

Growth of Juvenile Male (GIFT) Tilapia Fed 40% Black Soldier Fly (*Hermetia illucens*) Larval Meal in tanks of Fertilized or Unfertilized Water Sources

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Abstract: A simple farm-made feed utilizing Black Soldier Fly Larvae (BSFL), *Hermetia illucens*, was offered to all male juvenile GIFT tilapia, *Oreochromis niloticus*, in enclosed aquaculture systems, to test the effects of managing fish in three water sources of different quality. Two isonitrogenous and isoenergetic test diets were a Basal diet with protein concentrate or a strategically formulated diet with BSFL at 40% inclusion rate (400g kg⁻¹). A total of 240 fish (BW 1.95±0.01g) were placed in 12 tanks at a density of 20 fish to 1m⁻³ of tank volume in an open shed under tropical lowland conditions. The test diets were offered twice a day to satiety of fish-groups kept in tanks managed as two blocks of fertilized, natural stream and rain-tank water systems, replicated over three diet-water interchange periods (90 days), for the assessment of final body weight and length, biomass gain, feed intake/tank (FIT), FCR, PER, SGR, TGC and Condition Factor (K). Results were significant for diet, water source and by interactions ($P<0.05$). Higher feed intake on Basal diet ($P<0.001$) provided better FCR ($P<0.001$), PER ($P<0.001$), SGR ($P<0.01$) and TGC ($P<0.001$). Fertilized water afforded better growth ($P<0.001$) due to improved feed efficiency ($P<0.001$) when dietary feed intake was similar to tank water ($P>0.05$). Overall, fish-groups fed the BSFL diet in tank water performed the least (FIT=7.78g, FCR=4.20, PER=0.86, SGR=1.52% day⁻¹) while fish fed the Basal diet in fertilized water performed the best (FIT=8.91g, FCR=1.25, PER=3.00, SGR=3.53% day⁻¹). While, the nutritional value of BSFL catered for comparative SGR ($P<0.01$) in juvenile tilapia fed in fertilized water (FIT=7.07g, FCR=2.58, PER=1.38, SGR=2.45% day⁻¹) the diet palatability reduced feed intake ($P<0.001$), utilization ($P<0.001$) and protein efficiency ($P<0.5$), compared to tank water. Manipulating the digestibility of BSFL by including other feed ingredients may improve the utilization of this valuable on-farm produced protein feed.

Keywords: Black Soldier Fly larvae, GIFT tilapia, feed utilization efficiency

1. INTRODUCTION

Aquaculture in Papua New Guinea (PNG) is relatively new with mostly smallholder subsistence farmers. Expansion efforts have been limited to broodstock control, fingerling supply, poor husbandry skills and fish feeds (Booth, 2017). Despite these challenges, GIFT tilapia has emerged as the most preferred farmed fish species in PNG due to its low input requirements for pond rearing (Allen et al., 2007). However, the growth of the aquaculture industry in PNG has been hindered by the availability and quality of fish feed (Sammut and Wani 2015). Commonly used fish feed source is vegetable-based diets reared in natural water-fed earthen pond systems. It has been reported that less than 10 % of fish farms have access to pellet feed or farm-produced feeds; whereas 33 % of fish farms use organic manure for pond fertility (Smith, 2013). Although supplementing pellet-feeding is recommended to enhance small-scale fish farm productivity, it is rarely practiced alongside pond fertilization (Booth, 2017). A previous study using chicken manure for fish pond fertility showed that pellet feeds had no influence in the nutritional assimilation of GIFT in fertilized ponds (Narimbi et al., 2018), suggesting a response to food preferences or the lack of dietary protein in fish feed (Parata et al., 2020). These findings challenge the assumption that pond-based farming systems heavily rely on formulated diets and offer an opportunity to reduce farmers' dependence on feed inputs. However, the formulation of practical fish diets remains a highly technical task that is not well adopted by farmers.

Most fish farms, which are mostly rural and isolated, face constraints due to limited access to quality fish feed, ingredients particularly protein sources (Booth, 2017; Narimbi et al., 2018). Extensive work into the use of insect meal as an interchangeable animal protein source provides alternative protein sources in aquaculture diets (Barroso et al., 2014; Henry et al., 2015). The Black Soldier Fly Larvae (*Hermetia illucens*; BSFL) has been the most extensively researched insect meal alternative, offering the added benefit of converting organic waste material into high-quality protein (Surandra et al., 2016; Barragan-Fonseca et al., 2017). Promoting BSFL rearing for feed presents opportunities for remote and isolated fish farms who may have limited access to protein ingredients. In addition, the low input feeding system is mostly pond-based either utilizing green organic waste for pond fertility and feed to which BSFL may be supplemented for fish feeding systems in PNG.

Favorable waste medium conditions may result in BSFL containing up to 40% (400 g kg⁻¹) crude protein and 30% (300 g kg⁻¹) fat, providing a reasonably balanced amino acid profile similar to that of soybean and fishmeal (Diener et al., 2009; NRC, 2011). Additionally, BSFL is rich in minerals and vitamins, depending on the growth media used for feeding larvae (Wang and Shalomi, 2017). These farms can utilize BSFL not only as a source of insect meal protein, but also as a bio-converter of organic wastes.

BSFL insect meal has been successfully used in many feeding trials substituting fishmeal by 10 – 46% (100 – 460 g kg⁻¹) for Jian carp (*Cyprinus carpio*), Channel catfish (*Ictalurus punctatus*), Rainbow trout (*Onocorhynchus mykiss*) and Barramundi (*lates calcarifer*) in formulated test diets (Bondari and Sheppard, 1987; St Hilaire et al., 2007; Zhou et al., 2016; Katya et al., 2017). Whereas formulated tilapia diets successfully included BSFL meal at 80 g to 571g kg⁻¹ as a fishmeal replacement (Devic et al., 2018; Wachira et al., 2021; Tippayadara et al., 2021). It is known that water fertility improves pond productivity, it is necessary to investigate the effects on growth parameters treated in different water sources, either fertilized or unfertilized, using BSFL meal as a protein ingredient. The objective of this study is to assess the performance and feed utilization efficiency of a 40% formulated BSFL diet treated under different water sources for all-male juvenile GIFT in an aquaculture tank system.

2. MATERIALS & METHODS

Study location

The study was carried out at the National Agriculture Research Institute (NARI) aquaculture research facility in Lae Morobe Province. The facility is at 16 m above sea level at Latitudes 06° 41' south and Longitude 146° 04' east. The area experiences an average annual rainfall of 2,000 to 2,400 mm, and daily temperatures range between 27 to 30°C and relative humidity of 70 to 80% with a photoperiod of 12 hours (NARI Bubia Station weather data).

Experimental fish

Prior to stocking, all male GIFT tilapia fingerlings were obtained from the Highlands Aquaculture Development Centre (HAQDEC), Aiyura Eastern Highlands Province. A total of 240 male fish aged between 28 to 35 days old were selected and acclimatized for 14 days to fibreglass fish tank conditions prior to the start of the experiment. Fish were distributed randomly into tanks and fed twice a day on the basal test diet (Table 1) at 10% of their initial total body weight before introducing experiment treatments. Subsequent body weights and lengths for trial initiation was 1.95g (\pm STD 0.1g) with lengths of 4.88 cm (\pm STD 0.1 cm). Fish were placed into the 12 tanks at a stocking density of 20 to 1m⁻³ of tank volume.

Experiment facility

The experiment used 12 fibreglass cylinder-shaped tanks in an open shed facility designed as a flow through enclosed system with 2 rows of 6 tanks without a re-circulation component (Masser et al., 1999). The tanks hold a capacity of 100 L at depths of 70 cm. A household pump system and aeration blowers were installed to control water flow and aeration. The water pump (Sanyo® PDH255JP) system supplied water sources at an average of 9–27 L per minute. Aeration (Hailea® HAP 80 7200Lph Hi-blow) was supplied at 80 L per minute through cylinder air stones averaging between 6 – 8 mg L⁻¹ to each tank. The water sources used were chicken manured fertilized water (F), rain fed tank water, (T) and natural stream water (N). The fertilized water was stored in a 6, 000 L tank (water surface area of 3m²) fertilized with a dry matter (DM) chicken manure content of 40–50g placed at the bottom of the tank at an application rate of 12 g DM chicken manure to a 1 m² water surface area (El-Sayed, 2007). A 24-hour lighting installation was placed 1.5 m above the tank with screen netting placed around the tank. The tank was aerated and manually stirred on a daily basis to establish a homogenous mixture and encourage phytoplankton growth.

Trial layout design

The layout was designed to test a BSFL formulated diet against a Basal diet as the main factor offered to male juvenile fish maintained in three different water sources. The assigned three water sources F, N and T were channelled into designated tanks within the 2 rows of the 12 tanks. A blocking effect was applied to separate the 2 rows taken as blocks 1 and 2. Each block contained 6 tanks with 2 tanks within each block supplying F, N or T fed BSFL and Basal diet in 3 replicated feeding periods. Each period was 30 days with fish-groups measured for weights and lengths at the start and end of each period. Fish groups at the end of each period were assigned new treatments over the course of 90 days.

Treatment Diets

The feed BSFL was cultured on station and harvested from bio-digesters customised to collect migrating larvae feeding on kitchen waste, pig and chicken manure including waste chicken feed homogenised as organic farm wastes (Roberts et al., 2019). The larvae collected were from the last instar to pre-pupate stages. Harvested non-defatted larvae were chilled below 8 °C and left for thawing for about 4 - 8hrs prior to drying. Initial weights were collected to determine DM prior to drying in a forced air draft oven (Labec®) for 24 – 48 hrs at 95 - 105 °C. BSF larval meal calculated a 60 – 75 % moisture reduction for every 1 kg of fresh BSFL harvested. The dried larvae were grinded using a kitchen grinder turning the harvest larvae into mesh before milling. The dried larval mesh was further broken down using an electric hammer mill turning the mash into finer particulates (Table 2).

A universal feed concentrate was used as the main protein source for the basal diet containing a mixture of meat, blood, fish and soybean meal. Alternatively, BSFL meal provided a single protein constituent in the diet formulation. The universal concentrate and BSFL meal composition used a 40% as the inclusion rate (Table 2). This was based on BSFL meal being better utilized at an inclusion rate of 40% for tilapia juvenile fish (Devic et al., 2018; Tippayadara et al., 2021). The BSFL meal used a combination of wheat meal, copra meal, limestone, vegetable oil (palm oil), vitamin premix and methionine. The ingredients used in the formulation of

the BSFL and Basal diet were all sourced locally from Lae Feed Mills, Niugini Tablebirds Limited, International Food Corporation, and Lae main market. The ingredients used for both diets were all broken down into either flour form or fine particulates in preparations for the BSFL diet formulation and the Basal. Diet formulation followed UNE-form diet specifications presented by Thomson and Nolan (2001). The composition of BSFL and Basal diets was hand mixed into dough for further processing. The dough was passed through a mechanical pellet machine with a die size of 4mm in diameter. Each pellet diet was then placed into a forced air draft oven (Labec®) for 48 hrs at 95 - 105 °C. The pellets from each diet were later cooled down to ambient room temperature of 28 °C. Each pelleted diet was crumbled less than 2mm in diameter and packed into polythene bags stored in feed buckets in preparations for feeding at ambient temperature.

Table 1: Nutrient approximate analysis for BSFL larval meal and the universal concentrate used as the main sources for the tested formulated diets

*Nutrient proximate	Universal concentrate (%)	BSFL larval meal (%)
Dry matter	90.3	84.2
Digestible energy (Mj kg ⁻¹)	14.2	23.3
Crude protein	37.7	34.1
Crude fibre	4.7	8.8
Crude fat	9.2	30.4
Ash	8.2	16
Calcium	2	3.9
Available Phosphorus	1.06	0.1
Arginine	2.82	2.65
Histidine	-	-
Isoleucine	1.6	-
Lysine	2.43	2.6
Methionine	1.07	0.74
Phenylalanine	-	-
Threonine	-	-
Tryptophan	0.17	2.5
Valine	-	2.79
Nitrogen free extracts ¹	40.2	10.7
Carbohydrate ²	35.2	3.7

*Source: Approximate chemical analysis for BSFL larval meal samples conducted at the National Analytical Testing Services Laboratory (NATSL), University of Technology, PNG. Universal concentrate approximates based on Carey Universal Concentrate for broiler, pig and layer 26/04/2014; single mix formulation.

¹Nitrogen free extracts = 100 - (lipid + protein + ash + fibre)

²Carbohydrate = 100 - (moisture + lipid + protein + ash)

Fish feeding and sampling

Fish were fed for 7 days a week using a satiation feeding strategy broadcasted over the surface fed to visual satiety. The strategy was to have fish consume as much feed at each feeding schedule from pre-weighed feeding containers executed daily. An electronic balance scale (ACS-3H; 1, 000g \pm 1.0g) was used to measure total amount of feed-fed to fish with total offered recorded as feed intake. Feeding schedule was done twice a day at 7 – 9 am and 4 – 5 pm for the entire 90 day feeding period. At each of the 30-day feeding period, body weights and total fish length samples were taken at day 0, 15, and 30 using an electric balance scale (ACS-3H) and a standard ruler attached to a water board (30 cm \pm 1 mm). The body weights and lengths for the end of each period became the starting weights for the following sampling period. The samples taken for each period (i.e., 0, 15 and 30) became the recorded averages for that period with the end body and total fish lengths used as the starting weights and lengths for the subsequent periods. No anaesthetic was used to sedate the fish prior to sampling and handling.

Table 2: Ingredients used in the formation of a basal diet in comparisons to the BSFL test diet and estimated nutrient proximate for diets

Ingredients	Basal Diet A (%)	BSFL Diet B (%)
BSFL meal	-	40
Universal Concentrate	40	-
Fish meal (FAQ)	1	-
Wheat millrun	23	23
Copra meal	30	30
Vegetable cooking oil	1	2
Limestone	3	3
Vitamin premix	1	1
Methionine 50 %	1	1
Total (kg)	100	100
Nutrient proximate	Basal Diet A (%)	BSFL Diet B (%)
Dry matter (DM)	92.21	91.88
Digestible energy (DE, Mj kg ⁻¹)	18.84	16.13
Crude protein (CP)	26.11	26.39
Crude fibre	8.84	7.15
Crude fat	15.85	9.91
Ash	13.3	7.19
Calcium	2.44	2.07
Available Phosphorus	0.76	0.45
Nitrogen free extracts ¹	71.89	58.75
Carbohydrate ²	36.95	48.4
CP/DE ratio (g/Mj kg ⁻¹)	13.85	16.35

FAQ = fair average quality

¹Nitrogen free extracts = 100 - (lipid + protein + ash + fibre)

²Carbohydrate = 100 - (moisture + lipid + protein + ash)

Water quality management

Storage water tanks holding Fertilized, Natural and Tank water analysed weekly to maintain uniformed conditions for tilapia fish reared in tanks. Adjustments were gradually made after taking readings before introducing water sources into fish tanks. Table 3 shows average mean values for the bio-physical and chemical indicators collected during the course of the 90-day experimental period. The data was used to make adjustments before supplying the experimental fish tanks.

Table 3: Water quality mean values for storage water sump tanks holding treatment water sources with standard deviation (\pm S.D.) monitored prior to supplying experimental fish tanks over the 90-day period.

*Water parameters	Fertilized water	Natural water	Tank water	†Recommended
NH ₄ (mg L ⁻¹)	0.15 \pm 0.04	0.26 \pm 0.59	0.33 \pm 0.49	<1.0
pH	8.1 \pm 0.56	8.5 \pm 0.55	8.4 \pm 0.59	6 – 9.5
DO (mg L ⁻¹)	3.42 \pm 1.57	6.79 \pm 0.79	4.4 \pm 0.92	4 - 8
Temperature (C°)	25.6 \pm 1.17	25.9 \pm 1.15	26.5 \pm 1.6	24 – 30
TDS (mg L ⁻¹)	385 \pm 190	316 \pm 205	350 \pm 243	30 - 1500
EC (μs cm ⁻¹)	600 \pm 250	375 \pm 216	342 \pm 246	50 – 1500

*NH₄ = ammonia; DO = dissolved oxygen; TDS = total dissolved solids; EC = electric conductivity

†Published guidelines for best practice culture for tilapia in ponds (Rakocy, 1990).

Water quality parameters measured within experimental tanks followed guidelines for best practice in controlling ammonia, pH, aeration and temperature within tanks (Masser et al., 1999). A programmed wall switch timer (Intermatic© 701B) was used to schedule water intake from the three water sources to the tanks every 3 hrs with a 1 hr delay during feeding. Exceptions were made at 4-7am and 4-7pm where dissolved oxygen (DO) levels tend to drop rapidly. Daily water surface temperature (C°) and DO were recorded using an YSI Environment meter (550A-12) while an EcoSense® Tester (EC 1030A) was used to record pH, electric conductivity (μs cm⁻³) and total dissolved solids (mgL⁻¹). On average, the facility maintained a temperature of 26°C, DO ranging between 3–6 mg L⁻¹ and a mean of pH 7. Ammonia (NH₄) was recorded twice weekly using a Fluval® test kit (0.0–6.1 mgL⁻¹) ranging between 0.1-2.4 mgL⁻¹ for the entire duration.

Feed utilization

Total feed offered and daily feed intakes were averaged and analysed. Feed efficiency data were collected to determine the averaged weight gains, feed conversion ratio (FCR) and protein efficiency ratio (PER) on a dry matter basis using the formula below;

1. FCR = Dry Weight As-feed fed (g) \div Biomass gained per tank
2. PER = Weight gain (g) \div Protein intake (g)

Growth performance parameters

Fish performance was evaluated according to the following indices:

1. Body weight gain (BWG, g) = Final mean weight – Initial weight
2. Specific Growth Rate (SGR) (% day⁻¹) = {Exponent (Ln (final weight) – Ln (initial weight)) \div Experimental days (i.e., 90 days) \times 100 (Crane et al., 2019)}
3. Thermal unit growth coefficient (TGC) = FBW^(1/3) – IBW^(1/3) \times 1000 \div D \times C° (Cho, 1990)
4. Condition factor (K) of the fish was calculated using the formula:
K = 100W \div L^b (Pauly, 1983), where K= condition factor, W= fish weight (g), L= total length of fish (cm). The subscript b is the weight of unit length (slope) estimated from the linear regression transformed by taking the natural logarithm (Log) from both sides.

Statistical analysis

Mean data sets collected were summarized with standard errors calculated prior to statistical data analysis. A Shapiro-Wilk test for normality and a test of homogeneity using Levene's tests for means generated for the assumptions of an analysis of variance (ANOVA). A split plot ANOVA was used as the statistical design. Separation of means between treatment groups used a Least Significant Difference (LSD) test at 5% alpha value. All data generated used GENSTAT® 17th edition statistical software (VSNI, 2014).

3. RESULTS

Feed utilization and Growth Performance

The total feed intake was much lower when fish were offered the BSFL diet B than Basal diet A (Figure 1(1); $P<0.01$). Fish kept in the Tank water had a lower total feed intake than fish in Fertilized or Natural water ($P<0.01$). Biomass gain was higher for fish fed Basal diet (Figure 1(2); $P<0.001$). Fertilized water provided much larger increases in biomass for fish compared to Natural and Tank water (Figure 1(2); $P<0.001$). There were significant differences between the Basal and the BSFL diets between Water sources for total feed intake and biomass gains (Figure 1(1 and 2); $P<0.001$).

Diet effects were significant ($P<0.05$) for all growth parameters except for the final total length (FTL) of fish groups between water sources (Table 4). Basal diet A was clearly superior for all fish growth parameters. Keeping fish in Fertilized water resulted in heavier final body weights (FBW) than in either Natural and Tank water ($P<0.05$). Keeping fish in Fertilized and Natural water resulted in better feed conversion ratio (FCR) and protein efficiency ratio (PER). Thermal growth coefficient (TGC) of the fish was higher in Fertilized water but lowered in Natural and Tank water with significantly better values

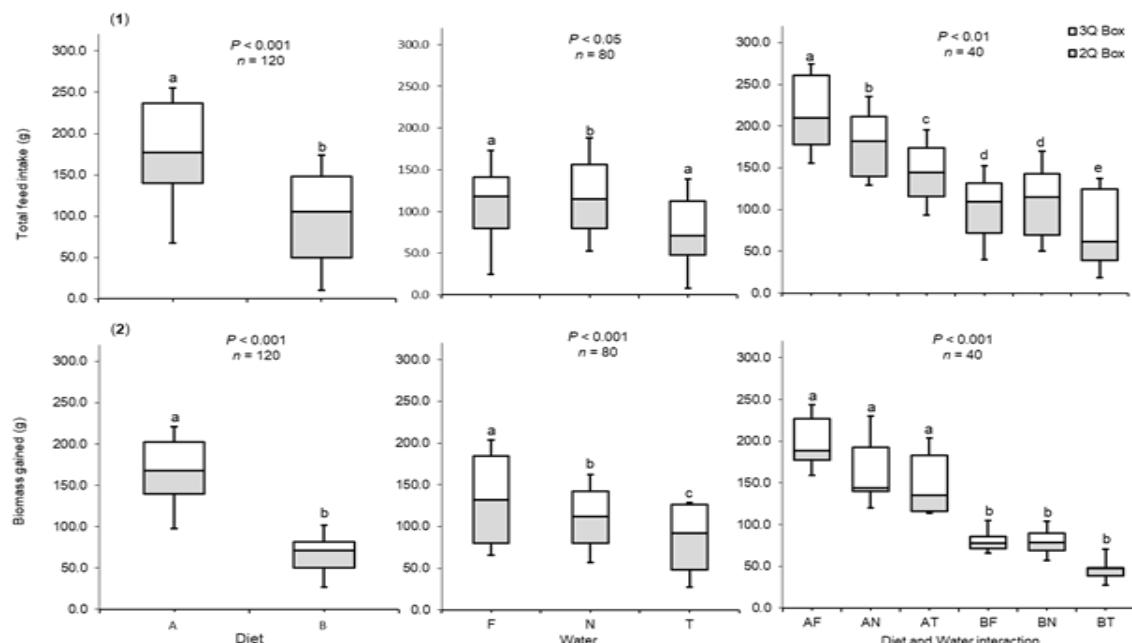


Figure 1: Box-plots showing Total feed intake (1) and Biomass gains (2) fed a Basal (A) and BSFL (B) formulated diet managed in fertilized (F), natural (N) and tank (T) water sources. Boxplots bearing different superscripts differ significantly.

Feeding fish with Basal diet A in Fertilized and Natural water resulted in increased total length (FTL, $P<0.05$). The final body weights were also heavier for fish when provided with the Basal diet in Fertilized water, but lowest for fish provided with BSFL diet B in Fertilized and Tank water. Fish in Natural water fed either Basal or BSFL diets achieved similar final bodyweights ($P<0.05$). In all three water sources the fish demonstrated consistently better FCR, PER, SGR and TGC for Basal than BSFL diet ($P<0.05$). In Tank water BSFL diet resulted in the lowest fish performance for FCR, PER, SGR and TGC. Condition factor (K) for Diets, water sources and the interactions were not significant ($P>0.05$) in growing conditions for fish at this level of testing.

Table 4: Growth performance indicators determined for mono-sex male juvenile GIFT fed a Basal (A) and BSFL (B) formulated diet treated in fertilized (F), natural (N) and tank (T) water sources for 30-day switch over periods in 90 days.

Growth Performance								
	FTL (cm)	FBW (g)	FIT* (g)	FCR	PER	SGR (% day ⁻¹)	TGC	K
Diet (D)	A	9.17 ^a	14.74 ^a	8.36 ^a	1.41 ^a	2.63 ^a	3.57 ^a	3.75 ^a
	B	8.32 ^b	10.59 ^b	7.26 ^b	3.12 ^b	1.19 ^b	2.03 ^b	1.40 ^b
Water (W)	F	8.93	13.9 ^a	7.99 ^a	1.91 ^b	2.19 ^a	2.99 ^a	2.94 ^a
	N	8.67	12.50 ^b	7.49 ^b	1.97 ^b	1.95 ^a	2.97 ^a	2.52 ^b
	T	8.64	11.61 ^b	7.95 ^a	2.91 ^a	1.58 ^b	2.45 ^b	2.27 ^c
D×W	AF	9.69 ^a	17.21 ^a	8.91 ^a	1.25 ^c	3.00 ^a	3.53 ^a	4.21 ^a
	AN	9.07 ^a	13.77 ^b	8.04 ^b	1.36 ^c	2.58 ^c	3.81 ^a	3.58 ^a
	AT	8.75 ^b	13.25 ^b	8.13 ^b	1.62 ^c	2.30 ^c	3.37 ^a	3.47 ^a
	BF	8.16 ^b	10.58 ^c	7.07 ^d	2.58 ^b	1.38 ^b	2.45 ^b	1.67 ^b
	BN	8.27 ^b	11.23 ^b	6.94 ^d	2.57 ^b	1.32 ^b	2.13 ^{bc}	1.47 ^b
	BT	8.53 ^b	9.96 ^c	7.78 ^{bc}	4.20 ^a	0.86 ^d	1.52 ^{bc}	1.06 ^b
	Grand Mean	8.74	12.67	7.81	2.26	1.91	2.80	2.58
Pooled SEM	0.18	0.47	0.26	0.44	0.10	0.42	0.55	0.12
LSD (D)	0.53	1.29	0.27	0.35	0.38	0.61	0.85	0.26
LSD (W)	0.44	1.39	0.43	0.20	0.10	0.24	0.16	0.20
LSD (D×W)	0.66	1.89	0.54	0.38	0.30	0.62	0.85	0.27
P-value (D)	**	***	***	***	***	**	***	ns
P-value (W)	ns	**	*	***	***	***	***	ns
P-value (D×W)	*	*	**	***	*	**	*	ns

Within-column means with different superscripts were separated by LSD at $P<0.05$; S.E.M = standard error of means; $P<0.05^*$; $P<0.01^{**}$; $P<0.001^{***}$; ns = non-significant; D = dietary treatment; W = water source treatment; D×W = diet and water interaction; FBW = final body weight; FTL = final total length; BWG = body weight gained; FIT* = feed intake per tank for 20 fish; FCR = feed conversion ratio; PER = protein efficiency ratio; SGR% = specific growth rate; TGC = thermal growth coefficient; K = condition factor

Water Quality

Diet alone had no measurable impact on the water quality apart from NH₃ for which Basal diet resulted in higher levels ($P<0.05$). Water quality parameters pH, temperature, TDS and EC were very different ($P<0.001$). Natural water provided higher mean pH and temperature and lower DO and TDS compared to Fertilized and Tank waters ($P<0.05$). Diet and Water interactions resulted in significantly different NH₃, pH and TDS ($P<0.05$). The mean ammonia content varied between the Water sources when different Diets were provided to the fish,

whereas, pH was distinctly higher when either A or B diet was supplied to the tanks ($P<0.05$). The interaction of both diet A and B with Natural water resulted in higher pH ($P<0.05$). Similarly, mean TDS and EC varied across the Water sources with different Diets. The TDS in Natural water were statistically lower ($P<0.05$) than for Fertilized and Tank water interaction with Diet while EC in Natural water was also lower with no significant differences ($P>0.05$).

Table 5: Water quality parameters determined for mono-sex male juvenile GIFT tilapia fed a basal and BSFL formulated diet treated in fertilized, natural and tank water sources.

Water Quality Parameters						
	NH_3 (mg L^{-1})	pH	DO (mg L^{-1})	Temperature ($^{\circ}\text{C}$)	TDS (mg L^{-1})	EC ($\mu\text{s cm}^{-1}$)
Diet (D)	A	0.18 ^a	8.11	4.54	25.71	323.10
	B	0.14 ^b	8.15	4.29	25.56	329.07
Water (W)	F	0.17	8.02 ^b	4.53	25.24 ^b	409.63 ^a
	N	0.15	8.30 ^a	4.31	26.40 ^a	260.11 ^b
D×W	T	0.16	8.06 ^b	4.41	25.26 ^b	308.52 ^b
	AF	0.19 ^a	8.02 ^c	4.69	25.37	375.88 ^{ab}
	AN	0.15 ^b	8.22 ^b	4.50	26.41	244.11 ^{ab}
	AT	0.19 ^a	8.09 ^{bc}	4.44	25.35	349.33 ^{ab}
	BF	0.16 ^a	8.02 ^c	4.36	25.12	443.38 ^a
	BN	0.14 ^b	8.38 ^a	4.12	26.39	276.10 ^b
	BT	0.12 ^b	8.03 ^c	4.38	25.17	267.72 ^b
	Grand Mean	0.16	8.13	4.42	25.64	326.09
Pooled SEM						
LSD (D)	0.03	0.09	0.36	0.14	87.86	90.83
LSD (W)	0.02	0.09	0.20	0.13	59.36	58.88
LSD (D×W)	0.03	0.13	0.39	0.19	100.86	102.67
P-value (D)	*	ns	ns	*	ns	ns
P-value (W)	ns	***	ns	***	***	***
P-value (D×W)	*	*	ns	ns	*	ns

Within-row means bearing different superscripts (a, b, c, d) differ significantly = $P<0.05^*$; $P<0.01^{**}$; $P<0.001^{***}$; S.E.M = standard error of means, means with different superscripts were separated by LSD at $P<0.05$; ns = non-significant; D = dietary treatment; W = water source treatment; D×W = dietary and water source treatment interaction; NH_3 = ammonia; DO = dissolved oxygen; TDS = total dissolved solids; EC = electric conductivity

4. DISCUSSION

The formulation of the BSFL diet was based on practical experience, where the vast majority of tilapia fish farmers tend not to provide protein sources to their fish, let alone pelleted fish feed. However, providing 40% of the diet with BSFL as the main source of insect protein and amino acids (Table 1) precluded it from providing the most optimum balance of other nutrients (Makkar et al., 2014; Henry et al., 2015), most evidently the digestible energy, fat, carbohydrates and phosphorus (Table 2). It was not likely that this simple BSFL diet would provide equivalent fish performance to match more complex diets such as the Basal diet. Nevertheless, the tilapia growth performances on the BSFL diet were comparable to the literature, specifically for similarly processed non-defatted BSFL meal (Aini et al., 2018; Tippayadara et al., 2021), and the results also afforded

insights on the influence of fertilized water sources, feeding and management systems on the growth performance of GIFT against different tilapia strains.

The overall significant effects of Diet, Water source and their interactions was notable in the feed intake and fish biomass boxplot comparisons (Fig.1). The 40% BSFL diet provided much lower total biomass gain on a lower total feed intake, suggesting that a longer growth period would be required to reach harvest size fish. However, the diet was resource efficient for utilizing farm-made or grown feed resources like BSF larval meal (Roberts et al., 2019), while also utilizing locally available plant protein feeds, wheat millrun and copra meal. Conversely, Basal diet pellets, which contained a protein concentrate plus additional fish meal (1% DM), provided superior feed intake, feed utilization efficiency and growth performance to GIFT tilapia. Furthermore, it was found that, while Diet alone provided the most significant effect on feed intake, feed utilization efficiency (FCR and PER), and tilapia growth performances (SGR% and TGC), the different Water sources appeared to modulate the diet effect so that fertilized and natural water provided clearly better environment for feed utilization efficiency and growth performance than clean tank water.

Effect of diet

Studies using BSFL meal to replace fishmeal at up to 571 gkg⁻¹ of diet compositions for feeding fish at differing growth stages had no adverse effects in growth parameters for tilapia (Devic et al., 2017; Wachira et al., 2021; Limbu et al., 2022). According to Wachira et al. (2021), an inclusion rate of 336 gkg⁻¹ BSFL meal diet composition provided the best growth performance for tilapia. Tippayadara et al. (2021) concluded that "BSFLM is regarded as one of the best alternatives for partial or complete replacement of FM in Nile tilapia diets". In comparison to other fish species, a 30% inclusion level adversely reduced growth performances in Turbot (*Psetta maxima*), Channel catfish and Rainbow trout (Newton et al., 2005; St-Hilaire et al., 2007; Kroekel et al., 2021); palatability and protein digestibility seemed to further reduce feed intake at 33% in Turbot (Kroekel et al., 2021).

Feed intake was reduced on the BSFL feed across all treatments (Table 4) and it was suspected that the chitinous components of higher inclusion rates of mature BSFL larvae, as was used in the current work, may have reduced the palatability of formulated diets to fish (Sealey et al., 2011; Makkar et al., 2014). It is plausible that other feed digestibility factors related to the presence of indigestible chitin (Sealey et al., 2011) or dietary fibre in copra meal (Knudsen, 1997), may have negatively affected the assimilation of nutrients by the juvenile tilapia. Nevertheless, replacing 100% of fishmeal in a diet combining BSFL meal with soybean and rice bran resulted in improved total feed intake and protein digestibility for tilapia (Tippayadara et al., 2021). This may indicate that a blend of more digestible plant protein ingredients at lower levels is advantageous rather than using BSFL as the sole protein source.

In fact, supplying two or more protein sources in fish diets is recognized to improve growth performance (Bondari et al., 1987) and a combination of either 40:60 or 60:40 fish meal to BSFL meal ratio were estimated to provide the best growth rates (Tippayadara et al., 2021). Moreover, it was found that dried cassava leaf provided an effective protein meal for blending with BSFL meal to replace 100% of fish meal (Aini et al., 2018). This should be expected since tilapia are omnivorous feeders. The inclusion of cassava leaf meal with BSFL in simplified diets for GIFT tilapia produced either at small-scale or for village farming would be practical and cost efficient but would probably pose metabolic challenges to juvenile fish.

Effect of water source

Fertilized water provided the best environment for fish performance followed by natural stream water and tank water the least. Since the water in all the fish tanks was replenished regularly to prevent buildup of organic waste and toxins (Table 3), it is evident that the effect of the Water sources was due to additional organic matter being available in the fertilized and natural water but not the tank water (Table 4). The higher level of TDS in fertilized water supports this observation. On the other hand, higher ammonia was found in tanks with the Basal diet, indicating that excess nutrient was released from feeding on this diet. However, the method of satiation feeding of the fish may have enhanced this effect for Basal diet, whereas reduced intake on BSFL made the effect less apparent. Satiation feeding was recommended for maximum growth performance of GIFT tilapia,

partly due to their improved protein utilization efficiency (Ng et al., 2008). Moreover, satiation feeding is more commonly practiced and most fish-stock kept in ponds are not measured for weight or length to assess their feed requirements.

It was demonstrated that GIFT tilapia has superior growth performances to hybrid red tilapia (Ng et al., 2008) and productivity in mono- and polyculture (Tran et al., 2021), in either fresh or brackish water (Moses et al 2021), and under low input (Kohinoor et al., 1999) or semi-intensive systems (Ahmad & Zulqurnain, 2018). When red hybrid tilapia fingerlings (about 1.5 g BW) were managed, at similar stocking density to the current work, in tank water that was cleaned daily, while feed was offered at 5% of BW, the feed utilization efficiency as FCR and PER was inferior for 30% BSFL substitution (Aisyah et al., 2022), although growth performance (SGR%) was just as effective. Also, when Nile tilapia fingerlings (about 21 g BW) were kept in cages in earthen ponds there was poorer FCR and SGR for 100% BSFL diets substituting soybean meal (Shati et al., 2022). It appears that a lower level of feeding may be offset by the diet composition and nutrient density or the availability of other feed resources for tilapia fish farmed in natural or fertilized water sources (Narimbi et al 2018; Parata et al 2020).

In earthen ponds utilizing diverted river water, juvenile Nile tilapia (about 35 g BW) had reduced feed efficiency for similar simple diets using BSFL meal for up to 100% replacement of fish meal (Wachira et al., 2021). On the other hand, Nile tilapia fry (about 6 g BW) was managed in cages off-shore in a deep lake (30-35 m) and appeared to thrive, with high SGR and slightly better FCR for diets with 80 g kg⁻¹ BSFL meal for substituting fish meal in complex diets (Devic et al., 2017). The results again point to the effectiveness of complex diets for feeding tilapia stock that may have restricted access to other feed resources and it may also reflect the different trophic status of standing lakes (or earthen ponds) compared to flowing streams (Dodds and Smith, 2006).

Effect of diet × water interaction

While the Basal diet provided juvenile tilapia with clearly superior performances for FCR, PER and SGR% in the Natural and Fertilized water sources, performance on the 100% BSFL-protein diet was still comparable with other reports in the literature (e.g., Limbu et al 2022 and References above). For instance, when BSFL meal replaced fish meal at 30% in diets that were offered at 5% of BW, the result for feed utilization efficiency (FCR=3.31 and PER=1.02) and growth performance (SGR%=2.31) were comparable to the current work (Muin et al., 2017). Certainly, the improved feed conversion and protein efficiency in natural and fertilized water indicates that our BSFL diet was effective to provide appreciable SGR% when tilapia may have other nutrient sources available in their environment.

It might also be suggested that the interchanging of diets and water sources in the methodology of the current work caused compensatory growth reaction in the fish-groups moving from lower to higher nutrient density diet or water source. However, the thirty-day feeding periods were expected to have catered for this recovery period for the fish. Comparison of the widely different thermal growth coefficients (TGC) was not reflected in the condition factor (K) ratio estimated from significantly different body length and weight of the fish under the different treatments.

The TGC exhibited higher values indicating metabolic efficiency under trial conditions. In comparison, the BSFL diet appeared to slightly improve with fertilized and natural water sources similar to TGC (1.01) attained in aerated ponds farming Nile tilapia (Mengistu et al., 2022). Similarly, K values were linear in weight and length when fed BSFL formulated diets (Shati et al., 2022); further suggesting that the diets and water interactions had no effect on fish condition (Muin et al., 2017), other factors such as dissolved oxygen and Temperature being stable (Table 5).

An additional outcome from this work was the evidence of high performance by juvenile tilapia as result of satiation feeding with the Basal diet in all three water sources. Since this farm-made pellet feed utilized locally available ingredients, fish meal (1%), wheat millrun (23%) and copra meal (30%), along with a tested and promoted universal concentrate (Sine et al., 2017; Solomon et al., 2016; Dom et al., 2023), the current Basal diet represents a significant improvement from a number of fish feed formulations reported by the same group (Glatz et al., 2017).

In this regard, the conclusions of Narimbi et al (2018) and Parata et al (2020), that tilapia in fertilized ponds consume more naturally available feeds than offered pellet feeds, thereby resulting in poor nutrition and a likelihood to cannibalise weaker fish, may be better contextualised. Specifically, the provision of farm-made formulated feeds, or more prized industrial pellet feed, should be adjusted to the pond environmental and management conditions, namely water quality and available aquatic life (Cho & Bureau 2001; Johansson, 2023). Appropriate feed formulation and feeding levels may then be provided in order for fish to achieve effective growth performance for attaining desired biomass gain more cost-effectively.

5. CONCLUSION

The tested 40% BSFL diet provided competitive feed intake, utilization efficiency and growth performance in juvenile fish. However, manipulating the digestibility of BSFL feed ingredients and studying the implication of other feed resources on fish growth and health in pond systems may improve the utilization of this valuable on-farm produced protein feed resource. It may also be suggested that a combination of the Basal diet ingredients with a higher proportion of BSFL meal is an alternative low-cost option to trial for more advanced small-scale fish farming enterprises. Whereas, the 100% BSFL diet is a marked improvement to local fish diet formulations and is recommended for further verification under controlled research conditions as well as complementary on-farm testing with subsistence farmers.

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