Evaluation of novel climate-friendly feed for Nile tilapia in Tanzania

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Masters project • 30 credits
Swedish University of Agricultural Sciences, SLU
Department of Animal Nutrition and Management
Master program Animal Science
SLU 2023

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Utvärdering av nytt klimatvänligt foder för Niltilapia i Tanzania

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Credits:	30 credits
Level:	First cycle, G2E
Course title:	Examensarbete Husdjursvetenskap
Course code:	EX0872
Programme/education:	Agronomprogrammet – Husdjur
Course coordinating dept:	Department of Animal Breeding and Genetics
Place of publication:	Swedish University of Agricultural Sciences, SLU
Year of publication:	2023
Cover picture:	Vilma Johansson
Keywords:	Black soldier fly, novel fish feed, circular aquaculture, sub-Sahara aquaculture, greenhouse gas emissions, LCA, Nile tilapia.

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Abstract

Tanzania is a country located in sub-Sahara Africa by the Indian Ocean. Tanzania's aquaculture sector is small but has the potential to grow. As the population grows, so does the demand for food. Nile tilapia is the most cultured fish in Tanzania due to its hardiness, fast growth and tolerance to many environmental conditions. Fish meal (FM) and soybean meal has traditionally been used as a protein source in fish feed. However, insects have been proposed as potential alternative protein sources to replace FM and soybean meal, which are expensive and negatively affects the environment and feed and food security. One of these insects is the black soldier fly (BSF) due to its high content of amino acids, lipids, vitamins and minerals. The objective of the current study is to examine and assess how a locally produced feed containing BSF meal will affect the growth performance of Nile tilapia. Physical feed quality and chemical composition were also evaluated. In addition, the environmental impact of using BSF larvae in tilapia feed was also evaluated by calculating the greenhouse gas (GHG) emissions from such a feed production system. The BSF diet was compared to a Control and commercially used diet at fish farms in Tanzania. Results show that growth performance could not be statistically evaluated due to variation in the different ponds used during the fish growth experiment. Nutritional composition and the chemical score showed low histidine, lysine, and methionine and a high phenylalanine content in the BSF meal compared to the FM. Physical feed quality showed that the BSF diet had the best water stability compared to other diets used in the experiment. However, the BSF diet had lower durability and hardness. GHG calculations showed that the BSF diet had the lowest emission of GHG.

Keywords: Black soldier fly, novel fish feed, circular aquaculture, sub-Sahara aquaculture, greenhouse gas emissions, LCA, Nile tilapia.

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Abbreviations

BSF	Black soldier fly
BSFM	Black soldier fly meal
BW	Body weight
CS	Chemical score
DO	Dissolved oxygen
EAA	Essential amino acid
FCR	Feed conversion ratio
FIFO	Fish in: Fish out ratio
FM	Fish meal
FMFO	Fishmeal and fish oil
GHG	Greenhouse gases
GWP	Global warming potential
LCA	Life cycle assessment
PUFA	polyunsaturated fatty acids
SCP	Single cell protein
SGR	Specific growth rate
WG	Weight gain

1. Introduction

Today, the world's aquaculture and fisheries industry has been recognised for its essential contribution to global food security (FAO 2022; Garlock et al. 2022). Global consumption of aquatic foods has increased at an average annual rate of 3% since the early '60s (FAO 2022). With this increase in global consumption, aquaculture production levels are predicted to grow another 32% by 2030 (FAO 2022). The main groups of fish species produced in 2020 were carp and other cyprinids. Asia is the primary global producer, accounting for 70% of total fisheries and aquaculture in 2020. Africa has the smallest share of the world's production, with only 7%. Although most African countries have a low production capacity, Africa's aquaculture production has almost doubled during the last 20 years (FAO 2022).

Tanzania is a country located by the Indian Ocean on the east coast of Africa. Aquaculture in Tanzania plays an important role in both economic and social development. The sector provides nutrition, food security, employment, income, and tourism to the country. Nile tilapia (*Oreachromis niloticus*), a freshwater fish native to Africa (El-Sayed 2020b), is today one of the most produced fish species in Tanzanian aquaculture (Alfanies & Nyambika 2008; Tanzania Fisheries Department 2019). Nile tilapia, often described as the 'aquatic chicken' (El-Sayed & Fitzsimmons 2023), is popular due to its hardiness (El-Sayed 2020a). Attributes such as fast growth, tolerance to a wide range of environmental conditions, resistance to diseases and stress, and the ability to reproduce in captivity are some factors that have made Nile tilapia popular (Alfanies & Nyambika 2008; El-Sayed 2020a).

Today, Tanzania's aquaculture sector is small, compared to other African coutries, such as Egypt, but with the potential to grow. In 2019, fisheries and aquaculture provided around 30% of the total animal protein in Tanzania (Tanzania Fisheries Department 2019). With a population of around 55 million (Tanzania Fisheries Department 2019), the demand cannot be met from the existing capture fishery (Alfanies & Nyambika 2008). In Tanzania, Nile tilapia is traditionally farmed in extensive non-commercial systems in earthen ponds. However, intensive culture systems are increasingly used to meet future demands (Mwanja & Nyandat 2013;

El-Sayed & Fitzsimmons 2023). Intensive culture systems require farmers to use commercially available formulated feed (Mwanja & Nyandat 2013), mainly imported from other countries (El-Sayed & Fitzsimmons 2023). Further development of aquaculture in Tanzania is constrained due to a lack of knowledge, poor management and nutritional and physical quality of feed, and insufficient supply of sustainable feed alternatives (Alfanies & Nyambika 2008; Mwanja & Nyandat 2013; Mmanda et al. 2020).

1.1 Aquaculture feeds

Fishmeal and fish oil (FMFO) are two important ingredients in fish feed. Fishmeal (FM), a protein-rich product (FAO 2022), is often used in fish feed due to its good composition of amino acids essential for fish growth, reproduction, and maintenance (Wilson 2003; NRC 2011; Fréon et al. 2017; Hua et al. 2019). Fish oil (FO) is used in fish feed due to its high content of polyunsaturated fatty acids (PUFA), which are essential for fish survival (NRC 2011; Froehlich et al. 2018; Davis 2022) and has proven health benefits in human consumption (Cottrell et al. 2020; Garlock et al. 2022). Because these two ingredients contain essential nutrients for fish, they are still regarded as the best sources of protein and fat for fish (FAO 2020).

Aquaculture has been the primary user of FMFO since the 2000s and continues using FMFO today to meet the world´s increasing demand for aquatic food (Froehlich et al. 2018). FMFO are made from whole fish or fish parts (FAO 2022). Small pelagic fish, also known as forage fish, is the most used group for producing FMFO (NRC 2011; Froehlich et al. 2018; FAO 2022). Peruvian anchoveta (*Engraulis ringens*) accounts for the most significant proportion of fish used in FMFO production, accounting for about 20% of capture fisheries in marine waters (FAO 2022). Simultaneously, marine captures have remained stagnant since the late 1980s (FAO 2022). Since the mid-70s, fishery stocks within biologically sustainable levels have decreased from 90% to around 65 % (FAO 2022). Overfishing causes negative impacts on biodiversity and ecosystem function, consequently reducing fisheries production and leading to negative economic and social consequences (Froehlich et al. 2018; Hua et al. 2019; FAO 2022). Better management and certifications are in place today, decreasing the volume of unsustainable catches targeted for FMFO production (FAO 2020). Even though these management strategies benefit oceans' biodiversity, they also negatively affect total FMFO production, decreasing the supply and leading to increased prices and an inability to satisfy an already upscaled demand (Froehlich et al. 2018).

Due to the high costs, limited availability and market forces, the aquaculture industry has started substituting marine-derived FMFO with other ingredients in fish feed, such as plant and animal-by-products (Couture et al. 2019; Kok et al. 2020). This is well illustrated through the Fish in: Fish out ratio (FIFO), which measures the amount of forage fish used to produce 1 kg of farmed fish (Kok et al. 2020). Since the 90s, there has been a steady decline in the FIFO ratio on total fed aquaculture, decreasing from 0,47 in 2000 to 0,19 in 2020 production (NRC 2011; IFFO 2020; Kok et al. 2020).

The largest replacers for FMFO on the market are plant-based alternatives such as soy. Soy is the most used feed ingredient due to its high protein, lipid content, and other essential nutrients (NRC 2011; Froehlich et al. 2018; Couture et al. 2019; Hua et al. 2019). However, soy as a substitute for FM has some disadvantages. Soybean meal contains antinutritional substances, which can cause enteritis in some fish species (NRC 2011; Couture et al. 2019; Hua et al. 2019). Also, the usage of soy products in fish feed puts additional strains on land, water and phosphorus resources (Couture et al. 2019; Hua et al. 2019). Plant-based materials are also used for direct human consumption and to feed aminals in the agriculture industry, leading to increased competition for these materials (Lock et al. 2015; Couture et al. 2019). Therefore, cost-efficient and sustainable novel protein sources are essential to secure future aquaculture production.

1.2 Black soldier fly as a novel protein source

Insects are considered an alternative to FMFO due to their high content of amino acids, lipids, vitamins and minerals (Barroso et al. 2014; Henry et al. 2015; Hua et al. 2019). Insects are also part of the natural diet of fish (van Huis et al. 2013), which makes them a good candidate for replacing FMFO. In 2017, the European Union approved the use of insect protein in aquafeed from seven insect species, Black Soldier Fly *(Hermetia illucens)*, Common Housefly (*Musca domestica*), Yellow Mealworm *(Tenebrio molitor)*, Lesser Mealworm (*Alphitobius diaperinus*), House cricket *(Acheta domesticus)*, Banded cricket *(Gryllodes sigillatus)* and Field Cricket *(Gryllus assimilis)* (Müller et al. 2017; Hua et al. 2019). Today most research on replacing FMFO with insects has focused on the common housefly, the yellow mealworm, and the black soldier fly (Hua et al. 2019).

The black soldier fly (BSF) is a small insect measuring up to 20 mm as an adult. The species is commonly found in tropical and subtropical regions, parts of southern Europe, and parts of the Balkan Peninsula (Müller et al. 2017). The fly prefers temperatures of about 28°C, and larvae hatch within 48 hours. After two weeks, the larvae are ready to pupate. The pupae hatch after two weeks, and the fly is fully grown (Müller et al. 2017). Furthermore, feeding activity is limited to the larvae stage, and fully grown flies do not have mouthparts and can, therefore, not eat, sting, or bite hance, do not spread vector-borne diseases (Müller et al. 2017). It has also been suggested that BSF larvae can reduce pathogenic bacteria such as *Escherichia coli* and *Salmonella enterica* in some animal manure and organic waste during rearing (Lalander et al. 2013; Müller et al. 2017). Depending on what kind of substrate the larvae are grown in, growth performance, chemical composition and waste reduction efficiency can differ (Meneguz et al. 2018; Agbohessou et al. 2021). The larvae can be grown on many different types of substrates, such as food waste (Lock et al. 2015), macroalgae, plant residues (Belghit et al. 2018) and different kinds of animal manure (St-Hilaire et al. 2007), and thereby do not compete with human resources (Barroso et al. 2014). The larvae´s ability to utilise industrial by-products and human waste contributes to increased circularity in the food system. BSF converts low-cost organic waste into animal biomass, rich in proteins and suitable for aquaculture production (Barroso et al. 2014). Additionally, BSF production leaves a small ecological footprint as production does not require as much input, e.g. energy, arable land and water, compared to soy and FM (Barroso et al. 2014; Henry et al. 2015; Müller et al. 2017; Panikkar et al. 2022). Life cycle analysis (LCA) is a tool that provides a comprehensive overview when evaluating a product´s environmental impact (Boakye-Yiadom et al. 2022). LCA on BSF products has shown that products from such production, e.g. protein, fertilizer and lipids, can be more sustainable than traditional products used today (Smetana et al. 2019; Boakye-Yiadom et al. 2022). However, there is a lack of LCA on using locally grown BSF in tilapia feed in East African conditions.

1.3 Feed quality and environmental impact

Extrusion processing is the primary global method used for fish feed production due to the feed's improved physical and nutritional quality. Research shows that the physical quality of feed varies with ingredient composition and processing conditions and may interfere with feed intake and nutrient digestibility, thus affecting the growth performance of the fish (Sørensen 2012; White 2013). However, the effects on physical quality are seldom included in research investigating novel ingredients' nutritional quality (Sørensen 2012).

Nevertheless, different physical pellet parameters, such as pellet hardness, durability and water stability, are essential when producing aquaculture feeds (Davis & Hardy 2022). Good pellet durability is essential during transport, handling and feeding, as the high presence of small fines in feed is undesirable (Davis & Hardy 2022:14; Hardy & Brezas 2022). The fish do not consume small fines, directly leading to economic losses for the farmer (Davis & Hardy 2022).

Additionally, fine feed particles can lead to pollution as they add additional nutrients to the environment (White 2013; Davis & Hardy 2022). Feed quality and strategy are also closely connected (Cao et al. 2007; Amirkolaie 2011). A combination of poor feeding management and low feed quality can lead to a large amount of excess feed in the water, contributing to organic and nutrient loading (Cao et al. 2007). Feed wastage can be as high as 38%, depending on feed type, practices, culture methods and species (Cao et al. 2007).

1.4 Aim and hypothesis of master thesis

The aim of this thesis was to investigate and assess how new, locally produced feed containing black soldier fly meal affects the growth performance of Nile tilapia (*Oreochromis niloticus*) under typical production conditions in Tanzania, compared to imported commercially available feed. Feed physical quality and chemical composition will also be evaluated on all tested feeds. In addition, the environmental impact of using BSF as a protein source in Nile tilapia feed will be assessed by analysing and evaluating the greenhouse gas emissions from such feed production. The hypothesis was that the diets would have no difference in fish growth performance, feed conversion ratio, and feed quality. Furthermore, it was also hypothesized that greenhouse gas emissions (GHG) from commercial and control feed are higher than from feed produced with Black soldier fly larvae (*Hermetia illucens*).

2. Materials and Methods

2.1 Dietary production and formulation

Control and BSF diet were produced by extrusion by Biobuu Limited, Dar-es-Salaam, Tanzania, whit a Jinan Shengrun twin-screw extruder equipped with a flow-through preconditioner (Jinan Shengrun Machinery Limited Company, Jinan, China). Both diets were extruded at high pressure (not recorded) and a temperature of 90, 120 or 100°C on barrel sections 1, 2 or 3, respectively, using a die head of 2 mm. The test diets have been formulated to provide both above minimal nutritional requirement levels based on NRC (2011) and IAFFD (IAFFD, 2023) and matched to the protein and energy levels of the commercial diet used in the experiment. The two test diets used were Control and BSF (Table 1). The BSF diet was formulated in order to maximize the inclusion level of BSF meal and the replacement of fish meal without compromising the dietary nutritional composition. An additional commercially available diet for tilapia was used as an extra control (Tonse Tilapia Grower Feed 3,0 mm; Tonse Fish Limited, Lusaka, Zambia). Ingredients and inclusion levels for the Tonse diet were not available.

Table 1. Formulation of the control and BSF feed used in the experiment, expressed on 'as is' basis.

Diet	**Control**	**BSF**
Ingredient	**%**	**%**
Fish meal, AC[a]	26.9	4.0
BSF defatted meal[b]	0	35.1
Whole maize[b]	20.7	19.1
Soy bean meal[c]	30.0	29.1
Lionpro blood meal[b]	8.3	1,6
Cotton seed cake[b]	8.3	6.0
Sunflower cake[b]	2.1	1,1
Sunflower oil	2.9	3.0
Vitamin mineral premix[b]	0.5	0.5
DL-methionine[b]	0.3	0.4
Monocalcium phosphate[b]	0.1	0.1
Total	100	100

[a] Sourced from Animal Care Ltd, Dar es Salaam, Tanzania, [b] Sourced from Tanzania, [c] Sourced from Zambia.

2.2 Tilapia growth experiment

The feeding experiment was conducted at Ruvu fish farm (WGS84; 6°36'40.1"S 38°47'07.8"E) in Pwani region, Tanzania, from the 31st of January, 2023, to the 26th of April, 2023 (total 85 days). Before the start of the experiment, the fish were reared in an earthen pond (30×15×1.5 meters) with a stocking density of eight fish per square meter. During this time, the fish was handfed three times daily with Koudijs Tilapia starter feed 2 mm (37% crude protein, 6-8% crude fat, 17.5 MJ/kg GE) (Koudijs Animal Nutrition B.V. Ede, Nederland).

One day before the start of the experiment, approximately 200 fish were captured with handheld nets and brought to a temporary holding tank (96×116×42 centimetre) in 20-litre buckets. Fish were held in the holding tank for a maximum of one hour. Ten fish at a time were then anaesthetized in a 10-litre bucket containing a solution of clove oil (*Syzygium aromaticum* L. Myrtaceae) (50 mg L^{-1}). When the fish had a general lack of consciousness (measured by the absence of reaction), the fish was individually weighed on an MH-666-A scale (Dongguan Ming Heng Electronic Technology Co., Ltd. 2019) or an SF-400A scale (*MCP SF 400A Scale* 2020). The total length (mouth to the tip of the tail) was measured with a ruler (Figure 2). The fish was then placed in a 20-litre bucket with fresh water for recovery. When around 50 fish had been placed in the freshwater bucket, they were transferred to their assigned experimental pond (30×15×1.5 meter) and hapa (7.5×2.1×1 meter). This procedure was repeated until a total of 150 fish was weighed per hapa.

Figure 1. Experimental hapas at Ruvu fish farm.

The three experimental feeds were fed to Nile tilapia. 1800 hormone-treated all-male Nile tilapias weighing 75±10 grams were placed in 12 hapas (150 fishes/hapa) in three earthen ponds in the configuration of 3 (Pond 14), 4 (Pond 12), or 5 (Pond 13) hapas per pond (Figure 13, Appendix). Each feed was randomly assigned to four hapas (n=12), where all three feeds were represented at least once in each pond. Fish was handfed twice daily at 10:00 and 16:00 with a daily feed rate of 3% of total body weight (BW) (NRC 1993). The fish that died during the experiment were collected immediately, and their weight and length were recorded. The daily feed allowance for the specific hapa was adjusted weekly. Temperature and dissolved oxygen (DO) were recorded daily in each pond using an OxyGuard® Handy Polaris 2 DO and temperature meter (OxyGuard International A/S, Farum Gydevej 64, Farum, Denmark). The pH in each pond was recorded three times during the experiment with ST20 Starter Pen Meters (Ohaus Corporation, 8 Campus Drive, Suite 105, Parsippany, USA).

Figure 2. Example of total length measurement.

At the halfway point in the experiment (day 43 of 85), thirty randomly selected fish from each hapa were individually weighed on an SF-400A (*MCP SF 400A Scale* 2020) scale without anaesthesia. A mean value of the individual fish weight per hapa was calculated, and the SGR value and the daily feed amount were corrected. In connection with the weighing, the hapa nets were cleaned by brushing the sides with a plastic brush to remove algal build-up. The cleaning procedure aimed to improve water flow through the hapas.

The final weight at day 85 was measured with the same method as at the start of the experiment. Fish from each hapa was brought to a holding tank (96×116×42 centimetre). Ten fish at a time were then anaesthetized in a 10-litre bucket containing a solution of clove oil (*Syzygium aromaticum* L. Myrtaceae) (50 mg L^{-1}). When the fish had a general lack of consciousness (measured by the absence of reaction), the fish was individually weighed. The fish was then placed in another holding tank (96×116×42 centimetre) with fresh water for recovery.

Due to the lack of technical ability, no feed waste was collected during the trial, and all feed was considered consumed.

2.3 Physical pellet quality analysis

Hardness (kgF) was determined on 20 pellets from each diet with a manual Amandus Kahl Pellet Hardness Tester with a scale from 0-25 kgF. The test was performed according to the manufacturer´s protocol.

The test for the water stability was done according to Baeverfjord et al. (2006) with some modifications. The BSF and Control diet was sifted through a basket with a 3 mm mesh size to remove fine particles before measurement started. Then, 10 grams of each diet were weighed into pre-weighed netting baskets with a 3 mm mesh size and a diameter of 8 cm. The baskets were then placed in 600 ml beakers, to which 300 ml of tap water was added. Three beakers per diet were incubated in a water bath at 28 °C and 100 shakings per min for 30, 90, and 180 min, respectively. Water temperature was noted at the start and the end of incubation.

After incubation, the beakers were gently dried with paper tissues and weighed. The contents of each beaker were then photographed with a Samsung Galaxy S22 phone with a camera setting of 3.0 zoom (Figure 14-18, Appendix). Baskets were placed in a heating cabinet at 105 °C for 18h. After drying, the baskets were weighed to determine each basket's residual dietary dry matter. The dried residuals were removed from the baskets, photographed and then frozen at -20 °C.

Pellet durability was determined with a Holmen NHP 100 potable pellet tester (TEKPRO Ltd, Willow Park, North Walsham, Norfolk, NR28 0BD, UK). The test was performed according to the manufacturer´s protocol. The test duration was 60 seconds with a pressure of 70 mBar.

2.4 Chemical analysis

Dry matter (DM) was determined by drying the sample at 103 °C for 16 h. The sample was then cooled in a desiccator before weighing. Ash content was determined by heating dried samples at 550 °C for 3 h, then cooling the samples in a desiccator before weighing. Total nitrogen (N) was determined using the Kjeldahl method using a 2520 digestor and a Kjeltec 8400 Analyser unit (FOSS Analytical A/S, Hilleröd, Denmark). Crude protein (CP) content was calculated as N × 6,25 (NMKL, 1976). Crude lipid content was analyzed according to the Official Journal of the European Union (2009), using a Soxtec 8000 Extraction Unit (FOSS Analytical A/S Hilleröd, Danmark). Neutral detergent fibre (NDF) was determined, according to Chai & Udén (1998), where 100% neutral detergent solution was used while amylase and sulphite were used to reduce starch and protein. Starch was analysed according to Larsson & Bengtsson (1983). Gross energy (GE) was determined in an isoperibol bomb calorimeter (Parr 6300, Parr Instrument Co. Moline, IL, USA). DM, ash, CP, Crude lipid, NDF, starch and GE were analysed in March and May of 2023 at the Department of Animal Nutrition and Management, Swedish University of Agriculture, Uppsala, Sweden. Amino acids were analysed according to the International Organization for Standardization (ISO) (2005). Amino acid analyses were performed by Eurofins Food and Feed testing, Lidköping, Sweden.

2.5 Calculations

Weight gain (WG), Specific growth rate (SGR) and feed conversion ratio (FCR) were the parameters used to estimate growth performance. These indicators were calculated according to the following equations:

$$WG\ (\%) = \frac{FW - SW}{SW} * 100$$

$$SGR\ (\%\ day^{-1}) = 100 * \frac{ln\,FW - ln\,SW}{T}$$

$$FCR = \frac{FA}{WG}$$

FW is the final weight (g) of the fish, SW is the start weight of the fish (g) at the beginning of the experiment, T is the duration of the experiment (days), and FA is the total feed administered (g) during the experiment period, WG is weight gain (g).

FCR has been corrected for any mortalities by subtracting the mean weight of dead fish from the start weight (SW). And subtracting the mean feed administered to dead fish from the total feed administered (FA).

The specific growth rate (SGR) was unknown in the first half of the experiment. Therefore specific growth rate (SGR) was set at 1.2 %, according to Nairuti et al. (2021). The daily feed allowance was predicted with the following equation:

$$(Biomass\ at\ start\ (g) * SGR\ ) * \left(\frac{Feed\ rate\ (\%\ BW)}{100} \right)$$

Halfway through the experiment, each treatment's midway specific growth rate (mSGR) was calculated, and feed rations were adjusted accordingly.

Due to the recorded high water temperature during the experiment (≥ 33 °C), which is not ideal for Nile tilapia (optimum 25-30 °C) (NRC 2011; Jun et al. 2012; El-Sayed 2020c), feeding rations were decreased to 80% (NRC 2011).

The chemical score (CS) for each essential amino acid (EAA) was calculated according to Veldkamp & Bosch (2015). Each amino acid in the protein source (in % of crude protein) was divided by this amino acid requirement of Nile tilapia (in % of required crude protein) and multiplied by 100. Calculations were done on a dry matter basis. EAA requirements for Nile tilapia (100-200g) were taken from the IAAFDs Nutrition Specification Database (ASNS) (IAAFD, 2023).

Pellet water stability was calculated with the following equation:

$$WS = \frac{((t + DS\ ) - t)}{(SM * DM/100)}$$

WS is water stability, t is tare (g), SM is sample weight (g), and DM is dry matter (g).

2.6 Statistical analysis

Statistical analysis for fish start weight, pellet hardness, durability and water stability was performed using GraphPad Prism version 9.5.1 for Windows (GraphPad Software, San Diego, California USA, www.graphpad.com). Fish start weight, pellet hardness, durability and water stability were evaluated using a one-way ANOVA followed by Tukey´s multiple comparisons tests. Additionally, one-way ANOVA was also performed on WG, SGR and FCR to test for possible pond and diet effects, followed by Tukey´s multiple comparisons tests.

Statistical analysis for WG, SGR and FCR was performed in R Statistical Software (v4.3.0; R Core Team 2021). For each dependent variable (FCR, SGR, and WG), two linear mixed-effects models were made using the “lme4”-package (v1.1.33; Bates et al., 2014). The first (main model) included diet as a fixed effect and was compared to a second (null model) excluding the diet component. All models used maximum likelihood and included pond as a random effect. The models were then pairwise compared using ANOVA from the “stats”-package (v4.1.2; R Core Team 2021).

2.7 Greenhouse gas calculations

GHG calculations were provided by the Research Institutes of Sweden. The standardised life cycle assessment (LCA) methodology, according to the International Organisation of Standardization; 14040 and 14044 (ISO, 2006a and 20006b), was used, and the functional unit was *one kg of feed at factory gate*. In the study, upstream resource use was split according to mass and, in cases of co-production, following the hierarchy of allocation methods presented in ISO 14040/14044. Co-products, i.e., products that have an economic value for the producer and are further utilized in another supply chain, share the same footprint per unit of product. Co-products that do not have an economic value were not assigned any impact.

Characterisation factors from IPCC (2021), a 100-year time horizon, were used to calculate the global warming potential (GWP). Background data was sourced from Ecoinvent 3 (Version 3.8) and the Agri-footprint database (Version 5.0). The LCA model was built in SimaPro Developer Multi User (version 9.4.).

2.7.1 System Boundaries

The system boundaries of the feed production include the production (fishing, agriculture or other and their primary processing to meals, oils and protein concentrates) and transport of feed raw materials to the feed factory, materials and energy in the feed factory (Figure 3). The model did not include feed and material used by adult BSF for egg production.

In the data used, material and energy use during operations for larvae and post-rearing processing were included, covering the production from cradle-to-gate. Infrastructure use of buildings for BSF production and feed factory were excluded. The BSF larvae were fed mixed waste (i.e. food waste from restaurants and raw fruit and vegetables from markets), and these ingredients were shredded before being fed to the larvae. An important methodological decision was the assumption that all feed ingredients used to feed BSF represented current waste streams and were therefore considered free of environmental burden. This builds on the assumption that these ingredients would have been thrown away otherwise and serve no alternative use in the current local food or feed systems.

Transports of waste from hotels and markets to insect production facility was set to an average of 35 km. The transport distances of the single feed ingredients for both feeds were calculated using Google Maps and seadistances.org. Inner Tanzanian transport of feed raw materials from the producer to the feed factory was assumed to be 250 km, and for imported feed materials from Zambia, 2000 km.

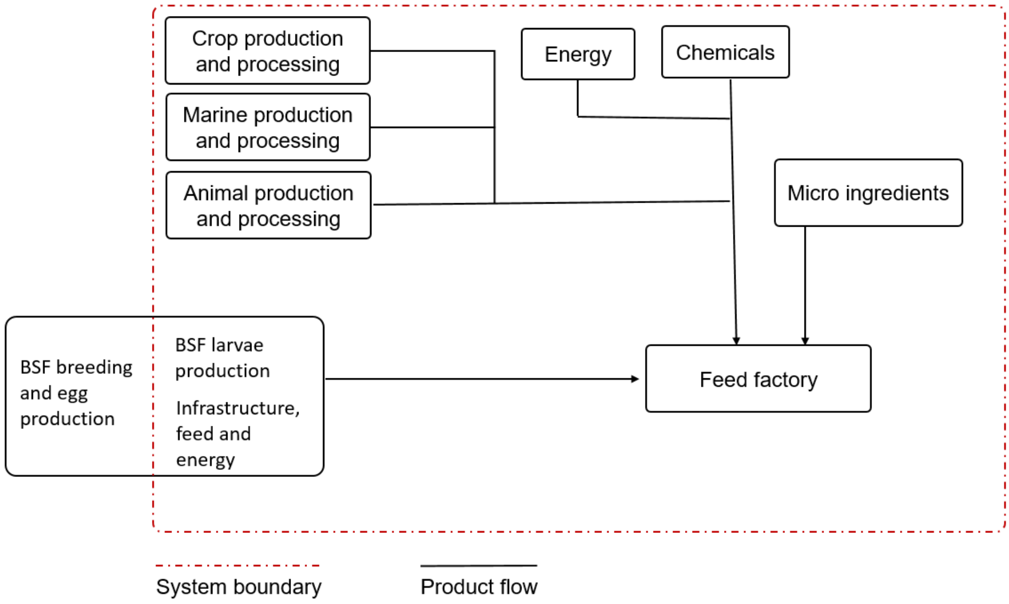

Figure 3. System boundaries of the studied BSF feed production.

3. Results

3.1 Nutritional composition and chemical score

The nutritional composition of experimental diets used in the feeding trial is shown in Table 2. The BSF diet had a higher amount of CP, CF, NDF and crude fibre, whereas the Tonse diet contained the highest amount of starch and the lowest amount of ash, CP and CF. The Control diet had the highest amount of ash and crude fibre. The Control diet had the lowest energy content, while BSF and Tonse diets had the same energy content. All diets supplied nutrients at or above the minimum requirement for Nile tilapia, according to NRS (2011).

The nutritional composition of fish meal and black soldier fly meal (BSFM) is shown in Table 3. In terms of EAA, the BSFM has considerably lower histidine, lysine and methionine content than the FM. The chemical score (Table 4 and Figure 4) for histidine, lysine and methionine were also low in BSFM, as they did not meet the EAA requirement for Nile tilapia. Furthermore, the BSFM had a considerably higher phenylalanine content than FM.

Table 2. Nutritional composition of experimental diets used in feeding trial expressed as g kg^{-1} DM if nothing else stated.

	Control	BSF	Tonse
DM (%)	98.0	97.9	90.4
Ash	158.5	107.1	76.5
Crude protein	382.1	389.3	356.5
Crude fat	61.5	65.5	48.2
NDF	145.9	182.8	113.6
Crude fibre	41.31	74.04	48.91
Starch	167.8	171.6	250.6
GE (MJ kg^{-1} DM)	18.7	19.1	19.1
Sum of AA	318.1	326.0	308.4
Essential amino acids			
Arginine	21.4	21.7	22.1
Histidine	10.7	9.6	8.4
Isoleucine	11.7	13.9	13.1
Leucine	29.7	27.5	25.9
Lysine	22.4	19.9	20.6
Methionine	8.0	7.5	7.9
Phenylalanine	18.0	16.4	15.9
Threonine	14.7	14.7	13.0
Valine	18.3	18.6	14.5
Non-essential amino acids			
Alanine	19.9	21.1	16.6
Aspartic acid	36.6	36.5	35.3
Cysteine +Cystine	4.0	3.9	4.4
Glutamic acid	51.4	54.1	56.8
Glycine	16.7	18.2	17.3
Hydroxyproline	<0.2 *	<0.2 *	<0.2 *
Ornithine	<0.01*	<0.01 *	<0.01*
Proline	17.8	24.3	19.9
Serine	17.0	18.1	16.7

* as is, g 100 g^{-1}

Table 3. Nutritional composition of fish meal and black soldier fly meal used in Control and BSF diet expressed as g kg^{-1} DM.

	FM	BSFM
DM (%)	83.3	95.5
Ash	312.3	166.8
Crude protein	641.6	525.3
Crude fat	63.6	121.2
Crude fibre	3.0	122.9
GE (MJ kg^{-1} DM)	170.0	206.6
Sum of AA	527.6	464.9
Essential amino acids		
Arginine	34.2	25.8
Histidine	16.9	<0.1
Isoleucine	24.7	36.6
Leucine	44.3	28.9
Lysine	46.3	8.3
Methionine	16.5	3.4
Phenylalanine	25.3	63.6
Threonine	26.2	21.4
Valine	30.9	31.5
Non-essential amino acids		
Alanine	37.5	37.6
Aspartic acid	57.2	49.6
Cysteine +Cystine	6.8	4.1
Glutamic acid	76.0	31.0
Glycine	35.0	14.8
Hydroxyproline	3.1	21.2
Ornithine	<0.1	20.2
Proline	22.9	41.5
Serine	23.9	25.6

FM, fish meal; BSFM, black soldier fly meal

Table 4. The chemical score[1] of essential amino acids on fish meal and BSF meal used in diets.

Essential amino acids	FM	BSFM
Arginine	117	108
Histidine	185	0
Isoleucine	153	277
Leucine	150	120
Lysine	141	31
Methionine	128	79
Phenylalanine	155	476
Threonine	122	122
Valine	144	179

FM, fish meal; BSFM, black soldier fly meal.

[1] For the chemical score, each amino acid in the protein source (in % of crude protein) was divided by this amino acid requirement of the target animal (in % of crude protein) and multiplied by 100.

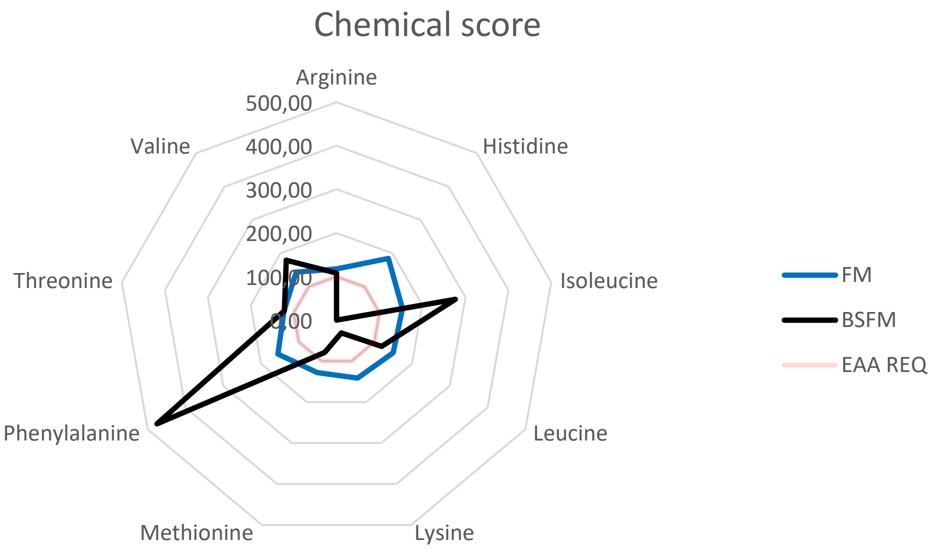

Figure 4. The chemical score of amino acids in fish meal and black soldier fly meal presented as a percentage of amino acid requirement (percent of crude protein) for Nile tilapia.
BSFM, Black soldier fly meal; FM, fish meal; EAA REQ, essential amino acid requirement

3.2 Fish growth experiment

There was no difference in start weight between the fish in the different hapas (*P*= 0.8955) (Table 7, Appendix). Fish mortality for Control, BSF and Tonse diets were 10, 8 and 6 %, respectively. The pairwise comparison revealed that the main and null models for WG did not significantly differ (*P*=0.8066). The same results were found for SGR (*P*=0.5068) and FCR (*P*=0.7082). This implies that the results for mentioned parameters can be better explained by inter-pond variation rather than diet. This was confirmed after testing for pond effect, where there was a clear difference between the ponds for all growth parameters (*P*<0.05). Results for pond effects are shown in Table 8 (Appendix).

WG (%), FCR and SGR are shown in Figures 5-7. Numerically, the Tonse diet had the highest, while the BSF diet had the lowest WG. Similarly, the BSF diet had the highest, while Tonse had the lowest FCR. Furthermore, the BSF diet had the numerically lowest SGR, while Tonse had the highest.

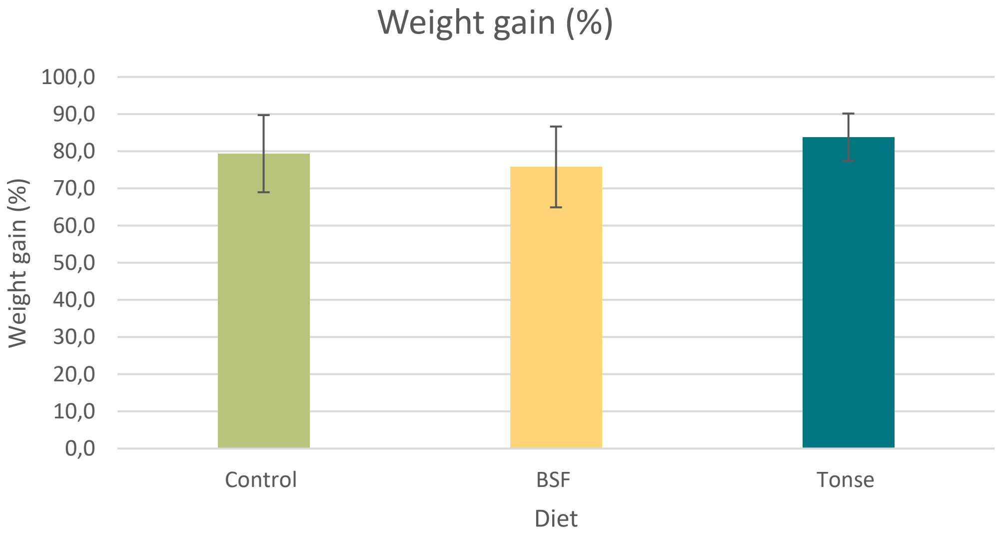

Figure 5. Weight gain of fish fed with experimental diets.

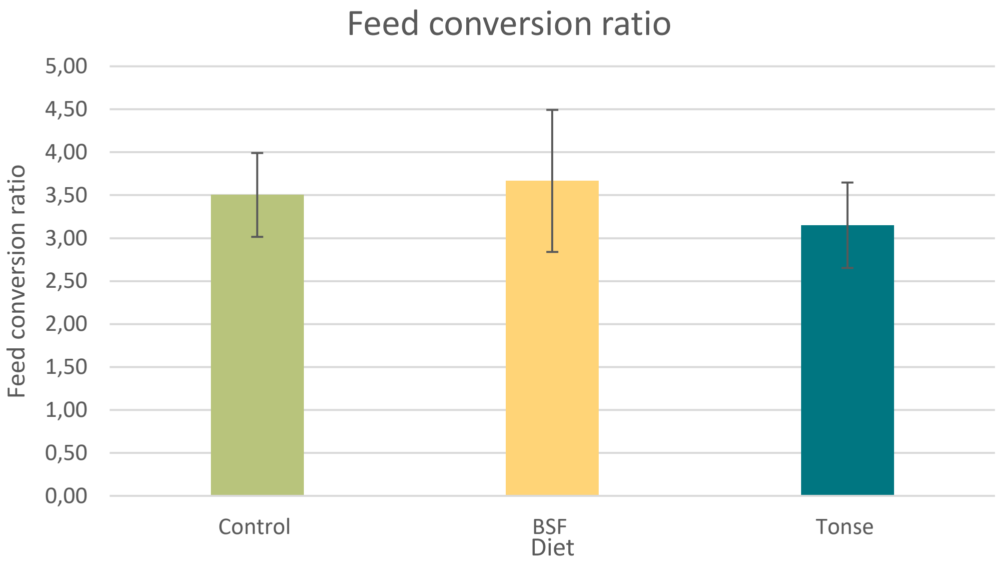

Figure 6. FCR of fish fed with experimental diets.

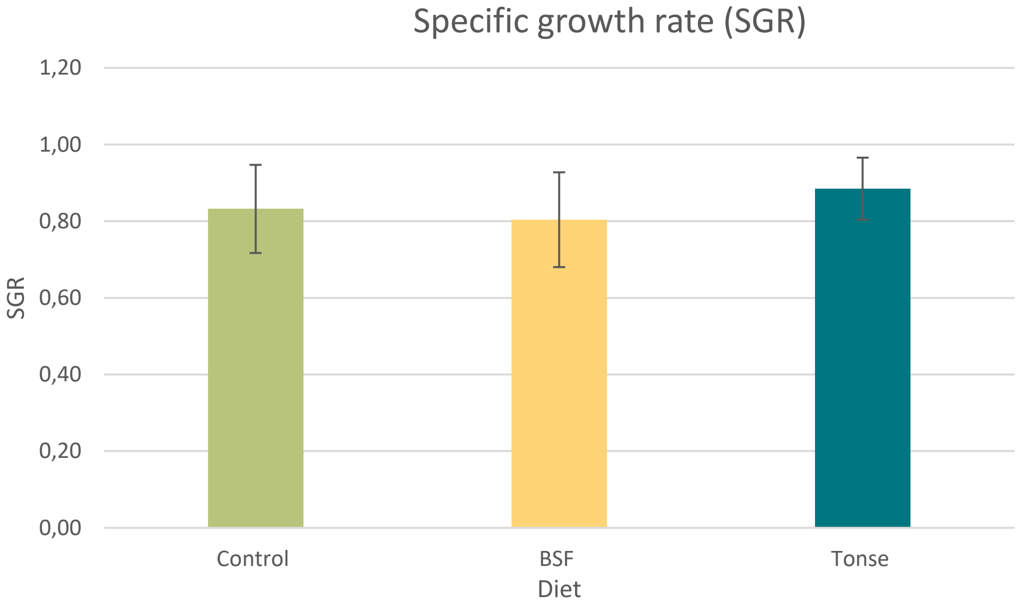

Figure 7. SGR of fish fed with experimental diets.

The mean water temperature of each pond is shown in Figure 8. All ponds had similar water temperatures throughout the experiment. Halfway through the experiment, the temperature increased, followed by a decrease in water temperature toward the end of the trial period. The mean temperature in all ponds during the experiment was 31.6 ± 2.8 °C. The mean dissolved oxygen for each pond is shown in Figure 9. Pond 12 had the lowest mean oxygen compared to ponds 13 and 14 throughout the experiment, whereas pond 14 had the highest mean oxygen concentration. Dissolved oxygen in pond 12 ranged between 3.4-6.3 mg/L, pond 13 between 4.0-6.6 mg/L and pond 14 between 4.7-6.7 mg/L. The water pH for each pond is shown in Figure 23 (Appendix). Pond 13 had the highest mean pH (8.9), whereas pond 12 had the lowest mean pH (8.6).

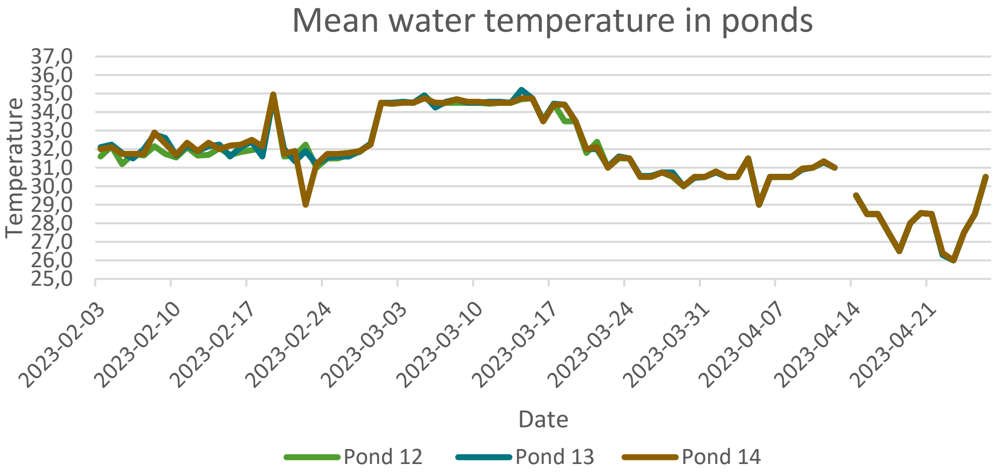

Figure 8. The mean temperature (C°) in experimental ponds during the experimental period.

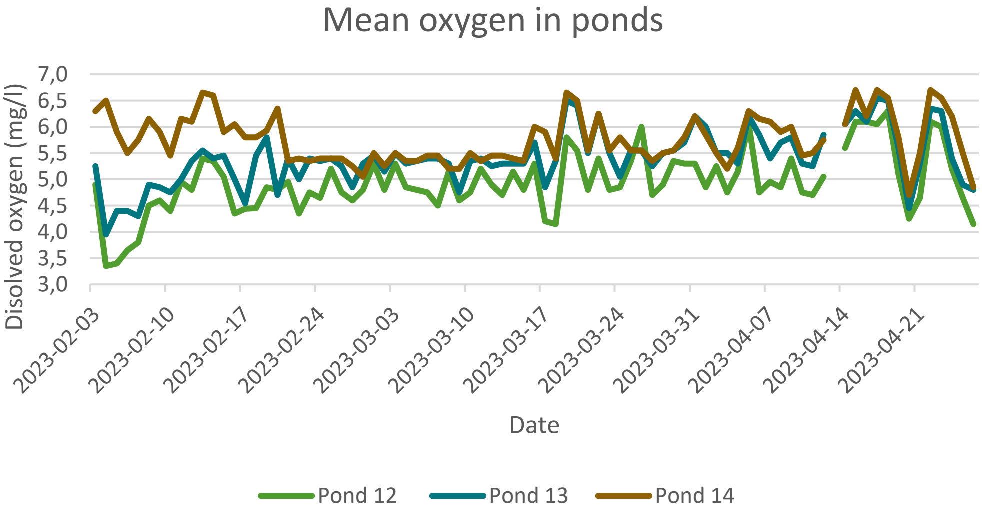

Figure 9. The mean dissolved oxygen in experimental ponds during the experimental period.

3.3 Greenhouse gas emissions

Quantified greenhouse gas emissions of the BSF diet and the control feed were 2.47 and 4.58 kg CO_2 eq/kg feed, respectively. The highest contribution for each diet was bloodmeal and soybean meal, respectively. Figures 10, 11 and Table 11 (Appendix 2) present greenhouse gas emissions for Control and BSF diet.

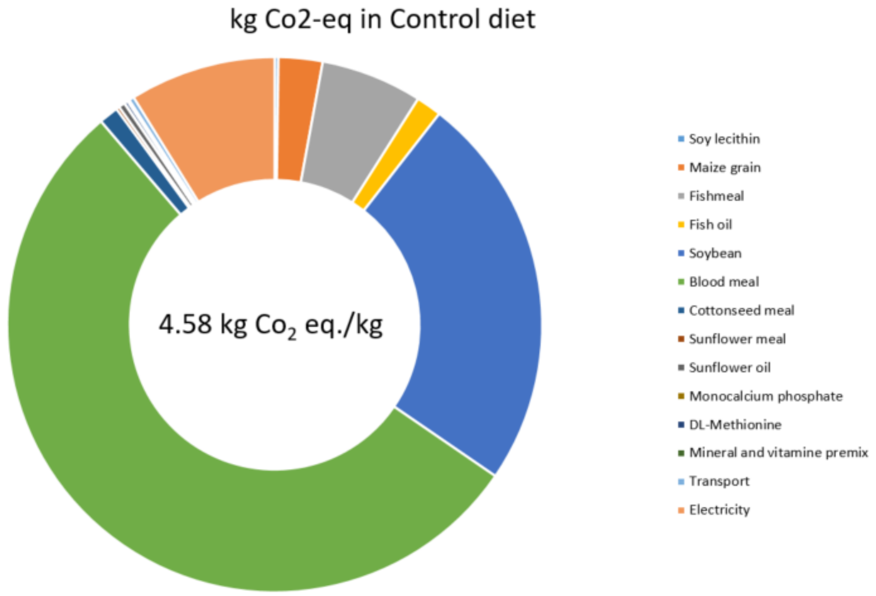

Figure 10. GHG emissions from the production of 1 kg Control diet.

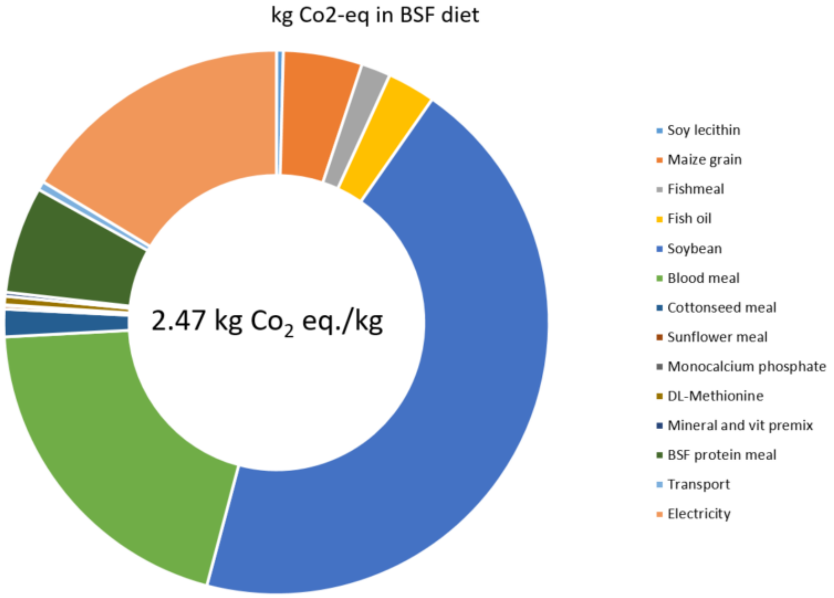

Figure 11. GHG emissions from the production of 1 kg BSF diet.

3.4 Physical pellet quality

Pellet hardness and durability are presented in Table 5. The BSF diet had lower pellet hardness than the control and Tonse diets. There were no differences in pellet durability index (PDI) between the diets. The water stability of the different diets is presented in Table 5 and Figure 12. The Tonse diet had lower water stability than the Control and BSF diets at all time points tested.

Table 5. Hardness and durability of diets used in feed trial.

Diet	Control	BSF	Tonse	SEM	*P*-value
Hardness test (Kg F)	4.070 ^{a}	3.365 ^{b}	4.130 ^{a}	0.2177	0.0012
PDI (%)	94.95	89.75	94.10	1.269	-
Water stability test					
30 min	87% ^{a}	84% ^{a}	68% ^{b}	4.000	0.0073
90 min	78% ^{a}	78% ^{a}	27% ^{b}	3.621	<0.0001
180 min	72% ^{a}	76% ^{a}	18% ^{b}	6.104	0.0001

a,b within rows corresponds to a significant difference ($P < 0.05$)

Hardness test, n=20; DPI, n=2. Water stability test 30, 90 and 180 min; n=3

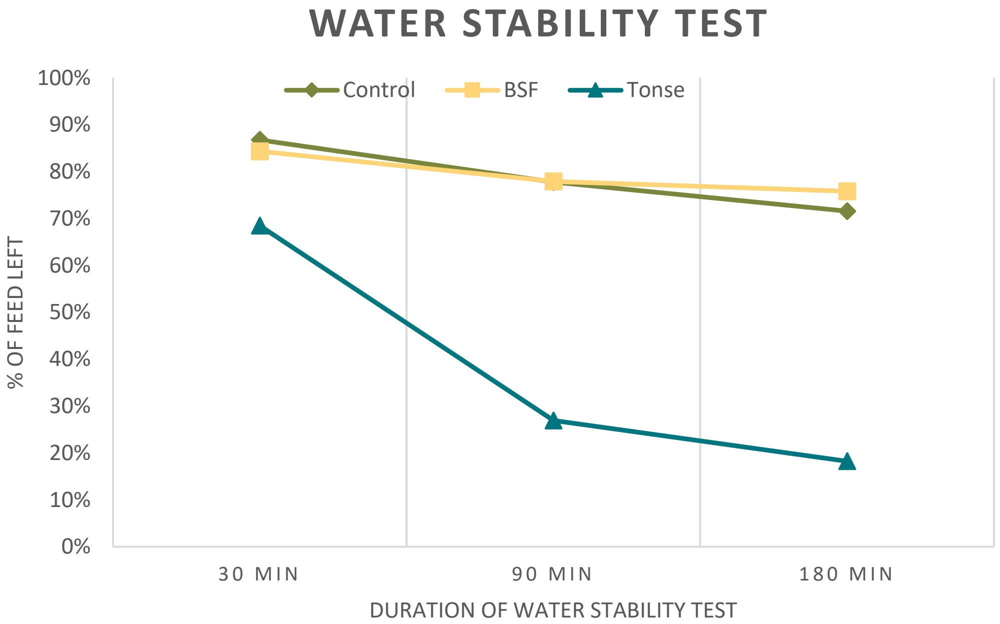

Figure 12. Water stability of diets used in feed trial.

4. Discussion

Fish growth experiment

The fish growth experiment showed that there was a clear difference between ponds, and that inter-pond variation rather than diets can better explain WG, FCR and SGR. Therefore, the fish growth experiment results are inconclusive as factors other than diet may have affected the results. The dissolved water temperature and pH for each pond were measured during the experiment. These variables have most likely affected the results. However, additional water quality variables may have influenced the growth performance results. Complex interactions influence water quality, including the weather and several other variables, such as ammonia, nitrite and nitrate concentration. Even though these variables can affect fish performance, few studies and limited information are available about the relationship between the water quality and growth performance in Nile tilapia. This issue is discussed more in two review articles by Mengistu et al. (2020) and Abd El-Hack et al. (2022). The current study did not measure these additional variables. Therefore, it is important to approach the results cautiously and consider other potential explanations for the observed outcomes.

In this study, the BSF diet had the lowest WG and SGR and the highest FCR numerically, which is not preferable. However, similar studies where FM has been replaced with BSF have shown promising results. In a study conducted by Panikkar et al. (2022), there was no difference in WG and SGR between a control diet containing FM and the BSF diet. Similar results were found in studies by Agbohessou et al. (2021) and Tippayadara et al. (2021), where final WG and SGR did not significantly differ when replacing FM with BSFM in different inclusion levels. Limbu et al. (2022) found that maximum growth performance in Nile tilapia fry, regarding WG and SGR, was achieved when replacing FM with 81% and 84% BSFM respectively. Nairuti et al. (2021) and Wachira et al. (2021) performed similar studies to the current, where the Nile tilapia growth experiment was performed in earthen ponds, replacing FM with BSFM. Nairuti et al. (2021) found no significant difference in WG, SGR, and FCR between the control and BSF diets with different inclusions of BSFM. Wachira et al. (2021) found that when replacing FM with BSFM at 33%, FCR and BW were improved compared to the control diet.

In addition, it is worth noting that the experiment mentioned above (Agbohessou et al. 2021; Nairuti et al. 2021; Tippayadara et al. 2021; Wachira et al. 2021; Limbu et al. 2022; Panikkar et al. 2022) was performed under optimum conditions in a controlled environment, i.e. stable water temperature, pH and DO. While the current study attempted to explore this alternative feed source, the considerable environmental variation created a challenge in obtaining statistically evaluated results. Therefore, further investigation under a better-controlled environment is necessary to fully understand the potential benefits and drawbacks of using BSFM in Nile tilapia feed. It is also important to note that the studies mentioned above have used smaller size fish (0,001 to 52,3 grams) than in the current study (75 grams). As nutritional requirements in fish change during different life stages (El-Sayed 2020d), the difference in size between studies emphasizes the need for more research on BSFM impact on growth performance in larger-size Nile tilapia.

All diets had a high FCR (2.4 – 4.9) (Table 6, Appendix) compared to an average FCR of 1.2 to 1.5 for Nile tilapia reared in similar conditions fed with extruded pellets (El-Sayed 2013). It should also be noted that feed waste from the hapas was not recorded during the experiment, which may have resulted in an overestimation of the FCR. An FCR above 2.0 can adversely affect the farm's profitability depending on current feed prices (Mengistu et al. 2020). The relationship between the feed input and the fish's weight gain determines the FCR. Therefore, mortalities occurring during production can affect the FCR. This problem was managed when calculating the FCR by correcting for any mortalities. However, mean values were used when correcting FCR. Therefore, calculating an exact FCR for each diet was not possible.

Moreover, a high FCR can depend on a variety of factors. Mengistu et al. (2020) found that when the water temperature was above 33°C, the FCR of Nile tilapia increased to above 2.0. Similar results were found by Jun et al. (2012), where temperatures over 32°C decreased feed utilization and growth. Both authors also concluded that water temperature has the most considerable effect on FCR in Nile tilapia (Jun et al. 2012; Mengistu et al. 2020). During the fish experiment at Ruvu, the water temperature was above 32 °C during large parts of the experiment period (Figure 8). Therefore, the water temperature of the ponds may have contributed to the high FCR. Mengistu et al. (2020) also found that a decrease in DO increases FCR. The optimum range of DO for Nile tilapia is between 5.5 to 6.5 mg/L (El-Sayed 2020c). During the current study, DO were under 5.5 mg/L in Pond 12 and 13 majority of the experiment. Therefore, the DO in these ponds may also have contributed to the high FCR.

The feed allowance used during the fish growth experiment was calculated using an SGR of 1.2 % (Nairuti et al. 2021) during the first half of the experiment. The calculated SGR in the current study was between 0.80% to 0.88 % (Figure 7). Therefore the feed allowance may have been overestimated, leading to overfeeding and poor feed utilization, thus affecting FCR negatively (increasing FCR).

Poor feed management can also contribute to a high FCR (White 2013). According to White (2013), a poorly adapted feeding frequency, delivery rate, feeding duration, and partial feed dispersal can all contribute to a high FCR. The fish were fed two times daily during the current study to mimic normal production conditions at Ruvu fish farm. Because of the species' feeding behaviour, NRC (1993) and Shiau (2002) recommend a feed frequency of three to four times a day for tilapia weighing 20-100 grams, while tilapia weighing >100 grams should be fed two to three times a day. With this in mind, a feed frequency of three times a day for the current study would perhaps have been beneficial for a decrease in FCR. On the other hand, Alemayehu & Getahun (2017) and Mengistu et al. (2020) found that FCR was not significantly affected by feeding frequency. Although, Alemayehu & Getahun (2017) found that growth performance was improved with a more frequent feeding regime.

Nutritonal composition

The BSF diet has the highest CP, CF and NDF compared to other diets (Table 2). Similar nutrient composition results for BSF diets were found in other studies (Tippayadara et al. 2021; Wachira et al. 2021; Limbu et al. 2022; Lu et al. 2022). The nutritional composition of the BSFM had a lower amount of histidine, lysine and methionine than FM. The essential amino acids lysine and methionine are usually the first limiting for Nile tilapia as they are generally deficient in plant protein sources used in fish diets (El-Sayed 2020d). In the chemical score calculations (Table 4, Figure 4), the BSFM composition of histidine, lysine, and methionine could not meet the EAA requirement for Nile tilapia. Therefore, it is crucial to be aware of these limitations when formulating recipes for Nile tilapia diets containing BSFM.

The nutritional composition of BSFM can vary depending on what kind of substrate the larvae grow on. In a study by Meneguz et al. (2018), BSF larvae were reared on four different substrates, fruit waste, fruit and vegetable waste, winery by-products and brewery by-products. The authors found that chemical composition varied depending on the substrate used for larvae production. Additionally, the time needed to reach the prepupal stage differed between substrates, where brewery by-products facilitated the fastest growth. Similar results were found in a study by Agbohessou et al. (2021), where BSF larvae were reared on either vegetable by-products or chicken feed or fish waste. As in Meneguz et al. (2018) study, chemical and fatty acid composition differed between the substrates. In the current study, BSF larvae were grown on raw fruit, -vegetables and restaurant waste. However, waste used for BSF production may change depending on season and supply, thus changing the chemical composition of the BSFM. This change in the chemical composition of protein and EAA can, in turn, lead to altered nutritional composition in the BSF diet, which can affect growth performance in Nile tilapia. Therefore, it is essential to emphasize having a homogenous substrate to maintain a uniform nutritional composition of the BSFM to achieve desired growth performance in the fish. Future research should also focus on testing different substrates' potential to achieve an amino acid profile in BSF larvae with a high content of limiting amino acids, such as lysine and methionine.

Phenylalanine was also considerably higher in the BSFM (Table 3) than in other studies (Lu et al. 2022). Phenylalanine is an essential amino acid for many insects as it plays an integral part in the hardening of the cuticle (sclerotization) (Behmer & Joern 1993; Behmer 2005). Therefore, the harvest time of the larvae, the larval development stage and the substrate source may cause the high phenylalanine content in the BSFM. However, more research is needed to determine where the high phenylalanine content originates and how this effect fish growth performance.

Physical feed quality

Using pellet quality assessment to illustrate a causal relationship between feed formulation and nutritional performance is important. During the water stability test, the BSF diet performed the best over time (Table 5, Figure 12). Fish fed with water-stable pellets have more time to ingest the feed before the pellets dissolve, thus minimizing excess nutrient load in the water (White 2013). Moreover, PDI during the current study was above 89% for all diets, whereas the BSF diet had the lowest PDI numerically (Table 5). Statistical analysis on PDI could not be performed due to the small sample size for each diet (n=2). However, a PDI over 89% is considered high, indicating good quality, which is important for minimizing fine particles and dust formation during transport, handling, and feeding, thus minimizing economic losses for the farmer and additional strain on the environment (White 2013; Davis & Hardy 2022). The BSF diet performed significantly lower on the hardness test (Table 5). A similar result was found in a study by Weththasinghe et al. (2021), where a reduction of hardness was seen with higher inclusion of BSFM in diets for Atlantic salmon (*Salmo salar*). Studies have also found that pellet hardness affects other fish species' apparent digestibility. A study by Aas et al. (2011) showed that apparent digestibility in rainbow trout (*Oncorhynchus mykiss*) was highest in the feed with the highest hardness. The authors suggested that a higher pellet hardness slowed gastric evacuation time, which may have resulted in slower digestion and a longer time for nutrient absorption resulting in improved digestibility. In the same study, the authors saw tendencies of fat belching in the feed with lower hardness, which can lead to unnecessary stress for the fish. Similar findings were presented in a study by Baeverfjord et al. (2006), where low water stability can potentially be a factor causing fat bleaching and other abdominal diseases in rainbow trout. However, these studies (Aas et al. 2011; Baeverfjord et al. 2006; Weththasinghe et al. 2021) are performed on carnivorous fish species, which have a different autonomy than Nile tilapia (Davis & Hardy 2022), which are omnivorous fish species. Therefore, future studies should investigate if pellet hardness has similar results in Nile tilapia.

Furthermore, the BSF diet and the BSFM had the highest inclusion of crude fat (Table 2 and 3). This high inclusion of fat may explain why the BSF diet had a lower PDI and hardness compared to the Control and Tonse diet, as fat is one factor that reduces pellet quality in extruded feeds (Sørensen 2012; Forte & Young 2016). This connection between a high inclusion of fat and poor pellet quality has also been seen in the study by Weththasinghe et al. (2021).

Good physical feed quality is essential for profitable fish production as it can affect fish growth performance (Baeverfjord et al. 2006; Aas et al. 2011; Weththasinghe et al. 2021) and minimize environmental impact (White 2013; Davis & Hardy 2022). Still, little research has been done on this subject, and the available research does not include Nile tilapia studies. Therefore, future research regarding novel feed's physical quality for Nile tilapia is necessary.

GHG calculations

The GHG calculations showed that the BSF diet had a lower GWP than the Control diet, as hypothesized. Because of the unknown inclusion rate of ingredients in the Tonse feed, GHG-emission calculations were not possible to perform. The BSF larvae were fed with food waste collected from surrounding restaurants and food markets that would otherwise be wasted, thus making BSF larvae, as an ingredient, a low contributor to GHG-emission in the diet. Due to allocation, if the BSF larvae, in the future, are fed with something else that is not food waste, the carbon footprint would be higher. Therefore, it is important to emphasize using waste streams as a substrate for BSF larvae to limit GHG emissions from production in future production, as food loss and waste represent half of total greenhouse gas emissions from food systems (Zhu et al. 2023). Soybean meal was the most significant contributor to GHG emissions in the BSF diet, followed by blood meal (Figure 11). Electricity consumption in the BSF diet production was also high (16%, Table 9, Appendix). Therefore, measures to reduce the inclusion of soybean meal and blood meal without affecting fish growth performance and physical pellet whilst streamlining the process to minimize electricity usage should be a focus in feed development. The functional unit used in this study was *one kg of feed at factory gate*. Using this functional unit, the GHG emission of 1 kg fish produced with different diets has not been evaluated. Therefore, future life cycle analysis should also evaluate this functional unit, as fish performance should be considered when determining GHG emission from a feed.

5. Conclusion

Few studies have been conducted on BSFM inclusion in Nile tilapia diets. In the current study, growth performance could not be adequately statistically evaluated due to inter-pond variation rather than diets. However, other studies performed under better-controlled environments have shown that replacing FM with BSFM has been successful on Nile tilapia. Nevertheless, these studies do not mimic typical production conditions in Tanzania. Therefore, future research must be performed in a better-controlled environment to avoid significant pond variation. Nutritional composition and the chemical score showed low histidine, lysine, and methionine and a high phenylalanine content in the BSFM. This may depend on the substrate used during BSF larvae production and the life stage of the larvae. However, more research is needed to determine the substrates effect on nutritional composition and how it affects fish growth performance. The BSF diet performed the best on the water stability test over time while simultaneously having a lower DPI and hardness than the other diets. To this day, physical feed quality evaluations on novel tilapia feeds are seldom made. Therefore, an assessment of the physical quality of feed in future research needs to be performed, as it is an essential instrument for optimizing fish performance and minimizing the environmental impact of feed waste. GHG calculations showed that the BSF diet had the lowest GWP. However, the functional unit used in this study was *one kg of feed at factory gate*. Future GHG calculations on the BSF feed should take fish production into account.

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Insekter, det nya proteinet för fisk i Tanzania

Fiskodling i Tanzania expanderar i takt med växande efterfrågan, där Niltilapia är den vanligaste odlade fisken. Odling av Niltilapia kräver proteinrikt foder för att de ska kunna växa snabbt. Proteinet i fodret kommer vanligtvis från fiskmjöl och sojabönor. Soja kräver mycket resurser för att växa samt att fiskmjöl är en begränsad resurs och har därför blivit dyr att använda i fisk foder. Därför har försök gjorts för att undersöka om insekter kan användas som protein ingrediens istället för fiskmjöl och soja.

Svart soldatfluga är en av de insekter som man försöker ersätta fisk och sojamjöl med. I ett experiment där miljön efterliknande vanliga produktionsförhållanden i Tanzania, byttes fiskmjöl ut mot 35 % malda larver från svart soldatfluga. I experimentet undersöktes även om de malda larverna hade en påverkan på fodrets kvalitet och om det släppte ut mindre koldioxid än vanliga foder som innehåller fiskmjöl. Insektsfodret testades på fiskar i tre olika dammar.

Resultatet, visade att fodret som innehöll svart soldatfluga hade ett lägre utsläpp av koldioxid än de vanliga fodren. Insektsfodret hade även bättre vattenstabilitet dvs. fodret löstes inte upp i vattnet lika snabbt som de andra fodren. Detta gör att fisken har mer tid på sig att äta upp allt foder och man slipper onödigt foderspill i vattnet som kan påverka miljön negativt. Däremot hade inte insektsfodret lika bra hårdhet och hållbarhet jämfört med de andra fodren. Detta kan bero på att fodersammansättning ser olika ut eftersom de innehåller olika ingredienser. När man gör utfodringsförsök på fisk är det viktigt att miljön är densamma för alla fiskar som är med i experiment. Olikheter i miljön kan göra att det inte går att utvärdera om de olika fodren skilde sig från varandra eller inte.

I detta experiment skilde sig miljön mellan de olika dammarna som fisken levde i och därmed gick det inte att statistiskt utvärdera om insektsfodret var bättre eller lika bra som de vanliga fodren. Därför behövs mer forskning i framtiden för att utvärdera om svart soldatfluga kan vara ett alternativ till fisk och sojamjöl i foder till niltilapia.

Acknowledgements

First of all, thank you to my supervisors, Aleksandar Vidakovic and Hanna Carlberg, for your support and valuable feedback. Your dedication and encouragement have motivated me to improve and challenge myself. Thank you for the time and effort you have invested in me.

Thanks to Pontus Gunnarsson, who helped me with statistics during this project. Thank you for your patience and guidance.

Thank you to Matthew, Alexa, Zola and Iris for your hospitality during my first stay in Tanzania.

Thank you, Kigen Compton, for good teamwork and your hospitality during my stay in Tanzania.

Thanks to the personnel at Ruvu fish farm for good teamwork and willingness to collaborate during this project.

Thank you to Markus Langerland and Yannic Wocken at RISE for providing calculations and more for the GHG-emissions chapter in this project.

Thank you to Astrid Gumucio for doing the absolute most to provide the chemical analysis of the feed needed during this project. Also, thank you to Jorge André and Camilla Andersson for doing the chemical analysis. Thank you for the great discussions and teamwork.

Thank you to Nils Lengquists scholarship fund for making it possible for me to do fieldwork in Tanzania.

And lastly, thank you to the Nordic Climat facility for providing the funds for this project.

Appendix

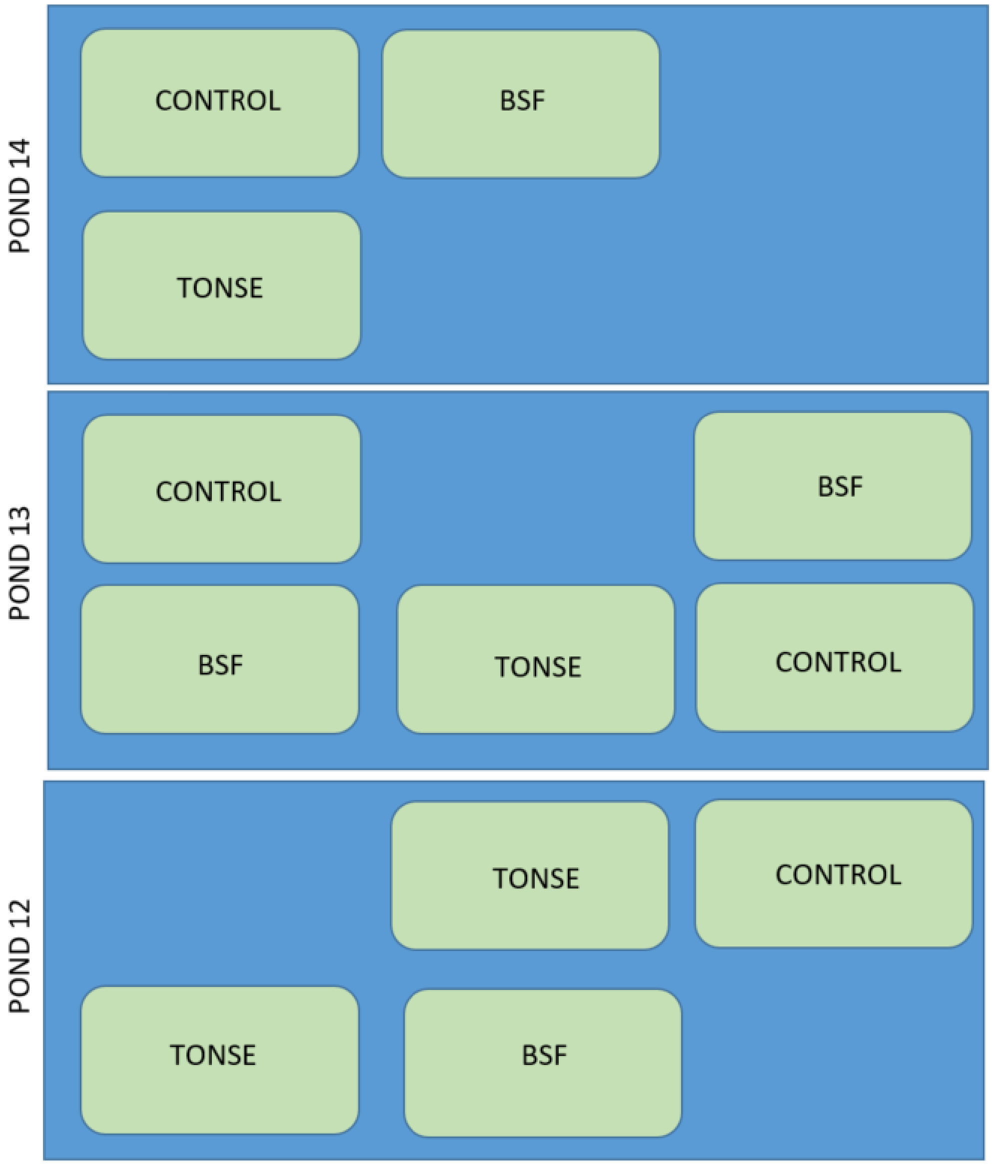

Figure 13. Map of hapa placement in experimental ponds in fish growth experiment.

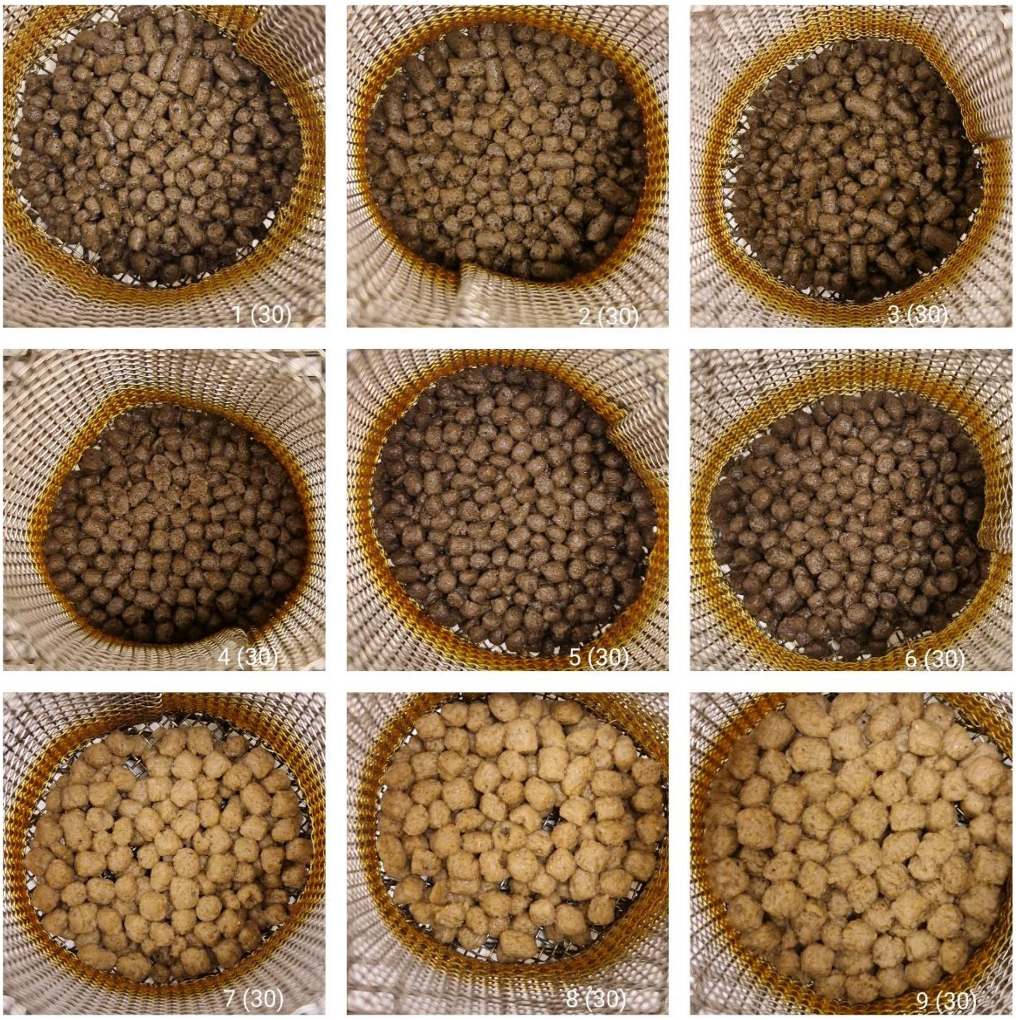

Figure 14. Pictures of experimental diets from water stability test 30min, wet samples.
Control diet: 1-3; BSF diet: 4-6; Tonse diet: 7-9.

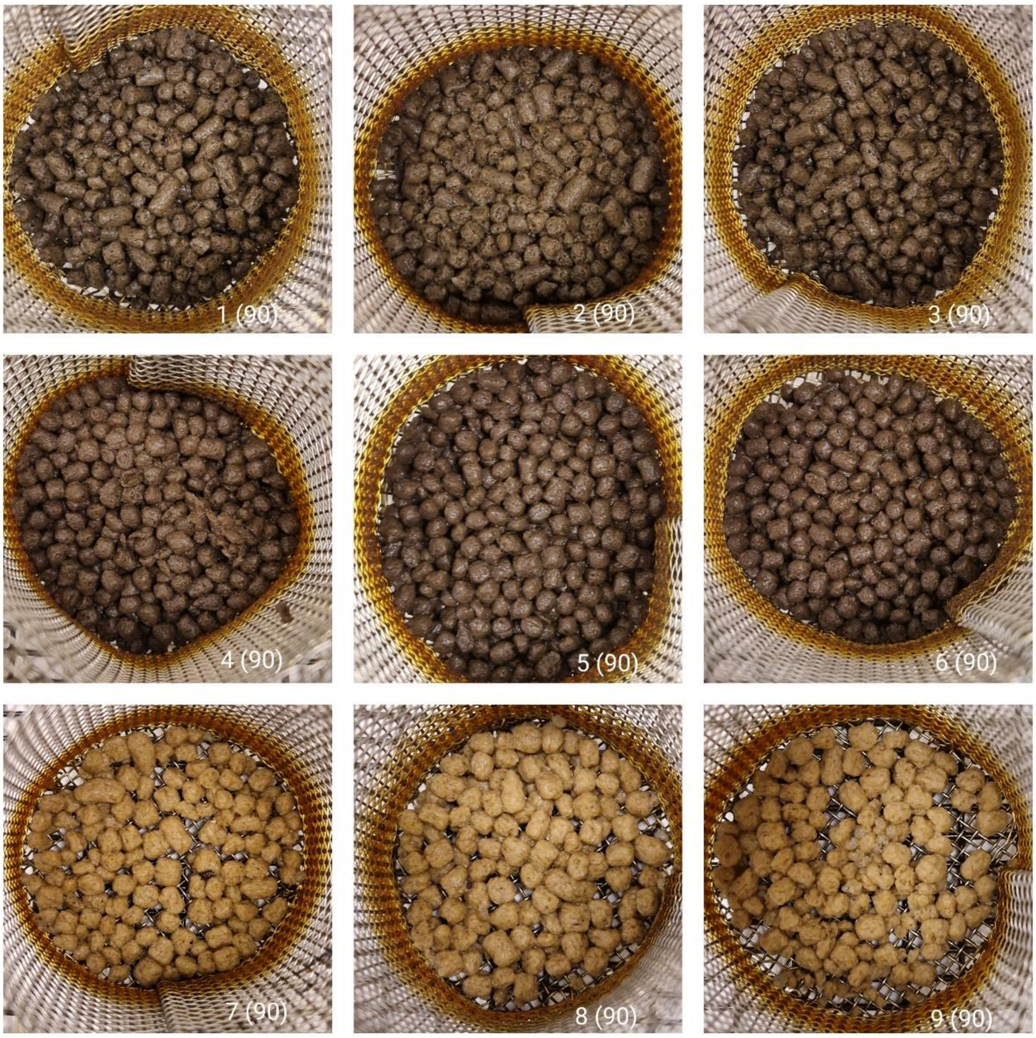

Figure 15. Pictures of experimental diets from water stability test 90min, wet samples.
Control diet: 1-3; BSF diet: 4-6; Tonse diet: 7-9.

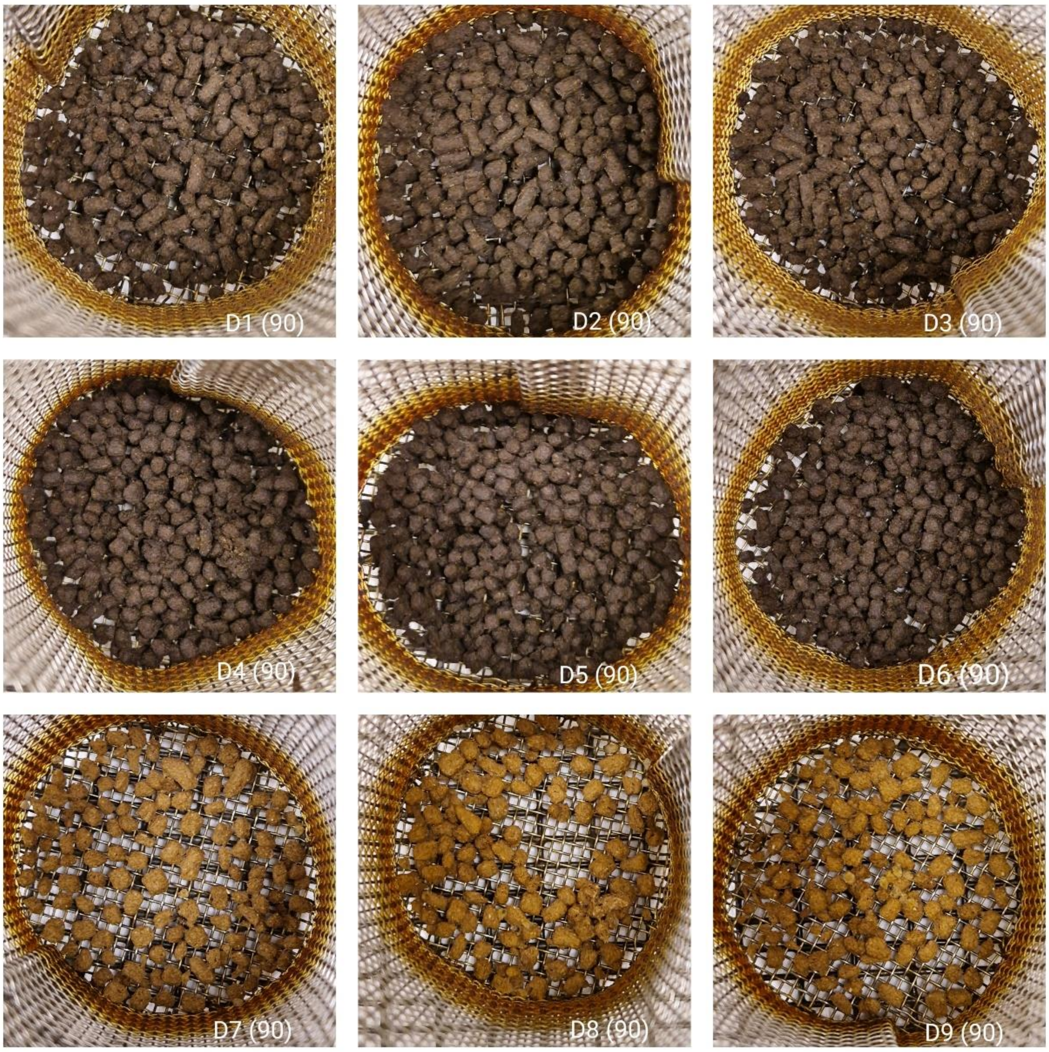

Figure 16. Pictures of experimental diets from water stability test 90min, dry samples.
Control diet: D1-D3; BSF diet: D4-D6; Tonse diet: D7-D9.

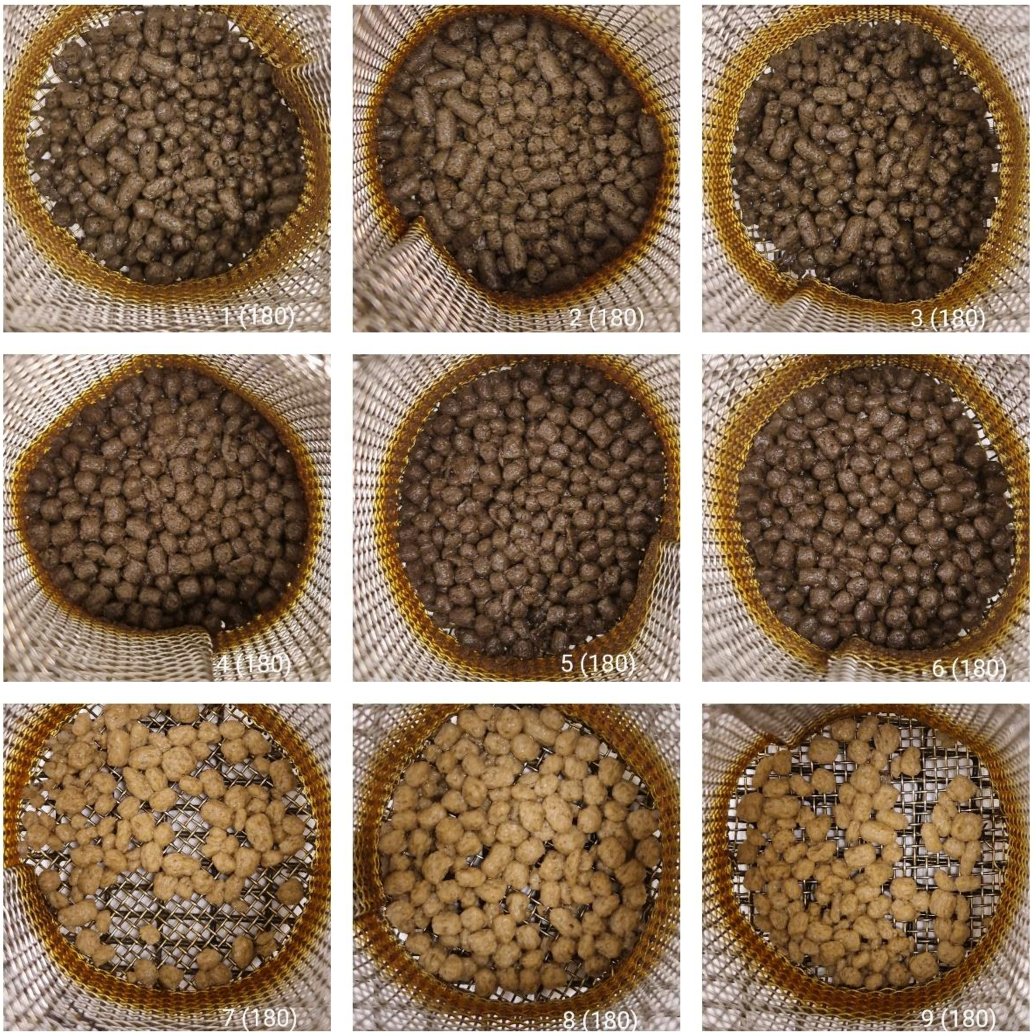

Figure 17. Pictures of experimental diets from water stability test 180min, wet samples.
Control diet: 1-3; BSF diet: 4-6; Tonse diet: 7-9.

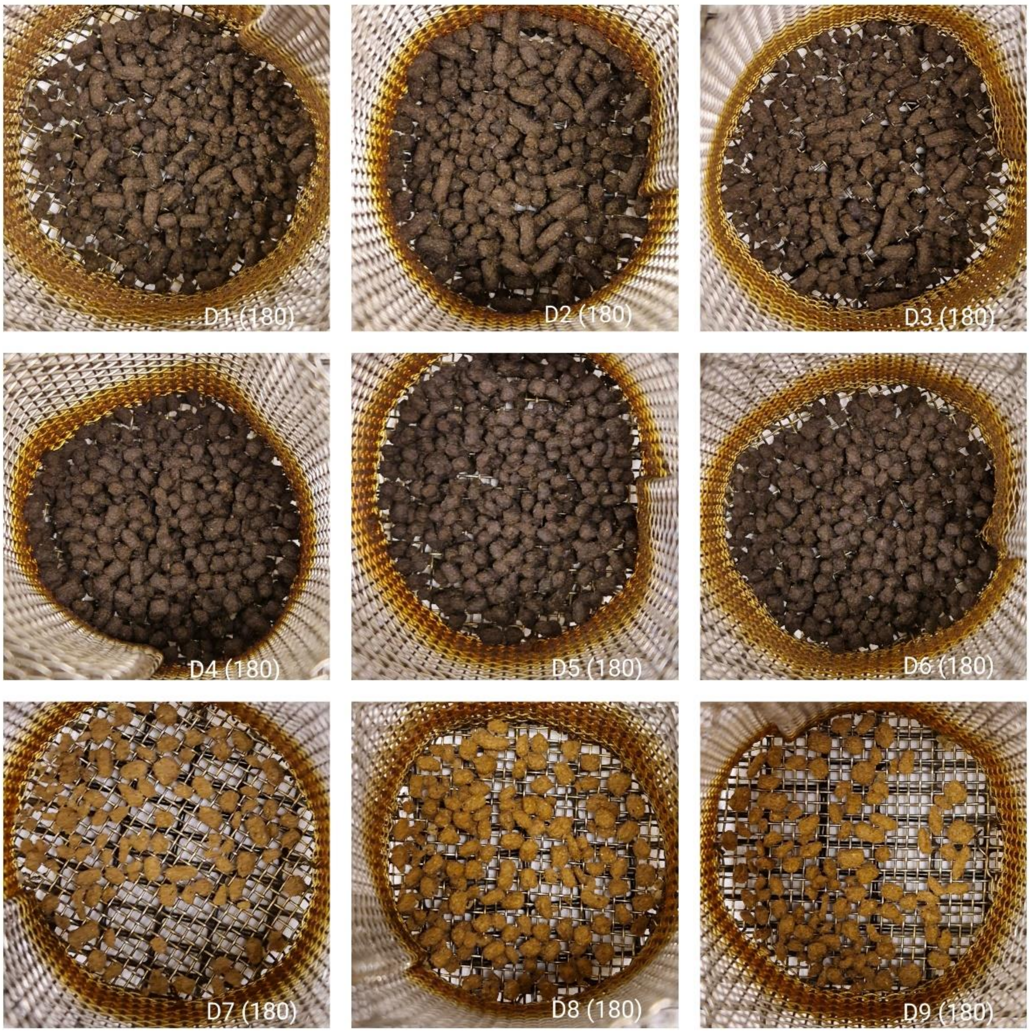

Figure 18. Pictures of experimental diets from water stability test 180min, dry samples.
Control diet: 1-3; BSF diet: 4-6; Tonse diet: 7-9.

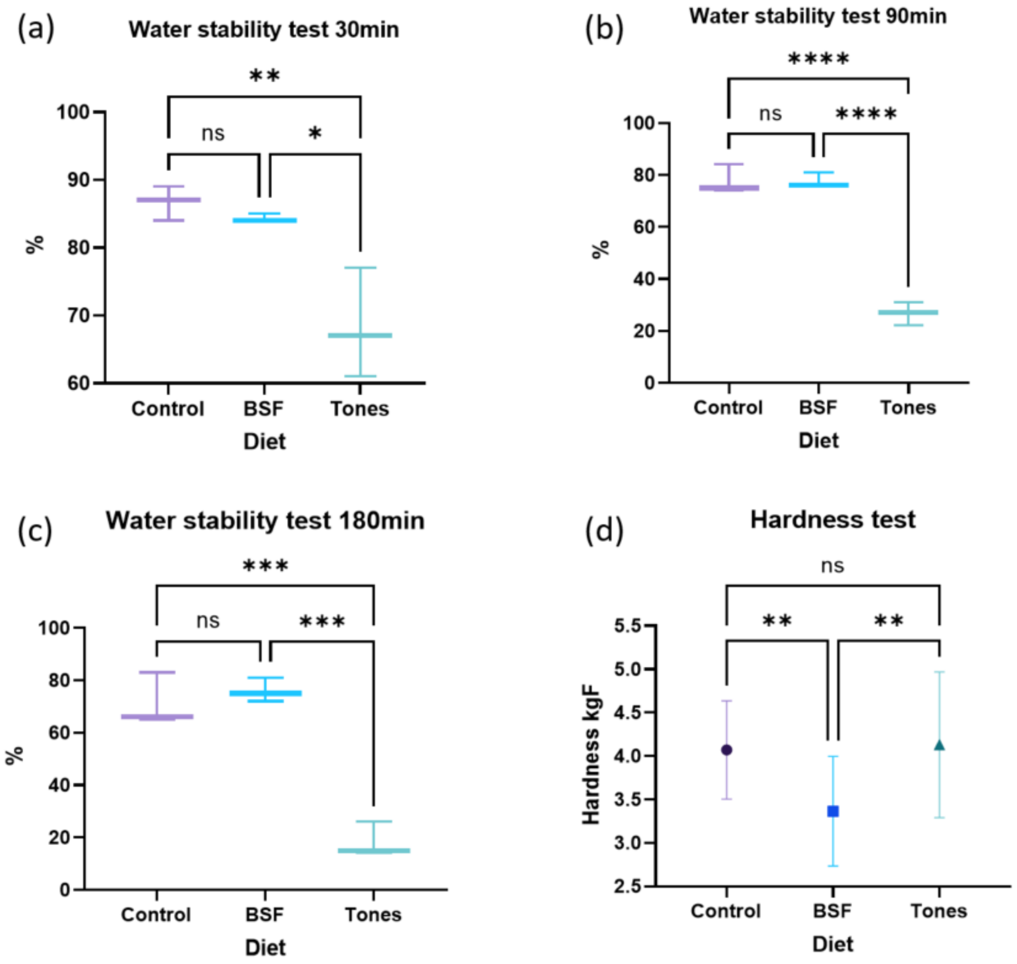

Figure 19. (a) Water stability test results after 30min. (b) Water stability test results after 90min. (c) Water stability test results after 180min. (d) The hardness test result of the experimental diet.
BSF, black soldier fly; ns, non-significant ($P < 0.05$)

Table 6. Growth performance indicators and mortality for each pond and diet, average ± SEM.

	Pond 12				Pond 13					Pond 14			
	BSF	Control	Tonse^{1}	Tonse^{2}	BSF^{1}	BSF^{2}	Control^{1}	Control^{2}	Tonse	BSF	Control	Tonse	SEM
Weight start (g)	72.5	74.8	74.7	74.0	74.5	74.6	73.2	74.6	74.6	74.7	74.6	74.0	0.1944
Weight end (g)	134.7	146.7	136.9	146.7	125.3	115.8	117.7	136.1	130.1	145.1	132.4	132.5	2.8221
Weight gain (g)	62.2	71.9	62.3	72.8	50.8	41.3	44.5	61.5	55.5	70.4	57.9	58.5	4.1540
Weight gain (%)	35	47	37	47	25	16	18	36	30	45	32	33	2.7869
SGR (%)	0.91	0.99	0.89	1.01	0.73	0.64	0.67	0.86	0.86	0.93	0.81	0.86	0.0327
FCR	2.8	2.9	3.0	2.4	3.8	4.9	4.2	3.2	3.7	3.1	3.7	3.5	0.1906
Mortality (%)	17	23	8	10	7	7	12	3	1	0	1	3	1.9341

Weight start n=150

Weight end, Pond 12; BSF n=125, Control n=116, Tonse^{1} n=138, Tonse^{2} n=135 Pond 13; BSF^{1} n=140, BSF^{2} n=139, Control^{1} n=132, Control^{2} n=146, Tonse n= 148 Pond 14; BSF n=150, Control n=149, Tonse n=146.

FCR, feed conversion ratio; BSF, black soldier fly; SGR, Specific growth rate

Table 7. Growth performance indicators for each diet, average ± SEM.

	Control	BSF	Tonse	SEM	*P*-value
Weight start	74.3	74.1	74.3	0.0565	0.8955
WG %	79.4	75.8	83.8	1.8934	0.7090
FCR	3.5	3.7	3.2	0.1243	0.6140
SGR %	0.83	0.80	0.88	0.0194	0.6507

BSF, black soldier fly; FCR, feed conversion ratio; SGR, specific growth rate; WG, weight gain.

Table 8. Growth performance indicators for each pond, average ± SEM.

	Pond 12	Pond 13	Pond 14	SEM	*P*-value
WG (%)	91	68	84	5.4514	0.0199
FCR	2.8	4.0	3.5	0.2821	0.0147
SGR	0.95	0.74	3.5	0.0507	0.0057

FCR, feed conversion ratio; SGR, specific growth rate; WG, weight gain.

Table 9. Greenhouse gas emissions from Control and BSF diet.

Ingredients/Elements	**Control**	**BSF**
	kg CO_2-eq	**kg CO_2-eq**
Fishmeal	0.28	0.04
BSF protein meal	0	0.16
Blood meal	2.48	0.50
Soybean meal	1.10	1.10
Cottonseed meal	0.05	0.04
Sunflower cake	0.01	0.01
Maize grain	0.12	0.12
Fish oil	0.07	0.07
Sunflower oil	0.02	0
Soy lecithin	0.01	0.01
Mineral and vitamin premix	0.01	0.01
DL-Methionine	0.01	0.01
Monocalcium phosphate	0.00	0.00
Transport	0.01	0.01
Electricity	0.40	0.40
Total	4.58	2.47

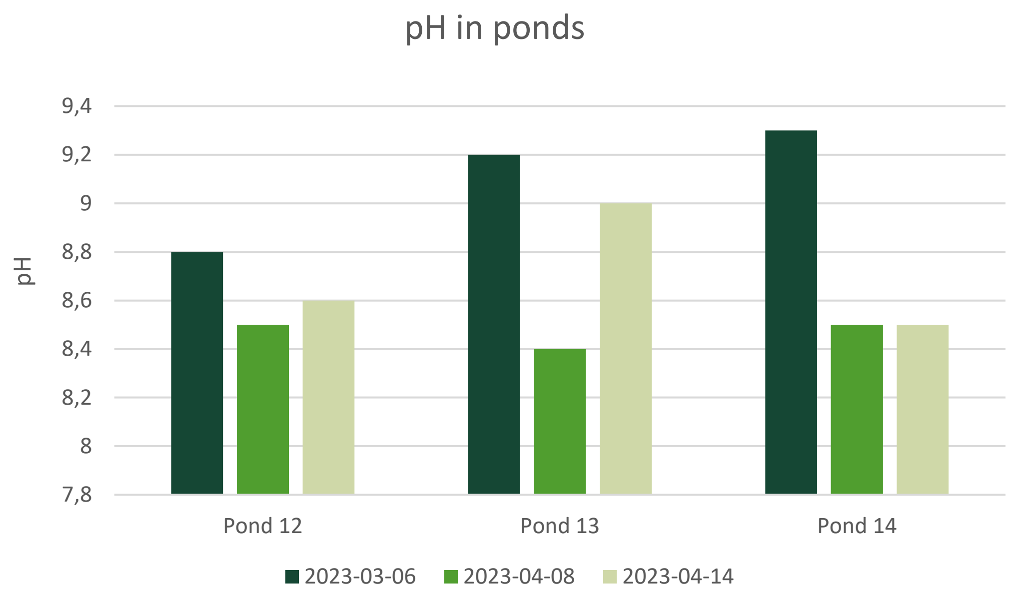

Figure 20. Water pH in experimental ponds taken during three time points.

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