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Valorisation of Organic Waste By-Products Using Black Soldier Fly (*Hermetia illucens*) as a Bio-Convertor

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Abstract: One third of food produced globally is wasted. Disposal of this waste is costly and is an example of poor resource management in the face of elevated environmental concerns and increasing food demand. Providing this waste as feedstock for black soldier fly (*Hermetia illucens*) larvae (BSFL) has the potential for bio-conversion and valorisation by production of useful feed materials and fertilisers. We raised BSFL under optimal conditions (28 °C and 70% relative humidity) on seven UK pre-consumer food waste-stream materials: fish trimmings, sugar-beet pulp, bakery waste, fruit and vegetable waste, cheese waste, fish feed waste and brewer's grains and yeast. The nutritional quality of the resulting BSFL meals and frass fertiliser were then analysed. In all cases, the volume of waste was reduced (37–79%) and meals containing high quality protein and lipid sources (44.1 ± 4.57% and 35.4 ± 4.12%, respectively) and frass with an NPK of 4.9-2.6-1.7 were produced. This shows the potential value of BSFL as a bio-convertor for the effective management of food waste.

Keywords: black soldier fly larvae; *Hermetia illucens*; bio-convertor; nutrient recovery; aquaculture feed; organic waste

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1. Introduction

It is estimated that the human population will exceed 9 billion by 2050. An increase in food production of around 50% will be needed to meet their needs [1]. Despite this rising demand, one third of all food produced globally is lost or wasted, equating to approximately 1.3 billion tonnes per year [2]. In the UK alone, 10.2 million tonnes of food waste was generated in 2015, of which 7.1 million tonnes was household waste and the remaining 3.1 million tonnes was from the post farm-gate supply chain [3]. The 'waste hierarchy' [4] illustrates destinations for waste ranked by environmental impact: prevention and minimisation (of waste generation such as redistribution), reuse (for other purposes, including use as animal feed and biomaterial processing), recycling (including composting and anaerobic digestion), energy recovery (including incineration for heat generation) and, finally, disposal. This hierarchy has been incorporated into UK law though the Waste (England and Wales) Regulations 2011, the Waste Regulations (Northern Ireland) 2011 and the Waste (Scotland) Regulations 2012 [5]. According to the waste hierarchy, prevention and redistribution have the lowest environmental impact; however, once food has started to spoil, recycling becomes the next best option. Anaerobic digestion (AD) is a go-to technology favoured globally for recycling food and other organic waste into bioenergy. However, these systems suffer from poor stability and low efficiency, due to the characteristics of food waste [6].

Some food waste can be recycled to make animal feed (within certain confines of the law). Bakery or confectionery products, providing they do not contain and or have not been in contact with meat, fish, or shellfish, can be used. Food or catering waste from kitchens which process meat, vegetarian kitchens which handle dairy products, restaurants and commercial kitchens producing vegan food and international catering waste cannot be used [7]. Animal by-products (ABPs) are subject to greater restrictions to maintain safe food supply chains and appropriate management of high-risk materials. ABPs are divided into three categories, categories one and two being high risk materials, while category three are low risk; category three materials can be processed into farm animal or pet feeds, among other products [8].

There is a growing interest in the use of insects as natural bio-convertors of organic waste valorising the waste by consuming the waste, incorporating it into their bodies and, in the process, converting it into valuable products. Life cycle assessment (LCA) has shown that insect bio-conversion is efficient and environmentally sustainable; direct greenhouse gas (GHG) emissions produced by insects are 47 times lower and the resulting global warming potential (GWP) is half that of open air composting [9]. Production of insects as a food also uses comparatively little space (reducing land use), but, usually, has a relatively high energy use (and GWP) for heating, to achieve a suitable culture environment and for drying the insects after harvest. This high energy demand may be exacerbated by the need to transport the food source to an insect production facility [10]. Overall, energy consumption can be reduced by using waste heat from other industrial processes, or using AD or incineration of other low category waste to heat the system [11]. Insect products can replace less sustainable products, such as fishmeal or soy products in aquaculture feeds; if this product replacement is also accounted for during LCA assessment, then the GWP of insect production decreases further [9,10].

Insects have previously been used in organic waste management strategies, recovering nutrients in the form of the constituent parts of the insect yielding high quality protein, lipid and chitin [12–15]. Insects, or their derivative proteins and fats, are utilized as food for humans [16,17] and in animal feeds [18,19]. Further, as a lipid-rich source, they can also be used for the production of biodiesel [10]. In this paper we focus primarily on the value of the products as constituents of animal feed.

Of the many insect species that have been studied [16,17], the black soldier fly (BSF) (*Hermetia illucens*) stands out. It has many characteristics that make it particularly attractive for commercial scale production in the UK. It is a species of true fly (Diptera) of the family Stratiomyidae. It originates from the Americas, although they are now more widespread in tropical and temperate regions [20–23]. They do not tolerate colder climates, such as those found in north-western Europe [24]. Therefore, if any escape from culture facilities, they are unlikely to survive the winter and become an invasive species. The BSF larvae (BSFL) are capable of consuming a wider range of organic materials than other fly species [25,26]. The adult stage is not a vector for human, animal, or plant pathogens. It does not possess mouth apparatus, so cannot bite [25,27,28]. BSF have an excellent nutritional profile, high in protein with a high quality amino acid profile and high levels of lipid, including economically and nutritionally valuable fatty acids [25]. *Hermetia illucens* was included in the seven insect species listed in the Commission Regulation (EU) 2017/893 [29] as safe for production for food use. This regulation permits the use of processed animal proteins (PAPs) derived from those insect species for aquaculture feeds, pet feeds and fur animal feeds in the EU. Insects grown for the production of processed insect proteins (PIPs) that are fed to other farmed animals are categorized as 'farmed animals' (Article 3(6) of Regulation (EC) No 1069/2009; [30]). As such, they are subject to regulations in the use of feed materials used to grow them [31]. The fact that they can be used in aquaculture feeds, their high-quality nutritional profile and their utility for bioconversion of waste strongly suggest that there will be an increase in the demand for BSFL and BSFL products.

Production of BSFL PIPs involves hatching BSFL, then growing them on an organic feed material, until they reach an appropriate life stage. They are then separated from the remains of the feed and larval residue (known as frass) and harvested. BSFL products have several potential uses, the primary use, that we discuss here, is the production of meal. Liu and Chen [32] concluded the early pre-pupae is the most appropriate life stage to harvest for meal production. This stage was, therefore, used during this study. BSFL meal can be further processed to concentrate protein while extracting the lipids for other uses as high-quality feed ingredients. In addition, lipids can also be used for biodiesel production [33] and chitin can be extracted from the meal for many uses, including food, pharmaceuticals, textiles, waste water treatment and cosmetics [34]. The BSFL lipid content is of particular interest because of the high content (approximately 28.6 ± 8.6% of insect mass) and because it is rich in useful fatty acids [25]. Spranghers and Ottoboni [14] showed that the fatty acid profile of BSFL is highly influenced by their diet, highlighting an opportunity for manipulation of the product via diet.

The 'frass' is a by-product that consists of faecal matter, residual growing substrate and shed exoskeletons from previous instars. It has value as a good quality, slow release organic fertiliser, with higher NPK values than other animal by-products recognized as fertilizers, such as composted poultry litter and worm castings [35]. Frass can also be processed via AD for further energy recovery, as it possesses suitable characteristics [36]. The anaerobic biodegradability fraction (fd) of BSFL frass is equal to that of food waste (89%); however, it has higher bio-methane potential (502 ± 9 mL CH_4/g VS) than food waste (449 ± 53 mL CH4/g VS) [36]. Food waste also causes two main problems for AD, poor stability, due to volatile fatty acids and low organic loading rates and effectively low efficiency [37,38], caused by high levels of easily biodegradable suspended solids [6]. However, significantly, utilising BSFL to bio-convert food waste into high quality feed ingredients can be classed as "prevention"; therefore, it is preferred over AD as a method for processing organic food waste in the waste hierarchy [4,39].

The increased interest in the use of BSFL as an organic waste management tool and a source of raw materials for the manufacture of animal feed has led to a better understanding of how nutrient density and feed substrate quality can influence the development and growth of BSFL [40,41]. BSFL have been shown to achieve a good feed conversion ratio (FCR), ranging between 1.4 and 2.6, when fed food waste materials [42]. Diets consisting of high protein and high lipid achieve the best results. The nutritional profile of the end larval material is also affected and, while protein levels do vary, the lipid levels and profile are more highly affected [14,43]. In this study, we look at the impact of seven potential organic waste streams (Table 1) from pre-consumer and manufacturing situations on BSFL bio-concentration of nutrients and, primarily, fatty acids. We evaluate which of our organic waste materials are most suitable for use in modulating and manipulating fatty acid profiles of BSFL meal and draw conclusions about the valorisation of organic waste by-products via BSFL treatment.

In response to the change in EU law [29] allowing use of insect meals and the growing interest in this area, it is very likely that these meals will become highly valued as aquaculture feed ingredients. Because fish lack the enzymes to completely synthesize polyunsaturated fatty acids (PUFA), or highly unsaturated fatty acids (HUFA) of the n-3 and n-6 series de novo [44], these must be provided preformed via the diet, making them essential fatty acids (EFAs): linoleic acid (18:2n-6), α-linolenic acid (18:3n-3), arachidonic acid (20:4n-6), Eicosapentaenoic acid (EPA, 20:5n-3) and Docosahexaenoic acid (DHA, 22:6n-3) [45]. This study pays close attention to these EFAs and their potential as feed ingredients.

Table 1. Pre-consumer and manufacturing organic waste streams identified for study.

Organic Waste	Source	Reason Waste Was Chosen for Investigation	Waste Category and Disposal Method	Median Gate Fezzze (GBP/tonne) [46]
Fish trimmings	Collected from local fish monger	Waste generated at fish processing facilities. Seeking to track long chain fatty acids in BSFL.	Fallen stock and digestive tracks—category II. Parts of stock unconsumed—category III.	Material recovery facilities (MRFs): all wastes, GBP 25, contracts from 2018 are GBP 35. In vessel composting (IVC): mixed food and green, GBP 50; all feedstock types, GBP 46. AD: all gates fees, GBP 27. Energy recovery: GBP 89. Landfill: non-hazardous waste, including landfill tax (standard rate for 2017/18 is GBP 88.95/tonne), GBP 113.
Sugar beet pulp	British sugar	Highly produced by sugar industry and meat-free.	Covered under fruit and vegetable waste.	
Bakery waste	Local bakery	Available in high volumes due to short shelf life and meat free	Non-animal by-product approved, depackaged and shred.	
Fruit and vegetable waste	Household waste (representative of supermarket waste)	Available in high volumes and meat-free	Non-animal by-product approved, depackaged and shred.	
Cheese waste	Harvey & Brockless (H&B) Cheese in London	Available in high volumes, meat-free and high in fat. Investigating how BSFL respond to high fat material.	Covered under dairy products. Treated as bakery and fruit and vegetable, depackaged and shred.	
Industrial fish feed waste	Skretting feed manufacturing facility	By product of aquaculture feed industry	As for fish trimmings.	
Brewer's grains and yeast	Firebird Brewery	Available in high volumes from brewing industry and meat-free.	Often used as animal feed or disposed via landfill [47].	

2. Materials and Methods

2.1. Outcomes

2.1.1. Growth Performance of BSFL Fed Identified Organic Materials

BSFL are produced under optimal conditions, fed on our identified feed materials and converted into BSFL meals. Growth performance is assessed to explore BSFL meal production and waste reduction potential of each organic material.

2.1.2. BSFL Bioconcentration and Modification of Fatty Acid Profile

Samples of feed materials and BSFL meals were collected and analysed for nutritional quality—protein, lipid and fatty acid profiles. Data were analysed to assess bioconcentration of nutrients during production of BSFL meals, in order to identify suitable organic by-products for nutrient recovery by BSFL.

2.1.3. Valorisation of Organic Waste By-Products via BSFL Treatment

The value of the BSFL outputs, meals and frass, was estimated to assess valorisation of the identified organic waste streams via BSFL treatment.

2.2. Processing Organic Waste Materials

Water was added to the sugar beet, bakery waste, cheese waste and fish feed waste to achieve 70% water content prior to feeding, while the fish trimmings, fruit and vegetable waste and brewer's grains and yeast already contained a high enough water content. All feedstock materials were homogenized, prior to feeding, in order to optimize processing by BSFL. Samples (100 g) of each material were frozen at −20 °C and sent to Nottingham University for proximate analyses and fatty acid analyses.

Material energy content was determined using a Parr 6300 bomb calorimeter connected to a Parr 6520 water recirculation system. One-gram Benzoic acid tablets standardized for bomb calorimetry (26.454 MJ/kg, Parr Instrument Co, item No: 3415) were used as standards. Material protein content was analysed using a Thermo Scientific FlashEA® 1112 N/Protein Analyzer in conjunction with the EAGER software. The lipid content of each sample was analysed using rapid Soxhlet extraction, using a Gerhardt Soxtherm. The extracted lipid samples were further analysed to determine the fatty acid profile of each sample by applying a direct method for fatty acid methyl ester (FAME) synthesis, in conjunction with GC analyses (Perkin Elmer Clarus 500 Gas Chromatograph), utilizing a Varian capillary column CP-Sil 88 for FAME; column length, 100 m, column width, 0.25 mm. Gas flow for air was 450 mL/min and hydrogen was 45 mL/min; the temperature set point was 250 °C. Ash was determined using the AOAC official method 942.05 [48]. Fibre content was analysed using the Gerhardt Fibrebag method.

2.3. BSFL Production

Each of the experimental organic waste stream materials were fed to five replicate groups of 25 larvae, for a total of 35 groups of BSFL. Larvae were grown under environmentally controlled conditions (28 °C and 70% relative humidity) and kept in the dark during production. BSFL growth and performance were assessed through feed conversion ratio (FCR), specific growth rate (SGR) and larval growth rate (LGR). Efficiency of conversion of ingested food (ECI) was assessed. Waste material reduction was assessed through waste reduction index (WRI) and substrate reduction (SR). All using the following equations:

$$FCR = TFI\ (kg) \div Weight\ gain\ (kg)$$

where *TFI* (total feed intake) = total feed given and *Weight gain* = weight at end of study period–weight at start of study period [49].

$$SGR\ (\%) = 100 \times (\ln W_2 - \ln W_1) \times (t_2 - t_1)^{-1}$$

where ln = natural log, W_1 = initial weight, W_2 = final weight, t_1 = starting time point (day one) and t_2 = end time point (final day number) [50].

$$LGR\ (g/day) = (W_2 - W_1)/number\ of\ days$$

where W_1 = initial larval weight (g), W_2 = final larval weight (g) [51].

$$ECI = B/(W - R)$$

where B = total biomass (larvae) (g), W = total amount of feed provided (g) and R = remaining substrate (g) [51].

$$WRI = (W - R/W)/days\ of\ trial\ (d) \times 100$$

where W = total amount of feed provided and R = remaining substrate [51].

$$SR = W - R/W \times 100$$

where W = total amount of feed provided and R = remaining substrate [51].

2.4. Nutritional Analyses of BSFL Pre-Pupae Fed Each Organic Material

BSFL groups were harvested at the pre-pupae stage and frozen at −20 °C. They were dried in a drying cabinet at 60 °C for 4 days, then ground into BSFL meal using a benchtop hand grinder. Samples of each meal were sent to Skretting UK for analysis—crude protein, crude lipid, amino acid profile and fatty acid profile. The BSFL meal nutrient content for crude protein, crude lipid and fatty acids were analysed as the feed materials above. Amino acid profiles were determined using hydrolyses and an amino acid analyser. These data were combined with Skretting UK's undisclosed data regarding protein digestibility of BSFL meal for value estimation as an aquaculture feed ingredient.

2.5. Data Analyses

A representative frass sample was collected during production of the *BSFL* and analysed by NMR laboratories for nitrogen (N), phosphorus (P), potassium (K) (NPK) and magnesium (Mg) to assess the quality of the waste product for use as a fertiliser.

Nutrient bioconcentration by *BSFL* from each of the organic waste materials was investigated by generating an apparent bioconcentration factor (*aBCF*) for each nutrient, i.e., crude protein, crude lipid, fatty acids and fatty acid groups, which are present in both feed materials and *BSFL* meals, calculated as follows:

$$aBCFi = \frac{(FAi/TDFA)BSFL\ meal}{(FAi/TDFA)Diet} \quad \text{or} \quad aBCFi = \frac{(Nutrienti)BSFL\ meal}{(Nutrienti)Diet}$$

where i = specific *FA* (g/100 g DM), or the sum of a group of *FA* (SFA, MUFA, MUFA trans, PUFA and branched *FA*) and *TDFA* = total detected fatty acids (g/100 g DM), or nutrient (g/100 g DM of crude protein or crude lipid) [43].

2.6. Value Estimation of BSFL Outputs

Each BSFL meal was inputted into Skretting's aquaculture feed formulation software programme; this programme assigned a value to each BSFL meal (as an ingredient), based on their nutritional qualities compared to the quality of all the other available feed ingredients and their current market prices (correct as of February 2019). The potential value of frass can be estimated based on N, P and K content along with current costs of those nutrients available through other marketed fertilisers, as described by Kissel and Risse [52].

2.7. Statistical Analysis

Tests for differences were carried out with 95% confidence levels ($p \leq 0.05$) between each test substance. The Kolmogorov–Smirnov tests for normality were carried out, with one-way ANOVA tests, followed by post hoc Tukey's tests, used for parametric data, and Mann–Whitney U tests, for non-parametric data.

3. Results

3.1. BSFL Growth, Performance and Substrate Reduction

Growth and performance of the BSFL varied significantly depending on the feedstock they were fed (Table 2). Sugar beet pulp and cheese waste were used the least efficiently by BSFL, attaining the lowest ECI, LGR and SGR, subsequently reaching the highest FCR. Bakery waste achieved the greatest performance (FCR and SGR), while fish feed waste was the most efficiently used (ECI). The BSFL consumed more cheese waste (WRI) than any other feedstock. The greatest reduction in feedstock substrate (SR) was seen with fruit and vegetable waste. BSFL mortality rate when fed fish feed waste (48.8%) was significantly higher ($p < 0.05$) than when fed all other feedstocks (4–12%).

3.2. BSFL Nutrient Bioconcentration

3.2.1. Organic Waste Material and BSFL Meal Profiles

The variety of organic waste materials used display a range in protein (8.4–54.0 g/100 g DM) and lipid (0.4–57.3 g/100 g DM) levels (full nutritional profile of the BSFL meal provided in Appendix A). Each material also displays varied fatty acid profiles (Table 3).

Table 2. Substrate reduction alongside growth and performance of BSFL when raised on the identified organic feedstocks.

Diet	Feed Conversion Rate (FCR)	Specific Growth Rate (SGR)	Larval Growth Rate (LGR) (mg/day)	Efficiency of Conversion of the Ingested Food (ECI)	Waste Reduction Index (WRI) (g/day)	Substrate Reduction (SR) (%)
Fish trimmings	5.98 ± 2.77 ^{ac}	16.92 ± 3.36 ^{a}	9.25 ± 4.94 ^{a}	0.32 ± 0.08 ^{a}	28.17 ± 5.26 ^{ad}	−54.49 ± 8.59 ^{a}
Sugar beet pulp	20.54 ± 8.68 ^{b}	9.95 ± 1.84 ^{b}	2.19 ± 0.54 ^{b}	0.11 ± 0.04 ^{b}	14.79 ± 0.68 ^{b}	−60.98 ± 8.31 ^{ac}
Bakery waste	4.84 ± 1.45 ^{a}	17.66 ± 1.68 ^{a}	9 ± 2.83 ^{a}	0.35 ± 0.08 ^{a}	22.66 ± 1.26 ^{ae}	−70.26 ± 9.49 ^{bc}
Fruit and vegetable waste	7.97 ± 1.1 ^{ac}	16.08 ± 1.21 ^{a}	6.45 ± 0.79 ^{ac}	0.15 ± 0.01 ^{b}	28.02 ± 2.21 ^{ad}	−79.28 ± 6.18 ^{b}
Cheese waste	12.92 ± 2.06 ^{c}	9.55 ± 0.99 ^{b}	2.71 ± 0.19 ^{c}	0.11 ± 0.03 ^{b}	45.17 ± 4.98 ^{c}	−63.86 ± 5.63 ^{ac}
Fish feed waste	6.42 ± 1.25 ^{ac}	17.39 ± 1.43 ^{a}	16.05 ± 1.9 ^{d}	0.55 ± 0.07 ^{c}	31 ± 1.42 ^{d}	−37.27 ± 5.4 ^{d}
Brewer's grain and yeast	6.78 ± 1.12 ^{ac}	16.59 ± 1.19 ^{a}	6.99 ± 0.38 ^{ac}	0.31 ± 0.04 ^{a}	21.19 ± 3.32 ^{be}	−52.04 ± 5.02 ^{a}

LGR, ECI, WRI and SR were calculated on a DM basis. Organic materials which do not share a common letter in each column are significantly different $p < 0.05$.

Table 3. (**a**) Nutritional profile of waste stream feed materials, proximate. (**b**) Fatty acid profiles of organic waste stream feed materials.

(**a**)

Parameter	Fish Trimmings	Sugar Beet Pulp	Bakery Waste	Fruit and Vegetable Waste	Cheese Waste	Fish Feed Waste	Brewer's Grains and Yeast
Dry matter (DM) (%)	31.07	52.65	58.63	12.47	53.54	93.93	21.66
Crude protein (g/100 g DM)	42.42	8.62	18.22	8.42	31.71	54.02	49.95
Crude fat (g/100 g DM)	36.47	0.36	2.66	1.68	57.27	10.40	6.56
Fibre (g/100 g DM)	0.00	4.21	0.65	0.10	0.22	1.63	0.88
Ash (g/100 g DM)	5.22	4.22	1.97	0.66	3.35	6.51	1.03
Energy (MJ/kg)	7.5	8.47	11.11	2.02	16.33	20.89	4.41

(**b**)

Fatty Acids (g/100 g DM)		Fish Trimmings	Sugar Beet Pulp	Bakery Waste	Fruit and Vegetable Waste	Cheese Waste	Fish Feed Waste	Brewer's Grains and Yeast
Caproic acid	C6:0	1.6	0.0	0.1	0.1	6.4	0.3	0.1
Caprylic acid	C8:0	0.01	0.01	0.01	0.00	0.31	0.01	0.00
Capric acid	C10:0	0.00	0.00	0.00	0.00	0.76	0.00	0.00
Undecanoic acid	C11:0	0.00	0.00	0.00	0.00	0.18	0.00	0.00
Lauric acid	C12:0	0.02	0.01	0.01	0.00	1.02	0.02	0.01
Tridecanoic acid	C13:0	3.03	0.05	0.77	0.55	0.88	1.46	3.06
Myristic acid	C14:0	0.95	0.01	0.01	0.01	3.74	0.63	0.01
Myristoleic acid	C14:1n-5	0.03	0.00	0.00	0.01	0.64	0.01	0.00
Pentadecanoic acid	C15:0	0.08	0.00	0.00	0.00	0.41	0.04	0.00
cis-10 pentadecanoic acid	C15:1	0.65	0.00	0.11	0.09	0.29	0.40	0.31
Palmitic acid	C16:0	2.62	0.08	0.29	0.05	10.75	1.55	0.35
Palmitoleic acid	C16:1n-7	0.71	0.00	0.00	0.00	0.74	0.42	0.01
cis-10 heptadecanoic acid	C17:1	0.03	0.00	0.00	0.00	0.03	0.01	0.01
Stearic acid	C18:0	0.50	0.02	0.04	0.01	3.45	0.28	0.03
Elaidic acid, Oleic acid	C18:1n-9	2.70	0.03	0.62	0.03	7.57	2.49	0.22
Linoleic acid	C18:2n-6	0.95	0.03	0.45	0.07	0.54	1.38	0.66
α-linolenic acid	C18:3n-3	0.20	0.00	0.06	0.01	0.16	0.38	0.07
Gamma-linolenic acid (GLA)	C18:3n-6	0.01	0.00	0.00	0.00	0.01	0.01	0.00
Arachidic acid	C20:0	0.04	0.00	0.01	0.00	0.06	0.05	0.00
Gondoic acid	C20:1n-9	4.42	0.00	0.03	0.00	0.03	0.98	0.07
Eicosadienoic acid	C20:2n-6	0.12	0.00	0.01	0.00	0.02	0.05	0.00
cis-11,14,17 eicosatrienoic acid	C20:3n-3	0.12	0.00	0.00	0.00	0.00	0.03	0.00
cis-8,11,14 eicosatrienoic acid	C20:3n-6	0.03	0.00	0.00	0.00	0.03	0.01	0.01
Arachidonic acid	C20:4n-6	0.03	0.00	0.00	0.00	0.03	0.04	0.00
Eicosapentaenoic acid (EPA)	C20:5n-3	0.24	0.00	0.00	0.00	0.01	0.56	0.01
Heneicosanoic acid	C21:0	0.02	0.00	0.00	0.00	0.03	0.01	0.03
Behenic acid	C22:0	0.02	0.00	0.00	0.00	0.04	0.06	0.01
Erucic acid	C22:1n-9	0.58	0.00	0.01	0.00	0.01	0.12	0.01
cis-13,16-docosadienoic acid	C22:2	0.07	0.00	0.00	0.00	0.02	0.01	0.01
Docosahexaenoic acid (DHA)	C22:6n-3	0.22	0.00	0.00	0.00	0.00	0.61	0.00
tricosanoic acid	C23:0	0.01	0.00	0.00	0.00	0.02	0.00	0.00
lignoceric acid	C24:0	0.03	0.00	0.00	0.01	0.03	0.01	0.01
nervonic acid	C24:1	0.60	0.00	0.00	0.00	0.00	0.08	0.01
Sum Sat FA		8.95	0.23	1.23	0.77	28.12	4.43	3.66
Sum unsaturated FA		11.73	0.06	1.30	0.22	10.13	7.56	1.39
Sum monoenes		9.73	0.03	0.78	0.14	9.30	4.50	0.64
Sum n-6 FA		1.14	0.03	0.46	0.07	0.63	1.48	0.68
Sum n-3 FA		0.79	0.00	0.06	0.01	0.17	1.57	0.07
Unsat/Saturated		1.31	0.28	1.06	0.29	0.36	1.71	0.38
n-6/n-3		1.44	13.47	7.17	6.09	3.66	0.94	9.29
n-3/n-6		0.69	0.07	0.14	0.16	0.27	1.06	0.11

The nutrient profiles of the BSFL meals (Table 4) were influenced by the different organic feed materials. However, there was less variation between the BSFL meals than there was between the organic waste stream materials, with average crude protein levels of 44.1% (±4.57) and lipid levels of 35.4% (±4.12), compared to 30.48% (±19.11) and 16.48%

(±21.86). Leucine, aspartic acid and glutamic acid were the three most prevalent amino acids across all the BSFL meals, with tyrosine being exceptionally higher in BSFL fed cheese waste, fish feed waste and brewer's grain and yeast. The fatty acids most prevalent across all the BSFL meals included Lauric acid, Myristic acid, Palmitic acid, Palmitoleic acid, Oleic acid and linoleic acid. BSFL fed fish trimmings also contained raised levels of EPA. BSFL fed brewer's grains contained the highest level of n6 fatty acids with a high level of n3 fatty acids, containing EFA's linoleic and α-linolenic acid, alongside a good level of EPA and small amounts of DHA, with only the fish trimmings and fish feed waste fed BSFL meals possessing higher levels of EPA and DHA.

Table 4. (**a**) Nutritional profiles of BSFL meals, proximate and amino acid analysis. (**b**) Nutritional profiles of BSFL meals, fatty acid analysis.

(**a**)

Parameter (g/100 g DM)		**BSFL Meals**						
		Fish Trimmings	**Sugar Beet Pulp**	**Bakery Waste**	**Fruit and Vegetable Waste**	**Cheese Waste**	**Fish Feed Waste**	**Brewer's Grains and Yeast**
Proximate	Crude protein	46.62	43.15	43.07	36.03	43.18	45.70	51.05
	Crude fat	35.05	35.49	37.63	40.30	36.94	35.22	27.00
Amino acids								
Essential	Arginine	2.06	1.79	1.84	1.43	1.95	2.14	1.96
	Histidine	1.43	1.34	1.31	1.01	1.30	1.44	1.34
	Isoleucine	1.97	1.84	1.76	1.45	1.82	1.99	2.11
	Leucine	3.85	3.58	2.79	2.28	2.85	3.15	3.30
	Lysine	2.41	2.19	2.41	1.97	2.38	2.53	3.15
	Methionine	0.87	0.78	0.79	0.63	0.82	0.80	0.85
	Cystine	0.29	0.27	0.33	0.29	0.23	0.22	0.37
	Phenylalanine	1.73	1.69	1.81	1.51	1.85	2.05	2.44
	Tyrosine	2.03	1.91	1.96	1.48	5.28	5.73	5.44
	Threonine	1.68	1.56	1.61	1.28	1.64	1.80	1.92
	Valine	2.67	2.52	2.62	2.11	2.64	2.91	3.04
Non-essential	Alanine	2.94	2.92	2.81	2.36	2.76	2.91	4.46
	Aspartic acid	3.86	3.76	3.89	3.10	3.69	4.12	4.05
	Glutamic acid	4.47	4.26	4.37	3.48	4.40	4.40	5.13
	Glycine	2.59	2.35	2.35	1.84	2.37	2.61	2.65
	Proline	2.65	2.47	2.32	1.91	2.88	2.97	3.35
	Serine	1.83	1.73	1.70	1.33	1.69	1.83	1.94
Sum of AA		39.31	36.95	36.68	29.47	40.56	43.61	47.51

Tryptophan was not tested for.

(**b**)

Parameter (g/100 g DM)		**BSFL Meals**						
		Fish Trimmings	**Sugar Beet Pulp**	**Bakery Waste**	**Fruit and Vegetable Waste**	**Cheese Waste**	**Fish Feed Waste**	**Brewer's Grains and Yeast**
Fatty acids								
Caprylic acid	C8:0	<LOD	<LOD	<LOD	<LOD	0.01	<LOD	<LOD
Capric acid	C10:0	0.34	0.35	0.34	0.28	0.37	0.48	0.23
Lauric acid	C12:0	12.59	20.37	19.80	15.39	12.35	16.71	7.18
Myristic acid	C14:0	2.33	3.69	3.88	3.39	3.59	2.97	1.83
Myristelaidic acid	C14:1n-5	0.06	0.09	0.08	0.28	0.31	0.08	0.06
Pentadecanoic acid	C15:0	0.13	<LOD	0.04	0.08	0.22	0.06	0.08
Palmitic acid	C16:0	4.71	4.29	4.74	5.72	7.39	3.61	4.60
Palmitoleic acid	C16:1n-7	2.23	0.97	1.05	2.06	1.80	1.18	1.34
	C16:2n-6	0.04	<LOD	<LOD	<LOD	<LOD	0.02	<LOD
Stearic acid	C18:0	0.55	0.55	0.68	0.73	0.95	0.44	0.69
	C18:1n-5	<LOD	<LOD	<LOD	<LOD	<LOD	0.02	0.01
Elaidic acid, Oleic acid	C18:1n-9	5.56	3.02	3.99	8.87	6.68	4.13	4.23
cis-vaccenic acid	C18:1n-7	0.42	<LOD	0.11	0.12	0.23	0.26	0.38
	C18:2n-4	<LOD	<LOD	<LOD	<LOD	0.04	0.01	<LOD
Linoleic acid	**C18:2n-6**	**2.29**	**1.28**	**2.11**	**1.65**	**1.27**	**2.00**	**3.57**
α-linolenic acid	**C18:3n-3**	**0.33**	**0.18**	**0.30**	**0.36**	**0.23**	**0.28**	**0.42**
Gamma-linolenic acid (GLA)	C18:3n-6	0.03	<LOD	<LOD	<LOD	0.02	0.01	0.01
Stearidonic acid (SDA)	C18:4n-3	0.23	<LOD	<LOD	<LOD	0.01	0.22	0.17
Arachidic acid	C20:0	<LOD	0.04	<LOD	<LOD	0.03	0.03	0.06
	C20:1n-8	0.09	<LOD	<LOD	<LOD	<LOD	0.24	0.20
Gadoleic acid	C20:1n-11	0.44	<LOD	<LOD	<LOD	0.11	0.34	0.30
Eicosadienoic acid	C20:2n-6	<LOD	<LOD	<LOD	<LOD	0.07	0.07	0.06

Table 4. *Cont.*

(b)

Parameter (g/100 g DM)		**BSFL Meals**						
		Fish Trimmings	**Sugar Beet Pulp**	**Bakery Waste**	**Fruit and Vegetable Waste**	**Cheese Waste**	**Fish Feed Waste**	**Brewer's Grains and Yeast**
Arachidonic acid	**C20:4n-6**	**0.14**	<LOD	<LOD	**0.04**	**0.05**	**0.03**	**0.02**
Eicosapentaenoic acid (EPA)	**C20:5n-3**	**1.20**	**0.09**	**0.04**	**0.08**	**0.18**	**0.78**	**0.53**
Behenic acid	C22:0	<LOD	<LOD	<LOD	<LOD	0.03	0.02	0.04
Cetoleic acid	C22:1n-11	<LOD	<LOD	<LOD	<LOD	<LOD	0.12	0.07
Docosahexaenoic acid (DHA)	**C22:6n-3**	**0.32**	<LOD	<LOD	<LOD	<LOD	**0.06**	**0.07**
Sum Sat FA		20.64	29.28	29.47	25.59	24.93	24.33	14.70
Sum unsaturated FA		13.37	5.64	7.68	13.46	10.99	9.85	11.43
Sum monoenes		8.79	4.08	5.23	11.32	9.12	6.38	6.58
Sum n-6 FA		2.50	1.28	2.11	1.69	1.41	2.13	3.66
Sum n-3 FA		2.08	0.27	0.34	0.44	0.42	1.34	1.20
Unsat/Saturated		0.65	0.19	0.26	0.53	0.44	0.40	0.78
n-6/n-3		1.20	4.70	6.22	3.82	3.35	1.59	3.06
n-3/n-6		0.83	0.21	0.16	0.26	0.30	0.63	0.33
Unknown		2.95	1.61	0.9	1.4	2.74	2.93	3.24

<LOD = below level of detection. Essential fatty acids highlighted in bold.

3.2.2. BSFL Bioconcentration of Nutrients

According to the method used to calculate aBCF, values higher than unity are considered to indicate nutrient concentration. These results show that BSFL bioconcentrate nutrients from most organic food materials very well. Lauric acid is the fatty acid which BSFL accumulate the greatest across all feed materials (Table 5). BSFL fed cheese waste is the only meal where lauric acid is not the most bioconcentrated FA; EPA is higher. BSFL achieve the greatest bioconcentration of the overall desired EFAs from fish trimmings, followed by brewer's grains and cheese waste.

Table 5. Apparent bioconcentration factor (aBCF) of nutritional parameters tested for and present in both feed materials and BSFL meals, achieved by BSFL during production feeding on each waste stream material.

Parameter (%DM)		**BSFL Apparent Bioconcentration Factor (aBCF)**						
		Fish Trimmings	**Sugar Beet Pulp**	**Bakery Waste**	**Fruit and Vegetable Waste**	**Cheese Waste**	**Fish Feed Waste**	**Brewer's Grains and Yeast**
Crude protein		1.1	5.0	2.4	4.3	1.4	0.8	1.0
Crude fat		1.0	98.4	14.1	23.9	0.6	3.4	4.1
Fatty acids								
Caprylic acid	C8:0	0	0	0	0	0.1	0	0
Capric acid	C10:0	216.4	0	43.4	6.4	0.7	57.4	0
Lauric acid	C12:0	561.9	21.1	114.6	157.9	18.8	230.2	329.0
Myristic acid	C14:0	2.6	4.9	29.1	15.2	1.5	1.4	57.2
Pentadecanoic acid	C15:0	1.7	0	1.4	2.7	0.8	0.4	8.2
Palmitic acid	C16:0	1.9	0.5	1.1	4.7	1.1	0.7	3.1
Palmitoleic acid	C16:1n-7	3.3	9.2	17.7	42.5	3.8	0.8	64.2
Stearic acid	C18:0	1.1	0.3	1.1	2.1	0.4	0.5	5.5
Elaidic acid, Oleic acid	C18:1n-9	2.1	1.1	0.5	10.6	1.4	0.5	4.6
Linoleic acid	C18:2n-6	2.5	0.5	0.3	1.0	3.6	0.4	1.3
α-linolenic acid	C18:3n-3	1.7	0.9	0.3	1.3	2.3	0.2	1.6
Gamma-linolenic acid (GLA)	C18:3n-6	2.4	0	0	0	4.8	0.2	0
Arachidic acid	C20:0	0.0	0.4	0	0	0.7	0.2	3.1
Eicosadienoic acid	C20:2n-6	0.0	0	0	0	5.2	0.5	3.0
Arachidonic acid	C20:4n-6	4.7	0	0	2.6	2.5	0.3	4.7
Eicosapentaenoic acid (EPA)	C20:5n-3	5.1	0	4.9	14.1	19.8	0.4	19.7
Behenic acid	C22:0	0	0	0	0	1.3	0.1	2.0
Docosahexaenoic acid (DHA)	C22:6n-3	1.5	0	0	0	0	0.0	0
Sum Sat FA		2.4	1.3	1.7	1.4	1.4	1.6	1.0
Sum unsaturated FA		1.2	0.9	0.4	2.5	1.7	0.4	2.0
Sum monoenes		0.9	1.2	0.5	3.4	1.5	0.4	2.5
Sum n-6 FA		2.3	0.5	0.3	1.0	3.5	0.4	1.3
Sum n-3 FA		2.7	1.4	0.4	1.6	3.8	0.3	4.0

3.3. Valorisation of BSFL Products

3.3.1. Value of BSFL Meals as Aquaculture Feed Ingredients

The predicted value of each BSFL meal, as feed ingredients within the aquaculture feed market, based on nutritional quality, is as follows:

- BSFL fed fish trimmings = GBP 824 per tonne
- BSFL fed sugar beet pulp = GBP 743 per tonne
- BSFL fed bakery waste = GBP 792 per tonne
- BSFL fed fruit and vegetable = GBP 792 per tonne
- BSFL fed cheese waste = GBP 787 per tonne
- BSFL fed fish feed waste = GBP 822 per tonne
- BSFL fed brewer’s grains and yeast = GBP 819 per tonne

(correct as of February 2019).

3.3.2. Quality and Value of BSFL Frass

Analyses of the BSFL frass revealed a magnesium level of 0.26% (2589 mg/kg) and an NPK of 4.9-2.6-1.7. Therefore, it would take approximately 5 tonnes of dry material or 8 tonnes of wet material (62.4% DM) to reach a maximum fertilizer application rate of 250 kg/ha of nitrogen. The BSFL frass contains high NPK levels compared to other manure fertilisers (Table 6). Utilising other fertiliser prices and NPK content (Table 7), the BSFL frass has been estimated to be worth a value of GBP 57.12/tonne. When the value of other manure fertilisers is calculated using the midpoint values for NPK taken from Table 5, the BSFL frass compares very favourable, achieving the highest value (Table 8).

Table 6. Comparison of BSFL frass NPK values with other manure fertilizers [53].

Fertiliser	Nitrogen (N) %	Phosphorus (P) %	Potassium (K) %
BSFL frass	4.9	2.6	1.7
Cow manure	0.5–2	0.2–0.7	0.4–2
Horse manure	0.7–1.5	0.2–0.7	0.6–0.8
Pig manure	0.4–2	0.5–1	0.4–1.2
Poultry manure	1.5–6	1–4	0.5–3
Sheep manure	2.2–3.6	0.3–0.6	0.7–1.7
Rabbit manure	3–4.8	1.5–2.8	1–1.3

Table 7. Cost of each nutrient (N, P and K) based on other fertiliser prices.

Fertiliser	Cost (GBP/Tonne)	Kg of Nutrient Per Tonne	Cost of Nutrient (GBP/Tonne)	Average Cost of Nutrient (GBP/kg)
Ammonium nitrite (34.5% N)	258	345	0.75	0.67
Granular Urea-standard specification (46% N)	272	460	0.59	
Muriate of Potash (MOP) (60% K_20)	283	600	0.47	0.47
Diammonium Phosphate (DAP) (46% P_2O_5)	350	460	0.76	0.71
Triple Super Phosphate (TSP) (46% P_2O_5)	302	460	0.66	

Prices correct as of October 2019 [54].

Table 8. Estimated value of BSFL frass compared to other manure fertilisers based on midpoint NPK content, taken from Table 5, and value of nutrients, taken from Table 6.

Fertiliser	Cost of Nutrient (GBP/Tonne)			Total Cost (GBP/Tonne)
	Nitrogen (N)	Phosphorus (P)	Potassium (K)	
BSFL frass	32.8	12.2	12.1	57.1
Cow manure	8.4	2.1	8.5	19.0
Horse manure	7.4	2.1	5.0	14.5
Pig manure	8.0	3.5	5.7	17.2
Poultry manure	25.1	11.8	12.4	49.3
Sheep manure	19.4	2.1	8.5	30.1
Rabbit manure	26.1	10.1	8.2	44.4

4. Discussion

The results achieved here clearly concur with that of other studies [32,55]—the nutritional profile of BSFL is highly influenced by their diet.

Protein is frequently the most expensive component of agricultural diets, especially in aquaculture diets [56]. The BSFL meals produced here are high in crude protein (>43%), except when produced using fruit and vegetable waste (36%). This level reaches as high as 51% when produced using brewer's grains. These meals are rich in the amino acids leucine, aspartic acid and glutamic acid and very rich in tyrosine, when produced with cheese waste, fish feed waste and brewer's grains, on an % DM basis. Fishmeal is considered a very high-quality protein source for aquaculture diets [57]. Compared to an average 65% (70.7% DM) seen in fishmeal [58], the quality of this BSFL meal is also high, with a well-balanced amino acid profile. However, BSFL meal contains relatively lower levels of the three common limiting amino acids, arginine, lysine and methionine, although levels of the latter two are still good; however, it is richer in histidine, isoleucine, phenylalanine, tyrosine, valine, alanine and proline, on a percentage protein basis (Table A1a). Soybean meal is the most commonly used plant protein in aquaculture feeds, despite its nutritional restrictions [59–61]. The BSFL meals are overall richer in the amino acids alanine, glycine, histidine, methionine, proline and valine, compared to high protein soybean meal [62], on a percentage protein basis (Table A1a).

The fatty acid profile is the nutritional aspect of the BSFL meal most affected by diet. The most prevalent fatty acids across all BSFL meals are lauric acid, oleic acid, palmitic acid and linoleic acid, both on a % DM (Table 3b) and % total fatty acid basis (Table A1b), although they do vary considerably, depending on the BSFL food source.

Lauric acid, the most prevalent fatty acid found in BSF meal, is credited with antimicrobial, antiviral and antifungal properties [63–65]. It has been shown to reduce *Campylobacter* spp. in broilers [66]. The other most abundant fatty acids found here also have many uses, including use in food [67], as emulsifiers in soap [68], as emollients in cosmetics [69] and as excipients in pharmaceuticals [67]. A high level of linoleic acid, which also has been credited with antimicrobial properties [70], along with the other essential FAs, is desirable in agriculture and aquaculture feed ingredients for many species.

The bioconcentration data we present indicate how efficiently each nutrient is recovered from the organic materials by BSFL treatment. The bioconcentration of each nutritional element varies with each organic feed stock. BSFL fed on fruit and vegetable waste had high EPA conversion rates; however, the final amount of EPA remained low in the BSFL meal, because the EPA levels were low in the fruit and vegetable waste material. The highest conversion rates for the essential fatty acids is seen in BSFL fed on fish trimmings, cheese waste and brewer's grains. BSFL fed fish trimmings and brewer's grains also had the highest levels of essential FAs. These food waste feed stocks, therefore, are likely the most viable that we tested for production of feed ingredients for use in aquaculture or agriculture feeds. The BSFL that were fed fish feed waste also had high levels of these essential FAs; however, the bioconcentration factor was low (viz. there was no apparent concentration during BSFL treatment), so the high levels of essential FAs were due to the high levels of these nutrients in the fish feed waste material.

These results provide evidence that manipulation of the fatty acid profile is achievable via diet. The BSFL meal that was produced, beyond use in feeds, has several possibilities for further refinement, such as lipid extraction. The high lipid content of BSFL meal, once extracted, would be suitable feedstock for biodiesel production [33]; BSFL that were fed fruit and vegetable waste generated the highest lipid levels. This meal also has the lowest crude protein level achieved here. While a detailed examination is beyond the scope of this study, we could speculate that lipid extraction would also provide an improved protein meal, as well as the richest biodiesel lipid feedstock.

Depending on the target nutrient or product and target use, we have identified several industrial and pre-consumer organic by-products that could be processed via BSFL treatment and provided data to show which of these generate high-value nutrients for feed production or other added value products, instead of simply sending this "food waste" for AD, composting, landfill, or incineration.

We have shown that all of our selected organic waste materials can be utilised as feedstock by BSFL; however, our growth and performance data indicate which are most suitable to accomplish higher levels of production of BSFL products. In all cases, waste reduction (DM basis) was achieved. Therefore, BSFL treatment could be a viable method of reducing all these pre-consumer organic waste materials, generating lower volumes of more valuable and accessible materials.

BSFL treatment, as well as being more sustainable according to the waste hierarchy, clearly provides opportunities for increased valorisation of organic by-products, especially as the frass produced from BSFL treatment is more suitable than many organic food materials for AD treatment, or, as discussed, has value as fertiliser. The value calculated here is purely based on NPK content compared to the costs of other manure fertilisers. However, when looking to source BSFL frass fertilisers, they attract a considerably higher value than that of its NPK content would suggest; Ecothrive charge is a frass soil conditioner selling at GBP 29.95 per 3.5 kg, which scales to GBP 8557.14 per tonne (correct as of April 2020 [71]), a vastly improved value from the estimated GBP 57 per tonne. BSFL frass qualities which contribute to this high value include slow release of nutrients, support of soil microbiota [72] and promotion of plant health and growth; BSFL frass has also indicated insecticidal properties against wireworm [35].

5. Conclusions

This study shows how seven organic waste by-products influence the nutritional profile of BSFL. We identify that fish feed trimmings, along with brewer's grains and yeast, are ideal organic waste materials for BSFL treatment in order to generate high quality BSFL meals which would be suitable for inclusion in aquaculture or agriculture feeds. We have also identified that fruit and vegetable waste is a potential candidate for BSFL treatment, followed by lipid extraction, for recovery and production of lipid feedstock for biodiesel due to the higher lipid content.

BSFL treatment is a viable option for recovering and recycling organic waste by-products, especially if it is traceable to source. It provides opportunity for the valorisation of these organic waste products as constituents of animal feed, providing a more environmentally friendly alternative route than landfill or AD.

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Conflicts of Interest: Mr Richard Small is the owner/CEO of Inspro Ltd.—a company that aims to become a supplier of BSFL meal. All other authors declare no conflict of interest.

Appendix A

Table A1. (**a**) Nutritional profile of BSFL meals, amino acid given as % protein, compared to an average 65% fishmeal and an average high protein soybean meal. (**b**) Nutritional profile of BSFL meals, fatty acids given as % total fatty acids, compared to an average 65% fishmeal and an average high protein soybean meal.

(**a**)

	Diet Component	**Average 65% Protein Fish-meal**	**High Protein Soy-bean Meal**	**Fish Trim-mings**	**Sugar Beet Pulp**	**Bakery Waste**	**Fruit and Veg-etable Waste**	**Cheese Waste**	**Fish Feed Waste**	**Brewer's Grains and Yeast**
Proximate analyses (% DM)	Crude Protein (% DM)	70.7	55.2	46.62	43.15	43.07	36.03	43.18	45.7	51.05
	Lipid (crude fat) (% DM)	10	1.7	35.05	35.49	37.63	40.3	36.94	35.22	27
Essential amino acids (% protein)	Arginine (Arg)	6.21	7.30	4.42	4.15	4.27	3.97	4.52	4.68	3.84
	Histidine (His)	2.50	2.7	3.07	3.11	3.04	2.80	3.01	3.15	2.62
	Isoleucine (Ile)	4.14	4.6	4.23	4.26	4.09	4.02	4.21	4.35	4.13
	Leucine (Leu)	7.17	7.7	8.26	8.30	6.48	6.33	6.60	6.89	6.46
	Lysine (Lys)	7.50	6.2	5.17	5.08	5.60	5.47	5.51	5.54	6.17
	Methionine (Met)	2.72	1.4	1.87	1.81	1.83	1.75	1.90	1.75	1.67
	Cystine (Cys)	0.86	1.6	0.62	0.63	0.77	0.80	0.53	0.48	0.72
	Phenylalanine (Phe)	3.90	5.1	3.71	3.92	4.20	4.19	4.28	4.49	4.78
	Tyrosine (Tyr)	3.04	3.5	4.35	4.43	4.55	4.11	12.23	12.54	10.66
	Threonine (Thr)	4.14	3.8	3.60	3.62	3.74	3.55	3.80	3.94	3.76
	Tryptophan (Try/Trp)	1.00	1.4	-	-	-	-	-	-	-
	Valine (Val)	4.98	4.8	5.73	5.84	6.08	5.86	6.11	6.37	5.95
Non-essential amino acids (% protein)	Alanine (Ala)	6.29	4.3	6.31	6.77	6.52	6.55	6.39	6.37	8.74
	Aspartic acid (Asp)	9.09	11.3	8.28	8.71	9.03	8.60	8.55	9.02	7.93
	Glutamic acid (Glu)	12.57	17.9	9.59	9.87	10.15	9.66	10.19	9.63	10.05
	Glycine (Gly)	6.65	4.2	5.56	5.45	5.46	5.11	5.49	5.71	5.19
	Proline (Pro)	4.34	5	5.68	5.72	5.39	5.30	6.67	6.50	6.56
	Serine (Ser)	3.89	4.6	3.93	4.01	3.95	3.69	3.91	4.00	3.80

Tryptophan was not analysed.

(**b**)

	Diet Component	**Average 65% Protein Fish-meal**	**High Protein Soy-bean Meal**	**Fish Trim-mings**	**Sugar Beet Pulp**	**Bakery Waste**	**Fruit and Veg-etable Waste**	**Cheese Waste**	**Fish Feed Waste**	**Brewer's Grains and Yeast**
Essential fatty acids (% total fatty acids)	C18:2n-6 (Linoleic acid)	2.1	54	6.53	3.61	5.61	4.09	3.44	5.68	13.22
	C18:3n-3 (α-linolenic acid)	1.9	7.2	0.94	0.51	0.80	0.89	0.62	0.80	1.56
	C20:4n-6 (Arachidonic acid)	2.4		0.40	<LOD	<LOD	0.10	0.14	0.09	0.07
	C20:5n-3 (Eicosapentaenoic acid (EPA))	9		3.42	0.25	0.11	0.20	0.49	2.21	1.96
	C22:6n-3 (Docosahexaenoic acid (DHA))	6.6		0.91	<LOD	<LOD	<LOD	<LOD	0.17	0.26

Table A1. *Cont.*

(**b**)

	Diet Component	**Average 65% Protein Fishmeal**	**High Protein Soybean Meal**	**Fish Trimmings**	**Sugar Beet Pulp**	**Bakery Waste**	**Fruit and Vegetable Waste**	**Cheese Waste**	**Fish Feed Waste**	**Brewer's Grains and Yeast**
Non-essential fatty acids (% total fatty acids)	C8:0 (Caprylic acid)			<LOD	<LOD	<LOD	<LOD	0.03	<LOD	<LOD
	C10:0 (Capric acid)			0.97	0.99	0.90	0.69	1.00	1.36	0.85
	C12:0 (Lauric acid)			35.92	57.40	52.62	38.19	33.43	47.44	26.59
	C14:0 (Myristic acid)	6	0.2	6.65	10.40	10.31	8.41	9.72	8.43	6.78
	C14:1n-5 (Myristelaidic acid)			0.17	0.25	0.21	0.69	0.84	0.23	0.22
	C15:0 (Pentadecanoic acid)			0.37	<LOD	0.11	0.20	0.60	0.17	0.30
	C16:0 (Palmitic acid)	17.8	11.2	13.44	12.09	12.60	14.19	20.01	10.25	17.04
	C16:1n-7 (Palmitoleic acid)	7.2	0.1	6.36	2.73	2.79	5.11	4.87	3.35	4.96
	C16:2n-6			0.11	<LOD	<LOD	<LOD	<LOD	0.06	<LOD
	C18:0 (Stearic acid)	3.6	3.8	1.57	1.55	1.81	1.81	2.57	1.25	2.56
	C18:1n-5			<LOD	<LOD	<LOD	<LOD	<LOD	0.06	0.04
	C18:1n-9 (Elaidic acid, Oleic acid)	12.3	23.1	15.86	8.51	10.60	22.01	18.08	11.73	15.67
	C18:1n-7 (cis-vaccenic acid)			1.20	<LOD	0.29	0.30	0.62	0.74	1.41
	C18:2n-4			<LOD	<LOD	<LOD	<LOD	0.11	0.03	<LOD
	C18:3n-6 (Gamma-linolenic acid (GLA))			0.09	<LOD	<LOD	<LOD	0.05	0.03	0.04
	C18:4n-3 (Stearidonic acid (SDA))	1.5		0.66	<LOD	<LOD	<LOD	0.03	0.62	0.63
	C20:0 (Arachidic acid)			<LOD	0.11	<LOD	<LOD	0.08	0.09	0.22
	C20:1n-8			0.26	<LOD	<LOD	<LOD	<LOD	0.68	0.74
	C20:1n-9 (Eicosenoic acid)	6.6								
	C20:1n-11 (Gadoleic acid)			1.26	<LOD	<LOD	<LOD	0.30	0.97	1.11
	C20:2n-6 (Eicosadienoic acid)			<LOD	<LOD	<LOD	<LOD	0.19	0.20	0.22
	C22:0 (Behenic acid)			<LOD	<LOD	<LOD	<LOD	0.08	0.06	0.15
	C22:1 n-9 (Erucic acid)	7.7								
	C22:1n-11 (Cetoleic acid)			<LOD	<LOD	<LOD	<LOD	<LOD	0.34	0.26
	C22:5n-3 (Docosapentaenoic acid (DPA))	2.6								

<LOD = below level of detection.

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