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Predicting Treatment Performance Using Ternary Plot

Macronutrients' impact on biomass conversion efficiency

Madeleine Gobl

ABSTRACT

PREDICTING TREATMENT PERFORMANCE USING TERNARY PLOT

Madeleine Gobl

Black soldier fly larvae (BSFL) waste treatment is becoming a more sought-after organic waste treatment since it produces larvae for animal feed as well as fertilizer, the need for prediction and optimization of treatment performance increases. This study aims to predict biomass conversion efficiency (BCE) for waste going into treatment, using only the nutritional data from other studies as it could give a good performance estimation while using available data. First, macronutrient fractions, based on a normalized total weight of volatile solids (VS), were calculated from nutritional data of other studies and of this present study. Using the results, coordinates of these samples were calculated and put on the ternary plot, revealing trends for different macronutrient compositions. To validate these trends, selected nutrient compositions were created as waste mixtures containing fish, bread and vegetable waste and then treated by BSFL. After treatment, BCE and waste reduction in both VS and wet weight basis were noted.

The results showed that a composition high in carbohydrate and low in fat content is favourable for a high BCE value while protein content remained similar across all treatments and only seemed to slightly increase BCE. Treatment A5 held the highest $BCE_{\%VS}$ (32.6 ± 3.5) with a composition of $73\%_{VS}$ carbohydrates, $19\%_{VS}$ protein and $8\%_{VS}$ fat and control treatment (A0) with no fish waste held the highest $BCE_{\%WW}$ (21.2 ± 1.8).

When comparing with the results of the other studies used to make the trends in the ternary plot, predictions of BCE were more accurate for a composition of $70\%_{VS}$ carbohydrates, $20\text{-}25\%_{VS}$ protein and $< 8\%_{VS}$ fat and also for compositions with similar waste substrates, as their percental changes were similar. This suggests that it is possible to predict high BCE values from nutritional data in a ternary plot and could be of use when optimizing larval production.

Keyword: Black soldier fly, organic waste treatment, biomass conversion efficiency, treatment performance, macronutrients, ternary plot, organic waste

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REFERAT

UPPSKATTNING AV BEHANDLINGSEFFEKTIVITETEN VIA TRIANGULÄR DIAGRAM

Madeleine Gobl

Fluglarvskompostering med den amerikanska vapensflugan (*Hermetia illucens*) är en avfallsbehandling för organiskt avfall som producerar larver och gödselmedel som kan används som djurfoder respektive gödselmedel. Många studier har undersökt hur makronäringsämnen påverkar larvproduktion eller behandlingseffektiviteten och kommit till olika resultat. I dagens läge skulle det underlätta om man kunde uppskatta den högsta möjliga effektiviteten baserat på data om avfallet som redan finns utan att behöva gå igenom en längre studie först. Den här studien har som syfte att undersöka om det är möjligt att förutspå biomassomvandlings-effektivitet (BCE) från makronäringsdata tagen från andra studier i ett triangulärt diagram. I denna studie användes studierna av Lopes *et al.*, (2020) och Isibika *et al.*, (2021) som basdata i triangulär diagrammet.

För att validera det trender som sågs i det triangulära diagrammet utfördes tester på valda kompositioner beståendes av ansjovis, bröd och grönsaksavfall med fluglarvkompostering. För att kunna applicera makronäringsämnernas data till triangulär diagrammet, beräknades mängden protein, kolhydrater och fett som en andel av den totala mängden glödförlust (VS). Efter behandling beräknades BCE på våtvikt (WW) och VS basis.

Resultaten tyder på att en avfallskomposition med hög halt kolhydrater och låg halt fett ($70\%_{VS}$ kolhydrater, $20–25\%_{VS}$ protein and $<8\%_{VS}$ fett) bidrog till ett högt BCE värde, där proteinhalten var snarlik i alla behandlingar och verkade ha ett litet bidrag till ett högt BCE värde. Behandlingen A5 hade det högsta $BCE_{\%VS}$ värdet (32.6 ± 3.5) med en komposition av $73\%_{VS}$ kolhydrater, $19\%_{VS}$ protein och $8\%_{VS}$ fett och kontroll behandlingen A0 (utan ansjovis avfall) hade högst $BCE_{\%WW}$ värde (21.2 ± 1.8).

Vid jämförelse mellan de andra studierna och resultaten, var uppskattningar av BCE mer precisa för en komposition med ca $70\%_{VS}$ kolhydrater, $20–25\%_{VS}$ protein and $<8\%_{VS}$ fett och för uppskattningar baserad på data från rapporter med liknande avfallssubstrat, då deras procentuella förändringar var liknande varandra. Detta stärker hypotesen att det är möjligt att kunna förutspå högt BCE värde med hjälp av näringsdata från avfallet och skulle kunna användas för att optimera av larvproduktion.

Nyckelord: amerikansk vapensflugan, triangulär diagram, organiskt avfallsbehandling, organiskt avfall, behandlingseffektivitet, biomassomvandlings-effektivitet, makronäringsämnen

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PREFACE

This study is a master’s thesis of 30 hp within the Master's Programme in Environmental and Water Engineering at Uppsala University and the Swedish University of Agricultural Sciences. This study was executed at the Institution for Energy and Technology in the Swedish University of Agricultural Sciences. Cecilia Lalander was supervisor and examiner was Björn Vinnerås for this study, both part of the institution of Energy and Technology at Swedish University of Agricultural Sciences.

I am grateful for Rena Hav providing the fish waste for this study, for the vegetable waste provided by Grönskashallen Sorlunda and the reclaimed bread from Fazer. I want to thank my supervisor Cecilia for great support and commitment, thoughtful feedback, for helping me with my experiments and discussing my work with me. I want to thank my examiner Björn for support and supervision of my study and also a big thank you to Victoria and Emillen for helping me with my experiments throughout treatment.

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POPULÄRVETENSKAPLIG SAMMANFATTNING

I linje med EU:s mål att skapa en mer cirkulär ekonomi inom EU, där produkter har en längre livslängd och mindre naturresursers behövs, forskas det mycket om hur samhället kan reducera sitt organiska avfall och även återanvända det. EU har direktiv för att nå sina mål för denna cirkulära ekonomi och har då lagt grund för avfallshierarkin. Avfallshierarkin visar metoderna förebyggande, återanvändning, materialåtervinning och energiåtervinning samt deponering av avfall i den ordningen där förebyggande är bäst och deponi är den sämsta avfallsbehandlingen för organiskt material för att uppnå EU:s miljömål. Specifika tekniker som finns idag för hantering av organiskt avfall är vanligast kompostering eller rötningen därav rötning energiåtervinner avfall till biogas och kompostering materialåtervinner och skapar gödslingsmedel. Kompostering som kan materialåtervinna avfall har större chans att förlänga materialets livslängd enligt avfallshierarkin och organiskt avfall, specifikt matavfall, kan ha möjlighet att bli djurfoder med rätt kompostering.

Fluglarvskompostering med den amerikanska vapensfluglarver (*Hermetia illucens*) är en organisk avfallsbehandling och har på senare tid fått mer uppmärksamhet från forskarvärlden och den privata sektorn. Fluglarvkompostering innebär att fluglarver förtär det organiska avfallet och till skillnad från vanlig kompostering som bara producerar gödselmedel, kan fluglarvskompostering producera larver som kan användas till djurfoder och gödselmedel som båda har ett ekonomiskt värde och gett ökat intresse för metoden. Studier har gjorts på hur man kan ökat effektiviteten av komposteringen och produktion av larverna genom att modifiera mängden makronäringsämnena proteiner, kolhydrater och fett i det organiska avfallet som larverna behandlar. De flesta rapporter har en slags konsensus om att protein påskyndar larvutvecklingen, kolhydrater ökar fetthalten i larverna och kan öka vikten och att fett är viktigt för larvernas utveckling till flugor.

Idag finns det inget sätt veta hur effektivt larver kommer bryta ned avfall tills hela behandlingen är klar vilket är en kunskapslucka som skulle vara nyttigt att fylla för att vidareutveckla och applicera fluglarvskompostering. Syftet med detta projekt är att undersöka huruvida det går att förutspå biomassomvandlings-effektivitet (BCE) med data av näringsinnehållet i avfallet på ett triangulärt diagram, där biomassomvandlings-effektivitet är ett mått på hur effektivt larven tar det den förtär in i sin biomassa. För att kunna förutspå effektivitet användes data från två andra studier, varav en använde fiskrens från kommersiellt fiske och återtaget bröd i sin studie för att se hur effektivt amerikanska vapensfluglarver kunde bryta ned fiskrens och den andre använde fiskkadaver med banan och apelsinskal för att undersöka om medkomposteringen av fruktskal och fiskavfall kunde öka biomassomvandlings-effektivitet.

För att kunna applicera dessa studiers makronäringsämnernas data till det triangulära diagrammet beräknades, mängden proteiner, kolhydrater och fett från varje studie som en andel av den totala mängden glödförlust i varje behandling. Dessa andelar användes sedan för att beräkna koordinater i det triangulära diagrammet och representerade då kompositioner som används i de två studierna.

Efter analys av det triangulära diagrammet med olika datapunkter som formade olika trender, så valdes från kompositioner ut av de möjliga för denna studie för validering. Substraten som användes i de utvalda kompositionerna bestod av ansjovisrens, bröd och grönsaksavfall i olika halter. För validering fluglarvkomposterades de utvalda kompositionerna, där 1785 larver användes för varje komposition och beräkningar utfördes för matdosering på 0,1 gram glödförlust skulle ges till varje larv. Efter behandling beräknades BCE på våtvikt och glödförlust basis. Kompositionerna som hade olika andelar av proteiner, kolhydrater och fett följde trender som tidigare observerats hos andra studier där protein visades påverkan på larvutveckling, kolhydrater visade påverkan på fetthalt och möjligen vikt samt fett var viktigt för flugutvecklingen. Resultaten visade att kontrollen utan tillsats av hade högst BCE$_{\%\text{våtvikt}}$ värde och A5 (5% tillsats av ansjovisrens) hade högst BCE$_{\%\text{glödförlust}}$ värde.

Generellt visade resultaten att en komposition av hög kolhydratshalt och låg fetthalt gav högre BCE, och proteinhalt som var snarlikt bland de flesta behandlingar, hade en mindre påverkan för ett högre BCE. Uppskattningen av BCE genom näringsdata på ett triangulärt diagram hade bra precision. Det är troligt att precisionen har ökat på grund av att substraten som användes i uppskattningen från baserad på de två andra studierna hade liknade protein och fett-källa som denna studie och att övriga substrat som återtaget bröd var liknade. Eftersom det gick att uppskatta BCE med näringsdata, är detta något som skulle kunna användas för att effektivisera fluglarvkomposteringen och dess larvproduktion.

WORDLIST

BSFL	Acronym for black soldier fly larvae
Black soldier fly treatment waste treatment /BSFL waste treatment	Organic waste treatment where black soldier flies consume material and convert it into biomass.
Frass	Product of BSFL waste treatment which can be used as plant fertilizer
Dry matter (DM)	Weight of mass that is dried
Volatile solids (VS)	Matter which vaporizes at high temperature leaving only ashes. It is calculated by amount dry matter minus the amount of ash.
Waste mixture/ feed mixture	The mixture of organic materials going into the BSFL waste treatment

TABLE OF CONTENT

1. INTRODUCTION

Climate change is one of our modern-day largest challenges. The increased greenhouse gas emissions has escalated the greenhouse effect and as the global average temperature rises and weather pattern changes, consequences of global warming drought, melting polar ices and rising sea levels will become more present in human life (United Nations, 2021)

As a response to the actions needed to combat climate change and become a more resilient and sustainable society, the European Commission launched the *Circular Economy Action Plan* (United Nations, 2021). One of the policies is the framework directive for waste management and has the principles of preventing, reusing, recycling, recovering and lastly disposing of waste. Prolonging the life cycle of a product or being able to reuse it in different forms will not only decrease our impact on the environment and climate change, it will also decrease the need for importing raw material (European Commission, 2021).

In their revision of the waste hierarchy for food waste, Teigiserova *et al.*, 2020 suggested that food waste could be reused as animal feed after proper safety treatment. Food waste includes food past their expiration date, defected food and food considered to be inedible for humans and reusing food waste as animal feed is a common practice in other countries such as Japan and South Korea (Salemdeeb *et al.*, 2017; Teigiserova, Hamelin and Thomsen, 2020).

One of the animal feeds being imported to the EU is soy meal. Soy meal production takes a heavy toll on the environment since it increases the deforestation of the Amazon rainforest in South America where the soybean is produced (Stolton and Dudley, 2014). The Amazon rain forest is known to be a carbon sink where carbon dioxide, a greenhouse gas, can be stored. As the amazon rain forest diminishes, more greenhouse gases are emitted into the atmosphere and contribute greatly to global warming (Gatti *et al.*, 2021). In addition, biodiversity is heavily reduced with the soy monocultures (Green *et al.*, 2019).

In the search of biodegradable waste treatments that follows the principles of a circular economy, black soldier fly larvae composting has emerged as an option. In black soldier fly larvae composting, larvae eat biodegradable waste and turn it into larval biomass and frass (Čičková *et al.*, 2015). The protein content found in the larval biomass could be compared with the soymeal protein content, a common animal feed, and the frass has a great potential as soil fertilizer (Čičková *et al.*, 2015; Klammsteiner *et al.*, 2020).

The black solider fly larvae could be filling two environmental needs with one deed, converting biodegradable waste into larval biomass used as animal feed while also producing high quality soil fertilizer.However due to current regulations and laws, insects are considered production animals and thus cannot be fed any animal by-products (ValuSect and Interreg, North-West Europe, 2020). Food waste from households is not allowed to be fed to production animals and thus most biodegradable waste treated by BSFL in Europe come from earlier in the food chain and are of a single source waste stream e.g., reclaimed bread or vegetables (Regulation (Ec) No 767/2009 Of The European Parliament, 2009; Gasco, Biancarosa and Liland, 2020; Lopes *et al.*, 2020; Isibika *et al.*, 2021).

Studies have shown that macronutrients such as proteins, fat and carbohydrates impact the waste- to-biomass conversion efficiency (BCE), an important measurement on BSFL treatment performance (Lalander *et al.*, 2019; Cohn, Latty and Abbas, 2022). Protein is considered to be an influential macronutrient when it comes to treatment performance. It has been demonstrated to increase the larval weight and is considered important for larval survival (Barragan-Fonseca, Dicke and van Loon, 2018; Gold *et al.*, 2018; Lalander *et al.*, 2019). Carbohydrates have been suggested to increase larval weight. When larvae are provided with a low-protein-high-carbohydrate diet, the carbohydrates are converted to lipids, which stores in the larvae fat and thus increasing its weight (Pimentel *et al.*, 2017; Gold *et al.*, 2018). Fat is necessary for larval development as the adult flies uses its nutrients for reproduction and has a lesser impact on larval weight than protein. However it has been suggested that fat does not affect process performance unless presented in excess (Nguyen, Tomberlin and Vanlaerhoven, 2013; Čičková *et al.*, 2015; Gold *et al.*, 2018). Excess amounts of fat have been demonstrated in Lopes et al (2020), Nguyen et al (2013) and Isibika et al (2021) to be detrimental to larvae survival and process performance.

Single source waste streams from the food industry can be BSFL composted alone, but studies have shown co-composting single source streams can increase treatment of substrates that does not have an optimal nutrient composition that support BSFL growth, as entire waste mixture being treated become more nutrient balanced (Gold *et al.*, 2020; Isibika *et al.*, 2021). Co-composting different single source waste streams has been explored, however the exact composition of the different streams for optimal performance is still unknown. Findings of BCE and their respective composition would help calculations before co-composting single source waste streams by knowing the fraction of macronutrients necessary is needed to achieve a high BCE score.

1.2 AIM AND GOAL OF THE STUDY

The aim of this study was to assess the possibility to predict black soldier fly larvae treatment performance when co-composting selected substrates based on the composition of the co-composting mixture in terms of content of protein, carbohydrate, and fat in the mixtures.

Specific objectives were:

- To establish ternary plots of macronutrient fractions with biomass conversion efficiency score.
- Verify the predicted BSFL composting performance of selected mixtures from the ternary plots in terms of biomass conversion efficiency and material reduction.

2. BACKGROUND

2.1 CLIMATE AND WASTE MANAGEMENT

In 2015, the European Union created The Paris Agreement, a universal and legally binding agreement to combat climate change by limiting the global average temperature to under 2°C (‘Paris Agreement’, 2016). In addition, the European union set up a strategy to become climate neutral by 2050, having net zero greenhouse gas emissions in the economy and working toward the goals of the Paris Agreement (‘Paris Agreement’, 2016; Directorate-General for Climate Action, 2019).

At this time, the European Commission launched an action plan called the Circular Economy Action Plan, with the aim of realizing the EUs commitment of a climate neutral economy and to transition the EU into a circular economy in order to create sustainable growth, where sustainable products and resources are promoted and the need for new natural resources is greatly reduced (United Nations, 2021). One of the largest policies in the Circular Economy Action Plan is the European Green Deal, where the goal is to make the EU a resilient and sustainable economy. The goals of this deal are “ensuring: no net emissions of greenhouse gases by 2050, economic growth decoupled from resource use, no person and no place left behind”(United Nations, 2021).

Figure 1: Waste hierarchy presented in an upside-down triangle. The top represents the treatment strategy that should be given highest priority (prevention) while the bottom presents the treatment strategy with that should be given lowest priority (disposal, e.g., landfill)(European Commission, 2021).

When it comes to the role of waste management in the new circular economy, the EU presented the waste hierarchy, a framework for how waste management should be conducted. According to the waste hierarchy, the prevention of waste generation has the highest priority, while disposal has the lowest (Figure 1). Landfills are considered to be an easy and inexpensive treatment of organic waste, However, biodegradable waste such as food and kitchen waste which has been landfilled produces methane, a greenhouse gas which contribute greatly to climate change (Blair and Mataraarachchi, 2021) .

Of the total generated biodegradable waste, one part is made out of food loss and wastage, defined as the edible food lost due to production, harvest and processing losses, as well as the food for human consumption discarded by retail or consumers (Gustavsson, 2011; Teigiserova, Hamelin and Thomsen, 2020). This type of losses are considered a waste of resources as it generates carbon dioxide emissions to produce it, but does not return any economic value when not consumed and should be kept to a minimum in all stages of the food chain (Gustavsson, 2011). However, a large proportion of the food waste is unavoidable and is often comprised of vegetable and fruit cuttings and peels among other things. The question thus is how the food waste can best be managed from a recycling and recovery point of view that are in line with the waste hierarchy framework.

Rajeh et al (2020) stated in a review that food waste can be reused in animal feed due to their nutritional value. Assisted by our knowledge and technology to conduct safe recycling schemes with e.g., heat treatments. This study's claim that food waste can be recycled as animal feed is also supported by Teigiserova et al (2020) who present an expanded version of the waste hierarchy for food waste (Rajeh *et al.*, 2020; Teigiserova, Hamelin and Thomsen, 2020).

2.2 BLACK SOLDIER FLY LARVAE

The black soldier fly (BSF) (*Hermetia illucens* L. (Diptera: Stratiomyidae)) is native to the American continents but can be found in tropical and subtropical climates. The flies lay eggs in dry creves, which take up to four days to hatch at 24 °C (Sheppard *et al.*, 2002; Čičková *et al.*, 2015). When the larvae have hatched, they begin to feed. The larval stage is on average three weeks, under optimal conditions in terms of ambient temperature and food availability, before the prepupae stage (Sheppard *et al.*, 2002; Myers *et al.*, 2008; Guo *et al.*, 2021)(Figure 2). As prepupae, they clear their digestive track and no longer feed, as their bodies reaches its maximum size and stores fat and nutrients needed for the metamorphosis (Newton *et al.*, 2005). The prepupae migrate from their food source with their newly developed mouth-hooks to find a place for the pupal stage (Sheppard *et al.*, 2002). Pupation takes 14 d under optimal conditions, while it can take a lot longer under sub-optimal conditions. From the pupae the fly emerges (Sheppard *et al.*, 2002). The fly has a large black body up 20 mm and spend most of its time sitting on vegetation.

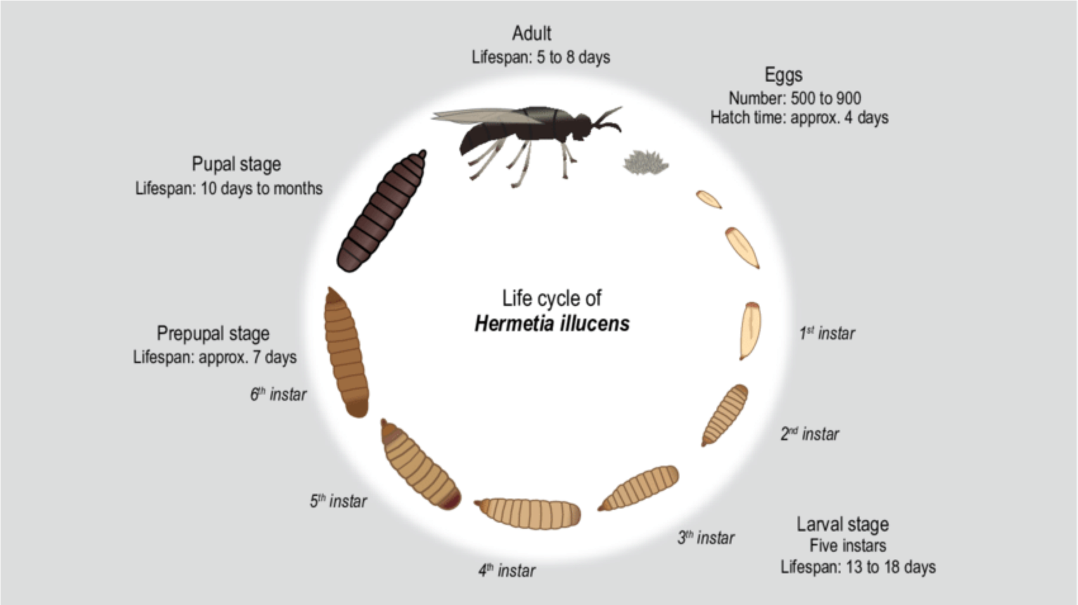


Figure 2: The life cycle of the black soldier fly (Lievens et al., 2021)

The fly doesn’t require food and survives only on water. As a consequence these flies do not approach humans or animals and thus are not considered vectors for disease transmission. (Sheppard *et al.*, 2002; Čičková *et al.*, 2015).

2.3 BLACK SOLDIER FLY LARVAE WASTE TREATMENT AND PRODUCTS

Black soldier fly larvae (BSFL) waste treatment is an organic waste treatment in which BSFL digest organic waste such as food waste, manure or food industry waste and convert it into their own biomass and treatment residue, called frass. The larval biomass can then be used for animal feed (Wang and Shelomi, 2017) while the frass can be used as fertilizer (Poveda, 2021).

Although BSFL composting are able to treat different types of organic waste such as manure, food or slaughter waste, the variation in performance and efficiency for different wastes, poses as a challenge for BSFL waste management (Gold *et al.*, 2020).

2.3.1 Frass

Insect frass is a part of the nutrient cycle in nature and contains nutrients that can be used for plant growth (Menino and Murta, 2021). Insect frass can be an important source of nitrogen for the plants, a resource that is often limited, as well as other nutrients, which can increase the microbial activity (Poveda, 2021). Frass has also been shown as a potential protein source for animal feed, showing notable increases in growth performance for catfish when mixed in with the diet up to 100g/kg (Yildirim-Aksoy, Eljack and Beck, 2020).

It has been shown that BSFL can increase the concentration of ammonia in the frass that they produce, a nutrient which plants use for growth (Green and Popa, 2012; Song *et al.*, 2021). Beesigamukarna *et al.*, (2020) compared BSFL frass fertilizer and commercial organic fertilizer for growing maize in field and demonstrated that maize grown with BSFL frass had an increased grain yield of up to 27% compared to the maize grown with commercial fertilizer and therefore has great potential to increase crop productivity in countries where commercial fertilizer is too expensive (Beesigamukama, Mochoge, N. K. Korir, *et al.*, 2020; Beesigamukama, Mochoge, N. Korir, *et al.*, 2020).

2.3.2 Larvae

The BSFL are the main workers in the BSFL waste treatment, digesting waste and turning it to biomass. Harvest of the larvae is done before the prepupal stage, when the color of the larvae color starts turning darker, due to the larval decrease in fat content in the later stages of their lives (Liu *et al.*, 2017).

The BSFL has a high protein and fat content and these nutritional qualities makes them qualified to be animal feed (Wang and Shelomi, 2017). Heuel *et al.*, (2021) fed laying hens either a larva or soybean-based diet and found no difference in nutritional value between the eggs from soybean or larvae-fed laying hens. It demonstrated that larvae could completely replace soybean meal in either commercial or organic diet for laying hens (Heuel *et al.*, 2021).

Katya *et al.*, (2017) demonstrated that BSFL meal could replace fish meal up to 50% in diet for rearing juvenile barramundi (*Lates calcarifer)* in fresh water and crude protein and moisture content were generally unaffected by the different diets.

2.3.3 Market potential of BSFL treatment and its products

Lohri *et al.*, (2017) analyzed 13 different organic waste treatment in four different categories such as direct use, biological treatment, physicochemical treatment and thermo-chemical treatment. One of the biological treatments reviewed was BSFL treatment and it was considered to have a high potential as an organic waste treatment in low- and middle-income countries, especially for countries with climates that BSFL can thrive in. However, there are some barriers e.g., knowledge gaps on biosafety and accumulation of hazardous substances e.g., heavy metals and hormones in the larvae. The review also states that universities and research groups are making progress towards relieving regulations and filling knowledge gaps, suggests that collaboration between the private sector and universities is an important steppingstone towards BSFL becoming a common practice.

Lalander *et al.,* (2018) studied the potential value of products created from different food waste and faeces treatments. In their assessment they examined thermophilic composting, BSFL treatment, anaerobic digestion (AD) and BSFL treatment followed by anaerobic digestion of frass. The study demonstrated that BSFL treatment followed by a anaerobic digestion treatment created the highest value products, however the authors' argued that this might not be the most feasible strategy everywhere (Lalander, Nordberg and Vinnerås, 2018). This assessment was made within a Swedish context and based on the fact that an AD infrastructure already existed, but these conditions may not always be present in low-income countries. Due to this, BSFL treatment alone was of higher interest, since the robust low-tech infrastructure and technology can produce highly valuable products.

2.4 BIOMASS CONVERSION EFFICIENCY AS A PERFORMANCE MEASUREMENT

One way of measuring the performance of the BSFL treatment is to calculate the biomass conversion efficiency (BCE), which gives an estimate for how well the larvae converts the waste into their own biomass and is presented as a percentage of dry matter (DM), volatile solids (VS) or wet weight depending on what is deemed relevant. Other important calculations for measuring performance are waste reduction, larval survival and final larval weight.

2.5 TERNARY PLOT

A ternary plot shows the proportion of three different components that add up to 100% of the composition of the three components. Each corner of the triangle is 100% of one fraction and 0% of the other substrates. (Weisstein, 2021). When applying a data point into the ternary plot, it gains coordinates, which creates a geometric centroids based on the three corners of the triangular diagram (Weisstein, 2021)

Ternary plots are commonly used for geochemical analysis, such as the classification of rocks. The composition of a selected stone can be determined using ternary plots (Figure 3), which also facilitate the naming of the rock from where the samples were taken (Fichter, 2000).

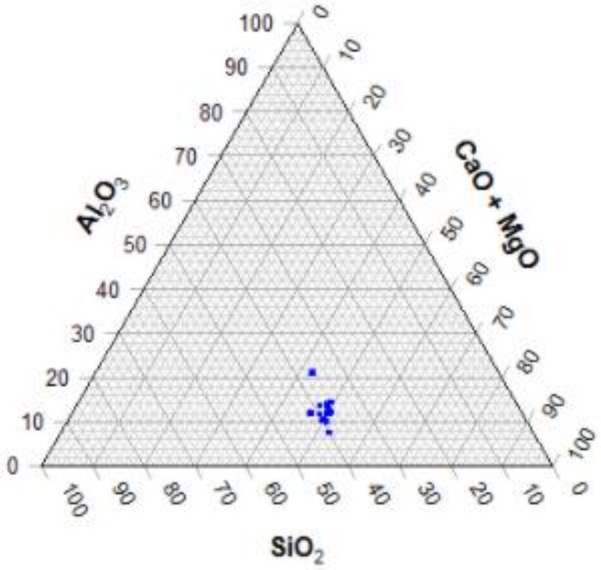


Figure 3: Representation an example of a ternary graph. This example shows a ternary plot used for rock classification (Stack Overflow, 2021).

3. METHODS

3.1 PREVIOUS STUDIES

As discussed in the introduction of this study, the composition of the feed mixture going into the treatment affects the treatment performance and through optimizing macronutrients in the feed mixture, treatment performance can be increased (Gold *et al.*, 2018, 2020; Lalander *et al.*, 2019; Isibika *et al.*, 2021).

Lopes *et al*., (2020) examined BSFL treatment performance of aquaculture waste when co-composted with bread waste. A positive correlation between fish waste inclusion rate and BCE was found. The fish waste added could be considered the main source of protein and fat in their experiment, and the bread waste were main source of carbohydrate. The highest BCE achieved were at small inclusions of fish waste (5-15% in wet weight) in the treatment.

Isibika *et al*., (2021) examined BSFL treatment performance on co-composted protein-rich fish waste and carbohydrate-rich fruit peel waste. The study demonstrated an increase in BCE when increasing the proportion of fish waste in the co-composting mixture. In their experiment, fruit peels were the main source of carbohydrates and fiber and the fish waste added protein and fat to the waste. Isibika *et al.*, (2021) were able to have higher inclusions of fish waste than Lopes *et al*., (2020), but demonstrated a larger degree of variance in treatment performance (i.e., a less stable process) with increased fish waste inclusion.

The hypothesis of this study was that predictions of treatment performance can be made from the correlations between macronutrient composition and BCE aspect of treatment performance. For the predictions, ternary plots would be based on data from earlier studies such as Lopes *et al*., (2020) and Isibika *et al*., (2021) and then be used to perform visual analysis of the BCE that could be expected at different compositions of the macronutrients protein, carbohydrate and fat.

To test the hypothesis, select data points in the ternary plots, representing co-composting mixtures available for this study, were selected and the performance in BSFL composting in terms of biomass conversion efficiencies between the experimental results and predicted values from the ternary plots were compared.

3.2 THE IMPACT OF MACRONUTRIENTS ON BSFL COMPOSTING PERFORMANCE

Studies have shown that the amount of protein, fat and carbohydrate in the feed mixture going into the treatment can significantly impact the larval growth and thus enhance performance of the BSFL waste treatment in terms of BCE (Gold *et al.*, 2018; Lalander *et al.*, 2019).

The substrates used in this study were anchovy, bread (unsweetened whole grain rye bread), and vegetable (lettuce and cabbage) waste and had different levels of macronutrients (Table 1).

Table 1: Nutritional value of the single waste streams and mixed waste, if the waste was single source or mixed and where source information came from. For example, A5%B15%V80% means a mixture composed by 5% anchovies, 15% bread and 80% vegetable waste in the table caption.

	Protein [g/100g]	Carbohydrate [g/100g]	Fat [g/100g]	Fiber [g/100g]	Single stream or co-composted	Macronutrient ratio (Protein:Carbohydrate: Fat:Fiber)	Source
Anchovy waste	9.12	2.6	14.0	-	Single stream	1:0.3:1.6	Eurofins
Bread waste	5.4	30.0	2.5	5.4	Single stream	1:6:0.5:1	Livsmedelsverket, SLU
Vegetable waste	0.97	3.6	0.11	1.3	Single stream	1:4:0.1:1.3	Livsmedelsverket
A50%B20%V30%	6.0	8.7	7.5	1.7	Co-composted	1:1.4:1.25:0.3	Calculated from nutritional data
A20%B15%V65%	3.4	8.0	3.2	1.9	Co-composted	1:2.3:0.9:0.6	Calculated from nutritional data
A10%B15%V75%	2.6	8.2	1.8	2.1	Co-composted	1:3.1:0.7:0.8	Calculated from nutritional data
A5%B15%V80%	2.2	8.3	1.1	2.1	Co-composted	1:3.8:0.5:0.95	Calculated from nutritional data
A0%B50%V50%	3.2	17.5	0.9	3.5	Co-composted	1:5.5:0.3:1.0	Calculated from nutritional data

3.2.1 Protein

Protein have been demonstrated to have a positive effect on larval development and larval weight (Barragan-Fonseca, Dicke and van Loon, 2018; Beniers and Graham, 2019; Lalander *et al.*, 2019; Gold *et al.*, 2020). Beniers *et al.*, (2019) feed BSFL with 25 different diets, varying in protein and carbohydrate concentration and saw that both dietary protein and carbohydrate increased larval weight, while dietary protein had a stronger influence on larval weight. Barragan-Fonseca *et al.*, (2018) examined the impact of protein-carbohydrate ratios on BSF development and composition and demonstrated that development time, larval yield and larval fat content were more impacted by the combined content of protein and carbohydrate given in feed than the protein-carbohydrate ratio itself. Moreover, they found that protein and carbohydrate rich diets resulted in flies of higher weight.

Studies (Oonincx *et al.*, 2015; Barragan-Fonseca, Dicke and van Loon, 2018; Beniers and Graham, 2019; Lalander *et al.*, 2019) examining the protein effects on BSF or BSFL saw positive effects such as increased final larval weight and shorter development time. Their findings suggest that protein is the most influential macronutrient when it comes to development time and final larval weight and thus important when planning formulations.

3.2.2 Carbohydrate

Carbohydrates have also been shown to influence larvae fat content and thus indirectly larval weight (Pimentel *et al.*, 2017; Spranghers *et al.*, 2017). Sprangers *et al.*, (2017), studied the nutritional composition of BSFL reared on different organic wastes and demonstrated that carbohydrates could be converted into fatty acids and therefore affect the fat content in larvae. Pimentel *et al.*, 2017, examined the metabolic adjustment of BSFL fat content when reared on different diets and found diet to affect the accumulation of protein and fat. Vegetable waste (a low-protein waste) increased fat accumulation in larvae while free sugars where converted into triacylglycerol in the larvae, a milk lipid (Pimentel *et al.*, 2017).

Cohn *et al.*, (2022), examined how different carbohydrates influenced larval waste reduction and bioconversion, by feeding larvae with chicken feed and adding of different carbohydrates. They found that treated waste containing simple sugars, sucrose and fructose, had higher waste reduction and bioconversion compared to xylan and arabinose. The authors' concluded that more complex carbohydrates, such as xylan and arabinose, were difficult for the larvae to digest due to their more complex molecule structure (Cohn, Latty and Abbas, 2022). The simple sugars are typically found in baked products, flour and products of food processing.

3.2.3 Fat

Fat is needed for larval development into adulthood, but if too much is present in the feed mixture it can decrease larval survival and prolong larval development time. Feeding larval only high fat fish waste has been shown to kill all larvae or result in very low survival rate, leaving the fish waste untreated (Craig Sheppard *et al.*, 1994; Nguyen, Tomberlin and Vanlaerhoven, 2013; Lopes *et al.*, 2020; Isibika *et al.*, 2021). It has also been suggested that mercury can inhibit growth in concentrations of 2000-3000 mg Hg/kg and can be accumulated in the larvae which should be considered when rearing larvae on fish waste (Attiogbe, Ayim and Martey, 2019; Dietz *et al.*, 2021).

3.2.4 Fiber

When examining degradation of fiber-rich waste such as fruit peels, it was shown that the fiber-rich waste could be processed by the BSFL if co-composted with a protein-rich waste such as fish waste (Isibika *et al.*, 2021). When examining different mixtures out of banana peels, orange peels and fish waste, Isibika et al (2021) found that BCE could be doubled if fish waste was included up to 25% in the feed mixture (Isibika *et al.*, 2021).

Fiber are generally difficult to digest for the larvae and it has been suggested that BSFL lack enzymes to fully degrade them, and thus fiber generally decrease BCE when in excess in waste treated (Gold *et al.*, 2018).

3.2.5 Single stream vs formulation performance

Gold *et al.*, (2020), demonstrated increased performance of the BSFL treatment when using mixed organic waste from a calculated formulation. The formula was based on mixing different substrates to achieve a 1:1 protein to carbohydrate ratio and then compared results from the mixed organic waste and from organic waste from a single source. The best performing single-stream waste, in terms of BCE, were human faeces high in protein and low in carbohydrate (21%$_{DM}$ fat, 2%$_{DM}$ carbohydrate and 20%$_{DM}$ protein), and vegetable canteen waste that had more carbohydrate than protein, but a high fat content (15.5%$_{DM}$ carbohydrate, 12%$_{DM}$ protein, and 29%$_{DM}$ fat) (Gold *et al.*, 2020). Single-stream wastes resulted in survival rates of 90-99% and BCE 3,8-23%$_{DM}$, while formulations resulted in survival rates of 97-100% and BCE of 14-32%$_{DM}$. This demonstrated an overall better performance from the waste formulation than single-stream wastes. The authors concluded that while the formulations had less protein, fat and carbohydrate than some of the individual waste, nutrient compositions in the formulations were balanced(Gold *et al.*, 2020)

3.3 BSFL TREATMENT

3.3.1 Data assessment

The ternary plot used in this study was created based on the information from the Tri-plot Excel spreadsheet created by David Graham and Nicholas Midgley (Graham and Midgley, 2000). Another source of information was David Advocate, a geologist at Colorado State University who also had made a Excel Spreadsheet on how to make the ternary plot and designing them (Advocate, 2019).

For clarification, the mixture A50%B20%V30% will be used as an example in every step of the data assessment.

Firstly, the nutritional facts from the anchovy, bread and vegetable waste were used to calculate how much protein, carbohydrates and fat content was from each of them in the waste mixture. Here, the mixture A50%B20%V30% had 6.0 g protein, 8.7 g carbohydrate and 7.5 g fat for every 100g waste mixture.

Then, the total VS weight was calculated, under the assumption that the total VS weight consisted mostly of protein fat and carbohydrates and was used to calculate coordinates on the ternary plot as well as protein$_{\%VS}$, fat$_{\%VS}$ and carbohydrate$_{\%VS}$ which would be used for composition analysis later on.

For example, the mixture A50%B20%V30% had a total VS content of 22.2 g and fractions of 27% protein$_{\%VS}$, 34% fat$_{\%VS}$ and 39% carbohydrate$_{\%VS}$.

A number for the top, left and right position in the ternary plot was calculated using Equation 1. In this study, the top number was based on the carbohydrate fraction, the left number was based on the protein fraction and the right number was based on fat.

$$TOP, RIGHT, LEFT = \frac{fraction\ in\ gram}{(sum\ of\ fractions \div 100)} \qquad \text{Equation 1}$$

The TOP, LEFT and RIGHT numbers for the waste mixture A50%B20%V30% were 39, 27 and 34.

Using the TOP and RIGHT position numbers, the x and y coordinate was created using (Equation 2) and (Equation 3) for each data point in the ternary plot. The left position number is not used in calculations for the coordinates, but it is relevant for reading the left side of the ternary plot when determining the fraction of the left side.

After the x and y coordinates were created the data points were applied in the ternary plot and grouped after their biomass conversion efficiency.

$$x - coordinate = \frac{RIGHT}{(TOP \div 2)} \qquad \text{Equation 2}$$

$$y - coordinate = TOP \qquad \text{Equation 3}$$

The coordinate for A50%B20%V30% using Equation 4 and Equation 5 were 53.35 on the x-coordinate and 39 on the y-coordinate and then put into the ternary plot.

Protein, carbohydrate, and fat contents in the feed mixtures reported in Lopes *et al.*, (2020) and Isibika et al 2021 (Table 2) were calculated from nutritional data and percentage of the macronutrients in volatile solids the co-composting mixtures was calculated as described above.

Table 2: Nutritional data of the substrates used in Lopes et al (2020) and Isibika et al (2021).

		DM [%]	VS [%$_{DM}$]	Protein	Carbohydrate	Fat	Fiber
Lopes *et al.*, (2020)	Bread waste	33.7 ± 0.4	97.2 ± 0.08	8.2 ± 1.0 %$_{DM}$	46.1 ± 4.4%$_{DM}$	-	8.17%$_{DM}$
	Fish waste	41.5 ± 0.9	95.7 ± 0.14	60.3 ± 2.3%$_{DM}$	-	32.5 ± 1.4%$_{DM}$	-
Isibika *et al.*, (2021)	Fish waste	28.2 ± 0.1	86.3 ± 0.9	15.9 ± 0.4 g $100g^{-1}$	2.1 ± 4.8 g $100g^{-1}$	5.8 ± 5.3 g $100g^{-1}$	0.2 ± 0.01 g $100g^{-1}$
	Banana peel	11.3 ± 0.01	86.3 ± 0.1	0.9 ± 0.1 g $100g^{-1}$	6.6 ± 0.3 g $100g^{-1}$	1.1 ± 0.5 g $100g^{-1}$	1.9 ± 0.4 g $100g^{-1}$
	Orange peels	18.8 ± 0.04	96.6 ± 0.6	1.1 ± 0.04 g $100g^{-1}$	14.1 ± 0.01 g $100g^{-1}$	0.3 ± 0.01 g $100g^{-1}$	2.6 ± 0.3 g $100g^{-1}$

Using percentages of volatile solids, the placement of each mixture in the ternary plot as calculated. This scatter plot was shaped to be a ternary plot. Ternary plots were created and used to visually display the different compositions of macronutrients present in all feed mixtures of Lopes *et al.*, (2020) and Isibika *et al.*, 2021, with each data point color corresponding to a feed mixture with a verified performance (BCE).

Three possible waste streams from primary production were used as model substrates, (anchovy waste, vegetable waste and bread waste) for this experiment. Different mixtures were created by incremental changes of 5 percentage points between bread and vegetable waste, while decreasing the inclusion of anchovy waste by 10%. For example, starting at mixture A100 which had 100% anchovy, 0% bread and 0% vegetable waste and then decrease the percentage of included anchovy waste to 90% while varying the bread and vegetable inclusion with 5 % points (Table 3).

Table 3: Example of how the incremental changes of inclusions in the feed mixture were made and inclusion of fish, bread, and vegetable.

Mixtures	Fractions of in feed [%]		
	Fish (A)	Bread (B)	Vegetables (V)
A100%B0%V0%	100	0	0
A90%B10%V0%	90	10	0
A90%B5%V5%	90	5	5
A90%B0%V10%	90	0	10
A80%B20%V0%	80	20	0
A80%B15%V5%	80	15	5
A80%B10%V10%	80	10	10

The composition of carbohydrate, protein and fat were calculated for all possible feed mixtures with incremental changes to these inclusions and then placed in a ternary plot (Figure 4). Comparing all data points with different compositions and relating them with respective BCE, five different compositions of the feed mixtures were selected to be verified in the BSFL treatment.

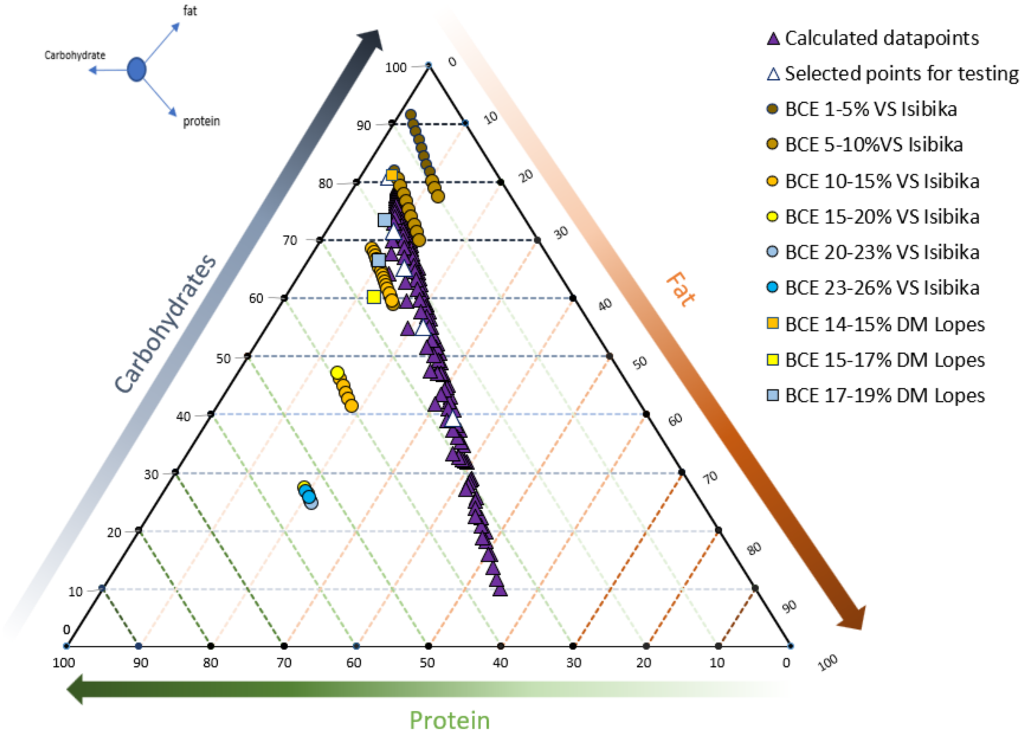


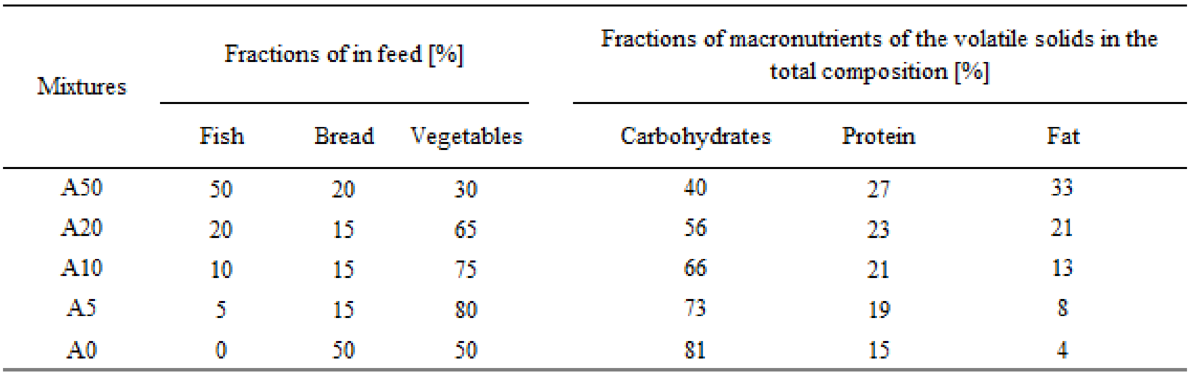
Figure 4: Ternary plot showing the feed mixtures composition of protein, fat and carbohydrate using data from Isibika *et al.*, (2021) (circles) and Lopes *et al.*, 2020 (squares) and all calculated feed mixture compositions in this study (purple triangels) as well as the feed mixtures selected for further investigation (white triangles).

3.3.2 Experimental setup

The feed mixtures chosen for validation were A50%B20%V30% (50% anchovy waste, 20% bread waste and 30% vegetables waste); A20%B15%V65% (20% anchovy waste, 15% bread waste and 65% vegetables waste); A10%B15%V75% (10% anchovy waste, 15% bread waste and 75% vegetables waste), A5%B15%V80% (5% anchovy waste, 15% bread waste and 80% vegetables waste), A0%B50%V50% (0% anchovy waste, 50% bread waste and 50% vegetables waste) (Table 4).

Each BSFL waste treatment was executed in a box (17x14 cm^2) without lid andplaced in a larger box. For each mixture, three replicates were made, giving a total of 15 replicates in the experiment. Each replicate had 1785 larvae, resulting in a larval density of 7.5 larvae/ cm^2.

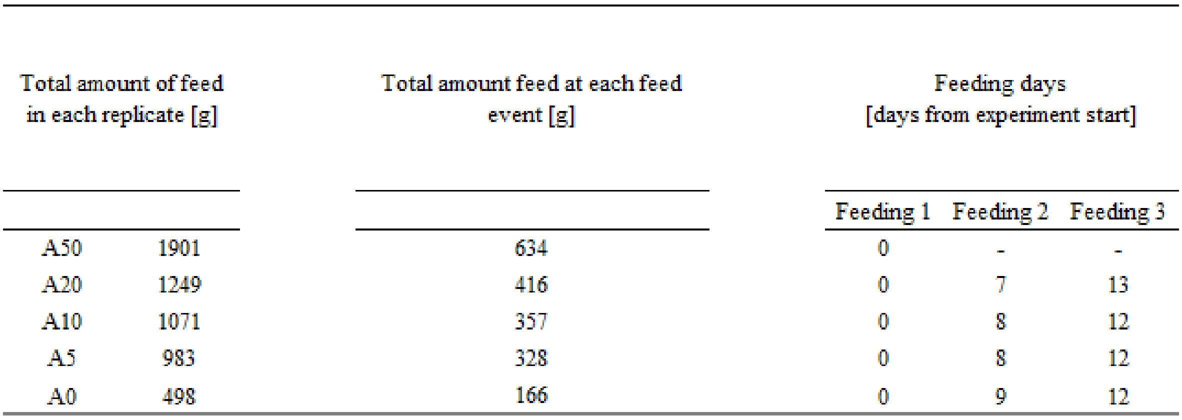
Table 4: Table showing fractions of substrates and macronutrients in feed.



Mixtures	Fractions of in feed [%]			Fractions of macronutrients of the volatile solids in the total composition [%]		
	Fish	Bread	Vegetables	Carbohydrates	Protein	Fat
A50	50	20	30	40	27	33
A20	20	15	65	56	23	21
A10	10	15	75	66	21	13
A5	5	15	80	73	19	8
A0	0	50	50	81	15	4

The total feed load provided to a replicate was based on giving each larva 0.1 g of volatile solids in total during the treatment. Feeding amount at each feeding can be found in Table 5 and feedings occurred on day 0 (experiment start), day 7-9 and day 12-13, depending on how much waste had been reduced in the replicate.

Table 5: Table showing total amount of feed given and feed given at each feeding and days of feeding since start of experiment.



Total amount of feed in each replicate [g]		Total amount feed at each feed event [g]	Feeding days [days from experiment start]		
			Feeding 1	Feeding 2	Feeding 3
A50	1901	634	0	-	-
A20	1249	416	0	7	13
A10	1071	357	0	8	12
A5	983	328	0	8	12
A0	498	166	0	9	12

The replicates were placed in a large box that was stacked in a rack inside a tent (Secret Jardin, Hydro Shoot 120) with the dimensions 120x120x200 cm. During the experiment, a car heater was attached to a temperature monitor to regulate the temperature and the average temperature was 27.4 ± 1.5°C. A circulation fan was placed on the floor of the tent to reduce a temperature gradient in the tent. The treatment period varied between 15-19 days. At the end of the treatment, the larvae and frass were separated using a Ø 200 mm sieve. The larvae were washed and dried with paper and then measured for the final total weight, average larval weight, while the frass was measured for total frass weight

3.4 SAMPLING

Samples from larvae, feed and frass were taken for measurement before and after treatment. Dry matter samples were taken for every replicate of their larvae and frass and treated as described above. The larvae were frozen in the freezer for 48 hours after the treatment before taking DM samples.

3.4.1 Dry matter (DM) and ash measurement

At the beginning of the experiment, three DM samples between 15-30 g were taken of the feed mixture. The samples were measured for weight and then placed in an oven at 70°C for 48 h, upon which they were measured again. For ash measurement, the DM samples were placed in a combustion oven. The heat was increased to 250°C, over the course of 1 h, and maintained for 2 h, before increasing the temperature to 550°C over the course of 1 h and maintained for 4 h. After ash treatment, the sample's ash weight was noted. After subtracting the ash weight from dry matter weight, the volatile solids weight was calculated and noted.

3.4.2 pH

The pH meter used was a SevenCompact pH meter S220. The pH Meter was calibrated a pH 4, pH 7 and pH 10 using pH-calibration solutions. pH samples of 5 g feed mixture mixed with 20 ml deionized water were taken at the beginning of the experiment and shaken and left in room temperature for 1 h before measurement. The same produce was done for pH measurement of the frass.

3.5 CALCULATIONS

3.5.1 Biomass conversion efficiency (BCE)

The percentage biomass conversion efficiency was calculated on a dry matter and volatile solids basis (Equation 6) as:

$$BCE_{DM\%} = \frac{final\ mass_{lv} \times DM\%_{lv}}{total\ mass_{feedmix.} \times DM\%_{feedmix.}} \qquad \text{Equation 6}$$

where the $final\ mass_{lv}$ is the mass of the total larval biomass, $DM\%_{lv}$ is the dry matter percentage of the larvae, the $total\ mass_{feedmix.}$ is the total mass of feed given to the larvae and $DM\%_{feedmix.}$ the dry matter percentage of the feed mixture. When calculating BCE on a volatile solid basis, the VS% of the larvae was added in the numerator and VS% of the feed mixture to the denominator.

3.5.2 Waste reduction

The percentage waste reduction was calculated as a percentage on a wet weight, dry matter and on volatile solids basis (Equation 7) as:

$$Waste\ reduction_{WW} = 1 - \frac{total\ mass_{frass}}{total\ mass_{feedmix.}} \qquad \text{Equation 7}$$

where the $total\ mass_{frass}$ is total mass of the frass and the $total\ mass_{feedmix.}$ is the total mass of feed given to the larvae. To calculate waste reduction on a dry matter or volatile solids basis, the DM% or VS% of the frass was added to the numerator and DM% or VS% of the feed mixture to the denominator

3.5.3 Larval survival

Larval survival was calculated as the percentage of the number of remaining larvae divided by the initial number of larvae as (Equation 8):

$$Larval\ survival\ [\%] = \frac{larvae_{end}}{larvae_{initial}} \qquad \text{Equation 8}$$

where $larvae_{end}$ is the number of larvae remaining at the end of the treatment and $larvae_{initial}$ is the number of larvae at the start of the treatment.

3.5.4 Statistical analysis

Three replicas of each mixture (n = 3) selected for testing were made in order to calculate the significance of the results. Statistical analyses were made in the software Minitab using ANOVA one-way analysis to test the significance of the results with a confidence interval of 95%.

All results were presented as average of the three replicas ± the standard deviation of the three replicas.

4. RESULTS

4.1 BSFL TREATMENT RESULTS

The moisture content of the inflow feed differed between the treatments, with the control treatment (A0) being driest ($41\%_{DM}$) and A5 being wettest ($17\%_{DM}$) (Table 4). Larvae had similar DM and VS values in all treatments. Moisture content of frass was lowest for A0 ($90\%_{DM}$) and highest for A20 ($56\%_{DM}$)

Table 6. Dry matter (DM) and volatile solids (VS) in the feed mixture, larvae and frass. pH was measured in the feed initial mixture and the frass. Values are presented as average ± standard deviation. Same letter within the same category in the column indicates no significant difference (p>0.05) in DM, VS, and pH.

	Initial feed mixture			Larvae		Frass		
	DM [$\%_{WW}$]	VS [$\%_{DM}$]	pH	DM [$\%_{WW}$]	VS [$\%_{DM}$]	DM [$\%_{WW}$]	VS [$\%_{DM}$]	pH
A20	22.2 ± 1.3^{b}	89.1 ± 0.7^{c}	4.23 ± 0.1^{a}	39.7 ± 0.3^{a}	93.3 ± 0.4^{b}	55.8 ± 2.5^{b}	74.9 ± 3.1^{a}	6.27 ± 0.2^{b}
A10	20.5 ± 1.4^{b}	92.3 ± 0.3^{b}	4.08 ± 0.02^{b}	35.8 ± 1.0^{b}	92.5 ± 0.2^{c}	60.0 ± 13^{b}	69.6 ± 1.0^{c}	7.09 ± 0.3^{a}
A5	16.9 ± 1.2^{c}	92.2 ± 0.4^{b}	3.98 ± 0.02^{c}	35.6 ± 1.3^{b}	93.2 ± 0.3^{cb}	82.4 ± 0.5^{a}	74.7 ± 1.1^{b}	6.64 ± 0.1^{b}
A0	40.7 ± 0.1^{a}	95.7 ± 0.1^{a}	4.26 ± 0.0^{a}	40.5 ± 2.1^{a}	95.4 ± 0.1^{a}	90.6 ± 0.2^{a}	86.5 ± 1.2^{a}	7.13 ± 0.1^{a}

All larvae in the treatments of A50 died early (3-4 days) into the experiment and with no waste visible treated and thus was omitted in the results. A0 was significantly (p=0.00) different compared to the rest of the treatments in BCE%ww and BCE%vs. The highest total biomass was in A20 (200 g in total) and the lowest in A0 (106 g in total) (Table 7). A0 had the highest $BCE\%_{WW}$ ($21\%_{WW}$) and also the lowest $BCE\%_{VS}$ ($21_{\%VS}$), while A5 had the highest $BCE\%_{VS}$ ($33\%_{VS}$) and lowest $BCE\%_{WW}$ ($15\%_{WW}$). A5 had the highest waste reduction ($95\%_{WW}$) and A10 had the highest waste reduction ($XX\%_{VS)}$, while A20 had the lowest waste reduction on both WW and VS basis ($85\%_{WW}$, $68\%_{VS}$).

Table 7. Total larva biomass, larval survival, $BCE\%_{DM}$ and $BCE\%_{VS}$, Waste reduction $\%_{VS}$ and Waste reduction $\%_{DM}$ made after treatment. Same letter within the same category in the column indicates no significant (p>0.05) difference.

	Larvae		Biomass conversion efficiency		Waste reduction	
	Total biomass [g]	Survival [%]	[$\%_{WW}$]	[$\%_{VS}$]	[$\%_{VS}$]	[$\%_{WW}$]
A20	200.3 ± 17.0^{c}	85.2 ± 7.3^{b}	16.0 ± 1.4^{b}	30.02 ± 2.3^{a}	68.0 ± 6.2^{b}	85.0 ± 2.0^{c}
A10	189.3 ± 12.0^{a}	96.3 ± 3.3^{a}	17.4 ± 1.1^{b}	30.5 ± 2.5^{a}	85.7 ± 0.8^{a}	93.3 ± 1.7^{a}
A5	150.6 ± 11.0^{a}	86.1 ± 4.0^{ab}	15.3 ± 1.1^{b}	32.6 ± 3.5^{a}	79.3 ± 1.6^{a}	94.8 ± 0.3^{a}
A0	106.4 ± 9.0^{b}	90.9 ± 5.4^{a}	21.2 ± 1.8^{a}	21.1 ± 0.9^{b}	78.5 ± 3.0^{a}	89.4 ± 1.4^{b}

4.1.1 Visual observations during treatment

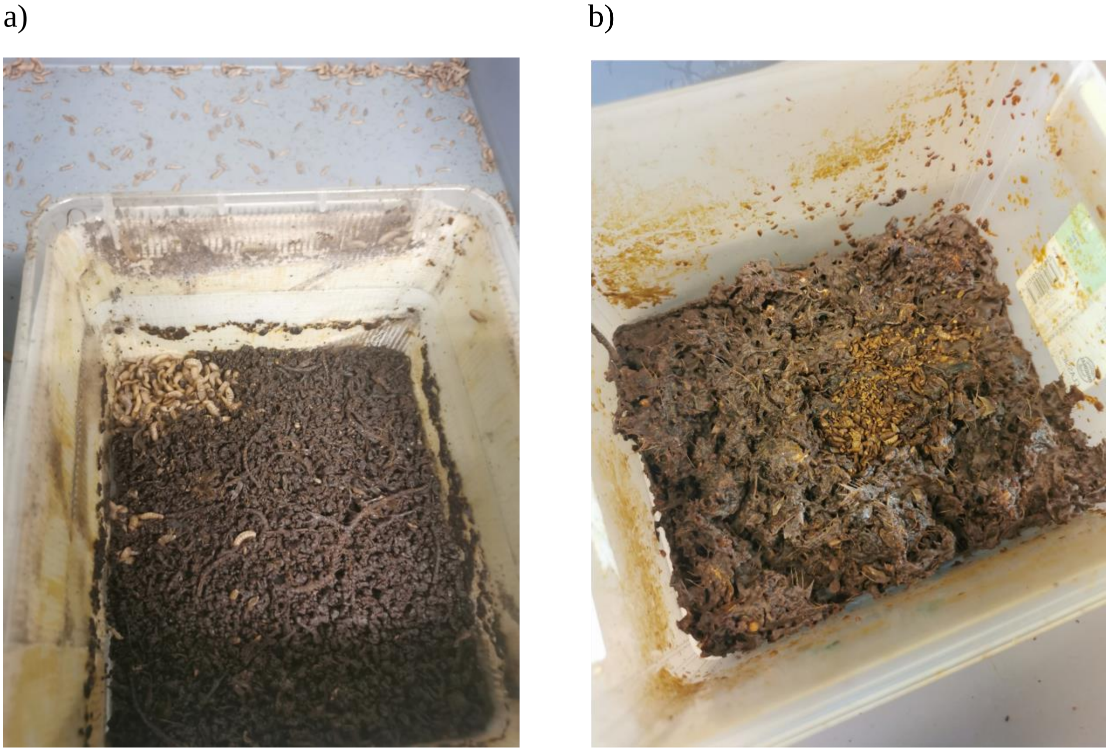


Figure 5: Pictures of replicates of treatment a) A20 after two feedings and b) A50 after one feeding, during BSFL composting.

Treatment A20 was, well processed by the larvae, judging from visual observation. However, the frass contained fatty clay-like material (Figure 5a). The lumps came in different sizes and made sieving the larvae from the frass very labor-intensive. During the experiment, the larvae escaped multiple times from the treatment box.

The larvae in A50 all died or escaped within three days of treatment in all three replicates (Figure 5b). When the escaping larvae were put back into the treatment they died soon after. The feed mixture was not treated by the larvae and no larval growth was observed.

4.2 TERNARY PLOTS

After performing the experiment, the selected data points were colorized with respect to the experimental BCE found (Figure 6). In the same ternary plot were the known BCE and macronutrient composition from Isibika *et al.*, (2021) and Lopes *et al.*, (2020), to compare previous BCE with the experimental one in this study.

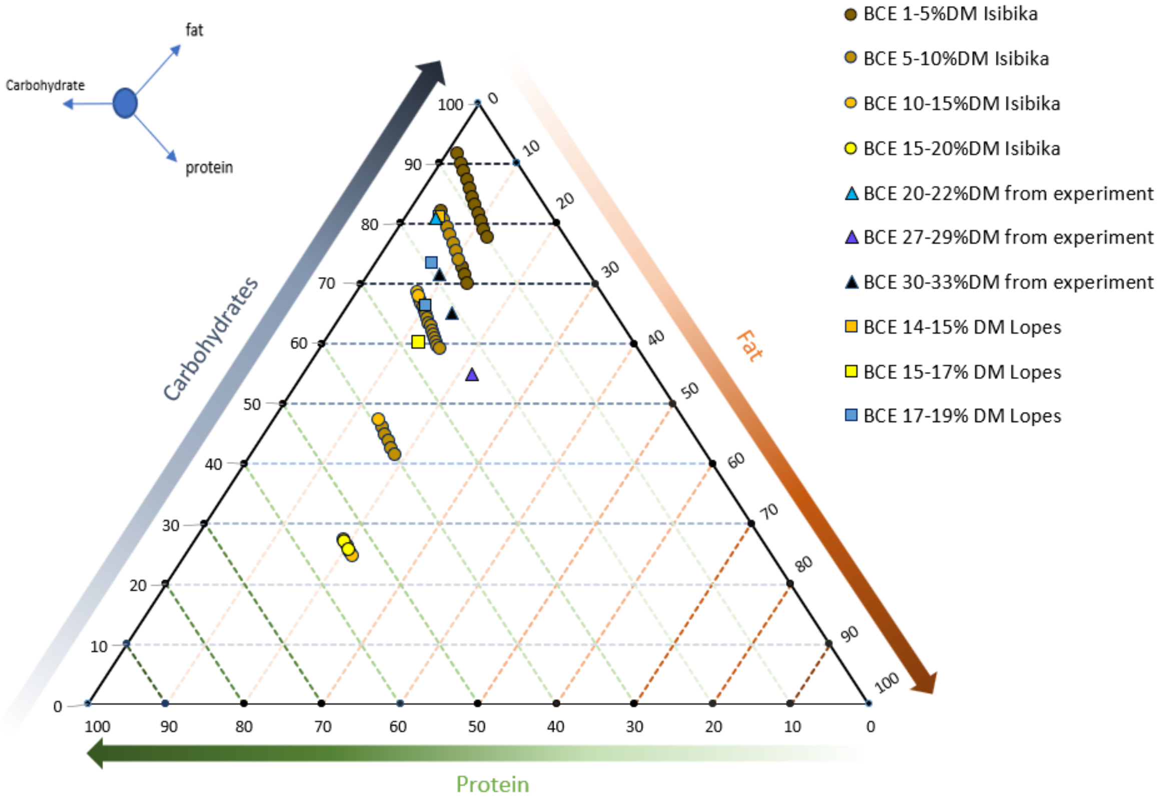


Figure 6: Ternary plot showing the composition of different mixtures, in terms of carbohydrate, fat and fibre content, included in this study (triangles), Lopes *et al.*, (2020) (squares) and Isibika *et al.*, (2021) (circles). The color of the symbols represent the BCE found for each mixture in the different studies.

5. DISCUSSION

5.1 BSFL TREATMENT

5.1.1 Effect of protein

All treatments had a higher $BCE\%_{VS}$ compared to the control treatment (A0), which had no fish waste (Table 7, Figure 6). Since the fish waste was the main source of protein in these treatments, this suggest that protein increases $BCE\%_{VS}$. The increase in protein from A0 (15% $protein_{\%VS}$) compared to A5 (19% $protein_{\%VS}$) and A10 (21% $protein_{\%VS}$) is quite large (27-40%), while the amount of carbohydrate among treatments (55-81% $carbohydrate_{\%VS}$) saw Table *4*an increase between 11- 45% and $fat_{\%VS}$ saw a incredily high increase of 500% from the control treatment A0 (4% $fat_{\%VS}$) to A20 (21% $fat_{\%VS}$)(Table *4*). This indicates that fat has a significant impact on BCE while carbohydrate might have a slightly bigger impact than protein, considering its wider percental increase. The treatments in this study seem to nonetheless have benefitted from the addition of protein, as BCE increased slightly.

Isibika *et al.*, (2021), using fish carcasses, and orange and banana peels, showed a correlation between increasing $BCE\%_{VS}$ and increased inclusion of fish waste to fiber-rich waste. However, an inclusion of more than 75% inclusion of fish waste in their mixtures resulted in high variation in BCE. In order to have a high BCE while keeping variance low, it was suggested that a 25% inclusion of fish waste was the most stable inclusion.

Lopes *et al.*, (2020), using fish waste and reclaimed bread, demonstrated an increase of $BCE\%_{DM}$ from mixtures with fish waste compared to the control without it. The highest larval final weight and $BCE\%_{DM}$ was achieved in T5, with a treatment with 5% inclusion of fish waste in the entire mixture. Fish waste in their study should be considered the main source of protein and thus showcases protein's influence on increasing $BCE\%_{DM}$. Lopes *et al.*, (2020) demonstrated increasing BCE with more fat and protein and less carbohydrate and found that the highest BCE was at 60-69% $carbohydrate_{\%VS}$,10-17% $fat\%_{VS}$ and 21-23% $protein\%_{VS}$. The values of BCE of either dry matter basis or volatile solids basis were roughly the same.

The effect of protein on larval growth have also been demonstrated by studies such as Beniers *et al.* (2018). They fed larvae 25 different diets with different levels of protein and carbohydrate and assessed final larval weight. The concentration of protein and carbohydrate were difficult to interpret for all the treatments, as they presented them in a table with incremental changes in terms of gram of protein and carbohydrate and not e.g., percentage of protein and carbohydrate in total composition. This way of presenting data made it difficult to compare Beniers *et al.* (2018) findings on protein concentrations effect on larval weight with other reports. However, they concluded that dietary protein had a significantly larger influence over final larval weight than carbohydrate. Beniers *et al.*, 2018 did not measure BCE or other treatment performances, as it was not the goal of their study. Nonetheless, final larval weight describes how large the larvae can grow in a substrate and would if the process parameters to obtain a higher larval weight were to be achieved, result in a high BCE.

Nguyen *et al.*, 2013, examined how well BSFL developed on different substrates and found that chicken feed produced the highest mean larval weight with kitchen waste being a close second place and beef liver on third. These three different types of wastes were high in protein (4.5-19.4 g/100g) and carbohydrate (1.2-16.3g/100g), which shows both to have an influence on larval weight. However, liver and kitchen waste had 59-79% mortality rate at pupal stage, which might indicate them as an unsustainable feed for larvae production.

Lalander *et al.*, 2019, examined larval development when reared on different types of waste and demonstrated larval development to be faster for substrates high in protein content e.g., poultry manure. However, as poultry feed, which was lower in crude protein content but higher in carboydrates, resulted in heavier larvae, it was suggested that protein might be more influential on faster larval development rather than the larval weight (Lalander *et al.*, 2019). This study also showed substrates such as fruits and vegetables to be low in protein but a high in VS content, resulting in larger larvae. This suggested VS to have a larger impact on weight than protein (Lalander *et al.*, 2019).

The suggestion of protein speeding larval development is also supported in *Flies, they are what they eat* by Barragán-Fonseca (2018). In their study they examined protein:carbohydrate ratios and summed up protein and carbohydrate content and their effect on performance of the BSFL when feed vegetable waste. The study showed protein having a stronger influence on development time rather than carbohydrate.

Most of the studies referenced here examined the effect of protein on larval development or on treatment performance in different ways with various measurements, making it difficult to do a comparison, since methods and parameters used to measure varied. Overall, there seem to be some consensus that protein can speed up larval development and may have some effect on increasing larval weight, but to what extent its effect on weight is, is difficult to say.

5.1.2 Effect of carbohydrate

Compared to the control treatment, a decrease in carbohydrate content seems to be beneficial for BCE as it decreases from 81% carbohydrate$_{\%VS}$ in the control to 72% carbohydrate$_{\%VS}$ in A5, which had the highest BCE$_{\%VS}$ (Table 6). The amount of carbohydrate (81%$_{VS}$) is still quite a high amount, and it is plausible that it is not the decrease in carbohydrate as much as the addition of fish waste ,the main source of protein and fat in A5, that makes the composition more balanced, thus resulting in higher BCE.

Aside from balancing the composition with more protein and fat compared to the control, there are also other factors that could have contributed to A5 having the highest BCE$_{\%VS}$. A5 had more carbohydrate and less fat than A10 (66% carbohydrate$_{\%VS}$, 21% protein$_{\%VS}$ and 13% fat$_{\%VS}$), which yielded the second highest BCE$_{\%VS}$. Both had similar protein content (19% in A5 and 21% in A10) and fat content (21-19% fat$_{\%VS}$), and thus it is more likely that the increase in carbohydrate (65-72% carbohydrate$_{\%VS}$) increased the BCE$_{\%VS}$.

In a carbohydrate-rich but low-protein diet, larvae will convert the carbohydrates to lipids in their biomass, increasing the proportion of fat in their body mass and thus increasing their weight. While encapsulation of fat is necessary for larvae development into adult flies, excess fat in the feed it can impede growth (Nguyen, Tomberlin and Vanlaerhoven, 2013; Gold *et al.*, 2018). It is more likely that the increase in carbohydrates resulted in an increased BCE$_{\%VS}$, and that the decrease in fat indirectly boosted that parameter.

Danieli *et al.*, 2019 also reported on the larvae's ability to convert carbohydrates to fat, where prepupae that were fed a high non-fibrous carbohydrate (NFC) mixed diet were 50% fatter compared to the rest of prepupae fed on a high fiber content or high protein content mixed diet.

Lopes *et al.*, (2020), had a high BCE$_{\%VS}$ with 66-73% carbohydrate$_{\%VS}$, 19-24%protein$_{\%VS}$ and 7-10% fat$_{\%VS}$. The highest BCE$_{\%VS}$ came from T5, a mixture of 5% fish waste and 95% bread compared to the control treatment T0 which consisted of 81% carbohydrate$_{\%VS}$, 15% protein$_{\%VS}$ and 4% fat$_{\%VS}$. It is likely that T5 was more nutritionally balanced (73% carbohydrate$_{\%VS}$, 19%protein$_{\%VS}$ and 7% fat$_{\%VS}$) for the larvae which increased the BCE more than a high amount of carbohydrate could. This was also demonstrated in this present study's treatments of A0 and A5.

Comparing with Isibika *et al.*, (2021) (Figure 6), the highest and more stable value BCE$_{\%VS}$ was found to be 60-70% carbohydrate$_{\%VS}$, 20-25% protein$_{\%VS}$ and 8-16% fat$_{\%VS}$. In this study, the BCE increased when more fat was added, and carbohydrate levels were decreased. The data from Isibika *et al.*, (2021) suggests that carbohydrates decrease the BCE; however, it is important to reconsider this first impression. In the study, orange and banana peels were used as a substrate in the feeding mixture. These peels are high in carbohydrate that might be categorized as non-fibrous carbohydrate (NFC) but can still be difficult for the larvae to digest as they may lack the enzymes for it (Gold *et al.*, 2018, 2020).

Cohn *et al.*, (2022), investigated the impact of different types of carbohydrates on BSFL treatment performance and found carbohydrates such as xylan and arabinose to be detrimental for BCE and waste reduction, especially xylan. Xylan and arabinose are hemicellulose carbohydrates that are common in fruits and vegetables such as orange and banana peels which were used in Isibika *et al.*, (2021). This deduction can be supported by the nutritional value of orange peels, where they have 21.8g of carbohydrate per 100g orange peels but none of them are monosaccharides, sucrose or other simple sugars, which results in higher bioconversion according to Cohn *et al.*, (2021)(Livsmedelsverket, 2021; Cohn, Latty and Abbas, 2022).

Carbohydrate might be the most complex macronutrient when it comes to examining their effect on BCE. They have some influence on larval fat content and larval weight and some carbohydrates can improve BCE in treatment performance as well as impede it. In order to gain clarity on the matter it might be useful for studies to better present what types of carbohydrates are present or perhaps use terms such as "easily available carbon" where the types of carbohydrates included are specified.

5.1.3 Effect of fat

The BCE increased as fat decreased or was present in small amounts. A50 (40% fat$_{\%VS}$, 30% protein$_{\%VS}$ and 30% carbohydrate$_{\%VS}$) had visible oil "pools" in the feed mixture. The larvae in this mixture either died or tried to escape. It has been suggested that excess amount of oil is difficult for the larvae to process and could be fatal as it likely makes it hard for larvae to move and it may obstruct breathing (Nguyen, Tomberlin and Vanlaerhoven, 2013; Spranghers *et al.*, 2017; Lopes *et al.*, 2020; Isibika *et al.*, 2021). No feed mixture from either this study or Isibika *et al.*, (2021) or Lopes *et al.*, (2020) had more than 26% fat$_{\%VS}$ in them (Figure 6). This might indicate a fat limit of $<$ 25% fat$_{\%VS}$ in the mixture before becoming detrimental for larvae survival.

This occurrence has been reported in other studies involving fish waste with high fat content. Both Lopes *et al.*, (2020) and Isibika *et al.*, (2021) observed high mortality rate when treating waste containing a high fat content, possibly due to low oxygen availability in the oil layers

(Lopes *et al.*, 2020). It is also possible that heavy metals in the anchovy decreased larval growth (Diener, Zurbrügg and Tockner, 2015). Heavy metals such as cadmium, lead and mercury can be present in food grade anchovy and cadmium had been found in higher concentrations than what is recommended (Storelli *et al.*, 2011; Bessa *et al.*, 2021). However, heavy metals were not measured in any of these studies. Also, the fact that they survived well up to a certain point suggest that perhaps the fat is the bigger issue.

Nguyen *et al.*, 2013, demonstrated larvae grown on fish renderings did not have a shorter development time compared to other high fat diets, such as kitchen waste, and suggested that a diet comprising only fish may contain too much fat. The fish diet also had a very high mean mortality percentage around 99-100% for the adult stage. High mortality rate was also demonstrated in Lopes *et al.*, (2020), where aqua culture waste inclusion increased mortality rate and Isibika *et al.*, (2021), where all larvae died from 100% fish waste-based feed.

5.1.4 Effect of fiber

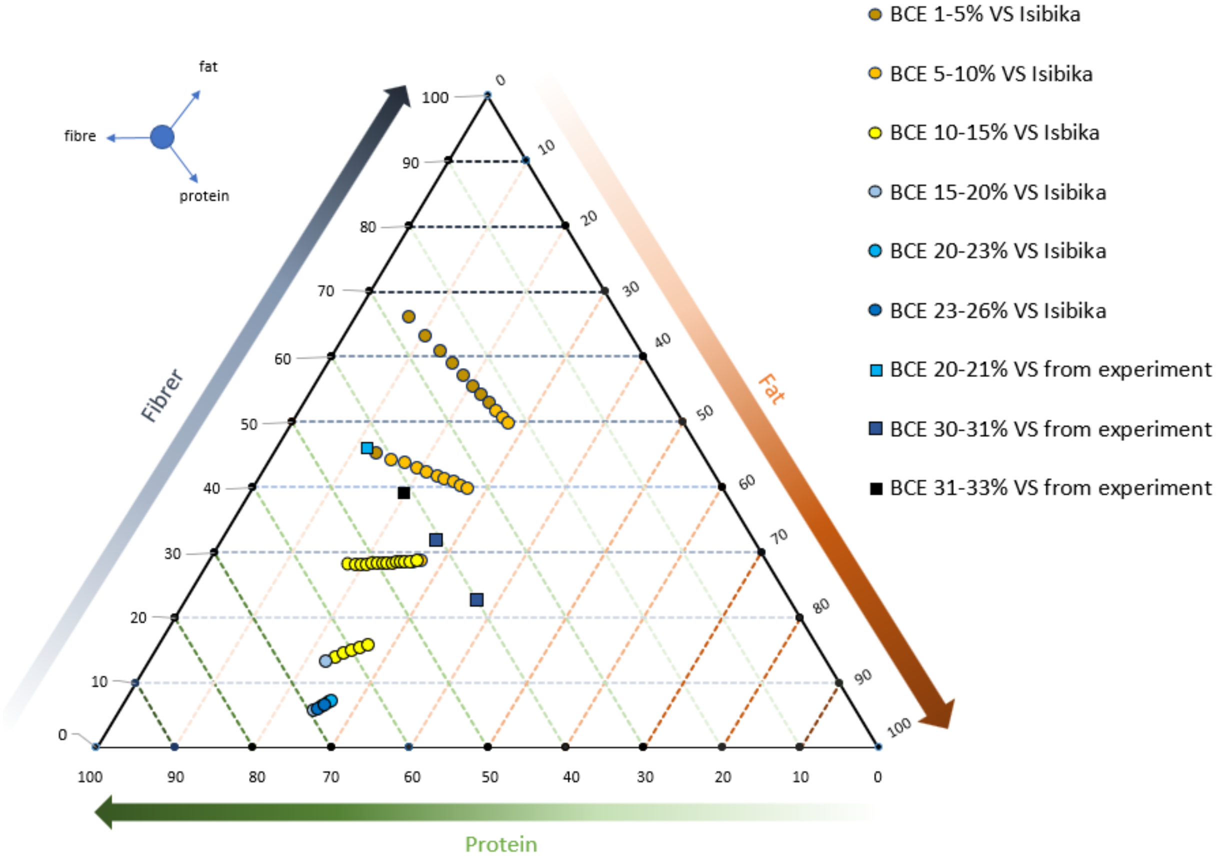


Figure 7: Ternary plot showing compositions of fiber, protein, and fat and their respective BCE values.

In Figure 6, it may seem that a high carbohydrate content was not beneficial for Isibika et al., (2021) and it was discussed above how it is likely due to the fiberous carbohydrates of fruit peels. In order to visualize this, Figure 7 was made and comparing Figure 7 with Figure 6, it appears that increasing fiber content results in a reduction of the BCE. This pattern in particularly clear for data points from Isibika et al., (2021). This seems to be the trend for the results of this study's' treatments as well.

However, Figure 7 has assumed fiber, protein and fat to be the main macronutirents in the composition which is a very rough estimate. Hence it is recommended to view Figure 7 as visual aid to help understand the idea of fiber being the limiting macronutrient instead of carbohydrate.

Fiber is likely difficult for the larvae to digest, as suggested by Gold et al., (2018), since the larvae may lack fiber degrading enzymes. Rehman et al., 2017, examined how well BSFL could degrade fibers present in manure. They demonstrated that BSFL could degrade hemicellulose, cellulose and lignin with a mixture of a ratio of 60% chicken manure and 40% dairy manure, while also producing larvae with a high nutritional content and achieving high waste reduction. However, this study did not do a mass balance for their experiment, making it unclear whether their fiber actually had degraded.

The fiber content might not be a component that increases the BCE directly, but it can still be of use. Isibika *et al.*, (2021) could explore higher inclusions of fish waste (25-75%), resulting in mixtures with high total fat content 22%$_{VS}$ fat, whereas Lopes *et al.*, (2020), could only include a maximum of 20-25% fish waste, resulting in 12%$_{VS}$ fat content. Isibika *et al.*, (2021) suggests that the excess of fat present in the mixtures of Lopes *et al.*, (2020) might cause weak structure in the waste and hinder larvae to movement through it. This might indicate that fiber can be a structure additive in waste with excess fat and other nutrients, as it likely takes longer for them to digest the fiber and the waste would have a more structure integrity for a longer period during the treatment. This indication should be further investigated in future research.

5.1.5 Waste reduction

A5 (73% carbohydrate, 19% protein and 8% fat), achieved the highest BCE$_{\%VS}$ and WR$_{\%WW,}$ but the lowest BCE$_{\%WW.}$ The feed mixture of A5 going into the treatment was very wet (16%$_{DM}$, Table 7) compared to the other treatments, while having similar WR$_{\%}$. It is probable that the reason A5 had the highest waste reduction on a wet weight basis is that water was evaporating from the very wet feed throughout the treatment. The high-water content likely also explains why the BCE$_{\%WW}$ was low. BCE$_{\%VS}$ is a better indicator for the larvae ability to digest the waste, while BCE$_{\%WW}$ gives a better idea of the waste substrates potential from a waste management perspective.

A20 had the lowest reduction on both a WW and VS basis. The composition of A20 was 56% carbohydrate, 23% protein and 21% fat and at the end of the treatment the feed had formed fatty and sticky lumps (Figure 5a). It is plausible that the fat content in the feed made the food more inaccessible at the end of treatment, encapsulating waste in fatty lumps and making it more difficult for the larvae to process.

Overall, the highest BCE and waste reduction on every basis was achieved by compositions with macronutrient fractions of 73-66% carbohydrate$_{\%VS}$, 21-19% protein$_{\%VS}$ and 8-13% fat$_{\%VS}$ in their total composition.

5.1.6 Protein:carbohydrate ratios

Studies such as Barragan-Fonseca *et al.*, (2018) examining the effect of protein and carbohydrate on the BSFL waste treatment or larval development usually refer to protein-to-carbohydrate ratio as a measurement. In their study they demonstrated that carbohydrate-biased prot:carb ratios increased the BCE when investigating performance on vegetable-reared BSFL. This was also supported in their other study in the same thesis, which investigated the effect of dietary carbohydrate on the life cycle of BSFL, where carbohydrate was demonstrated to have a larger influence over adult weight. Their results also showed that a prot:carb ratio between 1:2-1:4 gained the highest value in larval and adult performance such as larval crude fat, larval yield and adult weight.

The prot:carb ratio in Lopes *et al.*, (2020) suggest a carbohydrate biased ratio to be better for BCE as T5, a treatment with the highest BCE, had a ratio of 1:2.5.

In this present study, the best performing treatment A5 had a prot:carb ratio of 1:3.8 and comparing the ratio in Table 1 to the BCE results in Table 7, the trend suggest that carbohydrate-biased prot:carb ratio between 1:3 - 1:4 results in a higher BCE value as A5 had the highest $BCE_{\%VS}$ with the ratio 1:3.8 with A10 coming in second with the ratio 1:3.1. Supported by the findings from Barragan-Fonseca *et al.*, (2018) and Lopes *et al.*, (2020), it is likely that performance in both treatment and BSFL development is favored by a carbohydrate biased prot:carb ratio.

5.2 TERNARY PLOTS

5.2.1 Using ternary plots to predict BSFL composting performance

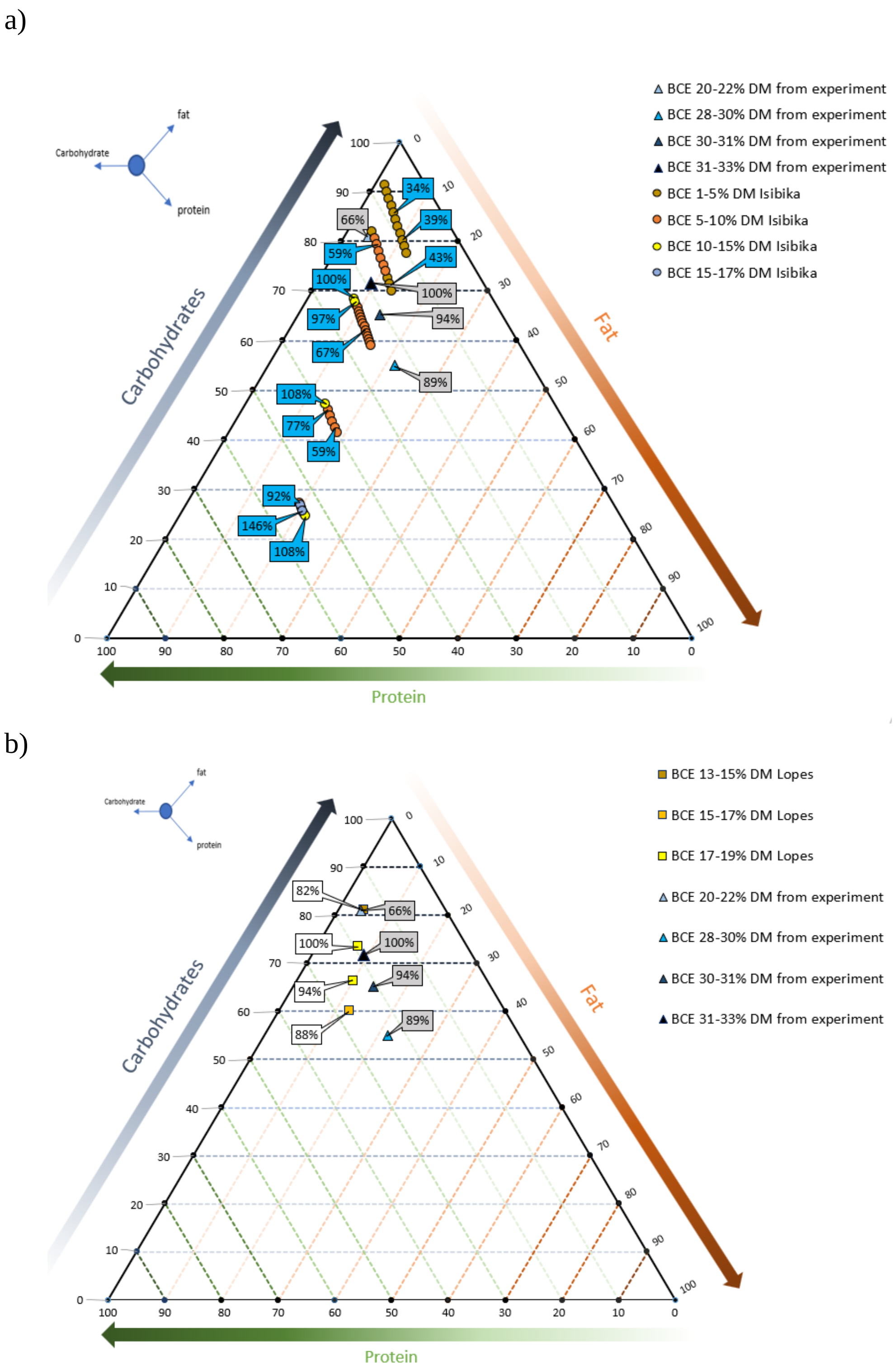


Figure 8: Ternary plot showing the treatments' compositions with their BCE. The data points are labeled with their percentage change from the highest BCE value achieved in all treatments from this study alongside with a) BCE and blue-labeled percentage changes of BCE from the highest BCE value achieved among 25% fish inclusions in Isibika *et al.*, (2021) and b) BCE and white-labeled percentage changes of BCE from the highest BCE value achieved in their Lopes *et al.*, (2020).

In order to see how well predictions could be made based on the ternary plot with BCE, the percentage change from the highest BCE value in each study was calculated for selected data points (Figure 8a, b). The 100% labels in Figure 7a) are based on the highest BCE value among the 25% fish waste inclusions in Isibika *et al.*, (2021). Isibika *et al.*, (2021) suggested a 25% inclusion as the more reliable inclusion of fish in their study for low variance and high BCE. Therefore, some the percentage changes in Figure 8a) are over 100%, since some treatments with inclusions higher than 25% sometimes resulted in a higher BCE than the 100% label was based on. Figure 7b) have it's 100% label based on the highest BCE value from Lopes *et al.*, (2020). All other data points are labeled with the percentage of based on the value which represents the 100% label.

The percentage change goes up to 100% from the controls (66% and 82,3%) as carbohydrate decreases and protein and fat increases (Figure 7b). Results from Lopes *et al.*, (2020) and from this study were very similar to each other in composition. This is likely due to both studies using very similar waste composition in the experiment. Lopes *et al.*, (2020) used reclaimed bread and fish waste from a commercial fish farm in Mora, Sweden, while this study used anchovy waste, reclaimed bread, and vegetable waste. Parra Paz *et al.*, (2015) found that parameters such as larval density and larval feed rate to affect BCE and with ideal density between 1.2-5.0 larvae per cm^2 and ideal feeding rate between 95 to 163 mg/larva per day for maximizing biomass production (Parra Paz, Carrejo and Gómez Rodríguez, 2015). In Lopes *et al.*, (2020), a density of 4.15 larvae per cm^2 was used while this study used a density of 7.5 larvae per cm^2: There were also different larvae feeding dosage, Lopes *et al.*, (2020) had 0.25 g VS feed to each larva, while larvae in this study got 0.1 g VS. These parameters could have made the BCE differ greatly between this study and Lopes, although the feed composition was similar.

The maximum BCE in both this study and for Lopes *et al.*, (2020) was found for the same composition of 73% carbohydrate$_{\%VS}$, 19% protein$_{\%VS}$ and 7-8% fat$_{\%VS}$. The only large difference in percentage change between Lopes *et al.*, (2020) and this study were the control treatments, 82%$_{BCE}$ from Lopes compared to 66%$_{BCE}$ from this study. Lopes *et al.*, (2020), used only bread in their controls, while this study used both bread and vegetables in the control treatment, which is most likely the cause of the difference.

Isibika *et al.*, (2021) and this study had their highest BCE value at similar compositions, 68% carbohydrate$_{\%VS}$, 24% protein$_{\%VS}$ and 8% fat$_{\%VS}$ for Isibika *et al.*, (2021) and 72% carbohydrate$_{\%VS}$, 19% protein$_{\%VS}$, 9% fat$_{\%VS}$ for this study. However, Isibika *et al.*, (2021) used fish waste based on fish carcasses from different types of fish and orange and banana peels in their treatments, which is different from the substrates used in this study and thus compositions with percental changes such as 66%$_{BCE}$, 94%$_{BCE}$ and 89%$_{BCE}$ from this study, had similar compositions with Isibika et al., (2021) but not similar percental changes (59%$_{BCE}$, 66%$_{BCE}$, 77%$_{BCE}$)(Figure 8).

Overall, predictions were accurate for macronutrient compositions of ca 70% carbohydrate$_{\%VS}$, ca 20-25% protein$_{\%VS}$ och <10% fat$_{\%VS}$ (Figure 9). Higher BCE might have been possible to achieve in this study, however the fish waste which was the main source of protein used in the waste compositions, was also the main source of fat and thus the high fat content could not be suppressed without decreasing protein content as well.

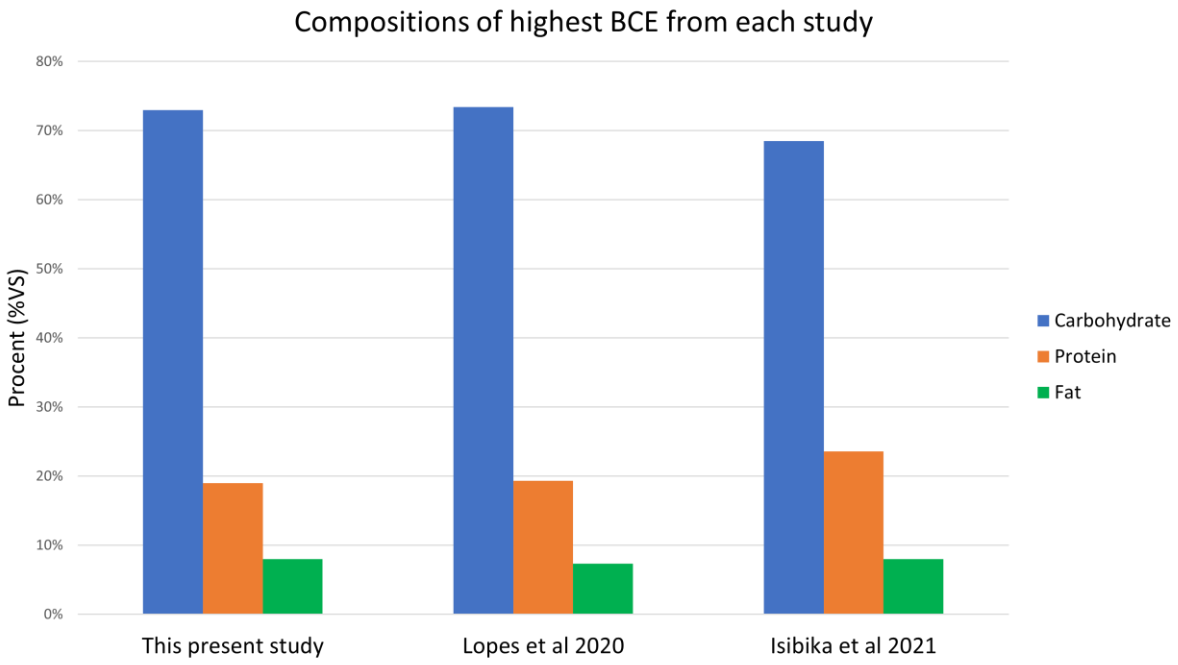


Figure 9: Bar chart of the composition for the highest BCE from Lopes et al., (2020), Isibika et al., (2021) and this study.

5.2.2 Ternary plot as an analysis tool

For this study, the ternary plot was a viable tool for performance. All treatments in Lopes *et al.*, (2020), Isibika *et al.*, (2021) and this study (excluding control treatments) had a similar main fat and protein source, and it is likely that had it been possible to solely add protein for this study's treatments, the predictions would have been less accurate for this study.

Since substrates used for Lopes *et al.*, (2020) and this study were similar and resulted in very similar percentage changes, ternary plots might be more suitable for predictions based on data from studies using the same substrates. Isibika *et al.*, (2021) had a similar composition as this study for the highest BCE, however their percentage changes had a very high variance and were not similar to this study's percentage changes which made predictions less precise. This high variance in percental changes is likely due to their relative higher fat content.

One of the limitations with the ternary plot is that it only can examine three variables of a composition at once. Nutritional compositions are not only dominated by the macronutrients such as protein, carbohydrate and fat but also contain fiber and other elements, which can affect the treatment performance. This became clear when Isibika *et al.*, (2021) showed results that seemingly decreased the BCE when more carbohydrates became present in the waste, but the decreasing result is most likely due to the high fiber content that came with adding more carbohydrates. Although it was possible to compare a ternary plot with fiber and a ternary plot with carbohydrate in order to compare and draw conclusions, a ternary plot might be a better analysis tool for substrates for feed mixture with easily available carbohydrates and protein, where fiber content is low, so that the BCE is affected by fewer variables other than the macronutrients. Further research could investigate feed mixtures of different substrates with the same composition of macronutrients to test the correlation between macronutrient composition and BCE more in depth.

6. CONCLUSIONS

Comparing previous reports' results of macronutrients effect on BCE can be complicated as different measurements. Based on the knowledge that was available and also on what was seen in the results of this present study, protein seem to benefit a high BCE value, carbohydrate can significantly increase BCE however more complex carbohydrates can be detrimental for BCE and knowing what types of carbohydrates are present would be valuable information. Fat is necessary for development but is only tolerable in small amount of the waste mixture wet weight (< 25% $fat_{\%VS}$) and fiber do not seem to be of benefit to BCE directly but could have other structural uses.

Highest BCE from each different study landed on a similar composition of protein, carbohydrate and fat (70% carbohydrate%VS, 20-25% $protein_{\%VS}$ and < 8% $fat_{\%VS}$), which supports the hypothesis that one can predict BCE using a ternary plot with nutritional data. It is likely the accurate estimation was further boosted by the similar substrates used in all studies. It would be of interest for other studies to further test this on waste compositions with very different substrates, to see with predictions remain accurate for high BCE scores

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