1. Introduction

Intensification and diversification of agricultural production into a profitable and competitive livestock enterprise is one of the options to increase food production and reduce poverty in Africa (FAO, 2012). The increasing demand for animal food products and the trends in consumption strongly suggest that much of the demand for meat can be met through increased poultry and fish production. However, high cost of animal feeds hampers sustained production of livestock, fish and their products. Feed costs account for about 60-70% of the total production costs (Teleu and Ngatchou, 2006). The current main sources of protein for animals such as fish meal/oil and soya beans are increasingly expensive and cannot be afforded by smallholder farmers (Tveterás and Tveterás, 2010). Insects are natural feed for fish and poultry, and scientists have long proposed using insect biomass as an alternative, high-quality feedstock (Dzepe et al., 2019; Van Huis, 2013; Van Huis et al., 2013).

Rumpold and Schlüter (2013) reviewed the nutritional compositions of 236 edible insects based on dry matter. Many of them were found to provide satisfactory amounts of energy, protein and to meet amino acid requirements for humans and animals even though significant variation...
was found in the data. Insects such as black soldier fly larvae (BSFL) thrive in large numbers on organic wastes (coffee bean pulp, vegetables, distillers’ waste and fish offal) and manure piles of poultry, pigs and cattle (Beskin et al., 2018; Karagodin et al., 2017; Nana et al., 2018). They can be used commercially to solve a number of environmental problems associated with manure and other organic waste (e.g. reducing manure mass, moisture content and offensive odours) (Diener et al., 2009; Dzepe et al., 2019; Nana et al., 2018).

BSFL producers are confronted with the problem of the production yield, which depends on the control of the conditions of the growing medium. Generally, the larval environment of holometabolous insects determine adult survivorship, longevity and fecundity rate. In the larval habitat, food and space regulate competition among conspecifics. For instance, developmental time and mortality increase while adult body size decreases if food is scarce (Bradshaw and Holzapfel, 1992; Hawley, 1985; Nekrasova, 2004). In BSFL, the larval protein, crude fat content and development time are strongly affected by nutrient concentration of substrate and larval density (Barragan-Fonseca et al., 2018). Density is also another regulator of body size and survivorship of holometabolous insects. Larval development rate, adult survivorship and body size decrease with increasing larval density (Schneider et al., 2000; Wiwatanaratanabat and Kittayapong, 2009). Lower larval densities are not always better to maximise growth rate. In some holometabolous insect species like black blowfly for example, larval aggregations provide adaptive benefits to individuals due to heat generation, which might enhance food assimilation (Green et al., 2002) and provide protection from low environmental temperature (Rivers and Dahlem, 2013).

Another neglected aspect of larval life history traits of the black soldier fly (BSF) is the impact of the substrate moisture content on their development and survivorship. Upon egg hatching, neonate larvae feed on moist decomposing organic matter until the post feeding stage at which they leave their food source in search of a place to pupate and complete metamorphosis. This natural life history of BSF is strongly controlled by the temperature and humidity condition (Sheppard et al., 1994; Tomberlin et al., 2009). For instance, BSF egg hatching and adult emergence success increase with increased relative humidity (Holmes et al., 2012).

There are few studies that evaluate the influence of rearing conditions on BSF larval survivorship. Therefore, this study was designed to assess the effects of rearing density, substrate moisture content and feedstock ratio on the production of BSFL.

2. Materials and methods

Study area

The present study was conducted in a greenhouse at the Agri-Business vocational training Center, Dschang, Cameroon. The centre is situated in the western region of Cameroon between 5°25'-5°30' north latitude and 10°-10°5' east longitude and at an average altitude of 1,410 m, with an equatorial climate. Data of the meteorological station of Dschang from 2001 to 2009 shows that there are two seasons: a long rainy season from March to October and a short dry season from November to February. The rainfall varies between 1,500-2,000 mm per year. The average annual temperature is around 21 °C with average annual sunstroke of 1,800 hours and a relative humidity varying between 40-97%. The air is perpetually fresh and tends to saturation early in the morning, hence the regular presence of fog or mist in the atmosphere before sunrise.

Establishment and maintenance of black soldier fly colony

The colony started with young larvae outcome from a local strain of BSF breed since 2016 at the International Institute of Tropical Agriculture, Cameroon. The larvae were maintained in a plastic container in a greenhouse where the ambient temperature ranged between 20-40 °C and were fed with domestic organic waste until they reach the post feeding stage, distinguished by the characteristic black cuticle, contrasting with the white larvae (May, 1961). The post feeding stages were collected and kept in a new plastic container with wood shavings in a dark cage, which facilitated pupation and emergence of adults. Emerged adults were released in a mating cage made of mosquito mesh and strongly lit by sunlight, making copulation and oviposition easier. In the mating cage, fermented chicken feed was used to attract ovipositing females. The method for egg collection was adapted from Dortmans et al. (2017). ‘Eggies’ were made with five clean wooden sheets (2x4x0.3 cm) separated with pushpins and held together by two rubber bands on both ends of the bundle. The pushpins created a small gap (1-2 mm) between the wooden sheets, allowing space for egg packages. Six clean ‘eggies’ were prepared and placed inside the mating cage over the attractant container half filled with fermented chicken feed mixed with water at 70%. The ‘eggies’ were collected every two days for eggs harvesting that were transferred into an incubation unit, where temperature and relative humidity were in ranges 25-35 °C and 40-80% RH, respectively. The incubation unit consisted of a hatching crate, a hatching container, a shelf (10×5×5 cm) made with wood and sieve, and a hygro-thermograph. The hatching container was filled with 2 cm thick chicken feed moistened to 70% with fresh water, above which the shelf with the sieve was put. The harvested eggs were spread on the sieve, and the dry feed...
was scattered around the container to prevent the hatched larvae from escaping. Finally, the container was put into the hatching crate. The eggs hatched after 3-4 days and the young larvae were fed in the same container for four days before using for further growth or experiments.

**Experimental design**

**Influence of larval density on BSF larval growth**

The densities were selected according to the minimal and maximal densities calculated from information presented in studies on BSF performed by Sheppard et al. (2002). In this study, six different rearing densities were obtained by placing 15, 30, 60, 90, 120 and 150 of four-day-old larvae per plastic container (Ø 4.38×5.53 cm) resulting in approximately 1, 2, 4, 6, 8 and 10 larvae/cm², respectively. Each density had four replicates and was fed ad libitum with commercial chicken feed mixed with fresh water at 60%. The plastic containers were covered with transparent muslin cloth to reduce drying in the medium and for ventilation.

**Effects of substrate moisture content on BSF larval growth**

These experiments were conducted in plastic container of Ø 11.30×5.53 cm, covered with a perforated lid fitted with a mesh which prevents larvae from escaping. The containers were inoculated with 100 of four-day-old larvae hand counted, resulting in 1 larva/cm² approximately. Larvae were fed ad libitum and the fresh water was added twice per day to adjust the substrate moisture content.

**Effects of feedstock ratio on BSF larval growth**

The feedstock ratios were also tested to determine their effects on the BSF larval life history parameters. Experiments were conducted in plastic containers (Ø 11.30×5.53 cm) covered with a perforated lid fitted with a mesh. The containers were inoculated with 100 of four-day-old larvae hand counted, resulting in 1 larva/cm² approximately. They were fed with five different daily diets of 25, 50, 100, 150 and 200 mg of feed per larva per day. In each container, 5, 10, 20, 30 and 40 g of commercial chicken feed mixed with fresh water at 60% were added every two days, to achieve the specific feeding rates of 25, 50, 100, 150 and 200 mg/larva/day, respectively. For each feedstock ratio, four replicates were used and every four days the larvae were removed from the containers and checked for weight gain and survivorship, then replaced in a new container with a new substrate.

**Life history traits of BSF larvae**

For each treatment, larvae were harvested at maturity in each container, cleaned, counted and weighed using an electronic scale (precision ±0.01 g). They were subsequently killed using hot water and their morphometric characteristics such as body size and body thickness were measured using a digital caliper (±0.01 mm). To determine survival rate, the number of live larvae at the end of the experiment was divided by the initial number of four days old larvae per replicate. The development time was considered to be the number of days between the start of the experiment and the observation of half of the post feeding stages in each container as previously described. The dry mass of the feed reduction was determined by subtraction the dry mass of the remaining from the dry mass of feed added to a rearing container after harvest of the larvae using an electronic scale (precision ±0.1 g).

**Statistical analysis**

The collected data were submitted to Shapiro normality test. Therefore, the Spearman correlation analysis was performed to establish relationships that may exist between larval densities, substrate moisture content and individual larval feed reduction, wet weight, development time and survival rate. While Pearson correlation analysis and analysis of variance (ANOVA) were performed for the effects of densities and moisture on larval body size and body thickness, respectively. On the other hand, to determine the possible differences in the larval feed reduction, development time and survival rate of the larvae subjected to different feedstock ratios, the non-parametric test of Kruskal-Wallis was used (Pairwise Wilcoxon test was used to determine the difference in rank means), while ANOVA was also used for the average larval wet weight, body size and body thickness. When a significant difference was found (P<0.05), Tukey post-hoc test was performed. All statistical analyses were carried out with 95% confidence interval, using R software, version 3.5.0.

**3. Results**

**Effects of larval density**

The larval feed reduction, wet weight, development time and survival rate were all affected by rearing densities (Table 1). The individual feed reduction showed a significant negative correlation with rearing densities (r=-0.849; P<0.05). It varied from 0.13±0.003 to 0.23±0.017 g. The individual larval wet weight (r=-0.845; P<0.05) and survival rate (r=-0.940; P<0.05) also showed a significant negative correlations. They varied from 0.11±0.008 to 0.15±0.003 g and from 96.67±0.907 to 100.00±0.000%, respectively. The larval development time on the other hand, was significantly
positively correlated with rearing densities \((r=0.920; \ P < 0.05)\) and, varied from 11.75±0.95 to 17.75±0.957 days.

The BSF larval body size and body thickness were also affected by rearing densities (Figure 1). The body size varied from 17.51±0.520 to 19.80±0.422 mm and was negatively correlated with rearing densities \((r=-0.775; \ P < 0.05)\), while body thickness varies from 4.55±0.069 to 5.02±0.081 mm and was also negatively correlated with rearing densities \((r=-0.700; \ P < 0.05)\).

**Effects of substrate moisture content**

The individual larval feed reduction, wet weight and development time have been negatively affected by the increase in moisture content (Table 2), while the survival rate was not significantly affected \((r=0.064; \ P =0.786)\). The larval feed reduction \((r=-0.582; \ P <0.05)\) and wet weight \((r=-0.594; \ P <0.05)\) showed a significant negative correlations with substrate moisture content. They varied from 0.12±0.008 to 0.26±0.005 g and from 0.12±0.006 to 0.19±0.005 g, respectively. The development time on the

<table>
<thead>
<tr>
<th>Density (larva/cm²)</th>
<th>Feed reduction (g/larva)</th>
<th>Wet weight (g/larva)</th>
<th>Development time (day)</th>
<th>Survival rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.23±0.017</td>
<td>0.15±0.003</td>
<td>11.75±0.957</td>
<td>100.00±0.000</td>
</tr>
<tr>
<td>2</td>
<td>0.17±0.016</td>
<td>0.13±0.008</td>
<td>13.29±0.957</td>
<td>100.00±0.000</td>
</tr>
<tr>
<td>4</td>
<td>0.14±0.006</td>
<td>0.12±0.003</td>
<td>14.75±0.957</td>
<td>98.33±1.360</td>
</tr>
<tr>
<td>6</td>
<td>0.14±0.003</td>
<td>0.12±0.001</td>
<td>15.50±1.290</td>
<td>96.67±0.907</td>
</tr>
<tr>
<td>8</td>
<td>0.14±0.005</td>
<td>0.12±0.003</td>
<td>16.25±0.500</td>
<td>94.58±2.590</td>
</tr>
<tr>
<td>10</td>
<td>0.13±0.003</td>
<td>0.11±0.008</td>
<td>17.75±0.957</td>
<td>90.17±1.000</td>
</tr>
</tbody>
</table>

1 Values within columns marked with same letters are not significantly different \((P<0.05)\).

<table>
<thead>
<tr>
<th>Moisture content (%)</th>
<th>Feed reduction (g/larva)</th>
<th>Wet weight (g/larva)</th>
<th>Development time (day)</th>
<th>Survival rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>0.15±0.003</td>
<td>0.15±0.002</td>
<td>12.25±0.500</td>
<td>86.75±10.242</td>
</tr>
<tr>
<td>50</td>
<td>0.20±0.008</td>
<td>0.17±0.012</td>
<td>12.75±0.957</td>
<td>94.75±2.829</td>
</tr>
<tr>
<td>60</td>
<td>0.26±0.005</td>
<td>0.19±0.005</td>
<td>13.25±0.957</td>
<td>99.25±0.957</td>
</tr>
<tr>
<td>70</td>
<td>0.14±0.005</td>
<td>0.14±0.003</td>
<td>16.00±0.816</td>
<td>94.25±0.957</td>
</tr>
<tr>
<td>80</td>
<td>0.12±0.008</td>
<td>0.12±0.006</td>
<td>18.75±0.500</td>
<td>89.00±9.591</td>
</tr>
</tbody>
</table>

1 Values within columns marked with same letters are not significantly different \((P<0.05)\).
Influence of abiotic factors on the growth of black soldier fly larvae

other hand, was positively correlated ($r=0.893; P<0.05$), and varied from $12.25\pm0.500$ to $18.75\pm0.500$ days.

The larval body size and body thickness varied from $18.98\pm0.453$ to $20.27\pm0.126$ mm and $4.79\pm0.133$ to $5.28\pm0.119$ mm, respectively and were significantly affected by the substrate moisture content ($P<0.05$). The larvae subjected to $60\%$ moisture content had recorded the highest body size and thickness, while those on $80\%$ had recorded the lowest (Figure 2).

**Effects of feedstock ratio**

Data analysis of individual feed reduction, wet weight, development time and survival rate of the larvae subjected to different feedstock ratios showed a significant variation ($P<0.05$) (Table 3). The larvae on diet $200$ mg/larva/day reduced an average of $0.29\pm0.013$ g of feed per larva, while those on $25$ mg/larva/day reduced an average of $0.07\pm0.007$ g. The values were intermediate for the larvae feed with $50$, $100$ and $150$ mg/larva/day diets. The lowest averages individual larval wet weight was also recorded with the larvae subjected to $25$ mg/larva/day diet ($0.09\pm0.006$ g), while the highest was recorded with those on diet $150$ mg/larva/day ($0.19\pm0.003$ g). The development time of larvae subjected to diets $100$, $150$ and $200$ mg/larva/day were relatively short and do not shows a significant variation ($P>0.05$), the values were $11.50\pm0.577$, $11.25\pm0.500$, and $11.75\pm0.500$ days, respectively. In contrast, they differ significantly from those of larvae subjected to $25$ and $50$ mg/larva/day diets ($P<0.05$), which were $17.50\pm0.577$ and $15.25\pm0.500$ days, respectively. The survival rate does not show a significant difference ($P>0.05$). It varied from $84.25\pm3.201$ to $96.50\pm1.290\%$ and, the larvae on $150$ mg/larva/day recorded the highest.

The individual body size and body thickness of larvae subjected to different feedstock ratios varied significantly ($P<0.05$) from $17.88\pm0.341$ to $20.55\pm0.335$ mm and from $4.28\pm0.112$ to $4.98\pm0.081$ mm, respectively (Figure 3). The highest average of body size and body thickness were recorded on the larvae subjected to $150$ mg/larva/day diet, while the lowest were recorded on those subjected to $25$ mg/larva/day.

### 4. Discussion

The BSF larval life history parameters were considerably influenced by the rearing density, substrate moisture content, and feedstock ratio. Previous studies have shown
that holometabolous insects including BSF are sensitive to several biotic and abiotic factors, especially larval habitat (Barragan-Fonseca et al., 2018; Khandaker and Bernard, 2013). The larval life history parameters of BSF were significantly improved with low rearing densities. This could be explained by the reduced competition among individuals that fosters larval feed intake and lead to a significant increase in larval weight gain which translates in an increase of body size and body thickness. These results are in line with Khandaker and Bernard (2013), who reported that lower larval density produced larger larval body sized in mosquitoes. However, Barragan-Fonseca et al. (2018) reported that the individual larval weight gain of BSF on high-nutrient concentrated diets was positively affected by higher rearing densities. Indeed, larval aggregation produces higher temperatures that may enhance feed assimilation in the insect (Rivers and Dahlem, 2013). For instance, blowfly larval aggregation has been reported to result in more efficient feeding because the increased concentration of tryptic and alkaline excretions from groups of larvae may release more nutrients from the diet and allow groups of larvae to forestall the toxic effects of some chemicals like mimosine that can interfere with conversion of amino acids and peptides into body mass (Green et al., 2002). In any case, it should be noted that these life history parameters may also be influenced by the quality or type of feed used. In this study, low densities positively affected individual larval weight. Parra Paz et al. (2015) found that larval density values up to 5 larvae/cm² do not significantly influence larval development time and wet weight if they are provided with enough foodstock. The low larval densities also act by increasing the accessibility of the larvae to the feed. Slansky and Wheeler (1989) observed that in higher densities the holometabolous insect can respond by extending the duration of larval stage. In the blowfly, Green et al. (2003) also observed that in higher densities the larvae ingested food for a longer period of time and increase their developmental time to obtain sufficient nutrition for pupation. The larval life history parameters have been negatively affected by the increase in substrate moisture content. This would be due to the fact that the high moisture contents levels result in a low concentration of nutrients in the substrate and make access difficult for the larvae. With low moisture contents, access to nutrients is easy and larval movements are reduced. The larvae can therefore efficiently use stored energy to build their biomass. Diener et al. (2011) reported that during food shortage or other unfavourable conditions, BSFL reduce their feed intake or cease to feed. In this study, the best larval life history parameters were recorded with 60% moisture content. Under this condition, the development time was about 13 days and the individual larval wet weight, body size and body thickness were greatly improved. These results concur with Spranghers et al. (2016) and Dortmans et al. (2017), who reported that, under favourable conditions, the development time of BSF larval stage is about two weeks and the suitable humidity ratio of the substrate for BSFL production is about 60%.

The larvae fed with low feedstock ratios needed longer time to reach maturity than those fed with high feedstock ratios. These results are in line with Diener et al. (2009), who found longer development time for BSFL reared in chicken feed when they were fed with 12.5; 25 and 50 mg of feed per larva per day, compared with those on 100 and 200 mg of feed per larva per day. Nana et al. (2018) also found that the larvae reared in fresh waste and high feeding ratio developed faster into large post feeding stages than those on low feeding ratio. The most favourable larval life history parameters were recorded with larvae subjected to 150 mg/larva/day diet. Their average feed reduction was significantly low compared to that of the larvae subjected to diet 200 mg/larva/day, but they weighed the most out of all four treatments. Regardless of the feedstock ratio, the larvae tend to migrate out of the substrate when they reach the post feeding stage. In the treatments with feed shortage, larvae were fed until they achieved the energy reserve required to perform pupal development. This energy reserve is defined as the minimal energy at which

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**Figure 3. Variation in individual (A) body size and (B) body thickness of black soldier fly larvae, Hermetia illucens** subjected to five feedstock ratios. Bars indicate the means, rectangles indicate the standard errors, and whiskers indicate 95% confidence intervals.
further feeding and growth are not required for a normal metamorphosis and pupation (Nijhout and Williams, 1974). As the BSF do not feed during pupal and adult stages, they need to accumulate enough energy during larval stage, to ensure adult survival and fecundity (Sheppard et al., 1994).

The highest larval growth performances were recorded at density 1 larva/cm², with a feedstock ratio of 150 mg/larva/day moistened to 60% with fresh water. The low rate of larval feed reduction in the first experiment, as well as the increase in development time of the larvae at density 1 larva/cm² and 60% moisture content in the second experiment, could be due to the feed shortage in the rearing medium. Similarly, the significant difference observed in the wet weight of the larvae reared at the same density and substrate moisture content in these two experiments which would certainly be due to the difference in the quantities of feed served. As previously described, holometabolous insects can respond to reduced nutrient levels in their diets by increasing either the rate of ingestion or extending the duration of ingestion (Slansky and Scriber, 1985; Slansky and Wheeler, 1989). During food shortage or other unfavourable conditions, BSFL reduce feed intake or cease to feed (Diener et al., 2011).

5. Conclusions

This study demonstrated that the larval life history characteristics of BSF were mostly controlled by the rearing conditions. Moreover, the characteristics of the larvae under low rearing densities have been greatly improved compared to those under high rearing densities. The best larval life history parameters in this study were recorded with 60% moisture content and with a feedstock ratio of 150 mg of feed per larva per day. These results could be useful and offer optimal solutions for small scale rearing of BSF for mass-production.

Acknowledgements

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