.60 ± 0.37.

Voluntary feed intake was not significantly different between dietary treatments. On the 29th day of the experiment voluntary feed intake was of 2.78% per day for the LI diet and 2.84% per day for the CI diet. On the 57th day, these values were 2.66% and 2.72% per day, respectively (Fig. 2).

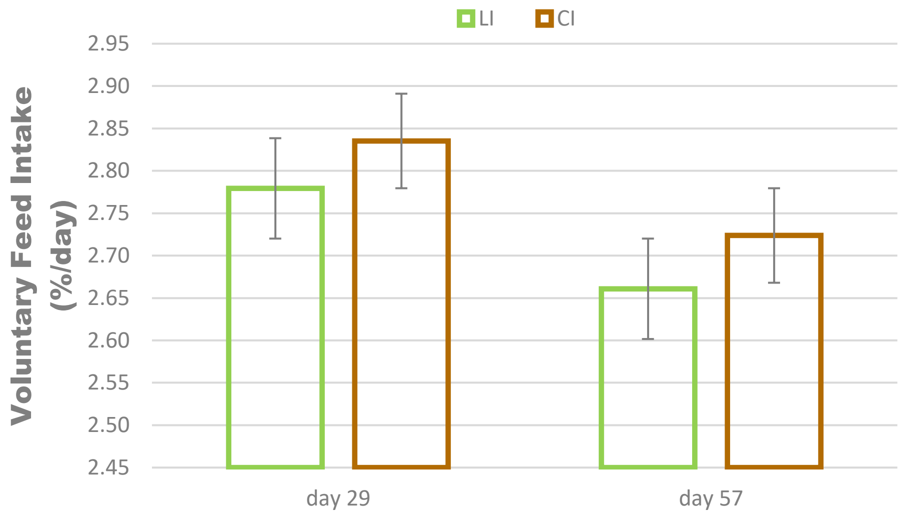

Figure 2. Voluntary feed intake, as percentage per day, in tilapia juveniles fed diets CI and LI. Values presented are the mean ± standard deviation (*n* = 3). Data corresponds to the middle (day 29) and end (day 57) of the experiment. Absence of superscript letters indicate no significant impact of the dietary treatments (p>0.05).

At the 29th day after the beginning of the experiment, FCR values registered were 1.06 ± 0.02 for the control group (CI) and 1.70 ± 0.05 for the experimental group (LI). At the end of the experiment, FCR was 1.23 ± 0.10 for the CI group and 2.01± 0.08 for the LI group (Fig. 3). The significantly lower FCR registered for CI individuals at the end of the experiment reflects a higher efficiency in feed conversion.

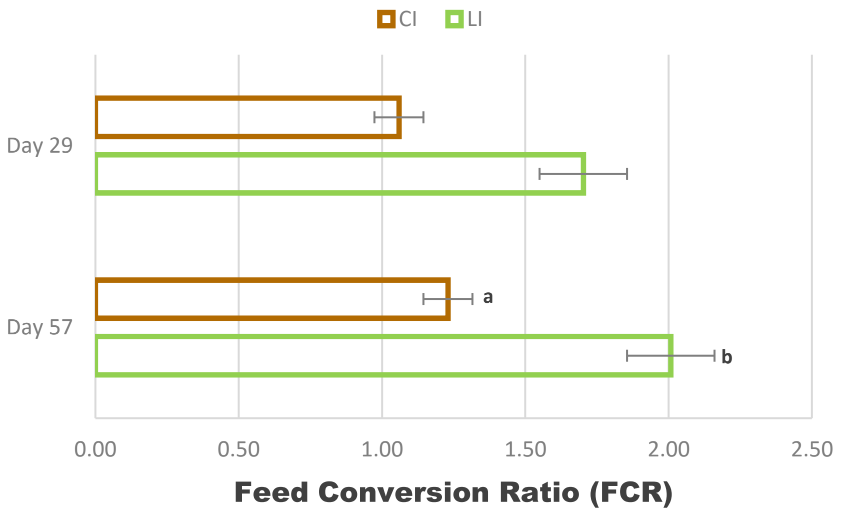

Figure 3. Feed Conversion Ratio in tilapia juveniles fed diets CI and LI. Data corresponds to the middle (day 29) and end (day 57) of the experiment. The values presented are the mean ± standard deviation (*n* = 3). Significant differences between diets within the same sampling day present different superscript letters (p<0.05).

Higher values of protein efficiency ratio (PER) suggest that least protein was required to ensure weight gain. Higher PER values were registered for the individuals fed the CI diet, at the middle of the experiment. On the 29th day of the essay mean PER values were 2.91 ± 0.07 for the CI diet (control) and 2.58 ± 0.08 for LI. At the end of the experiment these values were 2.51 ± 0.19 and 2.19 ± 0.09, respectively (Fig. 4).

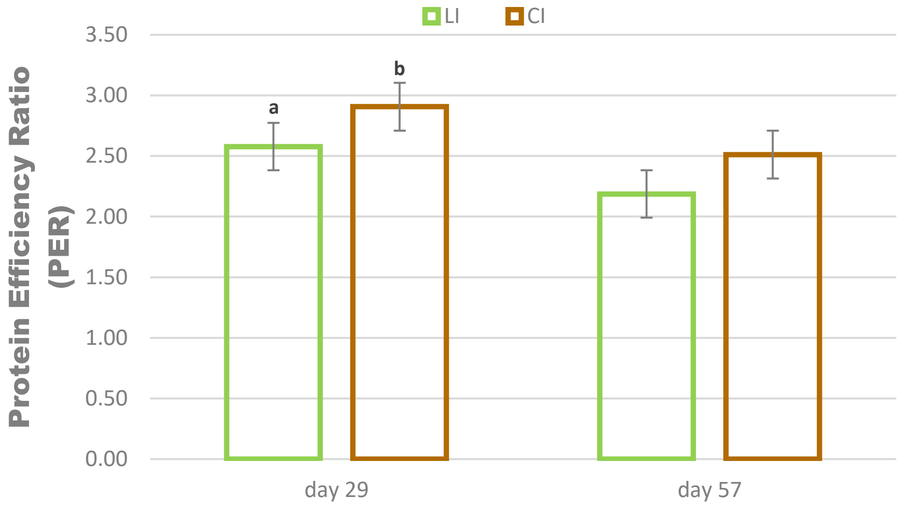

Figure 4. Protein efficiency ratio in tilapia juveniles fed diets CI (control) and LI. Values presented are the mean ± standard deviation (*n* = 3). Data corresponds to the middle (day 29) and end (day 57) of the experiment. Significant differences between diets within the same sampling day are signed with different superscript letters (p<0.05).

In Table 8, proximate composition of the individuals at the beginning and end of the experiment is reported. The differences in ash content between dietary treatments were statistically significant (p<0.05) indicates that CI individuals had a higher mineral content than LI ones. On the other hand, fish fed the LI diet had significantly higher gross energy levels and lipidic content. These also showed higher percentages of dry mater.

Table 8. Proximal body composition of Nile tilapia juveniles fed the experimental diets, at the beginning and end of the experiment.

	CI	LI
Dry Matter (%)	27.03 ±0.53[a]	28.63 ±0.88[b]
Crude Protein (% DM)	50.66 ± 2.10	49.39 ±1.07
Crude Fat (% DM)	17.95 ± 0.62[a]	35.90 ± 1.47[b]
Ash (% DM)	3.25 ± 0.82[a]	2.75 ± 0.31[b]
Phosphorus (% DM)	1.42 ± 0.24	1.03 ± 0.18
Gross Energy (MJ/kg DM)	22.98 ± 0.28[a]	26.19 ± 0.42[b]

Values are the mean ± standard deviation (n = 3). Different superscript letters within the same line indicate significant differences between dietary treatments (p<0.05).

Note: Initial values: **DM (%)** =24.33 ± 0.65; **Crude Protein (%DM)** = 54.31± 0.40; **Crude Fat (%DM)** = 18.13 ± 0.50; **Ash (%DM)** = 3.51 ± 0.22; **Phosphorus (%DM)** = 1.66 ± 0.02; **Gross Energy (MJ/kg DM)** = 22.18 ± 0.03.

Retention of protein, lipids and phosphorus was calculated, as a percentage of intake (Table 9). Retention of protein, phosphorus and energy was not significantly different between dietary treatments. Retention of lipids, however, was significantly higher in the LI than in the CI group.

Table 9. Nutrient and energy retention (% of intake) for Nile tilapia juveniles fed the diets CI and LI at the end of the experiment (day 57).

	CI	LI
Protein	34.74 ± 2.84	31.29 ± 2.29
Lipid	36.97 ± 3.27[a]	71.17 ± 9.66[b]
Phosphorus	45.07 ± 8.82	55.75 ± 14.74
Energy	25.90 ± 2.32	22.88 ± 2.92

Values are the mean ± standard deviation (n = 3). Different superscript letters within the same line indicate significant differences between dietary treatments (p<0.05).

Nutrient balances were calculated considering intake, gain and losses. Calculations were made for both nitrogen and phosphorus (Fig. 5). Starting with nitrogen (Fig. 5A), it can be seen that although intake was higher for CI, nitrogen losses were also higher for this group. All parameters – nitrogen intake, gain and loss - were significantly different between diets. Intake of nitrogen was of 1416.39 ± 59.22 mg/kg fish/day for the CI diet, while this value was only 988.89 ± 107.84 mg N/kg fish/day for LI. Individuals from the control group gained 490.97 ± 22.57 mg N/ kg fish/day, whereas the LI group gained only 307.48 ± 10.41 mg N/ kg fish/day. Regarding losses, individuals fed the CI diet lost 925.42 ± 77.69 mg N/ kg fish/day, while the LI group lost only 680.41 ± 97.47 mg N/ kg fish/day.

For phosphorus (Fig. 5B) differences between diets are more accentuated. All parameters – phosphorus intake, gain and loss - were significantly different between diets. The control group ingested 182.88 ± 7.65 mg P/ kg fish/day, and the LI group only 64.92 ± 7.08 mg P/ kg fish/day. Fish from the control diet gained approximately 82.43 ± 17.23 mg P/ kg fish/day, losing 100.45 ± 14.84 mg P/ kg fish/day, with losses surpassing the gains. The situation for LI individuals is the contrary, with gains overcoming losses. These individuals gained 36.19 ± 6.06 mg P/ kg fish/day and only lost 28.73 ± 13.03 mg P/ kg fish/day.

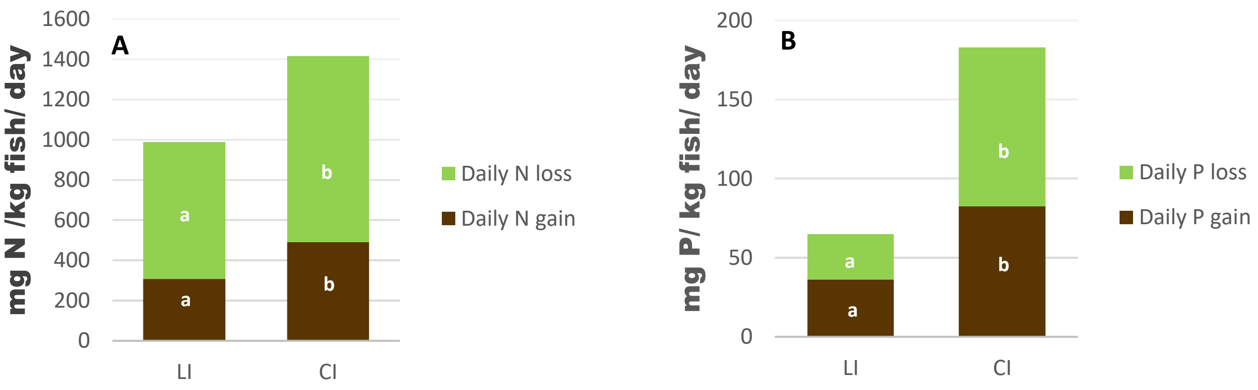

Fig. 5. Nitrogen (A) and phosphorus (B) balances in Nile tilapia juveniles fed the experimental diets over a period of 57 days. Intake is the sum of loss and gain. Values are expressed as mg N or P/ kg fish / day (*n* = 3). Significant differences (p<0.05) are indicated with different superscript letters.

4. Discussion

Nile tilapia juveniles require high levels of protein, lipids, vitamins, and minerals but low levels of carbohydrates (Lovell, 1989). For optimal growth performance of fingerlings and late juveniles, the recommended protein level in diets is around 35% (Abdel-Tawwab *et al.*, 2010). As for lipids, the dietary range recommended for Nile tilapia has been placed between 10 and 15% (He *et al.*, 2015). Plant oils (*e.g.*, sunflower oil, rapeseed oil) have been reported as suitable lipid sources for Nile tilapia (Lim *et al.*, 2011), but generally better growth performance is attained when using a mixture of plant and fish oils (El-Tawil *et al.*, 2019). Being a warm-water omnivorous fish, tilapia can efficiently use carbohydrates (Wilson, 1994), and inclusion levels of starch from 10-40% have been reported to support high growth rates in adults (Abro, 2014).

Starting with protein, the content of the experimental diet (24.60%) used in this study was lower than the optimal level recommended. The protein sources used in this diet were insect meal and fishmeal at low inclusion levels (2.50%), complemented with the use of local ingredients such as beans, leaves from moringa and cassava, corn, and peanuts. In previous studies, it has been reported that the protein content of raw beans is between 20 to 35%, depending on the type of bean (Toledo & Canniatti-Brazaca, 2008). The incorporation of this ingredient can, decrease the apparent digestibility of diet, hence, inclusion levels above 24% are not recommended for Nile tilapia juveniles (Paul, 2010). As for moringa and cassava leaves, data from Tanzania reports crude protein values of 31-35% for unprocessed moringa leaves (Madalla *et al.*, 2013) and 29% for cassava leaves. In their study in 2019, Doctolero & Bartolome concluded that inclusion levels of *Moringa oleifera* up to 20% provide acceptable growth in red Nile tilapia. Regarding cassava, other residues such as the root and flour can be efficiently used by Nile tilapia, as well (Boscolo *et al.*, 2002). The utilization of corn in diets for tilapia has also been described by Furuya (2010), who have emphasized the high digestibility of crude energy and protein. Peanuts, on the other hand, are used preferably as an energy source due to their high fat content (Suassuna *et al.*, 2006). Inclusion of roasted peanuts, for example, has been linked to higher digestibility of energy, and protein, and inversely, lower digestibility of fat (Paul, 2010).

Protein retention and protein content in the carcass from the individuals fed the experimental diet - that included the ingredients referred above - was similar to those of the individuals fed the control diet. Conversely, differences in protein utilization (PER) were only found in the middle of the experiment and not in the end. These results suggest that the protein sources used are suitable for the diets of Nile tilapia juveniles, as they did not impair protein retention neither utilization. Oppositely, lipid retention and lipid content in the carcass were higher for the individuals fed the experimental diet (LI). A similar trend has been described by Torelli *et al*. (2010) when using a diet formulated from agro-industrial residues in different fish species, including Nile tilapia. One of the ingredients used was, precisely, cassava. Many of these agro-industrial residues have been described as having high energy and lower protein values (Cyrino *et al*., 2010). In fact, an increase in fat content in Nile tilapia fed diets with increasing energy:protein ratios have been described (Gonçalves *et al*., 2009). An excess of energy can increase the deposition of body fat (Pereira Junior *et al*., 2013). Despite the overall energy content of both diets being similar, the experimental diet used in this experiment included high energy ingredients as corn and peanuts, which can explain the higher lipid deposition in the carcass of this individuals. Moreover, in Nile tilapia, energetic efficiency of digestible fat and carbohydrates have been proved to be higher than for protein (Schrama *et al*., 2018). The viscera and liver are other important fat storage tissues in fish (Cabral *et al*., 2013). The lower values of HSI reported for the experimental diet, paired with the higher lipidic content of the carcass, suggest that this diet formulation promoted fat deposition in the body, while for the control diet, fat was also diverted for the liver and viscera.

The inclusion of fishmeal in the experimental diet, at a very low level, was made to ensure feed acceptance by the fish. It is well known that plant-based diets have generally lower palatability due to higher levels of starch, and fibres (Abouei & Ekubo, 2011, Tusche *et al*., 2012). The purpose of fishmeal inclusion in the experimental diet seems to have been accomplished, as there were no significant differences in voluntary feed intake. Fishmeal is nonetheless a very costly ingredient (Bureau & Hua, 2010). So, to guarantee feed acceptance, farmers can use other animal by products from fisheries or cattle production. In fact, the use of fish-based hydrolysate proteins blended with other animal's processing wastes has yielded positive effects on palatability, digestibility, and others (Chotikachinda *et al*., 2013; Ovissipour *et al*., 2014; Srichanun *et al*., 2014; Silva *et al*., 2017). Protein hydrolysates from poultry and swine by-products have also been proved effective as palatability enhancers (Alves *et al*., 2019). Therefore, future development of less expensive techniques to transform animal farming by-products in palatability enhancers would be an advantage to artisanal farmers. The use of mixtures of plant and animal ingredients is a good strategy to reduce antinutritional factors and maintain a balanced amino acid profile (Agbo *et al*., 2015). This approach has yielded promising results in Nile tilapia diets, as can be seen in Agbo *et al*. (2015) and Al-Thobaiti *et al*. (2017).

Moving on to the overall growth performance, reflected by weight gain and daily growth index, both parameters were significantly lower for individuals fed the experimental diet. As stated above, the protein content of this diet is in the lower limit of the optimal range for Nile tilapia. Similar results were obtained by Liti *et al*. (2005) when comparing the effects of a commercial diet with a higher protein content (24%) with those of an alternative diet with lower protein content (18%). However, in fertilized ponds, natural occurring feed can supply fish with adequate protein levels until a certain limit of fish biomass (Diana *et al.*, 1991). In fact, different feeding strategies can be used in these occasions, with the lower protein diets to be used in fertilized ponds and higher protein or commercial diets only being used when fish reach a certain biomass, as a way to increase profitability (Diana *et al*., 1996). Since the goal in this study was not to use the experimental diet for intensive or commercial production, but instead for local consumption, the slight growth impairment registered between diets is not deterrent. In cage farming of Nile tilapia in Malaysia, production cycles have a duration of about 6 months, and individuals are harvested when they reach a body weight of 500g (Dullah *et al*., 2020). In the experiment developed for this thesis, with an approximate duration of two months, the individuals fed the experimental diet attained a final body weight of about 25g. Even if fish would require longer production cycles to attain an appropriate size for consumption, the adoption of longer production cycles would still enable farmers to obtain fish for direct consumption or to sell within the community as to generate supplemental income. It is important to bear in mind that Mozambique tilapia, unlike Nile tilapia, is often considered to have a slow growth rate, which inhibits its use for commercial aquaculture (Pickering, 2009; Harohau *et al*., 2016). However, in a scenario of community-based aquaculture, the species can still potentially play a role in mitigating protein shortages at the subsistence level, as it is accessible to impoverished consumers. In a study conducted by Harohau *et al*. (2016), pond productivity for *O. mossambicus* was estimated at 1267 kg/ha/year which is about four times more than the natural productivity recorded in the wild in Sri Lanka (280 kg/ha/year) (De Silva, 1988). Productivity levels as the one presented above are more than enough to yield food security and a safe animal protein source to low-income communities. Since in this thesis Nile tilapia was used instead of Mozambican tilapia, it is possible that in practice the results are not completely replicable. However, a diet following an analogous formulation to the one used in this study should still be suitable to produce *O. mossambicus* as a supplementary animal protein source to impoverished individuals.

Paired with lower growth rates, feed conversion ratio was higher for individuals fed the experimental diet. Low values of FCR reflect high feed efficiency, lower production costs and overall improved sustainability (Martinez-Cordova *et al*. 2016). The range of FCR values found for tilapia in the literature is very broad. For individuals grown in ponds and fed on-farm made feeds these values have been reported to be between 1.5-2.5 (Rana & Hassan, 2013). This is in line with the results obtained in the current work. At the end of the experiment, FCR values were 1.23 for the control diet (CI) and 2.01 for the experimental diet (LI), revealing lower feed efficiency for the latter. Regardless, both values fall under the range covered by the literature, meaning that the diets enable appropriate feed efficiency.

In fact, improving feed efficiency is key to minimize eutrophication (Aubin *et al*. 2009; Besson *et al*. 2016). In Africa, 28% of water bodies are threatened by eutrophication (Nyenje *et al*., 2010). In some areas, like Tanzania, legislation prohibits the establishment of human activities 60 m from riverbanks and lakeshores and the use of water is regulated with permits (Mulokozi *et al*., 2020). Nitrogen (N) and phosphorous (P) are the main contributors to eutrophication of water masses (Dupas *et al*., 2015), and so, it is extremely important that a diet promotes minimal excretion of both compounds, to prevent water quality deterioration and subsequent problems. Both P and N losses were lower for the individuals fed the LI diet. Previous studies have reported similar trends for phosphorus in diets rich in plant proteins or processed animal proteins (Dias *et al*., 2009; Cabral *et al*., 2011,2013; Campos *et al*., 2017). As for the lower nitrogen losses reported to LI, those are probably attributable to the lower dietary protein level. These results prove that the diet LI is sustainable, not only economically but also, environmentally. In earthen ponds that are often in close association with agricultural soils, eutrophication can jeopardize not only fish production, but also crop production. Such an outcome would completely disrupt the main goal of this study, which was to develop a diet that would provide communities with a safe and steady source of animal protein.

5. Conclusions

By 2050 it is possible that the population in developing countries increases by 2.4 billion people (Lipper *et al*., 2014). The dependence on agriculture to ensure the livelihoods and nutrition of so many people is unfeasible, since desertification and increasing salinization of agricultural grounds are major threats, with the tendency to aggravate over time (Godfray *et al*., 2010). The creation of sustainable food production systems must then rely on aquaculture. Within aquaculture, animal nutrition should be the main focus to ensure that fish have a good nutritional profile that is suitable for human consumption.

This way, the formulation of on-farm made aquafeeds seems promising as an alternative for rural aquaculture. On-farm made feeds can increase cost-effectiveness of production. Regardless the overall lower growth performance of the individuals fed the LI diet in this study, it can be stated that a formulation using a majority of household waste ingredients, if still tailored carefully, can be used to successfully produce low trophic fish species, such as tilapia, as a supplementary source of animal protein for human consumption in impoverished nations.

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