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The effect of an initial high-quality feeding regime on the survival of *Gryllus bimaculatus* (black cricket) on bio-waste

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RUNNING HEAD: BIOWASTE FEEDING REGIME AND BLACK CRICKET SURVIVAL

Abstract

Previous studies have led to claims that insects can offer a solution to several food security hurdles, one of which is the processing of food waste. However, although it has been demonstrated that some insects survive well on bio-waste (e.g. *Hermetia illucens*), no study, has to date, demonstrated success rearing species more commonly used for human consumption, such as crickets, on biowaste from hatching. This trial aimed to establish if the black cricket, *Gryllus bimaculatus*, can be reared successfully on bio-waste from hatching. Since, in other livestock sectors it has been established that nutritional requirements vary with age and that diet must be altered accordingly to achieve the best growth, e.g. chick feed to layer mash in chickens, the present trial used a similar feeding regime of an initially high-quality feed to see if this allowed the subsequent survival of crickets on low quality bio-waste products. Pilot trials have demonstrated poor to no survival on beer waste and cow manure and mid-level survival on unprocessed vegetable waste with chicken feed as the control. Based on this, feed regimes of either 1 or 2 weeks high quality feed (chicken feed) and then either 2 or 3 weeks of low quality feed (beer waste or vegetable waste) were tested. Results showed that even 1 week of high quality feed makes a significant difference in survival and end size of crickets subsequently reared on low-quality bio-waste.

Keywords: insect production, bio-waste feed, survival, growth, crickets

Introduction

The environmental impact of food production is increasingly being brought to the forefront of sustainability debates, particularly surrounding the reduction of carbon dioxide (CO_2) emissions. However, there are two equally important environmental factors which are sometimes ignored, water and land use. It is predicted that by 2025, at least 1.8 billion people will be living in regions without adequate fresh water supplies and a further two thirds of the global population will be in areas feeling pressure from dwindling water resources (FAO, 2012). Freshwater is a finite resource, of which an estimated 70% is used by the livestock and agriculture industries (Doreau et al., 2012). Agriculture uses water directly to grow crops and indirectly to grow fodder to produce livestock. Land availability is an issue which frequently arises in the discussion of sustainable agriculture. As the demand for meat grows there is increasing pressure on producers to farm more livestock which requires more land. It also requires more feed, which in turns leads to farmers increasing the amount of land being cropped, often via deforestation or an increase in fertilizer use. Currently the livestock sector uses about 80% of available agricultural land worldwide (Herrero et al., 2015).

Insects have been proposed an innovative alternative to farming traditional livestock by the United Nations (van Huis et al., 2013), as they are high in protein but assumed to require less water, land and feed input. Data looking at the water footprint of a commercial mealworm farm also found that when water use is controlled for percentage of edible protein (insects are 80-100% edible, while traditional livestock is 40-50% edible; Lundy and Parrella, 2015) mealworms have a lower water footprint than traditional livestock (Miglietta et al., 2015). One of the key arguments for the use of insects as food is that they have a better feed conversion efficiency because they are cold-blooded and depend on their environment to control metabolic processes (van Huis et al., 2013). Another argument also frequently attached to insects is their ability to make use of bio-waste feeds. Trials with *Tenebrio molitor* and *Teleogryllus testaceus* have demonstrated that diets can be successfully formulated from bio-waste sources (Caparros Megido et al., 2016; Miech et al., 2016; Ramos-Elorduy et al., 2002). However, to date only *Hermetia illucens* has been reared successfully on anything but chicken feed directly from hatching. For other insects such as *Acheta domesticus*, commercial scale trials have shown limited success with bio-waste products as feed (Lundy & Parrella, 2015) and lab scale trials with *Blaptia dubia*, *Tenebrio molito*, and *A. domesticus* found that survival and development rates were significantly negatively impacted on bio-waste feeds (Oonincx et al., 2015).

Bio-waste feed trials have demonstrated that black crickets, *Gryllus bimaculatus,* fed on a variety of bio-waste diets do not perform well (unpublished data). These trials found low to no survival in the two lowest quality feed groups (spent beer waste and cow manure), and for unprocessed vegetable waste the crickets only achieved 50% of the colony size of a control group fed on chicken feed. These findings were in line with previous findings from Lundy and Parrella (2015). Thus, alternative approaches are needed to make cricket rearing more sustainable successful, this is of particular importance because crickets are one of the most popular insects for human consumption second only to mealworms.

In traditional livestock production it has been demonstrated that nutrition in young animals has a significant impact on later development (Blaxter, 1957; Kilpatrick and Steen, 1995;

Pordomingo, 2002). For both livestock and domestic pets, it is standard practice to provide feed of varying nutritional content for different life stages, namely: infant, adolescent, adult, and senior (Kellems and Church, 2009). Crickets similarly have distinct life stages, egg, nymph and adult (Masaki and Walker, 1987) and research on diet restriction and impact on compensatory growth, aging and reproduction (Dmitriew and Rowe, 2011; Jobling, 2009; Lyn et al., 2011) shows that these too have differing nutritional requirements. Research has also shown that different life stages of the cricket, *Acheta domesticus*, metabolise food differently (Woodring et al., 1979).

Thus, it seems likely that using different feeds for different life-stages of crickets would allow a sustainable production system. This has not been tested experimentally and in this study, we report a feeding trial aimed at establishing if feeding crickets on a transition diet from high quality to low quality feed gives improved growth and survival when compared to the use of single low-quality feeding regimes.

Materials and Methods

Feed source and composition

All feeds were analysed for crude protein (Dumas), crude fibre, total oil and ash by Sciantec Analytical Services (North Yorkshire, United Kingdom).

The chicken feed was Fancy Feed Layers Pellets (Fancy Feed Company, Essex, UK) composed predominantly of wheat and soya bean meal comprised of 19.0% protein, 3.9% oil, 4.0% fibre and 5.9% ash.

The millet beer waste was sourced from local breweries in Burkina Faso, dried and frozen prior to transport and then ground for use as feed. Millet beer waste was chosen as the intended target of this research is developing communities, which are predominantly located in tropical climates where insect breeding is easier to facilitate and where millet beer is the brew of choice. It was comprised of 23.7% protein, 9.4% oil, 11.1% fibre and 5.3% ash.

The vegetable waste was ‘designed’ based on global composition records of food waste (WRAP, 2009). It consisted of 63.7% vegetables (56.0% root vegetables, 16.0% stem-leaf vegetables, 11.0% fruit-seed vegetables, 17.0% other vegetables e.g. leeks, peppers) and 36.3% fruit (28.0% bananas, 23.0% apples, 16.0% citrus fruit, 33.0% other fruit e.g. berries, melon and stone-fruit). All the fruits and vegetables were dried at 35°C for 36 hours and then ground to a homogenous mixture. It was comprised of 9.1% protein, 1.7% oil, 5.1% fibre and 6.0% ash.

All feeds were stored at -20°C when not in use to prevent spoilage.

Feeding trial

Each feeding trial was run for four weeks as this is the time it takes the crickets to reach sexual maturity and a size suitable for harvest, under ideal conditions according to current large-scale producers (private correspondence). The details are given in Table 1. The high-quality feed was chicken feed (CF) and the low quality was either dried and ground mixed vegetable waste (veg) or spent millet beer waste (BW) which in previous trials had given less than 50% colony survival.

Table 1. List of trial treatment conditions and replicate numbers

Name	N	Treatment Diet
$High_{CF}$ (H_{CF})	6	4 weeks on chicken feed
Low_{BW} (L_{BW})	6	4 weeks on spent millet beer waste
Low_{Veg} (L_{Veg})	5*	4 weeks on dried and ground vegetable waste
$1WH_{CF}$ $3WL_{BW}$	9	1 week on chicken feed followed by 3 weeks on millet beer waste
$2WH_{CF}$ $2WL_{BW}$	9	2 weeks on chicken feed followed by 2 weeks on millet beer waste
$1WH_{CF}3WL_{Veg}$	6	1 week on chicken feed followed by 3 weeks on ground dried vegetable waste
$2WH_{CF}2WL_{Veg}$	6	2 weeks ofnchicken feed followed by 2 weeks on ground dried vegetable waste

*One replicate was lost due to cage failure

For all treatments, hatchling (less than 24 hours old) *G. bimaculatus* which had not fed were supplied by Monkfield Nutrition Ltd (Ely, United Kingdom) and immediately subjected to treatment. Approximately 3.5 grams (±10%) of crickets were placed in large plastic cages (42cm H x 59cm W x 39cm D) covered with insect-proof mesh netting.

Each cage contained four egg cartons (30cm x 30cm x 30cm) to increase crawl space. Cages were kept in a climate-controlled chamber maintained at 30°C with 12-hour day/night and 30% humidity. Water was provided *ad libitum* in all cages via plastic tubes (50ml) with cotton balls placed in the end. Food was also provided *ad libitum* in the bottom of the cage.

After four weeks the crickets were killed by placing cages in a -20°C freezer. Then the following data were collected or calculated for each replicate of each treatment: total number of individuals, percentage survival, total colony weight, percent biomass accumulation and weight of 10 randomly selected individuals. To calculate percentage survival a starting number of individuals per treatment was needed and this was calculated based on the finding that on chicken feed 55% of the starting population survives (Miech et al., 2016; Oonincx et al., 2015). One H_{CF} group was run with each treatment and the final number to survive on H_{CF} was used in the following equation to estimate the starting number.

$$EH_{CF} \div R = SH_{CF}$$

Where EH_{CF} is the end survival number of the H_{CF} group, R is the known survival rate on H_{CF} (0.55), and SH_{CF} is the starting number of individuals present. This equation was calculated for SH_{CF} for 6 replicates of H_{CF} and the average taken for the survival rate calculation. As all replicates had the same starting weight of crickets and they were too young to display any significant size differences the same SH_{CF} result was used for all replicates.

Feed conversion efficiency

Feed conversion efficiency in animal production systems can be expressed in multiple ways but the most common is the Feed Conversion Ratio (FCR), which is the amount of feed (kg) needed to produce one kg of weight increase of the animal. For insect production the Efficiency of Conversion of Ingested food (ECI) is preferred which gives information on how efficiently feed is converted (Waldbauer, 1968). ECI is calculated as (weight gained/weight of consumed food) * 100%. In ideal scenarios one wants a high ECI (100% is perfect conversion), and a low FCR (1 is perfect conversion).

Both FCR and ECI calculations assume all provided feed is consumed, can be calculated on a fresh or dry matter basis and can be used for specific nutrient conversions. In this paper, FCR and ECI are both expressed on a fresh weight basis. An additional FCR was calculated for protein (P-FCR), using the amount (kg) of protein of a given feed, needed to produce one kg of cricket protein. The amount of protein was calculated as the amount of nitrogen (N) in each sample (as determined by the Dumas method) multiplied by the appropriate nitrogen-to-protein conversion factor for the feed source (Mariotti et al., 2008). For the crickets, 5.6 was used as the conversion factor based on previous research which has established that the conventional factor of 6.25 results in an overestimate of protein (Janssen and Vincken, 2017).

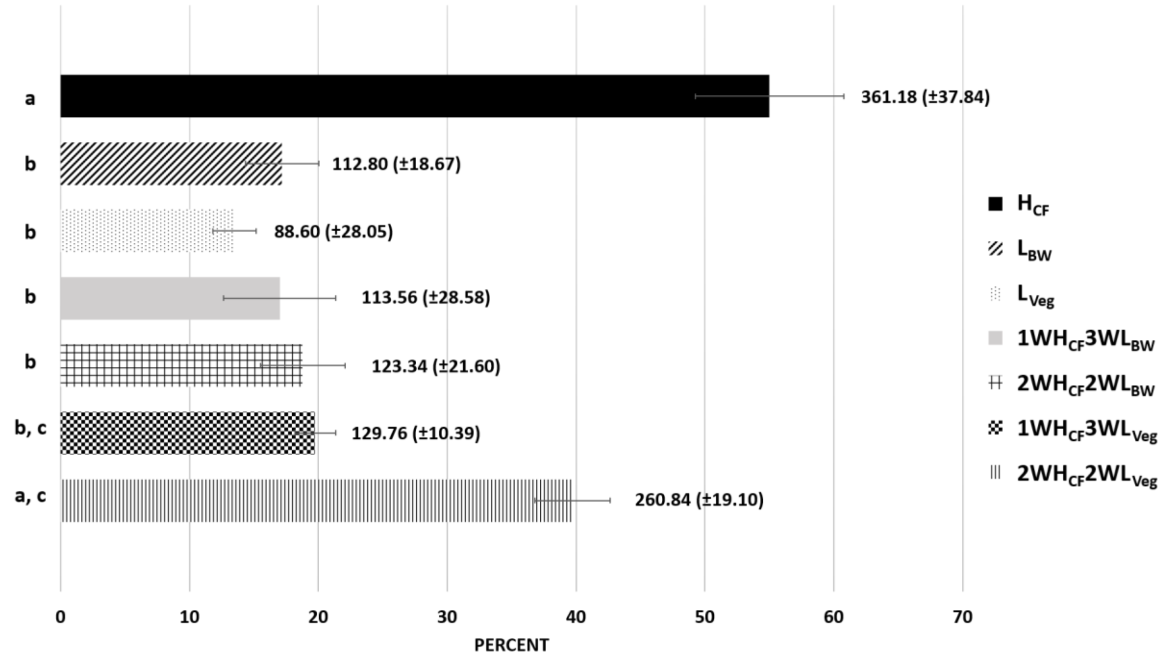
Statistical Analysis

Data were analysed using IBM SPSS Statistics 22 and Microsoft Excel to look for differences between groups, using one-way between subjects analysis of variance (ANOVA), Welch's F was used when Levene's test of homogeneity of variance was violated. The Scheffe test was used for post-hoc testing. The degrees of freedom were the number of compared treatments minus one. Standard deviation (SD) or error (SE) is reported where appropriate for averages.

Results and Discussion

To date the main problem in trying to rear crickets on bio-waste has been the very low survival rates coupled with low biomass accumulation. In the present study, we aimed to test whether early stage feeding with high energy feeds, before moving on to bio-waste could improve the production of crickets. This used a range of feeding regimes (see Table 1) and the results for survival across the groups and biomass accumulation are shown in Figures 1 and Table 2.

Figure 1. Average survival rate (%) of treatment groups with error bars and average survival number (standard error) listed by treatment, significant differences indicated with letters ($p < .05$)



Cricket colonies kept on the high-quality diet for all four weeks had a significantly higher survival rate (55% with ca. 360 individuals surviving) than all of the other treatment groups. However, the $2WH_{CF}2WL_{Veg}$ group had the second highest survival rate, with almost 40% and ca. 260 individuals surviving. Although this is approximately 15% behind the survival of the H_{CF} group, it is a step towards increased survival of crickets on bio-waste sources. There also appears to be a trend towards difference between $1WH_{CF}3WL_{Veg}$ and $2WH_{CF}2WL_{Veg}$. Overall these findings lend support to the previous theory that early nutrition has an influence on later survival.

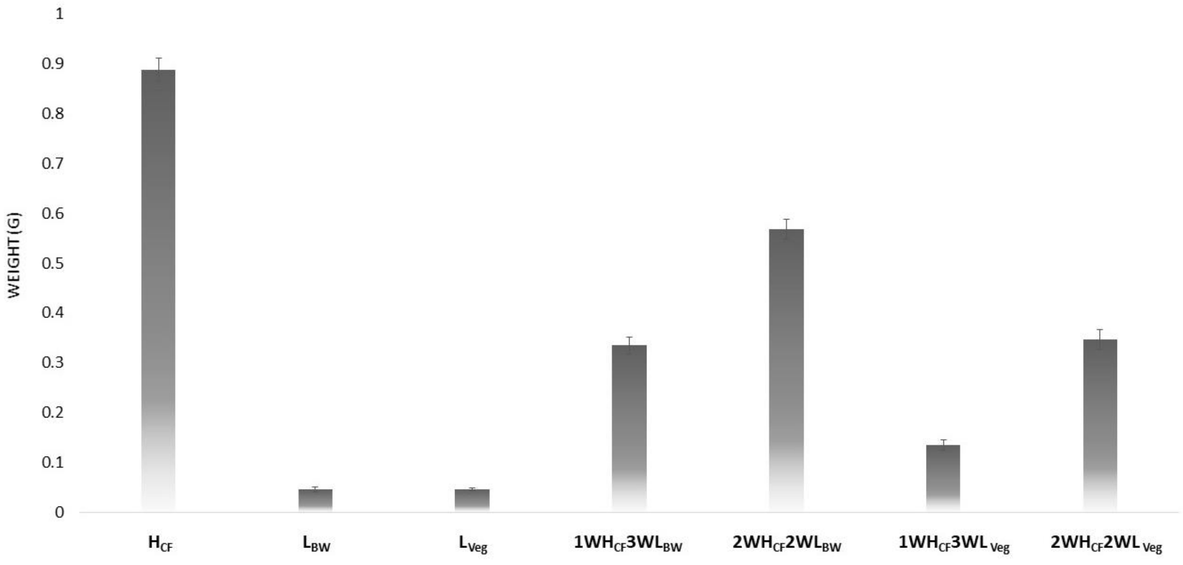
Table 2. Average ending colony weight and biomass accumulation across treatment groups, mean ± SD. Significant differences indicated with letters (p < .05).

Diet	N	End weight (g)	Biomass change (%)
H_{CF}	6	223.95 ± 54.73^{a}	6387.37 ± 1498.98^{a}
L_{BW}	6	4.56 ± 0.61^{b}	125.66 ± 13.71^{b}
L_{Veg}	5	4.71 ± 3.78$^{b, c}$	131.51 ± 104.92$^{b, c}$
$1WH_{CF}\ 3WL_{BW}$	9	27.32 ± 13.41$^{b, d}$	778.95 ± 380.67$^{b, d}$
$2WH_{CF}\ 2WL_{BW}$	9	47.39 ± 13.93$^{c, d, e}$	1298.92 ± 383.36$^{c, d, e}$
$1WH_{CF}3WL_{Veg}$	6	11.58 ± 1.18$^{b, e}$	341.54 ± 41.01$^{b, e}$
$2WH_{CF}2WL_{Veg}$	6	60.64 ± 5.86^{d}	1791.93 ± 255.46^{d}

Data for the cricket end weight yield and biomass accumulation support the findings of the survival data. The H_{CF} populations showed an average 6387% gain in biomass (fresh weight) significantly more than all other treatments ($p < .001$) and in line with previous work (Lundy and Parrella, 2015). $2WH_{CF}\ 2WL_{BW}$ and $2WH_{CF}2WL_{Veg}$ both accumulated over 1200% in biomass over the four weeks, however there was no significant difference in biomass accumulation except for between $2WH_{CF}2WL_{Veg}$ and the two bio-waste only treatments, L_{BW} and L_{Veg} ($p < .05$).

To better understand the performance of the cricket colonies, individual insect sizes were measured. This is important for insects bred for food or feed as they need to be a reasonable size for harvesting. Figure 2 shows the average individual size of crickets from the treatment groups.

Figure 2. Average size of individual crickets across treatments.



There was an overall significant impact ($p < .001$) of diet on the average individual size of crickets. Crickets fed on the high-quality chicken feed diet (H_{CF}) were significantly ($p < .001$) larger, weighing on average 0.64 (SE ± 0.084) grams more than the crickets from the other diet treatments. The two bio-waste only diets (L_{BW} & L_{Veg}) produced significantly ($p < .001$) smaller crickets than all the other diets, but there was no difference in individual size between them.

The size differences in crickets becomes more complex when looking at the high to low-quality switching regimes. From the survival data it would be expected that the longer crickets spent on the high-quality diet the better they would perform subsequently on low-quality feed. However, the crickets which were feed for 2 weeks on high-quality feed and then switched to 2 weeks of low-quality feed in the form of vegetable waste ($2WH_{CF}2WL_{Veg}$) had an average individual size which was not significantly different ($p = 1.00$) from crickets which were feed for 1 week on high-quality feed and then switched to 3 weeks of low quality feed in the form of beer waste ($1WH_{CF}$ $3WL_{BW}$). Furthermore, crickets fed for two weeks on high-quality feed and then switched to two weeks of low-quality feed in the form of beer waste ($2WH_{CF}$ $2WL_{BW}$) had a significantly larger ($p < .001$) average individual size than all the other high-low treatment groups.

A possible confounding factor to consider is which instar the crickets were in. Prior to becoming adults, crickets in the nymph stage develop through several instars (Masaki and Walker, 1987). Based on expected industrial timeframes, within four weeks crickets should have been in the final instar showing early signs of sexual development (in the form of wing and ovipositor growth). Crickets in the H_{CF} group were the only ones to consistently show these signs. This suggests that the lower nutritional quality of the other diets has stunted the development of the crickets resulting in smaller individual sizes at four weeks.

This highlights that perhaps the low-quality feed at any stage plays a larger role than previously thought and needs to be examined in more detail to ensure that a positive growth start is continued across the live span.

The differences between which feeding treatment groups performed best on overall survival vs. individual weight is interesting. Aside from those on the H_{CF} feed, the groups with the highest survival were not always the ones with the largest individuals. An example is $2WH_{CF}2WL_{Veg}$ which had the highest survival rate (behind H_{CF}) but was equal to $1WH_{CF}3WL_{BW}$ with regard to average individual cricket size. Whether it is better to have fewer larger insects or a larger quantity of smaller insects depends on the market. If a producer is selling insects by weight their size is irrelevant, as long as the entire colony weighs the most, however if a producer needs to sell a certain size of insects (e.g. as specialist food for lizards of certain sizes) it is of more importance what the characteristics of individuals in the colony are.

It is also worth noting that cannibalism can be a significant problem in mass-rearing of crickets (Harris and Svec, 1964). It is possible that in some groups, such as $2WH_{CF}2WL_{BW}$, which had a lower survival number but higher average individual size, the crickets within the colony ate each other rather than the feed provided. Although cannibalism was not directly observed in these colonies (as crickets were only observed for an hour each day); cannibalism has been previously observed in colonies which were subjected to stress due to lack of appropriate food.

The Efficiency of Conversion of Ingested food (ECI) and the Feed Conversion Ratios (FCR and P-FCR) are shown in Table 3.

Table 3. Average FCR and P-FCR across treatment groups, mean ± SD, FCR of 1 and ECI of 100 indicates perfect feed conversion. Significant differences indicated with letters ($p < .05$).

Diet	N	ECI	FCR	P-FCR
H_{CF}	6	33.15 ± 4.92^{a}	3.07 ± 0.46^{a}	1.24 ± 0.18^{a}
L_{BW}	6	8.65 ± 0.74^{b}	11.63 ± 1.02^{a}	$5.24 \pm 0.46^{a,\ c}$
L_{Veg}	5	4.66 ± 4.50^{b}	45.09 ± 45.63^{b}	$8.42 \pm 8.52^{b,\ c,\ d}$
$1WH_{CF}\ 3WL_{BW}$	9	$17.62 \pm 8.21^{b,\ c}$	6.77 ± 2.77^{a}	$3.00 \pm 1.23^{a,\ d}$
$2WH_{CF}\ 2WL_{BW}$	9	$25.02 \pm 10.01^{a,\ c}$	4.78 ± 2.32^{a}	2.03 ± 0.82^{a}
$1WH_{CF}3WL_{Veg}$	6	$10.13 \pm 2.68^{b,\ d}$	10.43 ± 2.54^{a}	$2.84 \pm 0.88^{a,\ d}$
$2WH_{CF}2WL_{Veg}$	6	$23.59 \pm 6.41^{a,\ c,\ d}$	4.51 ± 1.20^{a}	1.30 ± 0.26^{a}

Overall, there was a significant effect of feeding regime on ECI, FCR, and P-FCR ($p < .001$).

The crickets on the H_{CF} diet where the most efficient, converting 33% of feed into body mass. This was followed closely by the $2WH_{CF}2WL_{BW}$ (25% conversion) and $2WH_{CF}2WL_{Veg}$ (23.6% conversion) groups. These all had significantly higher ECI rates than the other groups, $p < .01$. The ECI rates are also higher than reported for *Acheta domesticus* but roughly on a par with rates for *Blaptica dubia* (Oonincx et al. 2015). The FCRs and P-FCRs for these three groups also show the same responses. H_{CF} still has the best FCR, but, given the differing protein content of the diets, the P-FCR is a more accurate measure of conversion and this shows that H_{CF} and $2WH_{CF}2WL_{Veg}$ are almost equal, indicating that crickets on these diets are equally able to convert protein directly into weight gain. The $2WH_{CF}2WL_{BW}$ group is not far behind with its P-FCR. It is worth noting that one of the diets (L_{Veg}) gave a high degree of variability in FCR and P-FCR (as seen by the large SD), likely due to the low survival numbers overall.

Crickets fed on the single bio-waste feeds, L_{BW} and L_{Veg}, had the lowest ECIs. Being at best 1/3rd as efficient at converting feed as the H_{CF} group and at worst only 1/7th as efficient. This rate is, however, not significantly worse than the $1WH_{CF}3WL_{BW}$ and $1WH_{CF}3WL_{Veg}$ groups. Demonstrating that once 2 weeks of a high-quality feed, prior to bio-waste are introduced, there is an improvement in overall ECI. This lends support to previous findings that different cricket life-stages metabolise food differently, as the crickets in the $2WH_{CF}2WL_{BW}$ and $2WH_{CF}2WL_{Veg}$ groups were one week older prior to being introduced to bio-waste (Woodring et al., 1979).

Conclusions

Although it is frequently suggested that crickets can be reared as a sustainable alternative to other protein sources, it is becoming increasingly evident that the question of what to feed crickets to ensure their sustainability is not straight forward. Research has shown that crickets struggle to survive at an industry scale on diets composed entirely of bio-waste (Lundy and Parrella, 2015; Oonincx et al., 2015) and to date chicken feed remains the best option for large scale production. However, in the present study it has been shown that incremental quality feeding regimes are a plausible alternative and could reduce the reliance on chicken feed in commercial scale cricket production. Thus, when crickets were provided with one week of a high-quality feed prior to being switched to a low-quality feed their survival and growth improved and this improved further when it was two weeks of high-quality feed. This could ultimately translate to a 50-75% reduction in the use of chicken feed.

The survival and growth of crickets on the incremental quality feeding regimes was still somewhat lower than crickets fed entirely on chicken feed, signifying that this is not yet the ideal solution. It is evident that a vital nutritional component necessary for cricket survival and growth is still missing from the low-quality bio-waste feeds. It is most likely that this is due to a lack of protein, since previous work with similar species has identified protein content of feed as a significant driver of survival and growth (Oonincx et al., 2015). Future research should try to identify sustainable, ideally waste-based, feed sources which are sufficiently high in protein to improve cricket survival. One option would be meat wastes and offal from local abattoirs, although an additional processing step would likely be needed.

Alternatively, other species of insects, which have lower nutritional requirements should be considered. *Hermetia illucens*, for example, has been demonstrated to thrive on a myriad of feed sources from food waste to manure (Oonincx et al., 2015; Banks et al., 2014). While crickets may be more desirable, future research should focus on species with the most sustainable production potential to avoid placing further stress on global resources. Ultimately, any intended insect production system will require a complete sustainability evaluation to examine potential social, economic and environmental impacts.

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