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Black Soldier Fly Larvae Meal as a Fishmeal Substitute in Juvenile Dusky Kob Diets: Effect on Feed Utilization, Growth Performance, and Blood Parameters

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Abstract: Using black soldier fly larvae meal (BSFM) in place of fishmeal is an ingenious strategy for sustainable fish aquaculture. However, BSFM has not been evaluated for dusky kob (*Argyrosomus japonicus*), an economically important fish in South Africa. Therefore, this five-week study investigated the effect of partially replacing fishmeal with BSFM on feed utilization, growth performance, and blood parameters of juvenile dusky kob in a recirculating aquaculture system. Four diets were formulated by replacing fishmeal in a commercial dusky kob diet with BSFM at the rate of 0 (BSFM0), 50 (BSFM50), 100 (BSFM100), and 200 g/kg (BSFM200). Fish length and weights were measured weekly, while blood analyses were performed at the end of Week 5. For fish length and weight gain, there were neither linear nor quadratic responses in Weeks 1–4, while quadratic trends ($y = 14.77\ (\pm\ 0.19)–0.11\ (\pm\ 0.05)x + 0.01\ (\pm\ 0.00)\ x^2$; $R^2 = 0.35$ and $y = 49.85\ (\pm\ 1.53)–1.22\ (\pm\ 0.39)x + 0.06\ (\pm\ 0.02)\ x^2$; $R^2 = 0.47$, respectively) were observed in Week 5 in response to BSFM levels. Quadratic effects ($y = 1.75\ (\pm\ 0.15) + 0.10\ (\pm\ 0.04)x - 0.01\ (\pm\ 0.00)\ x^2$; $R^2 = 0.39$) were also observed for feed conversion ratio (FCR) in response to BSFM inclusion levels. Fish fed BSFM200 had a similar overall FCR and specific growth rate as those reared on BSFM0. All blood parameters fell within the normal range for the dusky kob. We concluded that 20% dietary replacement of fishmeal with BSFM does not compromise feed utilization and growth performance of juvenile dusky kob.

Keywords: black soldier fly meal; dusky kob; feed conversion ratio; growth performance; haematology; serum biochemistry

1. Introduction

Wild fish stocks are a major source of animal protein for humans, but they have largely remained static [1]. Meanwhile, global fish consumption is increasing at a rate of 1.5% per year on a per capita basis, resulting in fish supply shortfalls [2]. This can be addressed by expanding aquaculture. In South Africa, the number of marine finfish farms for commercial production, and more especially for dusky kob (*Argyrosomus japonicus*), has remained low [3], indicating a labored response to the increasing local demand for fish. The growth of dusky kob aquaculture is mostly hampered by the high cost of fishmeal, a major dietary component [4], the supply of which is negatively affected by the static stocks of wild fisheries. Reliance on fishmeal-based diets affects aquaculture profitability [5,6] and environmental sustainability [7]. Indeed, overexploitation of wild fish stocks for fishmeal and oil causes progressive disruption of aquatic food webs and negatively affects the sustainability of these commodities. In addition, the supply of fishmeal and oil tends to be erratic because it depends on the availability of wild-captured forage fish, which cannot be guaranteed. The demand for fishmeal and fish oil on the South African market is high because it is also used in diets of other farmed animals. This has driven the search for economically and environmentally sustainable alternatives such as black soldier fly (*Hermetia illucens*) meal (BSFM).

Black soldier fly larvae are increasingly receiving attention as environmentally, socially, and economically sustainable alternative dietary protein sources for fish. The attractiveness of the larvae is based on their ability to efficiently utilize bio-waste [8] while producing a larvae meal that is rich in protein (42–63%) [9]. Indeed, BSFM has been used to replace fishmeal partially or completely in several fish species, albeit with contradictory growth performance results [10–12]. For example, Kroeckel et al. [13] reported reduced feed intake and lower protein digestibility and growth rate in turbot (*Psetta maximus*) when BSFM dietary inclusion levels reached 330 g/kg. On the other hand, no such negative impacts on feed intake and growth performance were observed when Atlantic salmon were fed BSFM at 50, 100, and 150 g/kg inclusion levels [14]. The inclusion of a black soldier fly frass (a heterogeneous mixture of larval excrement, exoskeleton sheds and residual feed ingredients, chitin, and beneficial microbes) at 50, 100, 200, and 300 g/kg improved feed intake and growth in hybrid tilapia [15]. This discordance across studies appears to be driven by variations in the industrial production of the flies, inclusion levels of BSFM in fish diets, and the fish species studied. Low levels of essential and non-essential amino acids in BSFM [16–18] compared to fishmeal [19] may explain the negative effects of BSFM when included at high levels in aquafeeds. Cummins et al. [16] further suggested that some negative effects of dietary BSFM on digestibility and growth of fish may be due to high proportion of chitin in this alternative protein source. The utility of BSFM as an alternative to fishmeal has not been evaluated for juvenile dusky kob, the only commercially farmed marine finfish species in South Africa. Consequently, the tolerance of dusky kob to the dietary inclusion of BSFM is unknown. The potential utilization of BSFM in future dusky kob diets could reduce farmers and feed manufacturers' dependency on fishmeal as a protein source. This could turn dusky kob farming into environmentally, socially, and economically sustainable enterprises. Therefore, this study investigated the effect of replacing dietary fishmeal with BSFM on feed utilization, growth performance, haematology, and serum biochemistry in juvenile dusky kob. Haematological and serum biochemical parameters of the fish are used to monitor the nutritional and health effects of BSFM on the fish. Given that the protein value of insect meals in other aquaculture species such as the Atlantic salmon has already been demonstrated [14], we hypothesized that partially replacing fishmeal with BSFM as a dietary protein source would not impair feed utilization, growth performance, and blood parameters in the dusky kob.

2. Materials and Methods

2.1. Experimental Diet Composition and Preparation

The ingredients and chemical composition of formulated experimental diets are shown in Table 1. A defatted commercial BSFM product was sourced from AgriProtein Technologies, Cape Town, South Africa. The BSF flies were kept in digitally controlled, bio-secure conditions that mimic the natural environment to maximize egg production and ensure optimal welfare. Upon hatching, the BSF larvae were reared on pumpkin and orange organic waste from food factories. The larvae were then killed in boiling water and dried in an oven at 60 °C until constant weight. The dried larvae were homogenized and defatted to produce a meal that was then analyzed for quality control purposes and packaged. Four isonitrogenous diets were formulated by replacing fishmeal with BSFM at the rate of 0 (BSFM0), 50 (BSFM50), 100 (BSFM100), and 200 g/kg (BSFM200) in a commercial dusky kob diet. Samples of formulated diets (BSFM0, BSFM50, BSFM100) were analyzed using the Association of Official Agricultural Chemists methods [20] for laboratory dry matter (method no. 930.15), ash (method no. 924.05), crude fiber (method no. 978.10), crude lipid (method no. 920.39), and crude protein (method no. 984.13). Table 1 shows that experimental diets were isonitrogenous (crude protein content: 469.5–487.6 g/kg DM) and isolipidic (crude lipid: 251.7−283.2 g/kg DM).

Table 1. Ingredients (g/kg) and chemical composition (g/kg DM, unless stated otherwise) of black soldier fly meal (BSFM) and experimental diets.

	Fishmeal	BSFM	Diets [1]			
			BSFM0	BSFM50	BSFM100	BSFM200
Ingredients						
Fishmeal [2]			682	632	582	482
BSFM			0	50	100	200
Bulk agent (cellulose)			219	219	219	219
Maize gluten			15	15	15	15
Premix [2]			7	7	7	7
Fish oil (mL)			77	77	77	77
Proximate composition						
Dry matter (g/kg)	914	950	936	972	944	951
Ash	210	93	107	110.5	104.8	133.4
Moisture	86	55	65	38	28	47
Crude protein	460	550.2	487.6	484.4	469.5	478.3
Crude fiber	15	57.8	13.4	15.6	10.4	19.6
Crude lipid	170	180	262	283.2	251.7	273.8

[1] Diets: BSFM0 = Commercial dusky kob diet in which fishmeal is the major protein source (control); BSFM50 = Dusky kob diet in which fishmeal replaced with BSFM at 50 g/kg; BSFM100 = Dusky kob diet in which fishmeal replaced with BSFM at 100 g/kg; BSFM200 = Dusky kob diet in which fishmeal replaced with BSFM at 200 g/kg. [2] Vitamins and minerals premix: procaine HCI (15 mg), methylsulphonylmethane (MSM) (300 mg), lecithin (300 mg), alpha-tocopherol (vitamin E) (30 mg), thiamine HCI (vitamin B1, 10 mg), riboflavin (vitamin B2, 3 mg), pyridoxine HCI (vitamin B6, 3 mg), nicotinamide (10 mg), calcium pantothenate (10 mg), choline (40 mg), magnesium (100 µg), chromium (25 µg), zinc amino acid chelate (10 mg), inositol (30 mg), manganese (75 µg) and iron (5 mg).

The dietary treatments were prepared and mixed at the Marine Research Aquarium as previously described by Madibana et al. [20]. Briefly, the powdered fishmeal was mixed with pre-weighed quantities of BSFM, cellulose (bulk agent), fish oil, maize gluten, and a premix of multi-vitamins and minerals. Water was added and the mixture was kneaded to produce a dough, which was then rolled out into a thin layer using a kitchen dough roller. The thin layer was dried to constant weight using a household floor fan. The flaked pieces of dried feed were then ground to different sizes ranging from 2 to 4 mm using a corn kernel hand grinder with an adjustable pressure disc for flake sizing.

2.2. Experimental System Components

The study was carried out at the Marine Research Aquarium of the Department of Environment, Forestry and Fisheries in Sea Point, Cape Town, South Africa. The experimental system was a recirculating aquaculture system consisting of 16 black, high-density polyethylene grow-out tanks (465 L capacity, 67 cm deep, and 94 cm in diameter) with flattened conical floors coated with white fiberglass resin to allow for better fish visibility. The sea water temperature was maintained at 25 °C [20,21] via a heat pump and dissolved oxygen at 5.5–6.0 mg/L via air lines [20,21]. The system design ensured a maximum water flow rate of 35 L per minute delivery to the fish holding tanks. The filtration system included a protein skimmer or foam fractionator, a sand filter, and biological filtration media. Ultraviolet lights (55 W) were fitted on the water route between the filtration system and the fish holding tanks. Ammonia was tested twice weekly using Sera ammonium/ammonia test kit (North Rhine-Westphalia, Germany). OxyGuard meter (OxyGuard international A/S, Denmark) was used to monitor both dissolved oxygen and temperature daily.

2.3. Transportation and Acclimatization of Experimental Fish

The handling of live fish was conducted in compliance with the South African Animals Protection Act, 1962 (Act 71 of 1962). Ethical clearance was obtained from the North-West University's Animal Research Ethics Committee (NWU-00691-17-S9). Dusky kob fingerlings were sourced from a commercial fish farm based in Paternoster, Western Cape Province, off the South African west coast. They were transported in 950 L of sea water, with a salinity of 34 ppt and a temperature of 26 °C. Upon arrival, 1280 fish were randomly and evenly distributed to each of the 16 tanks and left to acclimatize for a period of 2 weeks prior to trial commencement. Commercial fishmeal diet (SA Feed Pty Ltd., Western Cape, South Africa) was offered to the fish at 2.8% body weight during the acclimatization period. At the start of the experiment, the fish weighed, on average, 25.53 ± 0.50 g and were 12.14 ± 0.07 cm in length (mean ± standard error of the mean).

2.4. Feeding Strategy and Sampling

Each dietary treatment was allocated to four replicate tanks and fish were hand fed at the rate of 2.8% body weight per day, a rate that is sufficient for juvenile dusky kob and ensures that there are no refusals after feeding [20]. The daily ration was evenly split for 08h00 and 15h00 feeding sessions. Due to high photophobia tendencies and cannibalistic behavior, the fish were not exposed to any artificial lighting. Ten fish per replicate tank (40 fish per treatment) were randomly sampled weekly to measure standard length and body mass (Mettler Toledo electronic balance model: Viper SW 15). A 0.2 mL/L dose of 2–phenoxyethanol was used to anesthetize fish during the measurements. Sampled fish were returned to the tank of origin after measurements. Feed conversion ratio (FCR) was calculated based on the feed intake and weight gain of the fish for the duration of the feeding trial based on the following formula:

$$\text{FCR} = \frac{\text{Feed intake (g)}}{\text{Weight gained (g)}}$$

The specific growth rate (SGR) was also calculated at the end of the feeding trial using the following formula:

$$\text{SGR} = \left[\frac{(\text{Ln Final fish weight.} - \text{ Ln Initial fish weight.})}{\text{Time interval (days)}}\right] \times 100$$

Feed offered was adjusted on a weekly basis after body mass measurements. Tanks were continuously monitored before, during and after feeding for any mortality or behavioral changes in the fish.

2.5. Haematology and Serum Biochemical Analyses

At the end of the five-week feeding trial, a random sample of five fish per tank (20 fish per treatment) was anesthetized using 2-phenoxyethanol before blood was collected. Blood was collected and analyzed for haematology and serum biochemistry as previously described by Madibana et al. [21]. Manual blood count (light microscope (Olympus, Tokyo, Japan) under oil immersion at 100 × magnification) was performed for haematocrit, thrombocytes, lymphocytes, monocytes, neutrophils, basophils, and eosinophils. Alkaline phosphatase (ALP) activity was analysed according to Wright et al. [22] while aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were analyzed using the Reitman and Frankel [23] method. The total protein (method no. SM4-0147 K82), albumin (method no. SM4-0131 K82) and creatinine (method no. SM4-0141 K82) levels were analyzed, using an autoanalyzer (Technicon RA 1000, Bayer, Tarrytown, NY, USA) according to the manufacturer's instructions. The total globulin fraction was determined by subtracting the albumin from the total protein. Using the Boehringer Mannheim GPO-PAP kit, the standard RA-1000 enzymatic method was employed for triglycerides analyses [24]. The monocholesterol (CHOD-PAP) method was applied to determine cholesterol concentration [25], while urea was estimated according to the Crest Biosystems Modified Berthelot method [26].

2.6. Statistical Analysis

Measurements from multiple fish per tank were averaged before analysis, such that each replicate tank had one value. The NORMAL option in the Proc Univariate statement was used to test for the normality of measured parameters [27]. Data from all measured parameters were evaluated for linear and quadratic effects using polynomial contrasts. Response surface regression analysis [27] was applied to describe the responses of dusky kob to inclusion levels of BSFM using the following quadratic model: $y = c + bx + ax^2$, where y = response variable; a and b are the coefficients of the quadratic equation; c is intercept; x is dietary BSFM levels (%) and $-b/2a$ is the BSFM value at the vertex (optimal response).

3. Results

3.1. Experimental Diets, Feed Utilization and Growth Performance

There were no feed refusals after each feeding session throughout the experiment. There were neither linear nor quadratic trends for fish weight and standard length from Week 1 to 4 in response to incremental levels of dietary BSFM.

However, a significant quadratic response to BSFM levels was observed for fish length ($y = 14.77 (\pm 0.19) - 0.11 (\pm 0.05)x + 0.01 (\pm 0.00) x^2$; $R^2 = 0.35$) (Table 2) and fish weight gain ($y = 49.85 (\pm 1.53) - 1.22 (\pm 0.39)x + 0.06 (\pm 0.02) x^2$; $R^2 = 0.47$) in the last week of feeding (Week 5) (Table 3).

Table 4 shows that, over the five-week feeding period, there were no linear or quadratic effects of BSFM on feed intake ($p > 0.05$) and SGR. However, dietary BSFM had a significant quadratic effect ($y = 1.75 (\pm 0.15) + 0.10 (\pm 0.04)x - 0.01 (\pm 0.00) x^2$; $R^2 = 0.39$) on FCR (Table 4).

Table 2. Average weekly standard-length gains (cm) of dusky kob, *Argyrosomus japonicus*, in response to increasing levels of dietary black soldier fly meal (BSFM).

	Diets [1]				SEM [2]	Significance [3]	
	BSFM0	BSFM50	BSFM100	BSFM200		Linear	Quadratic
Week 1	0.31 [a]	0.18 [a]	0.20 [a]	0.03 [a,b]	0.09	NS	NS
Week 2	0.55 [a]	0.93 [a]	0.48 [a]	0.64 [a]	0.12	NS	NS
Week 3	0.20 [a]	0.48 [a]	0.93 [a]	0.44 [a]	0.10	NS	NS
Week 4	0.69 [b]	3.66 [b]	0.19 [a]	0.17 [a]	0.77	NS	NS
Week 5	0.79 [b,c]	0.21 [a]	0.90 [b,c]	1.21 [c]	0.11	NS	*

[1] Diets: BSFM0 = Commercial dusky kob diet in which fishmeal is the major protein source (control); BSFM50 = Dusky kob diet in which fishmeal replaced with BSFM at 50 g/kg; BSFM100 = Dusky kob diet in which fishmeal replaced with BSFM at 100 g/kg; BSFM200 = Dusky kob diet in which fishmeal replaced with BSFM at 200 g/kg. [a,b,c] Means in the same column with common superscripts do not differ ($p > 0.05$). [2] SEM: standard error of the mean. [3] Significance: NS = $p > 0.05$; * = $p < 0.05$.

Table 3. Average weekly weight gain (g) of dusky kob, *Argyrosomus japonicus*, in response to increasing levels of dietary black soldier fly meal (BSFM).

	Diets [1]				SEM [2]	Significance [3]	
	BSFM0	BSFM50	BSFM100	BSFM200		Linear	Quadratic
Week 1	3.31 ^{a}	2.35 ^{a}	2.11 ^{a}	0.03 ^{a,b}	0.58	NS	NS
Week 2	4.22 ^{a,b}	5.68b	2.52 ^{a,b}	1.48 ^{a}	0.88	NS	NS
Week 3	1.16 ^{a}	4.15 ^{a,b}	5.26 ^{a}	3.59 ^{a}	0.77	NS	NS
Week 4	6.16 ^{b}	4.88 ^{a,b}	0.39 ^{a}	2.42 ^{a,b}	1.31	NS	NS
Week 5	9.27 ^{b}	3.42 ^{a}	8.74 ^{b}	12.74 ^{b}	1.03	NS	*

[1] Diets: BSFM0 = Commercial dusky kob diet in which fishmeal is the major protein source (control); BSFM50 = Dusky kob diet in which fishmeal replaced with BSFM at 50 g/kg; BSFM100 = Dusky kob diet in which fishmeal replaced with BSFM at 100 g/kg; BSFM200 = Dusky kob diet in which fishmeal replaced with BSFM at 200 g/kg. ^{a,b} Means in the same column with common superscripts do not differ ($p > 0.05$). [2] SEM: standard error of the mean. [3] Significance: NS = $p > 0.05$; * = $p < 0.05$.

Table 4. Overall feed intake (g/fish/day), specific growth rate (SGR) and feed conversion ratio (FCR) of dusky kob, *Argyrosomus japonicus*, in response to increasing levels of dietary black soldier fly meal (BSFM).

	Diets [1]				SEM [2]	Significance [3]	
	BSFM0	BSFM50	BSFM100	BSFM200		Linear	Quadratic
Feed intake	0.30 ^{a}	0.30 ^{a}	0.28 ^{a}	0.28 ^{a}	0.05	NS	NS
SGR (% per day)	1.90 ^{a}	1.69 ^{a}	1.69 ^{a}	1.75 ^{a}	0.08	NS	NS
FCR	1.73 ^{a}	2.20 ^{b}	2.20 ^{b}	1.66 ^{a}	0.10	NS	*

[1] Diets: BSFM0 = Commercial dusky kob diet in which fishmeal is the major protein source (control); BSFM50 = Dusky kob diet in which fishmeal replaced with BSFM at 50 g/kg; BSFM100 = Dusky kob diet in which fishmeal replaced with BSFM at 100 g/kg; BSFM200 = Dusky kob diet in which fishmeal replaced with BSFM at 200 g/kg. ^{a,b} Means in the same column with common superscripts do not differ ($p > 0.05$). [2] SEM: standard error of the mean. [3] Significance: NS = $p > 0.05$; * = $p < 0.05$.

3.2. Haematological and Serum Biochemical Parameters

Table 5 shows that there were neither linear nor quadratic effects on haematological parameters ($p > 0.05$).

Table 5. Haematological parameters of dusky kob, *Argyrosomus japonicus*, in response to black soldier fly meal (BSFM).

Parameters	Diets [1]				SEM [3]	Significance [2]	
	BSFM0	BSFM50	BSFM100	BSFM200		Linear	Quadratic
Haematocrit (%)	43.63 ^{a,b}	46.40 ^{b}	38.83 ^{a}	41.68 ^{a,b}	1.05	NS	NS
Thrombocytes (/hpf)	1.50 ^{a}	11.53 ^{a}	6.63 ^{a}	5.43 ^{a}	1.53	NS	NS
Lymphocytes (%)	87.75 ^{a}	78.25 ^{a}	77.00 ^{a}	72.75 ^{a}	3.08	NS	NS
Neutrophils (%)	3.25 ^{a}	3.00 ^{a}	4.50 ^{a}	1.50 ^{a}	0.97	NS	NS
Eosinophils (%)	2.50 ^{a}	7.25 ^{a}	3.75 ^{a}	3.50 ^{a}	0.82	NS	NS
Basophils (%)	4.25 ^{a}	9.00 ^{a}	5.50 ^{a}	6.50 ^{a}	0.91	NS	NS
Monocytes (%)	2.25 ^{a}	3.79 ^{a}	9.25 ^{a}	15.75 ^{a}	3.32	NS	NS

[1] Diets: BSFM0 = Commercial dusky kob diet in which fishmeal is the major protein source (control); BSFM50 = Dusky kob diet in which fishmeal replaced with BSFM at 50 g/kg; BSFM100 = Dusky kob diet in which fishmeal replaced with BSFM at 100 g/kg; BSFM200 = Dusky kob diet in which fishmeal replaced with BSFM at 200 g/kg. ^{a,b} Means in the same row with common superscripts do not differ ($p > 0.05$). [2] Significance: NS = $p > 0.05$; * = $p < 0.05$. [3] SEM: standard error of the mean.

A linear trend ($p < 0.05$) was observed for alanine transaminase ($y = 14.36\ (\pm 5.84) + 0.32\ (\pm 1.48)x + 0.06\ (\pm 0.07)\ x^2$; $R^2 = 0.49$), aspartate aminotransferase ($y = 69.16\ (\pm 12.14) + 4.24\ (\pm 3.08)x - 0.10\ (\pm 0.14)\ x^2$; $R^2 = 0.31$) and triglycerides ($y = 1.44\ (\pm 0.\ 17) + 0.02\ (\pm 0.04)x - 0.00\ (\pm 0.00)\ x^2$; $R^2 = 0.28$)

(Table 6). No linear or quadratic trends were observed for the rest of the analyzed serum metabolites ($p > 0.05$) in response to BSFM levels.

Table 6. Effect of feeding black soldier fly meal (BSFM) on concentration of selected blood serum metabolites in dusky kob, *Argyrosomus japonicus*.

Parameters [4]	Diets [1]				SEM [3]	Significance [2]	
	BSFM0	BSFM50	BSFM100	BSFM200		Linear	Quadratic
Urea (mmol/L)	2.03 ^{a}	2.33 ^{a}	1.88 ^{a}	1.77 ^{a}	0.10	NS	NS
CREA (umol/L)	9.00 ^{a}	15.50 ^{a}	15.75 ^{a}	16.25 ^{a}	2.60	NS	NS
TP (g/L)	35.00 ^{a}	35.50 ^{a}	36.75 ^{a}	35.50 ^{a}	1.15	NS	NS
ALB (g/L)	12.50 ^{a}	12.50 ^{a}	12.7 ^{a}	12.50 ^{a}	0.30	NS	NS
GLOB (g/L)	22.50 ^{a}	23.00 ^{a}	24.00 ^{a}	23.00 ^{a}	0.88	NS	NS
ALB/GLOB	0.55 ^{a}	0.52 ^{a}	0.53 ^{a}	0.58 ^{a}	0.16	NS	NS
ALT (U/L)	13.00 ^{a}	21.00 ^{a,b}	20.50 ^{a,b}	43.75 ^{b}	4.07	*	NS
AST (U/L)	69.00 ^{a}	88.25 ^{a}	101.00 ^{a}	113.00 ^{a}	7.24	*	NS
ALKP (U/L)	59.75 ^{a}	62.25 ^{a}	62.75 ^{a}	62.25 ^{a}	2.15	NS	NS
CHOL (mmol/L)	1.78 ^{a}	1.48 ^{a}	2.08 ^{a}	1.91 ^{a}	0.11	NS	NS
TRIG (mmol/L)	1.49 ^{a}	1.42 ^{a}	1.77 ^{a}	1.98 ^{a}	0.10	*	NS

[1] Diets: BSFM0 = Commercial dusky kob diet in which fishmeal is the major protein source (control); BSFM50 = Dusky kob diet in which fishmeal replaced with BSFM at 50 g/kg; BSFM100 = Dusky kob diet in which fishmeal replaced with BSFM at 100 g/kg; BSFM200 = Dusky kob diet in which fishmeal replaced with BSFM at 200 g/kg. [a,b] Means in the same row with common superscripts do not differ ($p > 0.05$). [2] Significance: NS: $p > 0.05$; * $p < 0.05$. [3] SEM: standard error of the mean. [4] Parameters: ALB, albumin; ALKP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate aminotransferase; CHOL, cholesterol; CREA, creatinine; GLOB, globulin; TP, total protein; TRIG, triglycerides.

4. Discussion

4.1. Feed Intake, Feed Utilization and Growth Performance

No previous studies have been carried out to evaluate the utility of insect meals in diets for dusky kob. The current study, therefore, represents the first attempt at evaluating the use of this sustainable protein source in this important South African aquaculture fish species. In addition, the inclusion of BSFM or other insect meals in diets of other carnivorous fish has generated wildly conflicting results [11,12,28,29]. According to Henry et al. [30] and Barroso et al. [31], insect meals such as the BSFM have comparable nutritional qualities to fishmeal. However, the chemical composition of BSFM varies greatly. For example, the BSFM used in this feeding trial had higher protein content (550 g/kg DM) compared to BSFM previously used by Barroso et al. [31] (362 g/kg DM) and Devic et al. [32] (416.4 g/kg DM). The differences in protein content of these insect meals could be due to the different substrates used to grow the BSF larvae [33]. Vegetable waste, chicken feed and restaurant waste are some of the substrates that are used in the rearing process [33] resulting in varied nutritional composition of the BSF larvae. However, despite differences in protein content (399–431 g/kg) of BSF larvae, the same authors reported small substrate-induced differences in amino acid profiles.

No mortalities were recorded throughout the 35 days of feeding. Visual observations of the feeding behavior of the juvenile dusky kob showed that there were no palatability issues with the BSFM-containing experimental diets. The inclusion of BSFM in the diets of the current study revealed no linear or quadratic effects of BSFM on feed intake, which is in contrast with Kroeckel et al. [13] who recorded a significant reduction in feed intake, coupled with lower protein digestibility and growth performance at higher BSFM inclusion levels (330 g/kg fishmeal) in turbot (*Psetta maximus*) diets. However, substitution of fishmeal protein with BSFM at inclusion levels up to 14.75% did not compromise feed intake, growth performance or nutrient utilization in sea-water phase Atlantic salmon [14]. In the current study, fish weight increased by 85.3% from the start to the end of the feeding trial. In the final week of feeding, a quadratic response of fish weight to BSFM inclusion was evident. In addition, BSFM inclusion induced a significant quadratic and non-significant linear responses in terms of FCR in contrast with Dumas et al. [11], who observed a linear response in the FCR of rainbow trout. Dietary BSFM has been reported to have no effect on growth and feed efficiency in carnivorous fish such as gilthead seabream [34], rainbow trout [35] and Atlantic salmon [36]. Similarly, Iaconisi et al. [37] reported no changes in growth performance of blackspot seabream when reared on *Tenebrio molitor* larvae meal as a fishmeal replacement at levels up to 400 g/kg. Variations in terms of growth performance responses to insect meals among carnivorous fish can be attributed to several factors that include differences in terms of duration of experiments, water temperature, BSFM amino acid profile, and BSFM chitin content. Indeed, environmental conditions such as temperature have been shown to affect feed utilization efficiency and growth performance of arctic charr [38], European seabass [39], and Atlantic salmon [40]. Differences in habitats between the freshwater carnivorous fish and saltwater carnivorous fish may also influence BSFM utilization [41]. Variations in terms of BSFM amino acid profile and chitin content are caused by differences in the type of substrates used to produce larvae [33] and the age of the larvae when harvested for use [42], respectively.

In Week 5 of the study, juvenile dusky kob fed the control diet had statistically similar weight gain as those reared on the diet containing the highest level of BSFM. In addition, substitution of fishmeal with BSFM at 200g/kg resulted in similar overall feed intake, SGR and FCR. However, in younger (weeks 1–4) dusky kob, higher BSFM inclusion levels caused lower feed intake and weight gains compared to those reared on the control diet (BSFM0). Growth performance may also have been compromised by a suboptimal essential amino acid profile [31] in BSFM, which would have been inadequate to meet the higher amino acid requirements of the younger fish. Indeed, the protein value of insect meals is known to be inferior to fishmeal because it has limited quantities of essential amino acids such as histidine, threonine, and lysine [31]. In addition, chitin is a major component of black soldier fly larvae and has been reported to negatively affect the digestibility of BSFM in fish that lack chitinase activity [13,43]. The chitin content of BSF larvae has been reported to range from 6 to 9% [33,44], while chitinase enzyme activity has not yet been demonstrated in the dusky kob.

4.2. Haematological and Serum Biochemistry Parameters

Haematological parameters of experimental fish are an effective index to monitor physiological and pathological aberrations that may arise when novel dietary components are evaluated [21,45]. All haematological and serum biochemistry parameters from the current study were within the normal range for dusky kob [21,46]. These results suggest that feeding BSFM-containing diets did not compromise the physiological status of the dusky kob. Even though they fell within the normal range for dusky kob, serum AST and ALT linearly increased in response to BSFM levels in the current study. These observations were inconsistent with those of Belghit et al. [14] who observed no effect of BSF larvae meal on serum AST and ALT in the Atlantic salmon. Elevated (beyond the normal range) serum AST and ALT levels are known to be symptomatic of liver damage in *Clarias gariepinus* [47]. The cholesterol-reduction effect of BSFM reported in the European seabass [32] was not observed in the current study. This effect on cholesterol is attributed to the cholesterol-lowering properties of chitosan, which is found in high levels in BSFM [48]. However, it is likely that the dusky kob has low or no chitinase activity, which would explain the poor utilization of diets containing higher levels of BSFM in Weeks 1 to 4. Therefore, the fish would have been unable to breakdown and absorb enough chitin or its functional derivatives to induce a hypocholesterolemic effect as expected.

5. Conclusions, Limitations, and Future Research

Based on overall feed intake, SGR and FCR, substituting 20% of fishmeal with BSFM did not compromise feed utilization efficiency and growth performance in juvenile dusky kob. However, for higher gains in terms of environmental, economic, and social sustainability of dusky kob aquaculture, higher inclusion levels of BSFM in place of fishmeal may be necessary. Similarly, replacing fishmeal with BSFM in juvenile dusky kob diets did not compromise their haematological and serum biochemical parameters, which remained within the normal range. To this end, additional studies must evaluate higher BSFM inclusion levels in dusky kob diets. In these studies, supplementary dietary essential amino acids and/or pre-treatment with exogenous chitinase may be investigated as strategies to improve the utilization of BSFM-rich diets.

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