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Research Article

Which Protein Source is Best for Mass-Rearing of Asian Weaver Ants?

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ABSTRACT

The Asian weaver ant (*Oecophylla smaragdina*) is sometimes used as a biocontrol agent against pests in tropical agriculture as part of integrated pest management programmes. However, the effectiveness of weaver ants as a predator depends on the abundance and activity of colonies in naturally occurring populations. Mass-rearing is a method that could be useful both for getting lots of colonies and for maximising colony growth and aggressiveness. The diet of mass reared weaver ants potentially impacts their growth rate and behaviour. In this study, we investigate the effect of four common commercial protein diets (mackerel, tuna, cricket and mealworms) on *O. smaragdina* colony growth and aggressiveness during mass-rearing over a two-month experimental study at the Smart Research Institute, Riau, Indonesia. Colonies fed on mackerel, the cheapest protein source, and mealworm were able to grow significantly larger than those fed on tuna, even though colonies fed with mackerel showed the lowest amount of mass intake of protein food. In contrast, colonies fed with crickets (the diet with the greatest proportion of protein) had the greatest aggression index against experimentally placed bagworms (a common pest in oil palm plantations), with their aggression being significantly greater than that for mackerel-fed colonies. Taken together, our results show that there are trade-offs between different protein diets for weaver ant colonies in mass rearing facilities. Protein diet can be chosen depending on whether colony growth rate, colony aggressiveness, or price is the main factor driving decisions.

Keywords:
Oecophylla smaragdina, ant farming, biological control, aggression index, colony size, IPM

Introduction

In many countries in Southeast Asia, harvesting Asian weaver ants (*Oecophylla smaragdina,* Fabricius 1775) from nature is a common activity. People harvest ant brood (larvae and pupae) and use them for bird feed (Césard, 2004), or fishing bait. The adults are also sometimes eaten by humans (Itterbeeck, 2016; Offenberg & Wiwatwitaya, 2010). This wild harvesting of colonies in Java, Indonesia due to high demand for brood for use as birdsong food is potentially reducing the abundance of colonies in natural habitats, something not yet seen in other countries (Itterbeeck, 2016). Perhaps because of limitations on the numbers of wild colonies available, farming of weaver ants is popular in Indonesia, Thailand, and Laos, either on a household scale, or in larger commercial operations (Offenberg & Wiwatwitaya, 2010; Césard, 2004). Using such mass-rearing methods, the ant brood supply is relatively stable compared to the harvesting of brood from nature (Prayoga, 2015). Because weaver ants are also a biological control agent, we suggest that the methods used in weaver ant farming could be deployed to increase availability of colonies as part of integrated pest management programmes.

As a generalist predator, *Oecophylla* spp. are commonly used as biocontrol agents in several agricultural systems such as cacao, cashew, mango, coconut and citrus (Thurman et al., 2019). This ant species has been reported to control major pests such as true bugs (Coreidae and Miridae), beetles (Chrysomelidae), aphids (Aphididae), caterpillars (Lepidoptera), leaf miners (Coleoptera), leafrollers (Lepidoptera), fruit flies (Drosophilidae), leafhoppers (Cicadellidae) and shoot borers (Lepidoptera) (van Mele, 2008). Weaver ant populations fluctuate due to factors such as temperature sensitivity of the pupae development, mortality caused by disease, intraspecific and interspecific competition (Crozier et al., 2010), and abiotic environmental factors. Because of this, mass-rearing methods are potentially useful for increasing the availability of colonies. Such mass-reared colonies could be deployed in mass-release in response to pest outbreaks as part of an integrated pest management programme (IPM), potentially reducing the need to use other less sustainable control methods such as pesticides.

An important consideration besides nectary-food availability and prey abundance that determines colony development (Crozier et al., 2010) is the diet composition used in the mass-rearing system. This is because diet is likely to impact colony growth rate and colony aggressiveness, both of which are important for the effectiveness of ants as biological control agents. Ant colony growth and survival is likely to be determined by the balance of protein diet and carbohydrates (Dussutour & Simpson, 2012; Kay et al., 2012), while aggressiveness is likely to be determined by chemical cues and recognition systems (Newey et al., 2009; Newey et al., 2010). Moreover, synergistic effects between temperature and diet and its interaction can drive variation in aggressiveness within and between ant species (Barbieri et al., 2015). This study will investigate the effect of four common commercial protein diets on *O. smaragdina* colony growth and aggressiveness in a mass-rearing system.

Materials and methods

Weaver ant collection and husbandry

Weaver ant colonies were collected from oil palm plantations close to the mass-rearing facility. Only nests in which ants were present (confirmed using binoculars) and constructed from live green palm leaflets, were sampled. Nests were wrapped in a plastic bag, and then all connecting leaflets and fronds were cut using machetes and pruning shears. Ant nests from the field were transferred to racks in the mass-rearing facility. The mass-rearing facility was located at the Smart Research Institute (Smartri), (0º 55' 34.78" N, 101º 11' 38.57" E). The building was a simple 4 x 6 m square, wooden framed building with 60% black sun-shade nets covering the sides and a corrugated metal sheet roof (**Figure 1**). The interior ceiling was made with plywood with oil palm fibres used as insulation between the metal sheet and plywood. Colonies of *O. smaragdina* ants were reared in racks with plastic trays as shelves.

Figure 1. The mass-rearing facility used to propagate Asian weaver ant colonies.

Each colony was housed in a recycled 1.5 L plastic mineral water bottle (8 cm in diameter and 16 cm heights; **Figure 2**). The plastic bottles were thoroughly cleaned to remove any substance that may have negative effects on ants, including insecticide. The original leaf ball nests were broken open to facilitate migration into the empty bottles. At this time, water mist was sprayed into the nest with a hand-manual sprayer to protect the operator from the formic acid sprayed by ant workers into the air. We wore hand gloves and goggles during this work. Grease and used machine oil were used as barriers to avoid worker ants from attacking other colonies or from escaping from the facility. A Maxim iButton® datalogger that automatically recorded every three hours was installed in the centre of the facility to monitor ambient temperature and humidity. Overall mean temperature during the experiment was 27.2° C, with the mean daily maximum being 36.1° C and mean daily minimum being 22.6° C. The mean relative humidity was recorded at 85.7%, with mean daily maximum reaching 99% and daily minimum at 52.8%. After being transferred to mass-rearing racks, all the colonies were acclimated for three days. In the acclimation period, all colonies were fed only using sugar solution and fresh water.

Figure 2. A weaver ant colony growing in a recycled plastic bottle in the mass-rearing facility.

Experimental design

We conducted an experiment to see which protein food would be best for weaver ants. The commercial protein diets used in this experiment were: 1. canned tuna in vegetable oil (Kingfisher brand, claims to use *Katsuwonus* *pelamis* tuna). 2. fresh mackerel- *Euthynunus affinis* (local name ikan tongkol). 3. fresh yellow mealworm (*Tenebrio molitor*), and 4. fresh cricket (*Gryllus bimaculatus*). The canned tuna was supplied from the retail market, while mackerel was supplied from the traditional market. Mealworms and cricket were bought from a bird pet shop.

Diet treatments were assigned randomly to avoid biases due to gradients in the microclimate or other conditions in the rearing facility. All ant colonies were also given access to 30% sugar solution and freshwater administered *ad libitum*. Each diet treatment consisted of five replicates (n = 20 colonies in total). Experimental feeding and observations of colonies were conducted for two months.

The diet experiment started after the three-day acclimation period. Tuna, fresh mackerel, and fresh mealworms were fed at a rate of 4 g per day, while fresh crickets were fed at a rate of five individuals (average total of five individuals was 1.5 grams) every two days. Each two-day period in this experiment is referred to as a feeding period. Food was placed into a special feeding arena around the ant nest. The amount of leftover protein diet was weighed at the end of the feeding period to determine the amount consumed (mass of food remaining subtracted from mass of food placed). All the food in the feeding arenas were checked for fungal growth every day, especially the canned tuna and fresh mackerel. Any food with fungal growth or fungal contamination was replaced. Based on pre-trial observations, in 48 hours, there was decrease in the mass of tuna and mackerel when these were kept under control conditions in the mass-rearing facility without ant access, presumably due to evaporation. Hence, to analyse the actual amount eaten by ants and to avoid biased weight measurements, final tuna and mackerel weights were counted by reducing final mass with the average control mass.

Ant colony size observation

Observation of colony growth focused on occupation and colony survival in the plastic bottle or in any place within the plastic tray. Colony size was recorded on a scale ranging from zero (0) to five (5) (**Table 1**). The rating was assessed at the same time as the mass of food consumed was measured at the end of each two-day feeding periods.

Table 1. Scale used to assess ant colony size.

Rating	Definition
0	No ants in the colony area, and the colony failed to survive
1	Only major workers with a number less than or equal to 20
2	Only major workers with a number more than 20 to 50
3	Major and minor workers are present, foraging actively
4	Major workers, minor workers, and the queen are present, workers foraging actively
5	Major workers, minor workers, and the queen are present and workers forage actively. The colony has egg clusters (note that because this study uses transparent recycle-plastic bottle, the egg clusters are observable from outside).

Ant aggressiveness observations

As predacious ants, Asian weaver ants show aggressive behaviour toward foreign objects and other species of animals. The aggressive behaviour of the weaver ant was examined using an "aggression index" (Newey et al., 2009), covering specific behaviours such as threatening, pursuit, biting, or grappling by worker weaver ants. The index aggression index was formulated as:

$$A = f_{post} * 1 + f_{purs} * 2 + f_{bit} * 3$$

Where A = aggression index, f_{post} = frequency of aggressive posturing, f_{purs} = frequency of pursuit, and f_{bit} = frequency of biting or grappling. The tests used a single fresh *Clania tertia* bagworm that was 2 cm long. This species is an important pest of oil palm. The bagworm was always placed in a testing area 5 cm away from the ant nest. Ant behaviour was recorded within a 3 cm diameter area centred on the bagworm. Frequencies of each kind of aggressive response were recorded for three minutes for each colony. The test was conducted twice for each colony, with a ten-second rest between the two tests. A replacement of bagworm was used for every trial. This test was carried out every two weeks on all ant colonies.

Diet content and financial cost

The amount of protein in the four different diets of the weaver ant colonies is an important consideration in this experiment. Although this study did not conduct a proximate analysis for protein content, we gathered data from existing sources for the protein content. The canned tuna was labelled as having 70% tuna contents, comprising 16% protein. Fresh mealworm (*Tenebrio molitor*) comprises 45% – 51% protein content (Costa et al., 2020; Zhao et al., 2016). According to proximate analysis (**Figure 3**), mackerel tuna (*Euthynnus affinis*) contains 23% protein (Hizbullah et al., 2019).

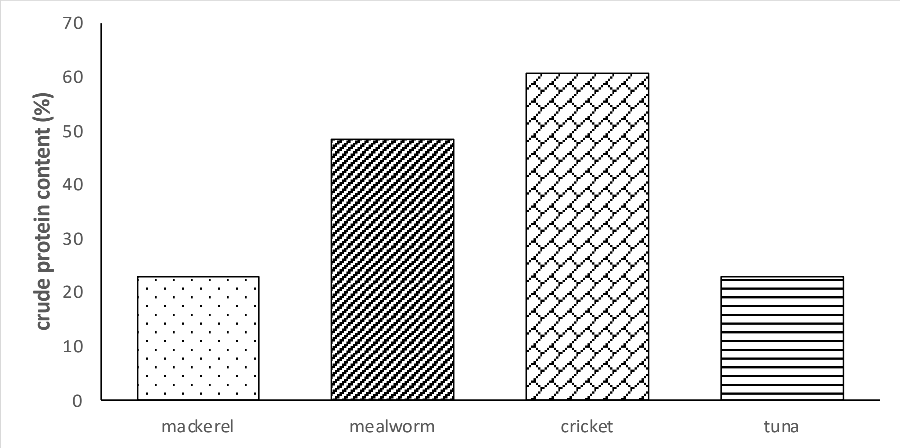

Figure 3. Proximate protein content of the different diets fed to the weaver ant colonies. These values are from previous laboratory analyses from Costa et al., (2020) and Zhao et al., (2016) for mealworm; Hizbullah et al., (2019) for mackerel; Jeong et al., (2021); Phesatcha et al., (2022); Udomsil et al., (2019) for cricket and nutritional data from Tuna King's Fisher brand for canned tuna. All the values relate to percentage of dry matter.

However, the highest protein content is in cricket *Gryllus bimaculatus*, with 61% – 69% protein (this species was used in this study) (Jeong et al., 2021; Phesatcha et al., 2022; Udomsil et al., 2019). The carbohydrate-protein ratio from that report is also compared directly from the references (**Figure 4**).

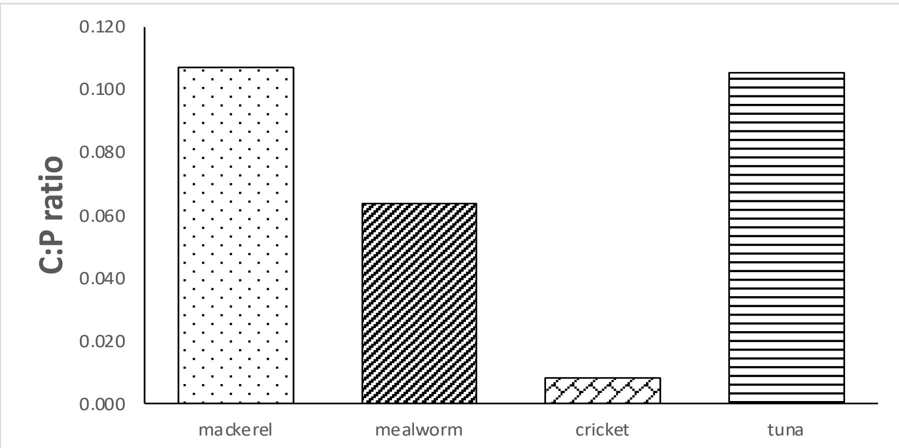

Figure 4. The carbohydrate-protein ratio for the different diets fed to weaver ant colonies used in this experiment. High values on the y-axis indicate diets rich in carbohydrate relative to protein. See Figure 3 legend for data sources.

We conducted cost comparison of commercial protein used in this experiment for cost and benefit consideration in weaver ant mass-rearing. The protein diet cost was analyzed by comparing each feeding session's protein intake (i.e food that was actually consumed, ignoring any not eaten at the end of the experiment; **Figure 5**).

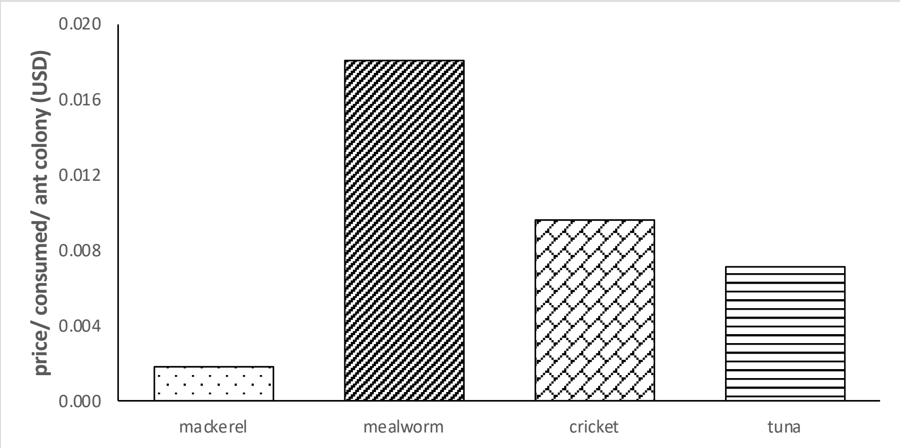

Figure 5. Comparison of the price of average protein diet intake per batch colony (in USD) per feeding session. Prices were recorded during the experiment, on the Indonesian market (July-September 2021).

Statistical analyses

Differences in the amount of food consumed by weaver ant colonies across different diets was determined using a linear model, with amount of food consumed as the response, diet type, room temperature during the feeding session, and observation series as fixed predictors. Colony identity was included as a random factor since multiple measurements were made for each colony.

Differences in the size of weaver ant colonies between treatments were tested statistically using an ordinal regression. Colony size was used as a response variable, while diet type and mass of food consumed were set as predictor variables. Colony identity was set as a random effect. Tukey pairwise tests were used to determine which treatments differed if differences were found among diet treatments. The best fit model was selected based on the lowest Akaike Information Criterion (AIC) (Aho et al., 2017).

All statistical models were performed using the R statistical programme version 4.0.2 (R Core Team, 2020) in R Studio version 1.1.423 (RStudio Team, 2020). Graphs were created using "*ggplot2*" and "*tidyverse*" R packages (Wickham et al., 2019). The linear mixed model (lmer function) was performed using the "*lme4*" package (Bates et al., 2015) and the ordinal model (clmm function) was performed using the "*ordinal*" package (Christensen RHB, 2019).

Results and discussion

All the protein diets were consumed to some degree by weaver ant colonies. Mackerel was consumed significantly less than other protein diets (t-value=-2.693, p=0.015, Table 2). Significantly more mealworm was eaten than cricket (t-value=2.657, p=0.016) but these did not differ significantly from tuna (t-value=0.522, p=0.606).

Table 2. The summary of the fitted model for the diet intake of weaver ant colonies.

Variable	estimate	Std.err	t-value	p > |z|
(Intercept)	1.6078	0.191	8.425	<0.001*
mackerel	-0.7265	0.270	-2.693	0.015*
mealworm	0.7183	0.270	2.657	0.016*
tuna	0.1551	0.297	0.522	0.606

Asterisks (*) indicate statistically significant comparisons at p<0.05.

We predicted that the kind of protein fed to ant colonies would affect their growth. The tuna-fed colonies had significantly smaller colony sizes compared to colonies fed on mackerel and mealworm (**Figure 7**). This result is interesting because the amount of tuna consumed was the same as cricket and mealworm (p>0.05, Figure 6), but tuna has lower protein content (**Figure 3**).

In this experiment, protein content may have a role in colony development such as supporting egg production (Crozier et al., 2010; Way, 1954), especially during early colony establishment. However, high protein content in the diet can also decrease the number of workers in an ant colony. This case is observed in black garden ant workers (*Lasius niger*), where workers die at a young age, causing the colony to collapse when it was fed a high-protein diet (Dussutour & Simpson, 2012).

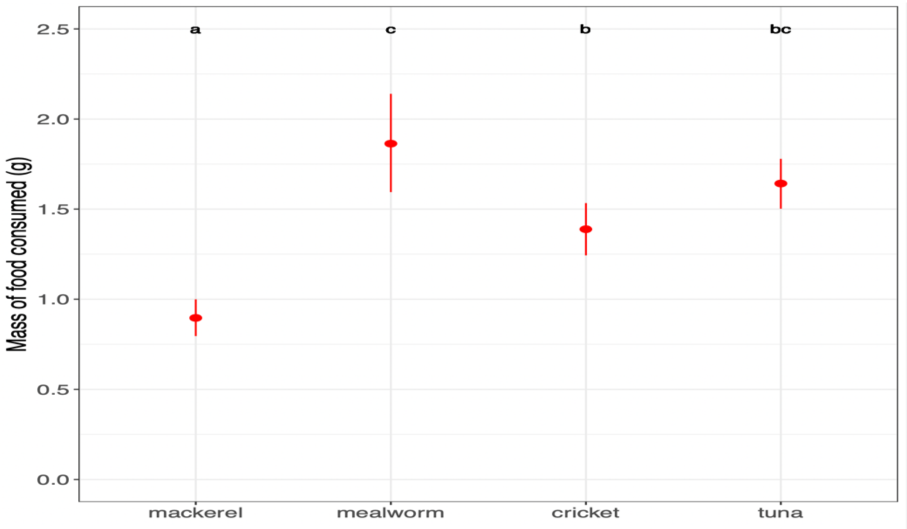

Figure 6. The mass of protein food consumed by weaver ant colonies during the mass-rearing experiment under different diet regimes. The dot and bar indicate means and standard error. The same letters at the top of the plot denote the diet that was consumed is not statistically significantly different (Tukey contrast, $p>0.05$).

The freshness of the diet is also likely to determine nutrient components, such as protein, fat and carbohydrate. A decrease in nutrient content levels occurs in fresh tuna stored in the fridge, with protein content decreasing to 21% from an initial value of 23% protein 14 days after it was caught and stored (Hizbullah et al., 2019). However, the nutrient content in canned commercial food undergoes food processing including use of preservatives, likely minimizing this loss. Moreover, there is a possibility that a preservative may have negative effects on ant colonies. Thus, the actual protein content in tuna might be lower than reported in the label. Consequently, tuna fed-colonies may need more protein content to build a bigger colony.

Table 3. The summary of the fitted model predicting weaver ant colony size in relation to type and amount of food consumed and temperature during mass-rearing.

Variable	mean	Std.err	z value	p > |z|
diet (cricket)	3.560	1.833	1.942	0.052
diet (mealworm)	4.318	1.872	2.306	0.021*
diet (mackerel)	5.620	1.890	2.974	0.003*
consume (gram)	0.103	0.145	0.709	0.479

The star (*) indicate significant at $p<0.05$.

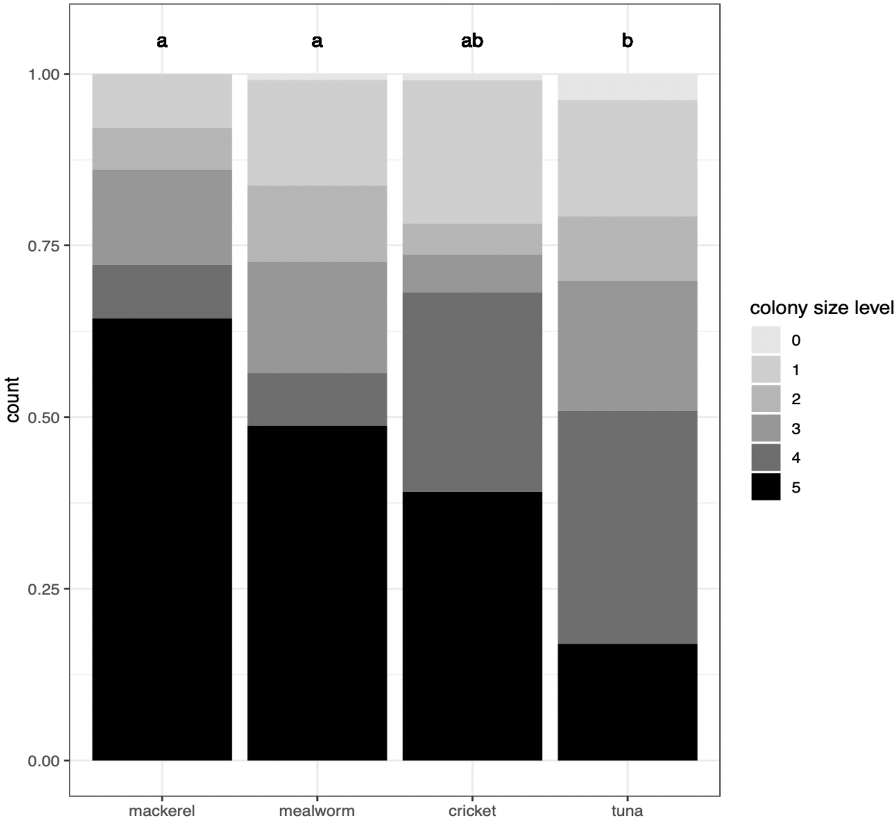

Figure 7. Colony size level in the mass-rearing experiment in relation to diet. The rating observation was a cumulative score from 25 observation sessions. Colony size was not different statistically for treatments with the same letter label shown in the upper plot (Tukey contrast at p=0.05).

In this study, the lowest ratio of carbohydrate: to protein was found in the cricket diet (**Figure 4**), which had the same effect on colony size as the tuna diet (z-value=1.884, p=0.06, **Figure 7**). In contrast, mackerel-fed colonies that ate a diet with higher carbohydrate-protein ratio (**Figure 4**) could grow bigger than the cricket-fed colony during the experiment, even though this difference was not statistically significant (p>0.05, **Table 3**). Dietary carbohydrates can increase the total larva mass and hence influence colony growth in a longer-term experiment (Kay et al., 2012). Likewise, the combination of protein and carbohydrate in diet may play an important role in maintaining colony size.

The cricket-fed colony had the highest mean aggression index against experimentally placed bagworms, although it only differed significantly from the mackerel-fed colony (Tukey contrast, t-value=2.818, p=0.034, **Figure 8**). The high aggression index in the cricket-fed colony is probably related to prey recognition (Newey et al., 2009), where ants are more likely to attack live intruders such as crickets and bagworms. The weaver ant colony use visual, auditory, tactile and olfactory cues to determine other organisms (Newey et al., 2009; Newey et al., 2010), which in this study is bagworm. The fact that the usual diet of these colonies was live insects might explain the high aggression index seen in the cricket-fed colony and the mealworm-fed colony (which were not significantly different: Tukey contrast, t-value=1.572, p=0.474, **Figure 8**). Nevertheless, the effect of diet on ant aggression level depends on the ant species. For instance, the aggression and activity of Argentine ants (*Linepithema humile*) are higher on colonies reared on low protein, high carbohydrate diets (Kay et al., 2012).

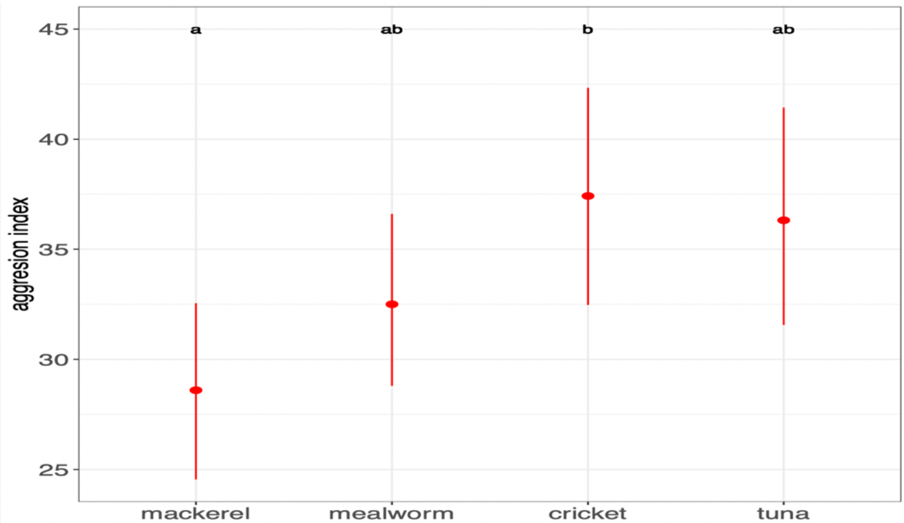

Figure 8. Differences in aggression between weaver ant colonies fed on different diets as part of a mass-rearing colony experiment. The dot and bar indicate means and standard errors (Tukey contrast, p<0.05).

Consideration of economic feasibility is important when conducting mass-rearing of biological control agents. In addition to labour costs, purchase of food is probably the highest cost for mass rearing facilities. Mackerel is the cheapest among the protein diets used in this experiment (**Figure 5**). Hence, when considering only cost of the food, mackerel can potentially support weaver ant colonies to build larger colonies at the lowest expense. However, this is potentially offset by the lower amounts of mackerel eaten by weaver ant colonies (**Figure 6**) and the lower aggression exhibited by those colonies (compared to those fed on crickets; **Figure 8**).

Three parameters should be considered when selecting food for conducting mass-rearing of Asian weaver ants: colony size/growth, aggressiveness level, and price. The most crucial aspect for the goal of the mass-rearing to enhance the Asian weaver ant as a biological agent for controlling leaf-eating caterpillars is the capability to produce large ant colonies. For this, mackerel should be selected as food. Second, colonies reared in captivity should have similar aggression levels to wild colonies. For this, crickets should be selected as food. Hence, there is a trade off between these two goals and diet choice can be tailored as needed. If the financing factor for weaver ant protein food is limited, then mackerel should be chosen as the cheapest source. Our results show that it is possible to maintain and grow these natural enemies of leaf-eating caterpillars, with future potential to implement natural enemy population augmentation as part of IPM programmes.

Conclusions

This study demonstrated that weaver ant colonies can be maintained in captivity with a sugar solution and various alternative protein sources and that they will grow larger while in captivity. There appear to be trade-offs between different protein regarding colony growth and aggression, with mackerel being best for the former and crickets for the latter. Furthermore, mackerel is the most economical protein food for weaver ants. A mixed-protein diet is probably best for ensuring growth of weaver ant colonies as well as to maintain their aggressiveness. The findings of this study could be useful in developing mass-rearing methods that uses the Asian weaver ant as a biocontrol agent in tropical agriculture systems.

Author contribution

ADA, KMY, and TMF contributed to the conception and design of the study. ADA collected the data. ADA, KMY, and TMF wrote the first draft of the manuscript. All authors contributed to the manuscript revision and have read and approved the submitted version.

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Conflict of Interest

The authors declare no conflict of interest.

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