

# Partial replacement of fishmeal with Chaetomorpha algae improves feed utilization, survival, biochemical composition and fatty acids profile of farmed shrimp *Penaeus monodon* post larvae

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## Research Article

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# Abstract

Shrimp is an economic important shellfish species for aquaculture in Tanzania, however its farming is constrained by reliant to costly feeds, attributed by fishmeal (FM) as main ingredient, which is expensive and scarce. This study determined the efficacy of plant protein derived from *Chaetomorpha* algae (CA) on survival, feed utilization, biochemical composition and fatty acids profile of the cultured *Penaeus monodon* as potential candidate for replacing FM in feed formulation. Four experimental feeds; F0, F1, F2 and F3 were made, denoting FM replacement levels of 0, 10, 20 and 30%, respectively. Post-larvae weighing  $0.49 \pm 0.06$  g were stocked at 15 individuals/m<sup>3</sup> in 2m<sup>3</sup> harpa nets in three replicates. F2 treatment showed significantly high survival rate. F2 and F3 improved growth significantly with F3 exhibiting the highest weight gain across treatments. Impacts of treatments on biochemical composition varied significantly across inclusion levels. The highest muscle somatic index (MSI) observed in F2 treatment. F3 treatment exhibited significantly high crude protein (CP) over the rest. Crude fat (CF) in F0 and F2 treatments were significantly high across inclusion levels. F0 and F1 treatments exhibited high proportion of saturated fatty acids (SFA) than the rest. In contrast, F2 and F3 treatments exhibited high levels of polyunsaturated fatty acids (PUFA) being superior to other treatments. Inclusion of CA at 20 to 30% levels in feed formulation improved feed utilization, biochemical composition and fatty acids profile of farmed *Penaeus monodon*. However, CA inclusion at 30% shown to impair survival rate and exhibited high saturated fatty acids. Thus, at 20% replacement level, CA is suitable for partial replacement of FM in *P. monodon* feeds formulation.

## 1. Introduction

With suitable land and vast water resources, Tanzania has the highest potential for aquaculture in Africa. However, aquaculture in Tanzania is typically a subsistence activity practiced mostly by small-scale farmers (Chenyambuga et al., 2012). Shrimp farming, is one of the aquaculture practices in the country, started in Bagamoyo District in early 1989 (Ngoile, 2012). However, as of now, there are only few extensive aquaculture systems along the coast and one farm practicing semi-intensive farming on Mafia island (MLF, 2018), an indication of local communities failure to adopt commercial farming. This failure is exacerbated in part by high operating costs associated with shrimp farming, primarily related to the production of high-quality shrimp feeds attributed by costs associated with FM which is the main source of protein, and the technical procedures on shrimp feed production. As a result, shrimp farming in the country is entirely dependent on imported feeds, which are generally expensive and difficult to access by most local farmers.

Protein is the key building block for feed formulation systems (FAO, 2003), as well as a major and expensive component of feeds (Shiau, 1998). The commonly used source of protein in shrimp feeds is FM (Smith et al., 2005), however, the decline in fish stocks (Akiyama et al., 1995), irregular availability, contribution to fisheries deterioration, potential for adulteration, biological pathogens (Fox et al., 2004), high costs (Hardy, 2006), and limited and unpredictable harvesting (Nagappan et al., 2021) have steered up research for its replacement. Consequently, several studies (Nasmia et al., 2022; Yıldırım et al., 2014)

have been conducted to replace FM partially or completely as main protein source in fish feeds to reduce costs and improve feed utilization efficiency in farmed organisms.

Despite the importance of algae, as candidate for replacement of FM in shrimp feeds formulations, its literature is currently scarce. However, several studies on the replacement of FM with algae in different culture species have been conducted (Hanel et al., 2007; Hussain et al., 2018; Nasmia et al., 2022). Nasmia et al. (2022) reported that, addition of *Caulerpa sp.* flour to the polyculture system of whiteleg shrimp and *Chanos chanos* feed improved feed utilization efficiency, survival rate and lowered feed conversion ratio. Addition of 30 gKg<sup>-1</sup> of *Caulerpa lentillifera* improved weight conversion rate, and feed efficiency of *P. monodon* (Putra et al., 2019). Meanwhile, Hafezieh et al. (2014) reported improved growth of *Litopenaeus vannamei* after addition of *Sargassum sp.* flour to the feed. Nonetheless, the utilization of CA on shrimp feeds formulation has not yet been reported. Meanwhile, the efficacy of FM replacement with algae on amino acids profile has been scarcely reported. Ju et al. (2009) reported increased fatty acid after addition of whole *Thalassiosira weissflogii* to *Litopenaeus vannamei* feed. González-Meza et al. (2022) reported on improved protein composition in *Penaeus vannamei* at 3% addition of a mixture of three different microalga species, *Tetraselmis suecica*, *Dunaliella salina*, and *Chaetoceros muelleri*. Nevertheless, the effect of replacing FM with CA on fatty acids profile of *P. monodon* has not yet been reported.

CA are abundant in Tanzania coastline and are rich in nutrients. Commonly harvested by locals during low tide for terrestrial animal feeds formulation. Generally, macroalgae are rich in nonstarch polysaccharides, vitamins, minerals (Wong & Cheung, 2000), proteins and lipids (Da Silva & Barbosa, 2008), and bioactive substances (Khan & Satam, 2003). Bioactive substance in algae include carotenoids, alkaloids, polyphenols, phycocyanins, terpenes and several enzymes (Maharana et al., 2015). According to Gokulakrishnan et al. (2015) and Kumar et al. (2008), algae is an excellent source of dietary protein.

Protein content in macroalgae varies from 10 to 47% of the dry weight across species, with red algae being superior to other (Cruz-Suárez et al., 2008; Setthamongkol et al., 2015). The protein content in green algae ranges from 9 to 26% in dry weight (Fleurence et al., 2018) while in CA has been reported to be about 20.4 g/100 g (Tsutsui et al., 2015). The species has also been reported to exhibit antioxidant properties (Gazali et al., 2019) making it impending candidate for promoting growth. These facts make CA a potential candidate in FM replacement trials. Co-culture trials of CA with *P. monodon* has been reported to enhance growth performance and reduce feed conversion ratio (Tsutsui et al., 2015).

The current study investigated the efficacy of partial replacement of FM with cheap green macroalgae CA in *P. monodon* rearing. The effect of feed formulated by incorporating CA on *P. monodon* was determined by assessing survival, feed utilization, biochemical composition and amino acid profile. We further, assessed most suitable replacement level to enable farmers to prepare feeds for their farming systems. The generated information is useful to the shrimp farmers globally in formulating low cost feeds and

hence increase profit made out of shrimp farming business. The information is also useful when production of low saturated fatty acids shrimp muscle of interest.

## 2. Material And Methods

### 2.1 Ethical Statement

This study was carried out in accordance with the Tanzania laws and guidelines of experimental animals' care. Shrimps were anaesthetized by immersing them in chilled water of 5°C to reduce pain during death.

### 2.2 Experimental Design

This experiment was conducted in harpa nets measuring 2 m<sup>2</sup>. Harpa nets were installed in triplicate in earthen pond measuring 4500 m<sup>2</sup>. Before stocking, ponds were treated with lime for seven days and filled with water that stayed for five days before stocking. On the day of stocking, water quality measurements (pH, salinity, temperature) were performed to control differences between acclimatization tanks and the culture ponds.

### 2.3 Preparation of Experiment Feed

Several kilogrammes wet weight) of the test ingredient, the green macroalgae CA, were collected from the ocean shore near Pangani River Estuary, where several algae, including CA proliferate during the dry seasons. The collected algae were sorted and washed to remove the unwanted materials including other plants. The algae meal was made by drying clean algae in an oven at 40°C and grinding it into powder. Except for the oil, all other ingredients used in this experiment, including silver cyprinid (*Rastineobola argentea*), soybean, wheat bran (WB), maize germ (MG), ground nuts (GN), were in dried form and mechanically grounded into powder using a locally available machine.

Four experimental feeds (F0 - control, F1, F2 and F3) with FM replacement levels of 0, 10, 15 and 20%, respectively, were formulated as shown in Table 1. The experimental feeds were ground again using a heavy-duty grinder to make a fine powder, which was then mixed with water in a food processor to form a dough. The dough was cooked for 10 minutes by steaming. The cooked dough was transformed into crumble pellets using a mechanical pellet machine. Pellets were oven dried at 90 °C for one minute, reducing the moisture to around 18%, and then dried further at 60 °C to reduce the moisture to about 8%. Dried pellets were stored at room temperature for feeding experiments.

**Table 1:** Composition of experimental diets

<b>Test Diets</b>	<b>F0</b>	<b>F1</b>	<b>F2</b>	<b>F3</b>
<b>Inclusion level (%)</b>	<b>0</b>	<b>10</b>	<b>20</b>	<b>30</b>
<b>Ingredients</b>				
Fishmeal	50	45	40	35
<i>Chaetomorpha</i> algae	0	5	10	15
Rice husks (energy)	16	16	16	16
Shrimp head	6	6	6	6
Vegetable oil	7	7	7	7
Wheat flour (binder)	14	14	14	14
Vitamin premix	4	4	4	4
Mineral premix	3	3	3	3
<b>Total</b>	<b>100</b>	<b>100</b>	<b>100</b>	<b>100</b>
<b>Proximate composition of diets</b>				
Crude Protein (%)	40.09	40.11	40.05	40.02
Crude Fat (%)	8.02	7.56	7.32	7.18
Crude Fiber (%)	3.5	3.5	4.4	4.9
Dry Matter (%)	88.9	89.3	89.1	89.8
Ash content (%)	9.2	10.0	9.9	10.3
Gross Energy (kcal/g)	2.69	3.07	3.18	3.34

## 2.4 Source of *Penaeus monodon* Post Larvae

*P. monodon* post-larvae fingerlings were collected from the wild between October and November 2021 which is the peak breeding season in Pangani Estuary and Tanga coastal waters. Soon after collection, the larvae were stored in 75-litres plastic buckets and supplied with oxygen from a portable aerator. The collected post-larvae were transported to a local fish farmer hatchery in Pangani. At the hatchery, the *P. monodon* were acclimatized for 7 days in 4 m<sup>3</sup> concrete tanks filled with brackish water from Pangani River Estuary. To reduce the sun rays, the tanks were covered by hard translucent plastic. During acclimatization, the water was fully aerated, and the behavior of the larva was observed. Dead post larvae were removed from the system. Prior to stocking, post-larvae were sorted and their weight and length were measured using a portable digital scale and a digital vernier caliper, respectively.

## 2.5 Feeding Experiments and Data Collection

Post-larvae weighing  $0.49 \pm 0.06$  g on average were stocked at the stocking density of 15 individuals per meter square in 2 m<sup>2</sup> harpa nets. Feeds were administered in descending order, from five times a day in the first week to two times a day in the fourth week, at a daily feeding ratio of 12% to 6% biomass, respectively. Shrimps were fed by placing the feed on the feeding trays. During the experimental period, sampling was conducted after every fifteen days by measuring their weight and length. Feeding experiments lasted for 45 days.

## 2.6 Determination of Growth Performance and Biochemical Composition

At the end of experiment, ten individuals were obtained at random from each experiment unit, and weight of each individual was recorded. The individuals were then frozen for 48 hours to facilitate peeling process, which followed by the removal of the exoskeleton. The obtained flesh was stored in -36 °C prior to biochemical composition analysis. Each biochemical value was determined in three replicates and expressed as % wet weight, based on the following: Protein (Latimer, 2016), lipid (Pombal et al., 2017), total ash (AOAC, 2000), fiber (AOAC, 2005), fatty acids (Kang & Wang, 2005; Kokotou, 2020), carbohydrate (Soga, 2000), dry mater and moisture (Jain & Singh, 2000). Minerals to include calcium (Ca) and phosphorus (P) were analyzed by following the method of AOAC (1995). Feed utilization parameters including Feed Conversion Ratio (FCR), Weight Gain (W) and Muscle Somatic Index (MSI) were analyzed using methods described by Kokotou (2020) and Sarlin and Philip (2016) as follows;

$$W = \textit{Average final biomass (g)} - \textit{Average initial biomass (g)}$$

$$SR = \frac{\textit{Number of individuals at harvest}}{\textit{Number of individuals at Stocking}} \times 100$$

$$MSI = \frac{\textit{Wet muscle weight (g)}}{\textit{Whole wet weight (g)}} \times 100$$

$$FCR = \frac{\textit{Amount of feed administered (g)}}{\textit{(Biomass at the end - Biomass at the begining)g}}$$

## 2.7 Statistical Analysis

Prior to statistical tests, data were checked for normality using Shapiro test followed with checking for homogeneity of variance using Levene test. The experiment was randomized in design with set of three

replicates for each of the experimental diets and control diet. Data were statistically analyzed by using one-way ANOVA carried out for the feed utilization, survival rate, biochemical composition and fatty acids within and across experimental diets at the end of the experiment. All statistical inferences were performed in R version 4.1.0 and based on a significant level of  $\alpha = 0.05$ .

## 3. Results

### 3.1 Survival Rate and Feed Utilization

The results on survival rate (SR) and feed utilization shown in Figure 1a-d. Both survival and feed utilization found to be impacted with replacement levels. The mean survival rate of shrimps fed with the F2 treatment ( $92.59 \pm 7.14$ ) was significantly higher ( $p < 0.05$ , Fig. 1a) comparing to other treatments. Shrimp fed with F3 treatment exhibited the lowest SR ( $89.62 \pm 10.50$ ). In contrary, the highest weight gain ( $8.02 \pm 0.26$  g) was observed in shrimps fed with F3 treatment, being slightly similar to shrimps fed with F2 treatment ( $7.80 \pm 0.49$  g), but differing significantly ( $p < 0.05$ , Fig. 1b) to shrimps fed with F0 ( $5.78 \pm 0.29$  g) and F1 ( $5.72 \pm 0.43$  g) treatments. The lowest weight gain was recorded in shrimps fed with F1 treatment.

Moreover, shrimps fed with F2 treatment exhibited lowest feed conversion ratio (FCR) of  $1.12 \pm 0.04$ , which was closely similar ( $p < 0.05$ ) to shrimps fed with F3 treatment ( $1.13 \pm 0.04$ ). Shrimp fed with F0 and F2 treatments exhibited low FCR ( $1.50 \pm 0.01$  and  $1.31 \pm 0.03$  respectively), which differed significantly ( $p < 0.05$ , Fig. 1c) to the rest. Shrimp fed with F0 treatment had the highest FCR ( $1.50 \pm 0.10$ ). Additionally, the muscle somatic index (MSI) was influenced by treatment levels, with shrimps fed F0 ( $60.94 \pm 5.68\%$ ) and F1 ( $62.50 \pm 3.15\%$ ) treatments exhibiting closely similar MSI differing significantly ( $p < 0.05$ , Fig. 1d) from shrimps fed with F2 ( $71.77 \pm 2.32\%$ ) and F3 ( $66.93 \pm 2.17\%$ ) treatments. The maximum influence on MSI was recorded in shrimps fed the F2 treatment.

### 3.2 Biochemical Composition and Fatty Acids Profile

Biochemical composition of shrimp muscle fed with experimental diets is summarized in Tables 2 and 3. The results show that the influence of replacement levels on biochemical composition of shrimp muscle and fatty acid varies across treatments. Crude protein (CP) of shrimp muscles differed significantly ( $p < 0.05$ ) across treatments, with shrimp fed with F1 ( $78.42 \pm 0.62\%$ ) and F2 ( $78.10 \pm 1.47\%$ ) treatments exhibiting closely similar composition. Shrimp fed with F3 exhibited the highest CP composition ( $79.11 \pm 1.05\%$ ), which differed significantly ( $p < 0.05$ , Table 2) from other treatments. The lowest CP composition was recorded in shrimps fed with F0 ( $77.44 \pm 1.08\%$ ) treatment.

Moreover, crude fat (CF) levels were significantly high ( $p < 0.05$ , Table 2) in shrimp fed with low treatments levels (F0 and F1) compared to high treatment levels (F2 and F3). The highest CF ( $3.81 \pm 0.43\%$ ) was recorded in shrimp fed with F0 treatment, while the lowest ( $2.20 \pm 0.37\%$ ) was recorded in shrimp fed with F3 treatment. Similarly, shrimps fed with F2 and F3 treatments had significantly high ( $p < 0.05$ , Table 2) crude fibre levels compared to shrimps fed with F1 and F0 treatments.

In contrary, there were no significant differences ( $p > 0.05$ , Table 2) in ash, dry matter or phosphorus across treatment levels. Carbohydrate content was low in shrimp fed with F0 ( $3.24 \pm 0.33\%$ ) and F1 ( $3.42 \pm 0.44\%$ ) treatments, which differed significantly ( $p < 0.05$ , Table 2) from shrimps fed with F2 ( $4.64 \pm 0.11\%$ ) and F3 ( $4.08 \pm 0.05\%$ ) treatments. Besides, calcium composition of shrimps fed with F3 treatment differed significantly ( $p < 0.05$ ) from the rest. The lowest calcium composition ( $62.76 \pm 0.43$  mg/100g) was exhibited by shrimp fed with F1 treatment, while shrimp fed with F2 treatment exhibited the lowest calcium to phosphorus ratio ( $0.84 \pm 3.022$ ).

The fatty acid composition of shrimps fed with different treatment varied significantly with treatment level. Shrimp fed with F0 and F1 treatments exhibited significantly high ( $p < 0.05$ , Table 3) proportion of saturated fatty acids (SFA) compared to shrimps fed with F2 and F3 treatments. In contrast, shrimps fed with F2 and F3 treatments had relatively high polyunsaturated fatty acids (PUFA) compared to shrimp fed with F0 and F1 treatments. However, mono unsaturated fatty acid (MUFA) levels in shrimps were similar ( $p < 0.05$ , Table 3) across treatments. The highest MUFA ( $9.17 \pm 0.25$ ) found in shrimps fed with F0, while the lowest ( $8.80 \pm 0.41$ ) was exhibited with shrimps fed with F3 treatment.

**Table 2:** Biochemical composition of *P. monodon* fed with experimental diets with fishmeal being partially replaced by algae at 0 (F0), 10 (F1), 20 (F2) and 30% (F3)

Parameter	Treatment			
	F0	F1	F2	F3
Crude protein (%)	$77.44 \pm 1.08^a$	$78.42 \pm 0.62^b$	$78.10 \pm 1.47^b$	$79.11 \pm 1.05^c$
Crude fat (%)	$3.81 \pm 0.43^a$	$3.56 \pm 0.33^a$	$2.55 \pm 0.39^b$	$2.20 \pm 0.37^b$
Ash (%)	$6.12 \pm 0.21^a$	$6.07 \pm 0.11^a$	$6.60 \pm 0.47^a$	$7.73 \pm 0.24^a$
Dry matter (%)	$89.11 \pm 1.66^a$	$89.99 \pm 1.71^a$	$88.87 \pm 0.93^a$	$89.38 \pm 0.64^a$
Crude fibre (%)	$0.03 \pm 0.01^a$	$0.04 \pm 0.02^a$	$0.11 \pm 0.01^b$	$0.10 \pm 0.01^b$
Carbohydrate (%)	$3.24 \pm 0.33^a$	$3.42 \pm 0.44^a$	$4.64 \pm 0.11^b$	$4.08 \pm 0.05^b$
Phosphorus (mg/100g)	$72.80 \pm 0.61^a$	$73.03 \pm 0.44^a$	$74.64 \pm 0.56^a$	$72.47 \pm 0.55^a$
Calcium (mg/100g)	$64.42 \pm 0.41^a$	$62.76 \pm 0.43^a$	$62.98 \pm 0.22^a$	$69.40 \pm 0.51^b$
Calcium/Phosphorus	$0.88 \pm 0.41^a$	$0.86 \pm 0.43^a$	$0.84 \pm 3.022^a$	$0.96 \pm 0.51^a$

Values are mean ( $n=12$ )  $\pm$  mean standard deviation, value with different superscript letters in a row indicate significant differences ( $P < 0.05$ ).

**Table 3:** Fatty acid composition of *P. monodon* fed with experimental diets with fishmeal being partially replaced by algae at 0 (F0), 10 (F1), 20 (F2) and 30% (F3)

Saturated Fatty Acids (SFA)	Treatment			
	F0	F1	F2	F3
Palmitic acid	6.61±0.03 <sup>a</sup>	6.38±0.29 <sup>a</sup>	4.93±0.24 <sup>b</sup>	4.17±0.12 <sup>b</sup>
Margaric Acid	2.00±0.08 <sup>a</sup>	2.03±0.10 <sup>a</sup>	1.46±0.08 <sup>b</sup>	1.18±0.06 <sup>b</sup>
Stearic acid	8.38±0.14 <sup>a</sup>	8.59±0.36 <sup>a</sup>	7.28±0.17 <sup>b</sup>	7.41±0.57 <sup>b</sup>
<b>Mono unsaturated fatty acids (MUFA)</b>				
Oleic acid	9.17±0.25 <sup>a</sup>	9.04±0.13 <sup>a</sup>	8.80±0.41 <sup>a</sup>	8.79±0.55 <sup>a</sup>
<b>Polyunsaturated fatty acids (PUFA)</b>				
Omega-6 fatty acids				
Linoleic acid	7.13±0.09 <sup>a</sup>	8.23±0.12 <sup>b</sup>	9.03±0.05 <sup>c</sup>	9.04±0.06 <sup>c</sup>
Omega-3 fatty acids				
Alfa linolenic acid	6.46±0.04 <sup>a</sup>	6.88±0.15 <sup>a</sup>	8.01±0.03 <sup>b</sup>	7.47±0.31 <sup>c</sup>
Stearidonic Acid	0.76±0.13 <sup>a</sup>	1.06±0.13 <sup>b</sup>	1.29±0.05 <sup>b</sup>	1.64±0.03 <sup>c</sup>
Eicosatrienoic Acid	5.99±0.05 <sup>a</sup>	6.09±0.03 <sup>a</sup>	6.21±0.03 <sup>b</sup>	6.78±0.11 <sup>c</sup>
Eicosapentaenoic acid	8.96±0.18 <sup>a</sup>	9.35±0.07 <sup>a</sup>	10.01±0.60 <sup>b</sup>	9.98±0.65 <sup>b</sup>
Docosahexaenoic acid	5.58±0.08 <sup>a</sup>	6.21±0.01 <sup>b</sup>	7.16±0.16 <sup>c</sup>	6.65±0.28 <sup>d</sup>

Values are mean (n=12) ± standard deviation of three replicates, value with different superscript letters in a row are significantly differences (P < 0.05).

## 4. Discussion

FM replacement has a significant impact on feed utilization at various treatment levels. The current study found different variations in response to CA treatment levels. For instance, weight increased with increasing treatment levels, indicating that CA supported weight gain and eventually growth of shrimp. Algae have been reported as good growth promoters due to presence of amino acids and compounds which can promote growth (Nasmia et al., 2022). Despite the fact that experimental feeds had similar protein content (approx. 40 CP), shrimps differed in protein composition due to varying sources of nutrients in experimental diet formulation. Weight gain is a function of feed utilization and efficiency. The maximum weight gain was observed in shrimp fed with 30% FM replacement level. CA contains complex

antioxidant compounds (Gazali et al., 2019), which contribute to protein turnover by reducing the oxidative damage in the skeletal muscle, and hence promoting growth (Xavier et al., 2020). Antioxidants also boost immunity (Datta, 2003), resulting in a healthier state of being. The effectiveness of marine algae in promoting the growth of farmed shrimps has been reported by several authors. Examples include those conducted by Penaflorida (1999) on small shrimp *P. monodon* fed with diet containing 5% *Kappaphycus alvarezii* meal, Rivera et al. (2002) on small shrimp *Litopenaeus vannamei* fed with diets containing 10% of *Macrocystis* meal; and Cruz-Suárez et al. (2008) on shrimp feeding trials with 3.3% supplemented *Ulva* meal.

Feed Conversion Ratio (FCR) was influenced by treatment inclusion levels. The FCR decreased with increasing treatment level up to 20% inclusion beyond which only slight change was observed. This finding is consistent with those reported by Yıldırım et al. (2014), who found that higher inclusion level of peanut meal replacing fishmeal in Tilapia fries resulted in low FCR. Similarly, Motte et al. (2019) reported lowered in FCR in *L. vannamei* of up to 50% replacement level FM replaced by insect meal. Moreover, different species of algae have been reported to vary on their impact in FCR. For example, Cruz-Suárez et al. (2008) showed *Ulva* meal significantly reduced FCR comparing to *Macrocystis* and *Ascophyllum* meal. The inclusion of *Hypnea cervicornis* and *Cryptonemia crenulata* in shrimp meals at rates of 39% and 26%, respectively, resulted in varying FCRs of 1.79:1 and 1.82:1, respectively (Da Silva and Barbosa 2008). Variations in the chemical composition of the algae may have led to the observed variation.

Muscle somatic index (MSI) increased across replacement levels up to F2 treatment level, with further increase in treatment levels showing insignificant change. When MSI increases, it is an indicating that diet administered to fish is well assimilated, and vice versa. The impact observed in this study indicates improved assimilation by supplemented algae.

The FM replacement with CA has an impact on SR. SR was influenced positively up to F2 treatment, beyond which SR was slightly negatively influenced. The presence of nutritional substance such as polysaccharides with the potential to improve shrimp immunity (Immanuel et al., 2012) may have contributed to the observed survival rate. The SR results reported in the present study are consistent with those reported by Nasmia et al. (2022), who found that SR of Whiteleg shrimp was high at 6% *Caulerpa spp.* replacement level and was significantly low in the control. Similarly, Suárez et al. (2009) reported significantly high survival in shrimp *L. vannamei* fed diets containing 4% *Sargassum spp.* replacement level. In this study, high levels of replacement have shown to reduce survival rate. However, other studies have reported high SR at high replacement levels, including findings by Alvarez et al. (2007) who reported SR of above 90% in FM substitution with soy bean in *Litopenaeus schmitti* culture. Similarly, Da Silva and Barbosa (2008) reported improved SR in *L. vannamei* at 39% replacement level of *Hypnea cervicornis* and *Cryptonemia crenulata* meals.

Shrimps are considered by a variety of health experts to be among the healthiest food in the world, by considering many nutritional benefits (Joseph, 2018). Nutrients from shrimp are useful to human body (Abdullah et al., 2009; Shakir et al., 1994) they are required for maintenance and growth (Dong, 2009). For

example, penaeids contains of protein rich in essential amino acids required for human body development (Yanar & Çelik, 2006), they are generally low in calories and are made up of extremely healthy cholesterol (Joseph, 2018), good quality Proteins (Yanar & Çelik, 2006), higher amounts of free amino acids (Banu et al., 2016), and lower amounts of fats and calories (Shalini et al., 2013) making it good candidate for human consumption. Also, Penaeids contains a protein which is low in saturated fat and rich in polyunsaturated fats especially the  $\omega$ -3 fatty acids namely Eicosapentaenoic acid (EPA, C20:5n-3) and Docosahexaenoic acid (DHA, C22:6n-3) making it superior to other types of meat (Bragagnolo & Rodriguez-Amaya, 2001; Priyadarshini et al., 2015). Moreover, shrimp contains numerous vitamins and minerals required by human body. Due to its nutritional value and worthiness to human body there are several works (For example, Wardiatno and Mashar (2010) and Dinakaran et al. (2009)) which encourage consumption of penaeids for good state of health. Generally, nutritive values of crustaceans depend upon their biochemical composition, such as protein, amino acids, lipids, fatty acids, carbohydrates, vitamins, minerals, related biomolecules (Banu et al., 2016).

It is well known that the biochemical composition of the edible tissues of marine invertebrates among other factors is influenced by their nutritional habits (Srilatha et al., 2013). In the current study the partial replacement of FM with algae has shown to improve nutritional value of shrimps across treatments. The results partly are in agreement with those reported by Jeyasanta and Patterson (2017) who showed higher protein, lipid, carbohydrate, fiber, ash in shrimps fed with experiment diet prepared from locally materials mainly trash fishes.

In the current study, shrimps fed with 30% replacement level showed highest protein gains, with intermediate replacement levels (10 and 20%) showing insignificant differences. This result is in agreement with reports on fresh water shrimp culture by Reddy and Reddy (2014) and Ferdose and Hossain (2011), which reported protein gains of 72.99 to 74.89% and  $74.85 \pm 0.65\%$ , respectively. The observed variation is related to factors such as sex, reproductive cycle, capture period, food availability, hydrologic level (Nargis, 2006). Moreover, diet composition influenced protein content, which probably contributed to the observed variations in protein composition.

In the present study, carbohydrate values increased with increasing treatment levels. Shrimp fed with treatments F2 and F3 had high carbohydrate content, which was probably contributed by high carbohydrate content from CA, which has been reported to contain up to 14% carbohydrate (Gazali et al., 2019). Carbohydrate values for shrimp fed at low treatment levels (F0 and F1) in the current study are similar to those reported by (Pratap et al., 2018) who reported carbohydrate content of  $3.38 \pm 0.05\%$  for *P. monodon* and (Gunalan et al., 2013) who reported carbohydrate content of  $3.2 \pm 0.3\%$  for *P. vannamei*. The findings of this study, however, contradicts those of Abdel-Salam (2013), who reported a carbohydrate composition of 1.89 to 1.91% in farmed *P. indicus*.

Ash is an indicator of minerals in shrimp flesh, the higher the ash, the higher the mineral content, particularly calcium. In the present study ash content was more or less similar across treatments, with high treatment levels exhibiting slightly higher values, indicating the impact of CA in mineral composition.

CA contains ash of up to 7.45 mg/g (Gokulakrishnan et al., 2015). Values of ash reported in this investigation contradicts findings of Islam et al. (2017) who reported ash of  $0.87\pm 0.06\%$  and G. Reddy et al. (2008) ash of 9.09%, but resemble those reported by Ali et al. (2017) in *P. monodon*. The trend of calcium composition across treatments in the present study is a mirror image of that of ash content. In contrast, the composition of phosphorus remained slightly consistent across treatment levels, similar to those reported by Abdel-Salam (2013) who reported phosphorus amounting to  $74.32\pm 1.99$  and  $75.45\pm 1.78$  mg/100g, for male and female shrimps, respectively in farmed *P. indicus*. However, calcium contents ( $45.09\pm 1.30$  and  $39.96\pm 2.20$  mg/100g) reported by the same author contradicts calcium content observed in the present study.

Fibre content varied with treatment levels but increased with inclusion levels, most likely due to the high fibre content of CA amounting to 21% (Tsutsui et al., 2015). Additionally, the crude fibre found in this study is similar to that reported by Liu et al. (2021), but differs from 8.2% of crude fibre in *P. indicus* flesh as reported by (Ravichandran et al., 2009).

In this study, all treatments exhibited lipid levels below 5%, similar to lipid value ( $4.72\pm 0.11\%$ ) reported by Pratap et al. (2018) for *P. monodon* caught in the wild. Lipid levels decreased with increasing treatment levels in the current study, which is likely due to the low fat content in algae, which is around 0.83 – 1% (Gazali et al., 2019). According to Abulude et al. (2006), the lipid composition of wild shrimp ranged between 5.0 and 9.0%, which is substantially higher than those reported by Pombal et al. (2017) value of 1.70% and 2.69% for natural and farmed *P. monodon*, respectively.

The fatty acids profile has been shown to be influenced by the partial replacement of FM. In low treatment inclusion levels, SFA were abundant, while PUFA were abundant in high treatment inclusion levels. MUFA levels were more or less similar across treatment levels. These findings are consistent with Banu et al. (2016), who found that PUFA predominated over SFA and MUFA in the shrimp muscle tissues. Food with high amount of unsaturated fats are generally preferred over high saturated fat foods, due to the fact that do contribute to raise of cholesterol levels in the body (Shalini et al., 2013). Consuming PUFA may reduce chances of coronary heart diseases, diabetes and cancer due to presence of eicosapentaenoic acid and docosahexaenoic acid which have both anti-atherogenic and antithrombotic effects as well as an important role in the control of hypertension and prevention of cardiac arrhythmias (Murphy et al., 2012; Siri-Tarino et al., 2010).

## 5. Conclusions

The replacement of FM with CA at different levels (0, 10, 20 and 30%) in the shrimp diet has varied impacts on the survival, feed utilization, biochemical composition and fatty acids profile of *P. monodon*. The replacement of FM with CA at 20 and 30% inclusion levels yielded closely similar results. However, 30% FM replacement has negative effect on survival rate and exhibit relatively high saturated fatty acids. Thus, at 20% replacement level, CA is suitable for partially replacing FM in *P. monodon* feeds formulation. The 20% FM replacement is also suitable when low saturated fatty acids shrimp muscle is of interest.

# Declarations

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The authors have no financial or proprietary interests in any material discussed in this article.

## Author Contribution

The experiment was conceived by Ambakisye Poland Simtoe who also set experiment design, executed the experiment and drafted the manuscript. Siwema Amran Luvanga planned, executed the experiment and analyzed data. Blandina Robert Lugendo supervised the study and critically reviewed the manuscript prior to submission. All authors approved the manuscript for submission to the journal.

## Data Availability Statement

The data that support the findings of this study can be made available by the corresponding author upon request.

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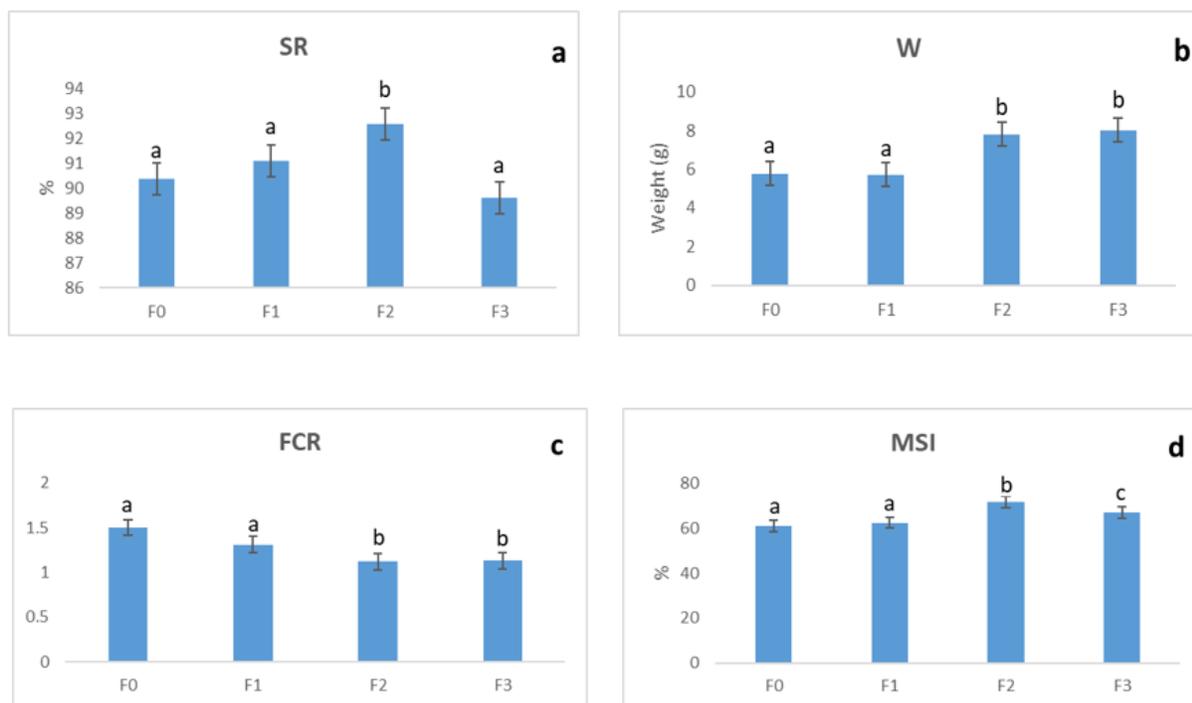
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## Figures



**Figure 1**

Effects of different treatments on a) SR (Survival Rate), b) W (Weight Gain), c) FCR (Feed Conversion Ratio) and d) MSI (Muscle Somatic Index). The data are presented as mean values and standard error

(mean  $\pm$  SEM) of three replicates. Columns with different superscript letters are significantly different ( $P < 0.05$ ).