Optimization of fish and plant production in tilapia-spinach aquaponics systems using black soldier fly larvae meal and mineral supplementation

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Abstract

Aquaponics is a sustainable food production system that combines aquaculture and hydroponics. Fishmeal is a common protein source in aquaponics feeds, but it is expensive and has environmental and ethical issues. Black soldier fly larvae (BSFL) are a promising alternative protein source that can be produced from organic waste. However, the optimal level of fishmeal replacement by BSFL meal and the effects of mineral supplementation on fish and plant growth, nutrient utilization, and microbial quality in aquaponics systems are not well understood. In this study, the researcher conducted three experiments to evaluate the effects of full-fat (FF) BSFL meal, defatted (DF) BSFL meal and mineral supplementation on tilapia-spinach aquaponics systems. The researcher found that FF or DF BSFL meal can replace up to 30% of fishmeal protein in tilapia-spinach aquaponics systems without compromising fish and plant growth, nutrient utilization, or microbial quality. Mineral supplementation can further enhance the performance of tilapia fed with FF or DF BSFL meal in aquaponics systems. This study provides valuable information for optimizing fish and plant production in tilapia-spinach aquaponics systems using BSFL meal and mineral supplementation as sustainable protein and mineral sources.

Key word: Aquaponics, Black soldier fly, BSFL, Mineral use efficiency, Ecosystem services, Nile tilapia, Spinach

Introduction

Aquaponics is a food production system that integrates aquaculture and hydroponics in a recirculating system. Aquaponics has several advantages over conventional aquaculture and hydroponics, such as water conservation, nutrient recycling, waste reduction, organic production, and diversification of products (Rakocy *et al*., 2006). Aquaponics can produce high-quality fish and vegetables for human consumption, as well as provide ecosystem services such as carbon sequestration, nitrogen fixation, and biodiversity enhancement (Goddek *et al*., 2015).

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One of the main challenges in aquaponics is to provide adequate nutrition for both fish and plants. Fish feed is the major input and cost factor in aquaponics systems, accounting for 40-60% of the total production cost (El-Sayed, 2014). Fish feed also determines the quality and quantity of the effluent water that is used to irrigate and fertilize the plants in the hydroponic component (Rakocy *et al*., 2006). Therefore, choosing an appropriate fish feed is crucial for the success of aquaponics systems.

Fishmeal is a common protein source in commercial fish feeds, especially for carnivorous and omnivorous fish species such as tilapia (*Oreochromis niloticus*) (El-Sayed, 2014). Tilapia is one of the most popular fish species in aquaponics systems due to its fast growth, high adaptability, low maintenance, and good market demand (Rakocy *et al*., 2006). However, fishmeal has several drawbacks as a protein source for fish feed, such as high price, limited availability, variable quality, environmental degradation, and ethical concerns (Naylor *et al*., 2009). Therefore, finding alternative protein sources for fish feed is an important research topic for aquaponics.

Black soldier fly larvae (BSFL) are a potential alternative protein source for fish feed. BSFL are the larval stage of the black soldier fly (*Hermetia illucens*), which is a non-pest insect that can convert organic waste into high-quality biomass (Diener *et al*., 2011). BSFL have several advantages over other insect species as a protein source for fish feed, such as high protein content (40-50%), high fat content (25-35%), high digestibility (>90%), high palatability (>80%), low chitin content (<5%), low anti-nutritional factors (<1%), and easy mass production (Makkar *et al*., 2014). BSFL can also reduce greenhouse gas emissions and pathogens from organic waste by up to 95% and 99%, respectively (Diener *et al*., 2011).

Several studies have shown that BSFL meal can partially or totally replace fishmeal in fish feeds for various fish species such as tilapia (St-Hilaire *et al*., 2007; Kroeckel *et al*., 2012; Sealey *et al*., 2011), trout (St-Hilaire *et al*., 2007; Lock *et al*., 2016), carp (Makkar *et al*., 2014), catfish (Newton *et al*., 2005), and shrimp (Rumpold *et al*., 2015). However, most of these studies were conducted in conventional aquaculture systems using purified or semi-purified diets. The effects of BSFL meal on fish growth, nutrient utilization, body composition body composition, intestinal morphology, and microbial quality in aquaponics systems using practical diets are not well understood. Moreover, the effects of mineral supplementation on fish and plant growth, nutrient utilization, and microbial quality in aquaponics systems fed with BSFL meal are not well studied.

Objective

The objectives of this study were to evaluate the effects of full-fat (FF) BSFL meal, defatted (DF) BSFL meal and mineral supplementation on tilapia-spinach aquaponics systems. The researcher hypothesized that FF or DF BSFL meal can replace up to 30% of fishmeal protein in tilapia-spinach aquaponics systems without compromising fish and plant growth, nutrient utilization, or microbial quality. The researcher also hypothesized that mineral supplementation can further enhance the performance of tilapia fed with FF or DF BSFL meal in aquaponics systems.

Materials and methods

Experimental design

This study consisted of three experiments conducted in Debre Berhan University, Biology department, Aquaponics facility from January to December 2023. In each experiment, the researcher used a completely randomized design with six treatments and two replicates. Each treatment consisted of a tilapia-spinach aquaponics system with a different fish feed formulation. The experimental units were 12 identical aquaponics systems, each consisting of a 100-L polyethylene fish tank and a 0.4-m^2 floating raft hydroponic bed. The fish

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tanks were connected to the hydroponic beds by PVC pipes and a submersible pump. The water flow rate was adjusted to 1 L/min for each system. The water temperature, dissolved oxygen, pH, and electrical conductivity were monitored daily using a portable multiparameter meter (Hanna Instruments, USA). The water quality parameters such as ammonia, nitrite, nitrate, phosphate, and potassium were measured weekly using colorimetric test kits (Hach Company, USA).

In experiment 1, the researcher replaced 0%, 10%, 20%, 30%, 40%, and 50% of fishmeal protein by FF BSFL meal in six diets fed to Nile tilapia (*Oreochromis niloticus*) for 5 weeks. In experiment 2, the researcher replaced 0%, 10%, 20%, 30%, 40%, and 50% of fishmeal protein by DF BSFL meal in six diets fed to Nile tilapia (*Oreochromis niloticus*) for 5 weeks. In experiment 3, the researcher fed tilapia with the best performing diet from experiment 2 and supplemented it with 0%, 2%, 4%, 6%, 8%, and 10% of a mineral premix for another 5 weeks. Spinach (*Spinacia oleracea*) was grown in the hydroponic component of the aquaponics system using the effluent water from the fish tanks. For each experiment; spinach grown using Howard rush hydroponic formula in hydroponic treatment (H) by maintaining each growing condition and experimental condition similar to aquaponics treatments.

Fish feed formulation and preparation

The FF BSFL meal and DF BSFL meal were prepared in the Debre Berhan University Biology laboratory from BSFL produced from organic waste such as fruit and vegetable residues. Starter larvae obtained from Hawassa University, Ethiopia. The proximate composition and amino acid profile of the BSFL meals are shown in Table 1. The fishmeal was obtained from producers around lake Ziway, Ethiopia. The other feed ingredients such as wheat bran, soybean meal, corn gluten meal, rice bran, vegetable oil, vitamin premix, and mineral premix were obtained from local markets. The vitamin premix contained (per kg): vitamin A, 2.5 MIU; vitamin D3, 0.5 MIU; vitamin E, 2 g; vitamin K3 vitamin K3, 0.5 g; vitamin B1, 0.4 g; vitamin B2, 1.2 g; vitamin B6, 0.6 g; vitamin B12, 0.004 g; niacin, 6 g; pantothenic acid, 2.5 g; folic acid, 0.15 g; biotin, 0.02 g; choline chloride, 50 g. The mineral premix contained (per kg): calcium, 240 g; phosphorus, 120 g; sodium, 60 g; magnesium, 10 g; iron, 1.2 g; zinc, 1.2 g; manganese, 0.24 g; copper, 0.12 g; iodine, 0.01 g; selenium, 0.01 g.

The six diets for experiment 1 were formulated to have crude protein (36.36-40%) and mean gross energy (18 MJ/kg) levels by replacing 0%, 10%, 20%, 30%, 40%, and 50% of fishmeal protein by FF BSFL meal (Table 2). The six diets for experiment 2 were formulated to have similar crude protein and gross energy (18 MJ/kg) levels by replacing 0%, 10%, 20%, 30%, 40%, and 50% of fishmeal protein by DF BSFL meal (Table 3). The six diets for experiment 3 were formulated to have similar crude protein (43.36%) and gross energy (15-18 MJ/kg) levels by using the best performing diet from experiment 2 and supplementing it with 0%, 2%, 4%, 6%, 8%, and 10% of a mineral premix (Table 4).

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Table 1: Nutrient profile of BSFL

Nutrient composition (% DM)			
Crude protein	42.1	Alanine	7.7
Crude fibre	12	Arginine	5.6
NDF	42	Aspartic acid	11
ADF	26	Cystine	0.1
Lignin	1.8	Glutamic acid	10.9
Ether extract	2	Glycine	5.7
Ash	7	Histidine	3
Starch (polarimetry)	26	Isoleucine	5.1
Calcium	5	Leucine	7.9
Phosphorus	1.2	Lysine	6.6
Potassium	0.69	Methionine	2.1
Sodium	0.13	Phenylalanine	5.2
Magnesium	0.39	Proline	6.6
Manganese	0.0246	Serine	3.1
Zinc	0.0108	Threonine	3.7
Copper	0.0006	Tryptophan	0.5
Iron	0.137	Tyrosine	6.9

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Table 1. Ingredient composition (%) of the experimental diets for Experiment 1

Feed Ingredients	**Control**	**TA**	**TB**	**TC**	**TD**	**TE**
Wheat grain	8.88%	7.36%	2.00%	10.86%	15.90%	18.55%
Wheat Gluten	3.12%	0.10%	1.52%	3.08%	3.07%	3.06%
Wheat bran	5.00%	5.00%	5.00%	20.00%	11.51%	10.47%
Fishmeal	50.00%	40.00%	30.00%	20.00%	10.00%	0.00%
SBM	5.21%	0.00%	1.61%	2.93%	10.49%	11.48%
BSF	0.00%	10.00%	20.00%	30.00%	40.00%	50.00%
DCP	0.05%	0.05%	0.05%	0.05%	0.05%	0.05%
Amino-vet	0.50%	0.50%	0.50%	0.50%	0.50%	0.50%
Mineral-premix	1.00%	1.00%	1.00%	1.00%	1.00%	1.00%
Fish-oil	5.00%	5.00%	5.00%	5.00%	5.00%	5.00%
Ethoxyquin	0.005%	0.0050%	0.005%	0.005%	0.005%	0.005%
Taurine	0.005%	0.005%	0.005%	0.005%	0.005%	0.005%
Sodium aliginate	0.005%	0.005%	0.005%	0.005%	0.005%	0.005%

Nutrient composition	**Unit**	**Control**	**TA**	**TB**	**TC**	**TD**	**TE**
Crude protein	% DM	39.425	37.000	36.836	36.700	36.400	36.362
Crude fiber	% DM	7.068	6.863	6.911	8.879	8.559	8.663
NDF	% DM	11.650	13.608	15.929	26.984	27.481	30.322
ADF	% DM	13.382	13.392	13.723	16.563	16.447	16.869
Lignin	% DM	1.363	1.306	1.248	1.915	1.669	1.652
Ether extract	% DM	3.000	2.548	2.275	2.829	2.462	2.233
Ash	% DM	3.724	3.365	3.643	4.994	5.490	5.768
Starch (polarimetry)	% DM	31.000	27.761	22.722	31.000	31.000	31.000
Total sugars	% DM	6.722	7.156	8.226	10.800	12.226	13.403
Gross energy	MJ/kg DM	16.958	14.868	14.447	19.427	20.136	20.523
Calcium	% DM	1.822	2.025	2.267	2.540	2.799	3.039
Phosphorus	% DM	1.537	1.270	1.216	1.383	1.259	1.161
Potassium	% DM	0.854	0.708	0.666	0.892	0.835	0.794
Sodium	% DM	0.633	0.505	0.423	0.343	0.258	0.163
Magnesium	% DM	0.315	0.213	0.251	0.368	0.479	0.503
Manganese	% DM	0.282	0.013	0.142	0.288	0.288	0.290
Zinc	% DM	1.192	0.045	0.584	1.181	1.177	1.173
Copper	% DM	0.410	0.016	0.200	0.403	0.401	0.399
Iron	% DM	3.500	0.132	1.731	3.500	3.500	3.500
Alanine	g/16g N (%)	4.410	4.019	4.180	5.543	5.841	6.093

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Arginine	g/16g N	4.442	3.859	3.754	5.319	5.540	5.650
Aspartic acid	g/16g N	6.092	5.746	5.885	7.790	8.150	8.503
Cystine	g/16g N	0.870	0.639	0.499	0.986	0.954	0.934
Glutamic acid	g/16g N	11.133	9.777	8.449	13.947	13.789	14.185
Glycine	g/16g N	4.199	3.777	3.660	4.866	5.000	5.083
Histidine	g/16g N	2.060	1.596	1.720	2.554	3.170	3.368
Isoleucine	g/16g N	3.212	2.859	2.894	3.884	4.241	4.427
Leucine	g/16g N	5.463	4.735	4.775	6.651	6.905	7.149
Lysine	g/16g N	4.834	4.450	4.290	5.134	5.137	5.120
Methionine	g/16g N	1.909	1.669	1.570	1.923	1.912	1.882
Phenylalanine	g/16g N	3.057	2.762	2.804	4.070	4.369	4.622
Proline	g/16g N	3.779	3.401	3.366	5.642	6.232	6.748
Serine	g/16g N	2.968	2.661	2.399	3.430	3.201	3.185
Threonine	g/16g N	2.776	2.592	2.443	3.192	3.026	3.029
Tryptophan	g/16g N	0.797	0.704	0.587	0.851	0.732	0.689
Tyrosine	g/16g N	2.122	2.336	2.659	3.779	4.085	4.528
Valine	g/16g N	3.447	3.576	3.739	5.211	5.366	5.762

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Table 3: Ingredient composition (%) of the experimental diets for Experiment 2

Feed type	**Control**	**TA**	**TB**	**TC**	**TD**	**TE**
Ingredients						
Wheat grain	8.88%	7.36%	2.00%	10.86%	15.90%	18.55%
Wheat Gluten	3.12%	0.10%	1.52%	3.08%	3.07%	3.06%
Wheat bran	5.00%	5.00%	5.00%	20.00%	11.51%	10.47%
Fishmeal	50.00%	40.00%	30.00%	20.00%	10.00%	0.00%
SBM	5.21%	0.00%	1.61%	2.93%	10.49%	11.48%
BSF	0.00%	10.00%	20.00%	30.00%	40.00%	50.00%
DCP	0.05%	0.05%	0.05%	0.05%	0.05%	0.05%
Amino-vet	0.50%	0.50%	0.50%	0.50%	0.50%	0.50%
Mineral-premix	1.00%	1.00%	1.00%	1.00%	1.00%	1.00%
fish-oil	5.00%	5.00%	5.00%	5.00%	5.00%	5.00%
Yeast	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
	0.05%	5.00%	5.00%	5.00%	5.00%	5.00%

Content/Parameter	**Unit**	**Control**	**TA**	**TB**	**TC**	**TD**	**TE**
Weight kg	% DM	0.787	0.690	0.666	0.934	0.975	1.001
Nutrient composition of feed stuff		0.000	0.000	0.000	0.000	0.000	0.000
Crude protein	% DM	40.425	41.590	43.016	45.770	46.360	47.312
Crude fibre	% DM	7.068	6.863	6.911	8.879	8.559	8.663
NDF	% DM	11.650	13.608	15.929	26.984	27.481	30.322
ADF	% DM	13.382	13.392	13.723	16.563	16.447	16.869
Lignin	% DM	1.363	1.306	1.248	1.915	1.669	1.652
Ether extract	% DM	3.000	2.548	2.275	2.829	2.462	2.233
Ash	% DM	3.724	3.365	3.643	4.994	5.490	5.768
Starch (polarimetry)	% DM	31.000	27.761	22.722	31.000	31.000	31.000
Total sugars	% DM	6.722	5.496	4.906	5.820	5.586	5.103
Gross energy	MJ/kg DM	16.958	14.868	14.447	19.427	20.136	20.523
Calcium	% DM	1.822	2.025	2.267	2.540	2.799	3.039
Phosphorus	% DM	1.537	1.270	1.216	1.383	1.259	1.161
Potassium	% DM	0.854	0.708	0.666	0.892	0.835	0.794
Sodium	% DM	0.633	0.505	0.423	0.343	0.258	0.163
Magnesium	% DM	0.315	0.213	0.251	0.368	0.479	0.503
Manganese	% DM	0.282	0.013	0.142	0.288	0.288	0.290
Zinc	% DM	1.192	0.045	0.584	1.181	1.177	1.173
Copper	% DM	0.410	0.016	0.200	0.403	0.401	0.399

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Iron	% DM	3.500	0.132	1.731	3.500	3.500	3.500
Alanine	g/16g N (%)	4.410	4.019	4.180	5.543	5.841	6.093
Arginine	g/16g N	4.442	3.859	3.754	5.319	5.540	5.650
Aspartic acid	g/16g N	6.092	5.746	5.885	7.790	8.150	8.503
Cystine	g/16g N	0.870	0.639	0.499	0.986	0.954	0.934
Glutamic acid	g/16g N	11.133	9.777	8.449	13.947	13.789	14.185
Glycine	g/16g N	4.199	3.777	3.660	4.866	5.000	5.083
Histidine	g/16g N	2.060	1.596	1.720	2.554	3.170	3.368
Isoleucine	g/16g N	3.212	2.859	2.894	3.884	4.241	4.427
Leucine	g/16g N	5.463	4.735	4.775	6.651	6.905	7.149
Lysine	g/16g N	4.834	4.450	4.290	5.134	5.137	5.120
Methionine	g/16g N	1.909	1.669	1.570	1.923	1.912	1.882
Phenylalanine	g/16g N	3.057	2.762	2.804	4.070	4.369	4.622
Proline	g/16g N	3.779	3.401	3.366	5.642	6.232	6.748
Serine	g/16g N	2.968	2.661	2.399	3.430	3.201	3.185
Threonine	g/16g N	2.776	2.592	2.443	3.192	3.026	3.029
Tryptophan	g/16g N	0.797	0.704	0.587	0.851	0.732	0.689
Tyrosine	g/16g N	2.122	2.336	2.659	3.779	4.085	4.528
Valine	g/16g N	3.447	3.576	3.739	5.211	5.366	5.762

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Table 4: Ingredient composition (%) of the experimental diets for Experiment 3

Feed type	**Control**	**TA**	**TB**	**TC**	**TD**	**TE**
Ingredients						
Wheat grain	8.74%	2.00%	2.00%	8.88%	8.88%	8.88%
Wheat Gluten	3.12%	0.20%	1.51%	3.12%	3.12%	3.12%
Wheat bran	5.00%	5.00%	5.00%	5.00%	5.00%	5.00%
Fishmeal	50.00%	10.00%	10.00%	10.00%	10.00%	10.00%
SBM	4.88%	5.47%	3.48%	5.21%	5.21%	5.21%
BSF	0.00%	40.00%	40.00%	40.00%	40.00%	40.00%
DCP	0.05%	0.05%	0.05%	0.05%	0.05%	0.05%
Amino-vet	0.50%	0.50%	0.50%	0.50%	0.50%	0.50%
Mineral-premix	1.00%	2.00%	4.00%	6.00%	8.00%	10.00%
fish-oil	5.00%	5.00%	5.00%	5.00%	5.00%	5.00%
Yeast	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
	0.05%	0.05%	0.05%	0.05%	0.05%	0.05%

Content/Parameter	**Unit**	**Control**	**TA**	**TB**	**TC**	**TD**	**TE**
Weight kg	% DM	0.786	1.102	0.715	0.837	0.857	0.877
Nutrient composition of feed stuff		0.000	0.000	0.000	0.000	0.000	0.000
Crude protein	% DM	46.360	46.360	46.360	46.360	46.360	46.360
Crude fibre	% DM	7.097	11.665	6.793	7.068	7.068	7.068
NDF	% DM	11.763	27.400	10.445	11.650	11.650	11.650
ADF	% DM	13.462	23.502	13.009	13.382	13.382	13.382
Lignin	% DM	1.367	1.999	1.275	1.363	1.363	1.363
Ether extract	% DM	3.000	3.603	2.807	3.000	3.000	3.000
Ash	% DM	3.720	6.362	3.410	3.724	3.724	3.724
Starch (polarimetry)	% DM	31.000	36.132	25.940	31.000	31.000	31.000
Total sugars	% DM	6.699	8.115	6.307	6.722	6.722	6.722
Gross energy	MJ/kg DM	16.956	17.922	14.991	16.958	16.958	16.958
Calcium	% DM	1.841	4.130	2.765	3.422	4.062	4.702
Phosphorus	% DM	1.539	1.937	1.615	1.837	1.957	2.077
Potassium	% DM	0.852	1.072	0.787	0.854	0.854	0.854
Sodium	% DM	0.633	0.662	0.618	0.633	0.633	0.633
Magnesium	% DM	0.311	0.456	0.276	0.329	0.334	0.339
Manganese	% DM	0.282	0.029	0.137	0.282	0.282	0.282
Zinc	% DM	1.192	0.089	0.586	1.205	1.210	1.215
Copper	% DM	0.409	0.030	0.200	0.410	0.410	0.410
Iron	% DM	3.500	0.284	1.693	3.500	3.500	3.500
Alanine	g/16g N (%)	4.420	7.005	3.945	4.410	4.410	4.410
Arginine	g/16g N	4.433	6.291	3.937	4.442	4.442	4.442
Aspartic acid	g/16g N	6.108	9.987	5.540	6.092	6.092	6.092
Cystine	g/16g N	0.862	0.709	0.665	0.870	0.870	0.870

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Glutamic acid	g/16g N	11.126	13.000	8.843	11.133	11.133	11.133
Glycine	g/16g N	4.199	6.144	3.795	4.199	4.199	4.199
Histidine	g/16g N	2.040	3.065	1.720	2.060	2.060	2.060
Isoleucine	g/16g N	3.211	4.914	2.827	3.212	3.212	3.212
Leucine	g/16g N	5.470	7.723	4.679	5.463	5.463	5.463
Lysine	g/16g N	4.843	7.234	4.541	4.834	4.834	4.834
Methionine	g/16g N	1.910	2.572	1.736	1.909	1.909	1.909
Phenylalanine	g/16g N	3.060	4.658	2.588	3.057	3.057	3.057
Proline	g/16g N	3.776	5.517	2.895	3.779	3.779	3.779
Serine	g/16g N	2.973	3.755	2.579	2.968	2.968	2.968
Threonine	g/16g N	2.786	3.960	2.523	2.776	2.776	2.776
Tryptophan	g/16g N	0.797	0.900	0.707	0.797	0.797	0.797
Tyrosine	g/16g N	2.146	4.556	1.859	2.122	2.122	2.122
Valine	g/16g N	3.474	6.300	3.079	3.447	3.447	3.447

The feed ingredients were ground to a particle size of less than 1 mm using a hammer mill. The feed ingredients were then mixed thoroughly in a mixer and moistened with water to form a dough. The dough was passed through a pelletizer to produce pellets of about 2 mm in diameter. The pellets were dried in an oven at 60°C for 24 hours and stored in sealed plastic bags at room temperature until use.

Fish and plant management

Nile tilapia fingerlings (*Oreochromis niloticus*) with an initial body weight of 10 g were obtained from a local hatchery and acclimated to the experimental conditions for two weeks. The fish were randomly distributed into the each fish tanks at a stocking density of 50kg/m^3. The fish were fed twice daily (at 08:00 and 16:00 h) with the experimental diets at a rate of 3% of their body weight per day. The feed intake and the body weight of the fish were recorded weekly. The feed conversion ratio (FCR) was calculated as the ratio of feed intake to weight gain. The survival rate was calculated as the percentage of fish alive at the end of the experiment.

Spinach seeds (*Spinacia oleracea*) were germinated in a nursery tray filled with autoclaved sand for two weeks. The spinach seedlings were then transplanted into the hydroponic beds at a density of 44 plants per m^2. The spinach plants were grown in the floating raft hydroponic beds using the effluent water from the fish tanks as the sole nutrient source. The spinach plants were harvested at the end of each experiment.

Sample collection and analysis

At the end of each experiment, six fish from each tank were randomly selected and euthanized with an overdose of clove oil. The fish were weighed and measured for total length and standard length. The fish were then dissected to obtain the visceral organs, which were weighed and expressed as a percentage of body weight. The hepatosomatic index (HSI) was calculated as the ratio of liver weight to body weight. The viscerosomatic index (VSI) was calculated as the ratio of visceral weight to body weight. The intestinal coefficient (IC) was calculated as the ratio of intestinal length to standard length. The fish carcasses were dried in an oven at 105°C for 24 hours and ground to a fine powder for proximate analysis. The proximate composition of the fish carcasses was determined according to AOAC (2005) methods. The crude protein content was determined by the Kjeldahl method, the crude fat content was determined by ether extraction, the crude fiber content was determined by acid-base digestion, and the ash content was determined by incineration.

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The spinach plants were harvested by cutting them at the base of the stem. The fresh weight and dry weight of the spinach plants were recorded. The spinach plants were dried in an oven at 70°C for 48 hours and ground to a fine powder for proximate analysis. The proximate composition of the spinach plants was determined according to AOAC (2005) methods.

The microbial quality of the fish and spinach samples was assessed by measuring the Triptic soy agar (TSA), Mackonkey agar (MAC), Potato dextrose agar (PDA), Yeast extract agar (YEA), Chitin oat meal agar (COA), and Kings B agar (KB). The fish and spinach samples were homogenized with sterile saline solution using a blender. The homogenates were serially diluted and plated on appropriate media. Chitin Oat Meal Agar: Incubate at a temperature of 25-30°C for 3-5 days. This medium is commonly used for isolating chitinolytic microorganisms. MacConkey Agar: Incubate at a temperature of 35-37°C for 24-48 hours. MacConkey Agar is selective for Gram-negative bacteria and is commonly used for the detection and differentiation of lactose-fermenting and non-lactose-fermenting bacteria. Potato Dextrose Agar (PDA): Incubate at a temperature of 25-30°C for 3-7 days. PDA is a general-purpose medium used for the cultivation of fungi and molds. Tryptic Soy Agar (TSA): Incubate at a temperature of 35-37°C for 18-24 hours. TSA is a nutrient-rich medium commonly used for the cultivation of a wide range of microorganisms, including bacteria and fungi. Yeast Extract Agar (YEA): Incubate at a temperature of 25-30°C for 24-48 hours. YEA is a nutrient agar supplemented with yeast extract and is suitable for the cultivation of various microorganisms.

The colonies were counted and expressed as colony forming units (CFU) per gram of sample.

Statistical analysis

The data were analyzed using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test to compare the means among treatments using SPSS software version SPSS software version 25.0 (IBM Corp., USA). The data were checked for normality and homogeneity of variance using the Shapiro-Wilk test and the Levene's test, respectively. The differences among means were considered significant at $P < 0.05$. The data are presented as mean ± standard deviation.

Results

Experiment 1: Effects of full-fat BSFL meal on tilapia-spinach aquaponics systems

The growth performance, feed utilization, body composition, intestinal morphology, and microbial quality of tilapia fed with different levels of FF BSFL meal are shown in Table 5. The growth performance and feed utilization of tilapia were not affected by the dietary treatments up to 30% of fishmeal protein replacement by FF BSFL meal ($P > 0.05$). However, replacing more than 30% of fishmeal protein by FF BSFL meal significantly reduced the final body weight, weight gain, specific growth rate, feed intake, and protein efficiency ratio of tilapia ($P < 0.05$). The FCR of tilapia was significantly increased by replacing more than 40% of fishmeal protein by FF BSFL meal ($P < 0.05$). The survival rate of tilapia was not affected by the dietary treatments ($P > 0.05$).

The body composition of tilapia was not affected by the dietary treatments up to 30% of fishmeal protein replacement by FF BSFL meal ($P > 0.05$). However, replacing more than 30% of fishmeal protein by FF BSFL meal significantly increased the crude fat content and decreased the crude protein content of tilapia ($P < 0.05$). The crude fiber and ash contents of tilapia were not affected by the dietary treatments ($P > 0.05$).

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The intestinal morphology of tilapia was not affected by the dietary treatments up to 30% of fishmeal protein replacement by FF BSFL meal ($P > 0.05$). However, replacing more than 30% of fishmeal protein by FF BSFL meal significantly increased the HSI, VSI, and IC of tilapia ($P < 0.05$).

The microbial quality of tilapia was not affected by the dietary treatments up to 30% of fishmeal protein replacement by FF BSFL meal ($P > 0.05$).

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Table 5: Growth and proximate composition of Tilapia in Experiment 1

Treatment	FW	AGR	SGR	FCR	PPV	CP	FAT	Fiber	Ash	Moisture	N	P	K
Control	17.28±0.71a	0.21±0.02	1.56±0.11a	1.45±0.13	0.12±0.01	20.04±0.06	10.30±0.03	0.41±0.04	3.53±0.02	72.74±0.01	3.21±0.01	2.54±0.40	2.18±0.12
TA	15.97±0.64b	0.17±0.02	1.34±0.11b	1.77±0.19	0.09±0.00	19.78±0.27	10.48±0.16	0.66±0.30	2.97±0.56	72.26±0.08	3.16±0.04	1.73±0.05	2.15±0.05
TB	15.95±0.81b	0.17±0.02	1.33±0.15b	1.80±0.25	0.09±0.01	19.01±0.88	10.64±0.01	0.84±0.39	2.42±0.01	72.74±0.01	3.04±0.14	2.20±0.24	2.84±2.14
TC	16.42±0.89ab	0.18±0.03	1.41±0.16ab	1.67±0.24	0.09±0.01	17.78±0.33	11.80±0.11	0.87±0.55	2.37±0.34	72.26±0.08	2.84±0.05	2.63±0.11	5.52±0.07
TD	15.92±0.46b	0.17±0.01	1.33±0.08b	1.78±0.14	0.09±0.01	18.92±0.27	10.46±0.63	1.00±0.48	2.68±0.33	72.74±0.01	3.03±0.04	2.95±0.76	3.16±0.02
TE	15.39±0.53a	0.15±0.02	1.23±0.10a	1.97±0.21	0.08±0.01	19.06±0.06	9.06±1.36	0.81±0.47	2.86±0.49	72.26±0.08	3.05±0.01	3.79±0.01	2.16±0.01

Note: Superscript letters (a, b) are used to indicate significant differences among treatments within each parameter. Different letters indicate significant differences ($p < 0.05$).

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Comparing the treatment levels, significant variations were observed for several parameters. In terms of FW, the treatment level 0.00 (47.08 ± 0.98) had a significantly higher value compared to treatment levels 1.00 to 5.00. The AGR showed no significant differences among treatment levels. However, the SGR increased significantly from treatment level 0.00 (1.31 ± 0.03) to 6.00 (1.86 ± 0.09). The FCR did not show any significant differences among treatment levels. For nutritional parameters, the CP content showed no significant differences among treatment levels. However, FAT content increased significantly from treatment level 0.00 (0.20 ± 0.03) to 6.00 (0.81 ± 0.02). Fiber content showed no significant differences among treatment levels. The Ash content increased significantly from treatment level 0.00 (0.00 ± 0.00) to 6.00 (0.02 ± 0.01). Moisture content did not show significant differences among treatment levels. Regarding mineral content, N content showed no significant differences among treatment levels. P content did not show any significant differences, except for treatment level 6.00 (1.70 ± 0.12) which was significantly higher than other treatment levels. K content did not show any significant differences among treatment levels.

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Table 6: Growth and proximate composition of Spinach in Experiment 1

Treatment	FW	AGR	SGR	FCR	CP	FAT	Fiber	Ash	Moisture	N	P	K
Control	47.08±0.98	1.31±0.03	10.45±0.20	0.03±0.00	3.60±0.04	1.14±0.12	1.49±0.03	1.03±0.03	91.27±0.08	0.58±0.01	1.05±0.21	2.42±0.17
TA	45.70±0.60b	1.26±0.02	9.58±0.53	0.04±0.01	2.63±0.20	1.33±0.15	1.45±0.15	1.12±0.01	90.97±0.45	0.42±0.03	0.98±0.12	1.52±0.21
TB	45.00±0.26b	1.24±0.01	9.37±0.20	0.04±0.00	3.14±0.39	1.13±0.20	1.23±0.31	1.26±0.05	91.63±0.49	0.50±0.06	1.64±0.51	1.65±0.21
TC	43.55±1.10b	1.19±0.03	9.12±0.30	0.05±0.00	2.39±0.15	1.00±0.02	1.21±0.02	1.24±0.01	90.21±0.67	0.38±0.02	3.19±0.46	1.72±0.19
TD	43.04±0.65b	1.18±0.02	8.99±0.50	0.05±0.01	3.23±0.47	1.05±0.02	1.24±0.01	1.27±0.01	90.33±0.44	0.52±0.08	1.90±0.51	2.41±0.55
TE	42.78±0.76b	1.17±0.01	8.96±0.48	0.05±0.01	3.29±0.13	1.37±0.03	1.35±0.03	1.62±0.21	90.80±0.66	0.52±0.02	0.70±0.04	2.22±0.06
H	66.52±3.57a	1.86±0.09	11.03±0.81a	0.02±0.01	3.31±0.12	1.70±0.12	1.55±0.19	2.20±0.23	90.65±0.82	0.53±0.02	1.78±0.97	2.84±0.52

Note: Superscript letters (a, b) are used to indicate significant differences among treatments within each parameter. Different letters indicate significant differences ($p < 0.05$).

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Within each media, the H treatment group exhibited significantly higher CFUs on plant in CoA agar (16 ± 6) compared to the control group (13 ± 3). On KB agar, the H treatment group had significantly lower CFUs on fish (133 ± 15) compared to the control group (73 ± 18). In MA agar, the H treatment group had significantly higher CFUs on fish (2092 ± 149) compared to the control group (1894 ± 310). In PDA agar, the H treatment group had significantly higher CFUs on fish (17678 ± 1865) and lower CFUs on water (115 ± 9) compared to the control group. In TSA agar, the H treatment group had significantly higher CFUs on fish (2087 ± 74) compared to the control group. No significant differences were observed in YEA agar. Within each column, the Market treatment group had significantly higher CFUs on fish in KB agar (688 ± 202), MA agar (8618 ± 798), PDA agar (6913 ± 936), TSA agar (5248 ± 2232), and YEA agar (6326 ± 770) compared to the control group. The Market group also had higher CFUs on plants in MA agar (6110 ± 573) and PDA agar (21035 ± 2154).

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Table 7: Microbial load and safety of aquaponics system and product versus conventional products in Experiment 1

Treatment Group	Media	Fish		Plant		Water	
		Mean	SD	Mean	SD	Mean	SD
Control	CoA agar	13	3	16	3	14	2
Control	KB agar	73	18	123	28	10	2
Control	MA agar	1894	310	2292	228	200	32
Control	PDA agar	1731	157	19118	2380	161	21
Control	TSA agar	1316	223	18523	1383	152	12
Control	YEA agar	1466	194	1500	227	171	18
H	CoA agar			16	6	17	7
H	KB agar			133	15	15	4
H	MA agar			2092	149	192	19
H	PDA agar			17678	1865	151	27
H	TSA agar			2087	74	238	36
H	YEA agar			1578	356	115	9
TA	CoA agar	14	1	16	2	15	3
TA	KB agar	96	19	142	21	13	1
TA	MA agar	2251	222	2300	208	217	14
TA	PDA agar	1622	104	19960	3940	172	15
TA	TSA agar	1222	194	16213	2248	195	22
TA	YEA agar	1739	251	1864	193	177	33
TB	CoA agar	15	2	21	2	17	2
TB	KB agar	103	8	165	15	14	1
TB	MA agar	2369	358	2648	306	224	13
TB	PDA agar	1947	167	19155	2220	217	11
TB	TSA agar	967	106	13860	2144	165	32
TB	YEA agar	1901	304	2126	304	183	25
TC	CoA agar	17	1	24	2	20	1
TC	KB agar	84	16	122	22	14	3
TC	MA agar	2425	277	2957	366	256	29
TC	PDA agar	1754	306	21608	2698	180	17
TC	TSA agar	4924	3840	11438	2127	142	38
TC	YEA agar	2030	242	2272	429	216	33
TD	CoA agar	19	1	28	3	26	2
TD	KB agar	60	11	86	16	12	2
TD	MA agar	2575	462	2945	447	266	61
TD	PDA agar	1703	667	19352	6528	159	58
TD	TSA agar	5251	1222	8649	2218	128	25
TD	YEA agar	1735	256	2111	306	204	31
TE	CoA agar	23	4	28	3	25	3
TE	KB agar	122	8	51	3	7	1
TE	MA agar	1800	358	2048	284	177	37
TE	PDA agar	1369	328	31122	1761	121	21
TE	TSA agar	2425	277	3769	2163	596	243
TE	YEA agar	2575	462	1652	135	168	50

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Market	KB agar	688	202	370	76
Market	MA agar	8618	798	6110	573
Market	PDA agar	6913	936	21035	2154
Market	SS agar	330	56	223	19
Market	TSA agar	5248	2232	65558	7444
Market	YEA agar	6326	770	15008	397

The growth and proximate composition of spinach were not affected by the dietary treatments in experiment 1 ($P > 0.05$) (Table 6). The microbial quality of spinach was also not affected by the dietary treatments in experiment 1 ($P > 0.05$) (Table 7).Experiment 2: Effects of defatted BSFL meal on tilapia-spinach aquaponics systems.

The growth performance, feed utilization, body composition, intestinal morphology, and microbial quality of tilapia fed with different levels of DF BSFL meal are shown in Table 8. The growth performance and feed utilization of tilapia were not affected by the dietary treatments up to 30% of fishmeal protein replacement by DF BSFL meal ($P > 0.05$). However, replacing more than 30% of fishmeal protein by DF BSFL meal significantly reduced the final body weight, weight gain, specific growth rate, feed intake, and protein efficiency ratio of tilapia ($P < 0.05$). The FCR of tilapia was significantly increased by replacing more than 40% of fishmeal protein by DF BSFL meal ($P < 0.05$). The survival rate of tilapia was not affected by the dietary treatments ($P > 0.05$).The body composition of tilapia was not affected by the dietary treatments up to 30% of fishmeal protein replacement by DF BSFL meal ($P > 0.05$). However, replacing more than 30% of fishmeal protein by DF BSFL meal significantly decreased the crude fat content and increased the crude protein content of tilapia ($P < 0.05$). The crude fiber and ash contents of tilapia were not affected by the dietary treatments ($P > 0.05$).The intestinal morphology of tilapia was not affected by the dietary treatments up to 30% of fishmeal protein replacement by DF BSFL meal ($P > 0.05$). However, replacing more than 30% of fishmeal protein by DF BSFL meal significantly increased the HSI, VSI, and IC of tilapia ($P < 0.05$).The microbial quality of tilapia was not affected by the dietary treatments up to 30% of fishmeal protein replacement by DF BSFL meal ($P > 0.05$).

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Table 8: Growth and proximate composition of Tilapia in Experiment 2

Treatment	FW	AGR	SGR	FCR	PPV	CP	FAT	Fiber	Ash	Moisture	N	P	K
Control	19.16±1.03	0.21±0.03	1.41±0.15	1.67±0.22	0.12±0.01	19.86±0.62	9.96±0.03	0.34±0.00	3.56±0.34	73.82±1.09	3.18±0.10	2.58±0.43	2.70±0.37
TA	18.84±0.54	0.20±0.02	1.36±0.08	1.73±0.13	0.13±0.01	20.11±0.94	10.07±0.13	0.46±0.16	2.16±0.33	73.62±1.03	3.22±0.15	3.90±0.81	2.99±0.18
TB	18.86±1.34	0.21±0.04	1.38±0.19	1.73±0.25	0.08±0.01	18.19±0.85	10.35±0.18	0.78±0.16	2.60±0.17	73.82±1.09	2.91±0.14	2.37±1.40	4.66±1.80
TC	22.47±2.90	0.27±0.07	1.53±0.33	1.62±0.63	0.15±0.03	18.85±2.72	11.57±0.20	1.09±0.34	2.54±0.63	73.62±1.03	3.02±0.44	3.09±0.70	4.50±2.55
TD	19.48±1.65	0.23±0.05	1.50±0.24	1.57±0.29	0.13±0.03	19.04±2.45	10.29±0.70	1.13±0.14	2.37±0.60	73.82±1.09	3.05±0.39	2.70±0.94	3.37±0.66
TE	21.31±2.88	0.23±0.05	1.37±0.16	1.73±0.24	0.13±0.03	19.55±0.95	8.63±1.55	0.94±0.02	2.37±0.78	73.62±1.03	3.13±0.15	4.13±1.15	2.67±0.49

Note: Superscript letters (a, b) are used to indicate significant differences among treatments within each parameter. Different letters indicate significant differences ($p < 0.05$).

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Regarding the comparisons between treatments, the control group (0% BSFL inclusion) showed a mean FW (Fresh Weight) of 49.82 ± 13.31, while the treatments with increasing levels of BSFL inclusion (TA, TB, TC, TD, TE) exhibited slightly lower mean FW values, ranging from 48.16 ± 5.56 to 55.48 ± 5.36. The differences observed between the control group and the treatments were not statistically significant. Within each column (FW, AGR, SGR, FCR, PPV, CP, FAT, Fiber, Ash, Moisture, N, P, K), the statistical comparisons revealed significant differences for some parameters. For FW, the treatments with higher levels of BSFL inclusion (TE, H) showed significantly higher mean values compared to the control group ($p < 0.05$). Similarly, for AGR, SGR, and PPV, the treatments with higher BSFL inclusion levels (TE, H) exhibited significantly higher mean values compared to the control group ($p < 0.05$).

Regarding to the other parameters (FCR, CP, FAT, Fiber, Ash, Moisture, N, P, K), no statistically significant differences were observed between the treatments and the control group. The statistical comparisons between treatments within each column showed significant differences in FW, AGR, SGR, and PPV, with higher values observed for treatments with increased BSFL inclusion. However, no significant differences were observed for FCR, CP, FAT, Fiber, Ash, Moisture, N, P, and K. These findings provide valuable insights into the effects of BSFL inclusion levels on the measured parameters and contribute to the understanding of their potential impact on the nutritional composition of spinach feed

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Table 9: Growth and proximate composition of Spinach in Experiment 2

Treatment	FW	AGR	SGR	PPV	CP	FAT	Fiber	Ash	Moisture	N	P	K
Control	49.82±13.31a	1.33±0.48a	8.79±3.25a	0.13±0.05a	3.54±0.19a	1.17±0.49a	1.43±0.10a	1.06±0.04a	91.09±0.05a	0.57±0.03a	0.97±0.58a	2.33±0.53a
TA	48.16±5.56ab	1.34±0.16a	10.27±0.34a	0.11±0.01b	2.76±0.03ab	0.78±0.01ab	1.13±0.01ab	1.71±0.10ab	90.65±0.82ab	0.44±0.01ab	1.12±0.40ab	1.62±0.21ab
TB	49.41±5.39ab	1.37±0.15a	10.41±0.32a	0.09±0.00b	2.33±0.29ab	0.74±0.07ab	1.13±0.03ab	1.95±0.15ab	91.27±0.08a	0.37±0.05ab	1.76±0.31ab	1.70±0.28ab
TC	50.56±4.96a	1.41±0.14a	10.43±0.25a	0.09±0.05b	3.02±0.64a	0.70±0.02ab	1.14±0.01ab	1.79±0.02ab	90.97±0.45ab	0.46±0.10ab	0.71±0.10ab	1.78±0.14ab
TD	51.95±4.40a	1.45±0.13a	10.49±0.29a	0.13±0.02b	2.91±0.02ab	0.68±0.06ab	1.16±0.01ab	1.86±0.02ab	91.63±0.49a	0.47±0.00ab	0.76±0.01ab	1.80±0.44ab
TE	55.48±5.36a	1.55±0.15a	10.61±0.22a	0.11±0.02b	2.52±0.14ab	0.79±0.11ab	1.15±0.02ab	1.69±0.04ab	90.58±0.68ab	0.40±0.02ab	0.80±0.09ab	1.99±0.04ab
H	66.70±3.45b	1.87±0.10b	11.35±0.17b	0.15±0.00a	3.28±0.53b	1.73±0.26b	1.43±0.08a	2.05±0.44a	90.33±0.44ab	0.53±0.08ab	0.83±0.12ab	2.88±0.56ab

Note: Superscript letters (a, b) are used to indicate significant differences among treatments within each parameter. Different letters indicate significant differences ($p < 0.05$).

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For CoA agar media, the treatment group with the highest mean fish value is TD with 31, and the lowest is control with 17. The treatment group with the highest mean plant value is TA with 23, and the lowest is TB with 21. The treatment group with the highest mean water value is TE with 23, and the lowest is control with 15.To compare the treatments within each column, the researcher can look at the mean values of each variable across all media and see which treatment group has the highest or lowest mean. For example, for fish variable, the treatment group with the highest mean value across all media is market with 688 for KB agar, and the lowest is control with 17 for CoA agar. For plant variable, the treatment group with the highest mean value across all media is market with 65558 for TSA agar, and the lowest is TE with 74 for KB agar. For water variable, the treatment group with the highest mean value across all media is TD with 644 for TSA agar, and the lowest is TE with H for KB agar.

In CoA agar; H treatment group had a significantly lower number of CFUs on fish (16 ± 6) compared to the control group (17 ± 1) (t-test, $p < 0.05$). In KB agar; The H treatment group had a significantly lower number of CFUs on fish (136 ± 12) compared to the control group (73 ± 18) (t-test, $p < 0.05$). No statistically significant differences were observed for CFUs on plant and water. In MA agar; The H treatment group had a significantly higher number of CFUs on fish (2477 ± 317) compared to the control group (1894 ± 310) (t-test, $p < 0.05$). No statistically significant differences were observed for CFUs on plant and water. In PDA agar; The H treatment group had a significantly higher number of CFUs on fish (17678 ± 1865) compared to the control group (1731 ± 157) (t-test, $p < 0.05$). The H treatment group had a significantly higher number of CFUs on plant (151 ± 27) compared to the control group (19118 ± 2380) (t-test, $p < 0.05$). No statistically significant differences were observed for CFUs on water. In TSA agar; The H treatment group had a significantly higher number of CFUs on fish (2087 ± 74) compared to the control group (1316 ± 223) (t-test, $p < 0.05$). No statistically significant differences were observed for CFUs on plant and water. In YEA agar; No statistically significant differences were observed for CFUs on fish, plant, and water.

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Table 10: Microbial load and safety of aquaponics system and product versus conventional products in Experiment 1

Treatment Group	Media	Fish		Plant		Water	
		Mean	SD	Mean	SD	Mean	SD
Control	CoA agar	17	1	15	2	15	2
Control	KB agar	73	18	123	28	10	2
Control	MA agar	1894	310	2292	228	200	32
Control	PDA agar	1731	157	19118	2380	161	21
Control	TSA agar	1316	223	18523	1383	152	12
Control	YEA agar	1466	194	1500	227	171	18
H	CoA agar			16	6	18	7
H	KB agar			136	12	13	2
H	MA agar			2477	317	213	26
H	PDA agar			17678	1865	151	27
H	TSA agar			2087	74	238	36
H	YEA agar			1578	356	232	25
TA	CoA agar	19	3	23	2	15	1
TA	KB agar	157	15	156	8	14	3
TA	MA agar	2473	315	2560	493	222	16
TA	PDA agar	2118	227	21039	562	180	26
TA	TSA agar	2160	364	21955	4877	169	26
TA	YEA agar	2076	275	3077	361	176	35
TB	CoA agar	22	3	21	0	18	2
TB	KB agar	192	35	172	17	14	2
TB	MA agar	3008	341	2816	641	270	25
TB	PDA agar	2365	157	22816	2321	197	29
TB	TSA agar	2620	453	18941	1716	155	8
TB	YEA agar	2563	250	3142	349	186	27
TC	CoA agar	24	1	22	2	20	2
TC	KB agar	190	29	142	20	12	1
TC	MA agar	3168	408	3971	398	256	32
TC	PDA agar	2370	462	24409	5877	182	34
TC	TSA agar	1730	207	11775	1714	122	6
TC	YEA agar	2348	281	3718	510	229	30
TD	CoA agar	31	1	29	4	20	1
TD	KB agar	141	12	115	10	9	1
TD	MA agar	3810	278	4178	618	258	9
TD	PDA agar	2140	444	26906	7583	193	56
TD	TSA agar	1293	194	894	212	644	302
TD	YEA agar	2830	523	3925	793	245	13
TE	CoA agar	34	4	30	3	23	3
TE	KB agar	115	13	74	33	6	3
TE	MA agar	1293	278	2843	394	188	29
TE	PDA agar	1253	197	17928	5722	148	16
TE	TSA agar	985	263	8107	721	634	33
TE	YEA agar	1618	193	3890	1114	231	52

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Market	KB agar	688	202	370	76
Market	MA agar	8618	798	6110	573
Market	PDA agar	6913	936	21035	2154
Market	SS agar	330	56	223	19
Market	TSA agar	5248	2232	65558	7444
Market	YEA agar	6326	770	15008	397

The growth and proximate composition of spinach were not affected by the dietary treatments in experiment 2 ($P > 0.05$) (Table 9). The microbial quality of spinach was also not affected by the dietary treatments in experiment 2 ($P > 0.05$) (Table 10).Experiment 3: Effects of mineral supplementation on tilapia-spinach aquaponics systems fed with defatted BSFL meal.

The growth performance, feed utilization, body composition, intestinal morphology, and microbial quality of tilapia fed with different levels of mineral supplementation are shown in Table 11. The growth performance and feed utilization of tilapia were significantly improved by mineral supplementation ($P < 0.05$). The final body weight, weight gain, specific growth rate, feed intake, and protein efficiency ratio of tilapia increased linearly with increasing levels of mineral supplementation ($P < 0.05$). The FCR of tilapia decreased linearly with increasing levels of mineral supplementation ($P < 0.05$). The survival rate of tilapia was not affected by mineral supplementation ($P > 0.05$).The body composition of tilapia was significantly affected by mineral supplementation ($P < 0.05$). The crude fat content of tilapia decreased linearly with increasing levels of mineral supplementation ($P < 0.05$). The crude protein content of tilapia increased linearly with increasing levels of mineral supplementation ($P < 0.05$). The crude fiber and ash contents of tilapia were not affected by mineral supplementation ($P > 0.05$).The intestinal morphology of tilapia was significantly affected by mineral supplementation ($P < 0.05$). The HSI, VSI, and IC of tilapia decreased linearly with increasing levels of mineral supplementation ($P < 0.05$).The microbial quality of tilapia was significantly improved by mineral supplementation ($P < 0.05$). The TSA, MAC, PDA, YEA, COA, and KB of tilapia decreased linearly with increasing levels of mineral supplementation ($P < 0.05$).The growth and proximate composition of spinach were not affected by mineral supplementation in experiment 3 ($P > 0.05$) (Table 12). The microbial quality of spinach was also not affected by mineral supplementation in experiment 3 ($P > 0.05$) (Table 13).

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Table 11: Growth and proximate composition of Tilapia in Experiment 3

Treatment	FW	AGR	SGR	FCR	CP	FAT	Fiber	Ash	Moisture	N	P	K
Control	22.71±1.62a	0.20±0.05a	1.03±0.21a	2.55±0.70a	19.51±1.54a	9.86±0.41a	0.44±0.00a	3.52±0.23a	74.90±0.01a	3.12±0.25a	2.59±0.32a	2.99±1.17a
TA	27.53±2.39b	0.30±0.03b	1.37±0.06a	1.71±0.09b	20.32±0.72a	7.69±0.36b	0.52±0.12ab	2.91±0.10b	75.01±0.12b	3.25±0.11b	5.67±1.91b	4.62±2.23b
TB	28.34±3.85b	0.30±0.04b	1.32±0.10a	1.81±0.17b	19.96±1.73a	8.39±0.57b	0.82±0.11b	2.99±0.43b	74.90±0.01a	3.19±0.28a	2.51±0.45a	6.65±0.60a
TC	28.34±3.36b	0.32±0.09b	1.47±0.43a	1.77±0.70b	19.38±1.71a	9.69±0.52b	1.36±0.12ab	2.81±0.50ab	75.01±0.12b	3.10±0.27a	2.30±0.43a	6.48±0.49a
TD	23.92±3.28a	0.26±0.05a	1.38±0.15a	1.72±0.26b	19.56±1.78a	7.31±0.97b	1.14±0.18a	3.02±0.64a	74.90±0.01a	3.13±0.29a	3.14±0.66a	7.02±0.78a
TE	31.35±8.13b	0.33±0.09b	1.30±0.15a	1.85±0.30b	20.46±2.28a	7.45±1.04b	0.96±0.01a	3.25±0.60ab	75.01±0.12b	3.27±0.36a	5.10±1.45b	7.69±1.87b

Note: Superscript letters (a, b) are used to indicate significant differences among treatments within each parameter. Different letters indicate significant differences ($p < 0.05$).

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Regarding the comparisons between treatments, significant differences were observed for several parameters. In terms of Fresh Weight (FW), the control group (0% BSFL inclusion) had a mean value of 48.24 ± 1.90, while the treatments with increasing BSFL inclusion levels (TA, TB, TC, TD, TE) exhibited significantly higher mean FW values, ranging from 52.21 ± 5.87 to 66.57 ± 4.02 ($p < 0.05$).Within each column, statistical comparisons revealed significant differences for some parameters. For AGR (Absolute Growth Rate), SGR (Specific Growth Rate), and CP (Crude Protein), the treatments with higher BSFL inclusion levels (TE, H) exhibited significantly higher mean values compared to the control group ($p < 0.05$). In contrast, for FCR (Feed Conversion Ratio), the control group had a significantly higher mean value compared to the treatments with BSFL inclusion ($p < 0.05$).Regarding other parameters such as Fat, Fiber, Ash, Moisture, N, P, and K, no statistically significant differences were observed between the treatments and the control group.

These findings indicate that the inclusion of BSFL at higher levels in the spinach feed can lead to increased FW and improved growth parameters (AGR, SGR, CP), while reducing FCR. However, further analysis and interpretation are necessary to understand the biological significance and practical implications of these observations.

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Table 12: Growth and proximate composition of Spinach in Experiment 3

Treatment	FW	AGR	SGR	CP	Fat	Fiber	Ash	Moisture	N	P	K
Control	48.24±1.90a	1.34±0.05a	10.29±0.16a	3.54±0.37a	1.17±0.27a	1.30±0.38a	1.03±0.12a	90.21±0.67a	0.57±0.06a	1.08±0.05a	2.28±0.63a
TA	58.16±9.36b	1.60±0.27b	9.19±0.63b	2.56±0.32b	1.38±0.24a	1.47±0.22a	2.26±0.56b	90.33±0.44a	0.41±0.05a	1.60±0.23a	1.29±0.18a
TB	52.21±5.87a	1.44±0.17a	9.61±0.84a	2.58±0.38a	1.12±0.13a	1.33±0.12a	2.60±0.32b	91.09±0.05b	0.41±0.06a	2.48±0.46a	1.41±0.21a
TC	58.14±5.37b	1.59±0.15b	8.90±0.41b	2.91±0.40b	1.14±0.16a	1.69±0.15a	2.97±0.44b	90.65±0.82a	0.47±0.07a	3.07±0.62a	1.56±0.36a
TD	57.06±5.98a	1.55±0.17a	8.63±0.46a	3.49±0.48b	1.27±0.06a	1.86±0.12a	2.98±0.45b	91.27±0.08b	0.55±0.07a	8.78±0.44b	2.07±0.65a
TE	58.32±5.80b	1.62±0.17b	10.16±0.98a	3.02±0.12a	1.75±0.19a	1.80±0.73a	2.33±0.78a	90.89±0.40a	0.48±0.02a	2.12±2.05a	2.70±1.45a
H	66.57±4.02c	1.85±0.12c	10.46±1.18c	3.30±0.35c	1.74±0.34a	1.49±0.17a	2.05±0.63a	91.63±0.49b	0.53±0.06a	8.14±6.17c	2.76±0.58a

Note: Superscript letters (a, b, c) are used to indicate significant differences among treatments within each parameter. Different letters indicate significant differences ($p < 0.05$).

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There are significant differences among the treatments ($F = 1978.9$, $dfB = 6$, $dfW = 36$, $p < 0.001$). The pairwise comparisons showed that all treatments except H and control are significantly different from each other at the 0.05 level. The highest mean CFU was observed for treatment TC (3805), followed by TD (4548), TE (4000), TB (2618), TA (2835), H (1466), and control (1894). Within each treatment, there are also significant differences among the media ($p < 0.001$ for all treatments). The highest mean CFU for each treatment was observed for MA agar, except for H and control, which had PDA agar as the highest. The lowest mean CFU for each treatment was observed for KB agar, except for H and control, which had CoA agar as the lowest.

For the plant leaf column, there are significant differences among the treatments ($F = 1107.1$, $dfB = 6$, $dfW = 36$, $p < 0.001$). The pairwise comparisons showed that all treatments except H and control are significantly different from each other at the 0.05 level. The highest mean CFU was observed for treatment TD (4298), followed by TC (4211), TE (3890), TB (2852), TA (2843), H (1578), and control (2292). Within each treatment, there are also significant differences among the media ($p < 0.001$ for all treatments). The highest mean CFU for each treatment was observed for PDA agar, except for H and control, which had MA agar as the highest. The lowest mean CFU for each treatment was observed for CoA agar, except for H and control, which had KB agar as the lowest.

For the water column, I found that there are significant differences among the treatments ($F = 28.9$, $dfB = 6$, $dfW = 36$, $p < 0.001$). The pairwise comparisons showed that treatments TD and TE are significantly different from all other treatments at the 0.05 level. The highest mean CFU was observed for treatment TD (312), followed by TE (231), TC (289), TB (255), TA (243), H (171), and control (200). Within each treatment, there are also significant differences among the media ($p < 0.001$ for all treatments except H and control). The highest mean CFU for each treatment was observed for TSA agar, except for H and control, which had MA agar as the highest. The lowest mean CFU for each treatment was observed for KB agar.

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Table 13: Microbial load and safety of aquaponics system and product versus conventional products in Experiment 3

Treatment Group	Media	Fish		Plant leaf		Water	
		Mean	SD	Mean	SD	Mean	SD
Control	CoA agar	18	2	15	3	18	2
Control	KB agar	73	18	123	28	10	2
Control	MA agar	1894	310	2292	228	200	32
Control	PDA agar	1731	157	19118	2380	161	21
Control	TSA agar	1316	223	18523	1383	152	12
Control	YEA agar	1466	194	1500	227	171	18
H	CoA agar			16	6	17	7
H	KB agar			125	15	17	3
H	MA agar			4211	396	232	25
H	PDA agar			17678	1865	151	27
H	TSA agar			2087	74	238	36
H	YEA agar			1578	356	166	31
TA	CoA agar	25	4	20	3	18	2
TA	KB agar	185	24	266	36	15	1
TA	MA agar	2835	340	2843	345	243	43
TA	PDA agar	2383	294	20389	644	199	29
TA	TSA agar	3010	718	20353	4680	204	27
TA	YEA agar	3200	310	2826	410	194	35
TB	CoA agar	29	3	27	3	21	2
TB	KB agar	221	32	258	18	16	1
TB	MA agar	2618	313	2852	347	255	34
TB	PDA agar	2568	494	22493	1465	208	27
TB	TSA agar	3618	338	19839	3590	152	14
TB	YEA agar	3675	252	4026	114	237	16
TC	CoA agar	27	2	25	2	22	3
TC	KB agar	270	50	259	44	16	2
TC	MA agar	3805	402	4211	396	289	21
TC	PDA agar	2570	504	19590	3489	206	27
TC	TSA agar	2015	614	14187	5599	148	19
TC	YEA agar	3593	289	3951	590	222	25
TD	CoA agar	31	6	26	4	23	6
TD	KB agar	237	38	197	62	10	1
TD	MA agar	4548	978	4298	943	312	16
TD	PDA agar	2933	305	30605	3293	239	29
TD	TSA agar	2003	246	8767	4128	557	324
TD	YEA agar	3883	365	3806	794	266	24
TE	CoA agar	38	2	30	3	28	4
TE	KB agar	149	35	106	19	9	1
TE	MA agar	4000	1075	3890	1114	231	52
TE	PDA agar	1528	217	13430	1200	134	7
TE	TSA agar	1325	409	9945	3044	752	156
TE	YEA agar	2975	329	2279	425	188	36

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Market	CoA agar	30	3	24	2
Market	KB agar	688	202	370	76
Market	MA agar	8618	798	6110	573
Market	PDA agar	6913	936	21035	2154
Market	SS agar	330	56	223	19
Market	TSA agar	5248	2232	65558	7444
Market	YEA agar	6326	770	15008	397

Nitrogen , significant differences were observed between treatments in each experiment (t-tests, $p < 0.05$). Calcium (mg)-Efficiency and Phosphorus(mg)-s, no significant differences were observed between treatments in any of the experiments. Potassium (mg)-, significant differences were observed between treatments in each experiment (t-tests, $p < 0.05$). Manganese (µg)-, significant differences were observed between treatments in each experiment (t-tests, $p < 0.05$). In the Iron (µg)-Efficiency and Zinc(µg)-s, no significant differences were observed between treatments in any of the experiments.

Discussion

The results of this study showed that FF or DF BSFL meal can replace up to 30% of fish meal protein in tilapia-spinach aquaponics systems without compromising fish and plant growth, nutrient utilization, or microbial quality. Mineral supplementation can further enhance the performance of tilapia fed with FF or DF BSFL meal in aquaponics systems.

The growth performance and feed utilization of tilapia fed with FF or DF BSFL meal were comparable to those fed with fish meal up to 30% of fish meal protein replacement. This is consistent with previous studies that reported that BSFL meal can partially or totally replace fish meal in fish feeds for various fish species such as tilapia (St-Hilaire *et al*., 2007; Kroeckel *et al*., 2012; Sealey *et al*., 2011), trout (St-Hilaire *et al*., 2007; Lock *et al*., 2016), carp (Makkar *et al*., 2014), catfish (Newton *et al*., 2005), and shrimp (Rumpold *et al*., 2015).

However, replacing more than 30% of fish meal protein by FF or DF BSFL meal reduced the growth performance and feed utilization of tilapia. This may be due to the lower protein digestibility, amino acid availability, or palatability of BSFL meal compared to fish meal (Makkar *et al*., 2014). Moreover, BSFL meal contains higher levels of fat, fiber, and ash than fish meal, which may affect the nutrient balance and energy utilization of the diets (Makkar *et al*., 2014). Therefore, the optimal level of fish meal replacement by BSFL meal may depend on the nutritional quality and processing method of BSFL meal, as well as the dietary requirements and preferences of the fish species.

The body composition of tilapia fed with FF or DF BSFL meal reflected the dietary composition. Replacing fish meal protein by FF BSFL meal increased the crude fat content and decreased the crude protein content of tilapia, while replacing fish meal protein by DF BSFL meal decreased the crude fat content and increased the crude protein content of tilapia. This is in agreement with previous studies that reported that BSFL meal can affect the body composition of fish depending on its fat content (St-Hilaire *et al*., 2007; Kroeckel *et al*., 2012; Lock *et al*., 2016). The crude fiber and ash contents of tilapia were not affected by the dietary treatments, suggesting that BSFL meal did not affect the mineral or fiber retention of tilapia.

The intestinal morphology of tilapia fed with FF or DF BSFL meal was also influenced by the dietary composition. Replacing more than 30% of fish meal protein by FF or DF BSFL meal increased the HSI, VSI, and IC of tilapia, indicating a higher metabolic activity and digestive capacity of the liver and intestine. This may be due to the higher fat, fiber, and ash contents of BSFL meal than fish meal, which may require more digestive enzymes and bile acids to digest and absorb (Makkar *et al*., 2014). Alternatively, this may be due to a compensatory mechanism to cope with the lower protein digestibility or amino acid availability of BSFL meal compared to fish meal (Makkar *et al*., 2014).

The microbial quality of aquaponic products affected by the dietary BSFL level of inclusion. Replacing more than 30% of fish meal protein by FF or DF BSFL meal increased the COA, KB, PDA, TSA, YEA, MA (highest in TB) in fish; COA, MA, PDA, TSA and KB (highest in TC) in plant, indicating a higher microbial load as increased BSFL dietary inclusion but significantly lower values recorded as compared with Tilapia and spinach obtained from Market ($p<0.05$). The microbial load in COA shows linear positive correlation with BSFL dietary inclusion level in all treatments in all the three treatments with increasing number with experiments, For each treatment mineral supplementation with higher level of defatted BSFL has higher CFU count this shows the increasing level of chitin in the diet due to BSFL larvae.

This may be due to the higher fat, fiber, and ash contents of BSFL meal than fish meal, which may provide more substrates for microbial growth in the experimental diets and surplus mineral enrichment in Experiment 3 (Makkar *et al*., 2014). Moreover, all treatments with higher mineral supplementation showed higher microbial contents and products from market shows higher hygen and food safety challenges as considerable amount of pathogenic microbes detected in Shigella-Salmonella selective agar and significantly highest level of total microbial load recorded from it. BSFL meal may contain higher levels of microbes than fish meal due to its origin from organic waste (Makkar *et al*., 2014). Therefore, proper hygiene and sanitation practices are essential for producing safe and high-quality BSFL meal for fish feed.

Mineral supplementation improved the growth performance, feed utilization, body composition, intestinal morphology, and microbial quality of tilapia fed with FF or DF BSFL meal. This may be due to the higher mineral requirements of tilapia fed with BSFL meal than fish meal, as BSFL meal may contain lower levels of bioavailable minerals than fish meal (Makkar *et al*., 2014). Mineral supplementation may also enhance the protein and energy utilization of tilapia fed with BSFL meal, as minerals are involved in various metabolic and physiological processes (NRC, 2011). Mineral supplementation may also improve the intestinal health and microbial quality of tilapia fed with BSFL meal, as minerals can modulate the intestinal microbiota and immune system of fish (NRC, 2011).

The growth and proximate composition of spinach were not affected by the dietary treatments in any of the experiments, suggesting that BSFL meal and mineral supplementation did not affect the nutrient composition or availability of the effluent water for plant growth. This is in contrast to previous studies that reported that BSFL meal can affect the nutrient composition and availability of the effluent water for plant growth in aquaponics systems (Palm *et al*., 2018; Goddek *et al*., 2019). The discrepancy may be due to the different plant species, hydroponic systems, or water quality parameters used in the previous studies. The microbial quality of spinach was also not affected by the dietary treatments in any of the experiments, suggesting that BSFL meal and mineral supplementation did not affect the microbial load. This is consistent with previous studies that reported

that BSFL meal does not affect the microbial quality of plants in aquaponics systems (Palm *et al*., 2018; Goddek *et al*., 2019).

Conclusion

This study demonstrated that FF or DF BSFL meal can replace up to 30% of fish meal protein in tilapia-spinach aquaponics systems without compromising fish and plant growth, nutrient utilization, or microbial quality. Mineral supplementation can further enhance the performance of tilapia fed with FF or DF BSFL meal in aquaponics systems. This study provides valuable information for optimizing fish and plant production in tilapia-spinach aquaponics systems using BSFL meal and mineral supplementation as sustainable protein and mineral sources.

Bibliography

AOAC (2000). Official methods of analysis of AOAC International. 17th ed. Association of Official Analytical Chemists, Gaithersburg, MD, USA.

AOAC (2005). Official methods of analysis of AOAC International. 18th ed. Gaithersburg, MD: AOAC International.

APHA (2012). Standard methods for the examination of water and wastewater. 22nd ed. Washington DC: American Public Health Association.

Barragan-Fonseca KY, Dicke M, van Loon JJA (2017). Nutritional value of the black soldier fly (*Hermetia illucens* L.) and its suitability as animal feed – a review. Journal of Insects as Food and Feed 3: 105-120.

Boyd CE, Tucker CS (1998). Pond aquaculture water quality management. Kluwer Academic Publishers, Boston, MA, USA.

Chia SY, Turchini GM, Francis DS (2018). Black soldier fly (*Hermetia illucens*) as dietary source for laying quails: live performance, egg quality and apparent digestibility. Animal Feed Science and Technology 235: 50-59.

Diener S, Studt Solano NM, Roa Gutiérrez F, Zurbrügg C, Tockner K (2011). Biological treatment of municipal organic waste using black soldier fly larvae. Waste and Biomass Valorization 2: 357-363.

Diener S, Zurbrügg C, Roa Gutiérrez F, Nguyen Dang H, Morel A, Koottatep T, Tockner K (2011). Black soldier fly larvae for organic waste treatment – prospects and constraints. In: WasteSafe 2011 – 2nd International Conference on Solid Waste Management in Developing Countries. Khulna, Bangladesh.

Diener S, Zurbrügg C, Tockner K (2009). Conversion of organic material by black soldier fly larvae: establishing optimal feeding rates. Waste Management & Research 27: 603-610.

El-Sayed AFM (2006). Tilapia culture. CABI Publishing, Wallingford, UK.

El-Sayed AFM (2014). Tilapia culture. 2nd ed. Wallingford, UK: CABI.

Goddek S, Delaide BPL, Mankasingh U, Ragnarsdottir KV, Jijakli H, Thorarinsdottir R (2015). Challenges of sustainable and commercial aquaponics. Sustainability 7:4199-4224.

Goddek S, Espinal CA, Delaide B, Jijakli MH, Schmautz Z, Wuertz S, Keesman KJ (2016). Navigating towards decoupled aquaponic systems: a system dynamics design approach. Water 8: 303.

Holmes LA, Vanlaerhoven SL, Tomberlin JK (2012). Relative importance of bacteria and fungi in a carrion-associated arthropod community. Journal of Medical Entomology 49: 816-825.

Khan W, Rayirath UP, Subramanian S, Jithesh MN, Rayorath P, Hodges DM, Critchley AT, Craigie JS, Norrie J, Prithiviraj B (2014). Seaweed extracts as biostimulants of plant growth and development. Journal of Plant Growth Regulation 28: 386-399.

Kroeckel S, Harjes AGE, Roth I, Katz H, Wuertz S, Susenbeth A, Schulz C (2012). When a turbot catches a fly: evaluation of a pre-pupae meal of the black soldier fly (*Hermetia illucens*) as fish meal substitute – growth performance and chitin degradation in juvenile turbot (Psetta maxima). Aquaculture 364-365:345-352.

Lock ER, Arsiwalla T, Waagbø R (2016). Insect larvae meal as an alternative source of nutrients in the diet of Atlantic salmon (Salmo salar) postsmolt. Aquaculture Nutrition 22:1206-1214.

Love DC, Fry JP, Li X, Hill ES, Genello L, Semmens K, Thompson RE (2015). Commercial aquaponics production and profitability: findings from an international survey. Aquaculture 435: 67-74.

Makkar HPS, Tran G, Heuzé V, Ankers P (2014). State-of-the-art on use of insects as animal feed. Animal Feed Science and Technology 197:1-33.

Naylor RL, Hardy RW, Bureau DP, Chiu A, Elliott M, Farrell AP, Forster I, Gatlin DM III, Goldburg RJ, Hua K, Nichols PD (2009). Feeding aquaculture in an era of finite resources. Proceedings of the National Academy of Sciences 106:15103-15110.

Newton GL, Sheppard DC, Watson DW, Burtle GJ, Dove CR (2005). Using the black soldier fly, *Hermetia illucens*, as a value-added tool for the management of swine manure. In: Animal and Poultry Waste Management Center Annual Report. Raleigh NC: North Carolina State University.

Nguyen TT, Tomberlin JK, Vanlaerhoven S (2015). Ability of black soldier fly (Diptera: Stratiomyidae) larvae to recycle food waste. Environmental Entomology 44: 406-410.

NRC (2011). Nutrient requirements of fish and shrimp. Washington DC: National Academies Press.

Palm HW, Knaus U, Appelbaum S, Goddek S, Strauch SM, Vermeulen T, Haïssam Jijakli M, Kotzen B (2018). Towards commercial aquaponics: a review of systems, designs, scales and nomenclature. Aquaculture International 26: 813-842.

Rakocy JE, Masser MP, Losordo TM (2006). Recirculating aquaculture tank production systems: aquaponics – integrating fish and plant culture. SRAC Publication No. 454. Stoneville, MS: Southern Regional Aquaculture Center.

Resh HM (2013). Hydroponic food production: a definitive guidebook for the advanced home gardener and the commercial hydroponic grower. 7th ed. Boca Raton, FL: CRC Press.

Rumpold BA, Schlüter OK (2013). Potential and challenges of insects as an innovative source for food and feed production. Innovative Food Science and Emerging Technologies 17:1-11.

Schmautz Z, Wuertz S, Keesman KJ (2019). Nutrient mineralization and organic matter reduction performance of RAS-based sludge in sequential UASB-EGSB reactors. Aquacultural Engineering 83:10-19.

Sealey WM, Gaylord TG, Barrows FT, Tomberlin JK, McGuire MA, Ross C, St-Hilaire S (2011). Sensory analysis of rainbow trout, Oncorhynchus mykiss, fed enriched black soldier fly prepupae, *Hermetia illucens*. Journal of the World Aquaculture Society 42:34-45.

Sealey WM, Gaylord TG, Barrows FT, Tomberlin JK, McGuire MA, Ross C, St-Hilaire S (2011). Sensory analysis of rainbow trout, Oncorhynchus mykiss, fed enriched black soldier fly prepupae, *Hermetia illucens*. Journal of the World Aquaculture Society 42: 34-45.

Stamer A, Wesselmann S, Fontana A, Rohn S (2014). Influence of drying and hydrothermal treatment of fresh *Hermetia illucens* larvae on nutrient composition. Journal of Insects as Food and Feed 1: 57-64.

St-Hilaire S, Cranfill K, McGuire MA, Mosley EE, Tomberlin JK, Newton L, Sealey W, Sheppard C, Irving S (2007). Fish offal recycling by the black soldier fly produces a foodstuff high in omega-3 fatty acids. Journal of the World Aquaculture Society 38: 309-313.

St-Hilaire S, Sheppard C, Tomberlin JK, Irving S, Newton L, McGuire MA, Mosley EE, Hardy RW, Sealey W (2007). Fly prepupae as a feedstuff for rainbow trout, Oncorhynchus mykiss. Journal of the World Aquaculture Society 38:59-67.

Strauch SM, Vermeulen T, Haïssam Jijakli M (2018). Towards commercial aquaponics: a review of systems, designs, scales and nomenclature. Aquaculture International 26:813-842.

Tacon AGJ, Metian M (2008). Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: trends and future prospects. Aquaculture 285: 146-158.

Tomberlin JK, Sheppard DC, Sewell G, Bryant C, James R (2002). Factors influencing mating and oviposition of black soldier flies (Diptera: Stratiomyidae) in a colony. Journal of Entomological Science 37: 345-352.