Vol. 17(10), pp. 1330-1335, October, 2021
DOI: 10.5897/AJAR2021.15742
Article Number: C23B0CF67963
ISSN: 1991-637X

http://www.academicjournals.org/AJAR

ACADEMIC JOURNALS

African Journal of Agricultural Research

Full Length Research Paper

Nutritive value of common housefly (*Musca domestica*) prepupae reared on broiler by-products as source of animal feed

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Received 8 August, 2021; Accepted 20 September, 2021

Common housefly (*Musca domestica*) is one of the insects that is found in almost all parts of the world, it is found in diverse habitats making its life cycle stages such as larvae and pupae readily available for foraging poultry on free range systems. Large commercial poultry farms world-wide are experiencing low supply of animal based proteins and excess organic wastes generated from the farms. Common housefly prepupae has proved to be the solution based on their ability to valorize the organic wastes and a potential to be used as source of protein for fish, poultry and pigs. The study-site was Farhan Farm in Msabaha, Kilifi County, Kenya, and the main objective was determining the nutritive value of *M. domestica* prepupae reared on selected broiler by-products. Completely Randomized Design (CRD) replicated three times was used. The selected broiler by-products in the study which served as treatments were manure (droppings), blood, crop content, visceral rejects and broiler carcasses. Chemical analysis was done at Kenya Agricultural and Livestock Research Organization (KALRO) Laboratory in Kakamega. Results showed that *M. domestica* reared on carcasses had the highest crude protein (45.83%), compared to those reared on blood (41.54%), crop content (33.18%), visceral rejects (33.14%) and manure (30.65%). The difference in growth performance and nutritive value of prepupae from the five treatments showed that there is effect of substrate on nutritive value of prepupae. In conclusion, the best performing treatments in terms of nutritive values were carcasses, blood, visceral rejects, crop content and manure in that order.

Key words: *Musca domestica* prepupae, nutritive value, protein.

INTRODUCTION

Insects for the past years have been known as source of high quality, efficient and sustainable source of protein (Van Huis, 2013). Their use as an alternative source of protein has great potential since they can be reared on the organic side stream. In addition, insects are efficient feed converters, a trait associated with them as ectotherms (Veldkamp and Bosch, 2015). In feed industry, insects such as common housefly (*Musca domestica*), black soldier fly (*Hermetia illucens*) and yellow mealworm (*Tenebrio molitor)* have been given

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much attention due to their ability to valorize organic wastes as they convert them to protein (Anankware et al., 2015).

Common housefly (*M. domestica*) are predominatant all over the world, their ability to live in diverse habitats has made some of its life cycle stages such as larvae and pupae to be readily available for foraging poultry on free range systems (Hu et al., 2017). In large commercial livestock farms rearing broiler, layers, dairy animals and pigs, farmers often experience challenges of disposing agricultural wastes (agro-by products) such as manure, blood, offal, dead animals and feed waste (van Zanten et al., 2015). Research has been conducted on the use of common housefly larvae and pupae in bioconversion of organic wastes for formulating feed in poultry, pigs and farmed fish (Pretorius, 2011).

Studies on use of common housefly larvae as feedstuff has been done on broiler and pig production (Veldkamp and Bosch, 2015). Results proved that *M. domestica* larvae meal has essential amino acid pattern comparable to fish meal (Pieterse and Pretorius, 2014). However, (Obeng et al., 2015) observed that the development and various life-history traits of common housefly are highly dependent on the growing substrates used. Therefore, this study focused on determining the nutritive value *M. domestica* prepupae reared on broiler by-products as source of animal feed.

MATERIALS AND METHODS

Experimental set-up

This study was conducted at Farhan Broiler Farm in Msabaha, Kilifi-county at the coastal Kenya which lies between 3° 30' 2" S and 40° 1' 29" E. Msabaha has an average annual temperature of 26.3°C and an average rainfall of 1094 mm per annum (Maina et al., 2015). Adult houseflies were trapped using four improvised plastic flytraps where fermented broiler starter crumbs were used as the bait, similar to the method adopted by Kleiding and WHO (1986), the attracted flies were used to establish breeding colonies. Six improvised, plastic, fly cages measuring (100 × 100 × 100 cm) were used to house adult flies. Approximately 1000 houseflies were kept in each cage for a period of 3 weeks for each breeding cycle. After this period, cages were cleaned and disinfected for fresh young breeders. Water in cages was provided using containers that had protruding cotton wicks as drinking sites. A mixture of powdered milk and granulated sugar (1:1 ratio) was provided *ad libitum* in open containers as food for the flies. Cages were located in a room with a photoperiod of 12 h light and 12 h darkness, temperature of 25 ± 2°C, and ambient humidity (40-60%). From approximately 5 days after emergence, flies were provided with oviposition substrate in the form of 100 g of wetted broiler starter crumbs in a 100 mm petri dish. Flies were allowed to oviposit for a 4-hour period every two days over 3 weeks. Eggs were separated using a modified suspension method. In brief, egg masses were transferred to a 0.8 Mole sucrose solution, agitated and buoyant eggs were collected from the top layer. Eggs were then washed in water three times to remove any impurities and sucrose. The eggs were finally rinsed using a 75 μm stainless steel filter and briefly air dried. Eggs were suspended in water (5 mg eggs/ml, approximately 75 eggs) to facilitate distribution immediately before use (Pretorius, 2011).

Treatments

Five different substrates were tested for their effects on nutritive value of *M. domestica* prepupae; broiler manure, broiler crop content, broiler intestinal rejects, dead broiler and broiler blood. The broiler breed used in the study was Hubbard Efficiency plus, supplied by Supreme Chicks Company in Kenya to Farhan Farm. The manure(droppings) was taken from broiler shades that had been used for thirty five days and above, crop content was taken from broilers which were feeding on finisher pellets and were thirty five days and above, visceral rejects were taken from birds which were thirty five days and above, it mainly consisted of bursa, kidney, lungs and fats, dead bird are broiler that died from day thirty five and above and broiler blood which was collected from the broilers above thirty five days at the farm slaughter house. Water was added to crop content and dead broiler at the rate of 20 ml/100 g and manure at the rate of 80 ml/100 g to attain a moist condition for the eggs and the larvae to develop into quality prepupae. For blood and visceral rejects, water was not added since the moisture content was sufficient.

Prepupae rearing

About 500 of 3- to 6-h-old eggs were placed in triplicate onto 2000 g fresh material of each substrate in plastic buckets. The buckets (10 L) were screened with fine-mesh cotton gauze and covered with a lid provided with three ventilation holes. The buckets were placed in a containment structure with standard height and width to ensure uniform temperature. The larvae were subjected to feeding regime of 2000 g, until they reached the prepupal stage. All prepupae per each treatment were harvested on the same day; prepupae for crop content were harvested on the 7^{th} day, for blood were harvested on the 5^{th} day, for manure were harvested on the 15^{th} day, for visceral rejects were harvested on the 6^{th} day and finally for carcasses were harvested on the 9^{th} day. The variation of the prepupae harvesting days was associated with difference in the molting time observed from the five treatments as per the pilot experiment conducted to determine the effect of broiler by-products on the molting time of *M. domestica* prepupae.

Harvesting of prepupae

Harvesting of prepupae was done by filling the buckets with water half-way, then stirred with a rod in a clock-wise direction continuously to develop centrifugal force, all the prepupae that floated were collected and the remaining few were collected manually by use of forceps after draining water. The collected prepupae were washed with tap water and frozen at (−20°C) overnight. Then, they were vacuum packed and stored at (−20°C) until all chemical analysis were performed at KALRO Kakamega Laboratory.

Proximate analysis

The nutritional contents of prepupae were analyzed in triplicate. The moisture content was determined by drying the defrozen samples in an oven at (100 –105°C) to constant weight. Total lipids were determined by continuous extraction in a Soxhlet apparatus for 8 hours using hexane as solvent. The crude protein contents were evaluated through nitrogen determination by the Kjeldahl method and conversion to proteins by multiplying the quantity of nitrogen by the coefficient 6.25 (Horwitz, 2010). Crude fibres by sequential hot digestion of the defatted sample with diluted acid and alkaline solutions (Horwitz, 2010).

Minerals analyses

The samples were properly mixed and homogenized before weighing 1 g into a crucible (Foss- Borosilicate 3.3, Por. 2). Thereafter, the samples were dried in an air forced oven (MRC-Scientific instruments, Model PF120, Serial Number 21-302357) for 1 hour at 105°C. The samples in the crucible were then taken to a pre-heated muffle furnace (Barnstead Thermolyne-type 48000 furnace, Model No. F48010) and heated at 550°C for 3 h until all the organic materials were ashed. The residues were visually viewed to ensure they are free from carbonaceous particles. The crucibles were cooled in a desiccator, the ash was transferred into a 250 ml volumetric flask and 1 ml of nitric acid (HNO_3) was added to dissolve it. The volume was then made up to 250 ml mark using distilled water. The ash solution was then filtered using filter papers (F1001 grade, size 125 mm).

For phosphorus determination, the Ultra-Violet (UV) visible spectrophotometer (Model UV mini-1240 Shimadzu) was preheated for 1 h prior to sample analysis. The wavelength was set at 400 nm and the absorbance was adjusted to 0 with 0 µg/ml standard. Thereafter, the test samples and the standards were run under the same conditions. The concentration of phosphorous in the samples was determined in mg/100 g against 5 standard solutions. For determination of potassium, flame photometer (Model 420) was adjusted to 0 absorbance with 0 ppm standard and the test samples were run along with standards under the same conditions. The concentration of potassium in the samples was determined in mg/100 g against 5 standard solutions. In the case of iron concentration, Shimadzu, Atomic Absorption Spectrophotometer (Model, AA-6200) was set to warm up for 10 minutes with flame and source lamp lit prior to sample analysis. The wavelength was set at 248 nm and the lamp current at 10 mA for iron. The absorbance was adjusted to 0 with 0 µg/ml standard.

Thereafter, the test samples and the standards were run under the same conditions. The concentrations of iron in the samples were determined in mg/100 g against 5 standard solutions. For calcium and magnesium determination, Shimadzu, Atomic Absorption Spectrophotometer (Model, AA-6200) was allowed to warm up for 10 minutes with flame and source lamp lit prior to sample analysis. The wavelength was set at 422 nm and the lamp current at 10 mA for Calcium determination and 285 nm and 10 mA lamp current for Magnesium respectively. The absorbance was adjusted to 0 with 0 µg/ml standard. Thereafter, the test samples and the standards were run under the same conditions. The concentrations of Calcium and Magnesium in the samples were determined in mg/100 g against 5 standard solutions.

Statistical analysis

All data were analyzed using R Software version 3.5.3. One-way ANOVA was used to analyze the effects of broiler by-products used as substrates on nutritive value of *M. domestica* prepupae. Shapiro-Wilk test and Bartlett's test were used to check for normality of data and homogeneity of variance respectively. Since significant difference in the nutritive value of the prepupae reared on the five selected broiler by-products was expected, a post-hoc test was done using Least Significant Difference (LSD) to identify the treatments which had significant different values in their performance.

RESULTS

In this study, the nutritive values were analyzed in two sets as proximate and mineral analysis. The analysis was done in KALRO Kakamega, at the Animal Feed Laboratory from August to September 2020. For proximate analysis the focus was on prepupae dry matter, moisture content, crude fibre, crude fat and crude protein. The minerals analyzed in the study were; phosphorus, calcium, potassium, magnesium and Iron. The laboratory findings were recorded in Table 1. On the seventh column are the p-values that denote the probability of the null hypothesis; all the selected broiler substrates had the same proximate and mineral analysis result. Table 1 summarizes the proximate and mineral analysis of *M. domestica* prepupae reared on selected broiler by-products.

Proximate analysis

There was significant difference in the dry matter content of *M. domestica* prepupae whereby prepupae reared on visceral rejects had the highest dry matter content (26.50%) while prepupae reared on manure had the lowest dry matter of (14.25%), on the same note prepupae reared on manure had the highest moisture content (85.75%) while prepupae reared on visceral rejects had the lowest moisture content (73.50%) as captured in Table 1. There was a significant difference in crude fibre content of the prepupae whereby prepupae reared on blood had the highest crude fibre (44.50%) while prepupae reared on manure had the lowest (17.00%) which had no significant difference with prepupae reared on visceral rejects (20.00%) and crop content (18.50%). There were no significant differences in the crude fat content of *M. domestica* prepupae reared on all the five broiler-by products with the following averages of prepupae fat detected; blood (17.00%), crop content (19.75%), carcasses (23.33%), visceral rejects (24.30%) and manure (12.00%). There was significant difference in the crude protein content of *M. domestica* prepupae whereby prepupae reared on carcasses had the highest crude protein (45.83%) while prepupae reared on manure had the lowest crude protein (30.65%).

Mineral analysis

The minerals that were tested in this study were phosphorus, calcium, potassium, magnesium and iron. From Table 1 there was significant difference in the mineral composition of *M. domestica* prepupae reared on different broiler by-products. Prepupae reared on broiler visceral rejects had the highest concentration of phosphorus (211.7 mg) while prepupae reared on broiler carcasses had the lowest concentration of phosphorus (88.3 mg). There was significant difference in calcium concentration of *M. domestica* prepupae whereby prepupae reared on visceral rejects had the highest concentration of calcium (2.71 mg) while prepupae

Table 1. Proximate and mineral analysis results of *M. domestica* Prepupae reared on selected broiler by-products.

Parameter	Blood	Crop content	Carcasses	Visceral rejects	Manure	P values
D.M (%)	21.50±0.71^{d}	23.25±0.35^{c}	24.75±0.35^{b}	26.50±0.71a	14.25±0.35^{c}	<0.0001
M (%)	78.50±0.71^{b}	76.75±0.35^{c}	75.25±0.35^{d}	73.50±0.71^{e}	85.75±0.35^{a}	<0.0001
C.F (%)	44.50±0.71^{a}	18.50±0.71^{c}	38.50±2.12^{b}	20.00±0.0^{c}	17.00±1.41^{c}	<0.0001
C.Fat (%)	17.00±6.60^{a}	19.75±1.41^{a}	23.33±9.43^{a}	24.30±1.41^{a}	12.00±0.0^{a}	0.2600
C.P (%)	41.54±1.30^{b}	33.18±0.25^{c}	45.83±1.30^{a}	33.14±0.92^{c}	30.65±0.50^{d}	<0.0001
P (mg/100 g)	144.7±0.49^{b}	165.1±13.5^{b}	88.4±21.13^{c}	211.8±0.64^{a}	156.5±2.45^{b}	0.0010
Ca (mg/100 g)	1.61±0.27^{b}	1.97±0.21^{b}	0.76±0.18^{c}	2.71±0.01^{a}	1.96±0.09^{b}	0.0009
K (mg/100 g)	66.43±0.00^{d}	105.77±0.0^{a}	47.67±0.00^{e}	100.42±0.01^{b}	99.04±0.00^{c}	<0.0001
Mg (mg/100 g)	17.39±0.00^{d}	43.40±0.00^{b}	15.81±0.00^{e}	37.41±0.00^{c}	63.97±0.00^{a}	<-0.0016
Fe (mg/100 g)	3.56±0.00^{e}	5.39±0.01^{d}	10.61±6.00^{b}	10.58±0.01^{c}	13.46±00^{a}	<-0.0016

Means (n = 5) in the same row followed with different superscript letters are significantly different at 95% confidence level; D.M-Dry Matter; M-Moisture, C.F-Crude Fibre, C.Fat-Crude Fat, C.P-Crude Protein; P-Phosphorus, Ca-Calcium, K-Potassium, Mg-Magnesium and Fe-Iron. The table also has column for the P-Values.

reared on carcasses had the lowest concentration of calcium (0.76 mg). There was significant difference in the potassium concentration of *M. domestica* prepupae whereby prepupae reared on crop content had the highest concentration (105.77 mg) while prepupae reared on carcasses had the lowest concentration of potassium (47.67 mg). There was significant difference in the concentration of magnesium at 95% confidence level in the *M. domestica* prepupae whereby the concentration in prepupae reared on manure was the highest (63.97 mg) and the lowest concentration was recorded for prepupae reared on carcasses (15.81 mg). Finally, for iron, there was significant difference in the concentration of iron in prepupae, whereby *M. domestica* prepupae reared on manure had the highest concentration (13.46 mg) of iron while prepupae reared on blood had the lowest concentration (3.56 mg).

DISCUSSION

Proximate analysis

There was significant difference in the dry matter content of *M. domestica* prepupae whereby prepupae reared on visceral rejects had the highest dry matter content an indication that it had less free water compared to prepupae reared on manure which had the lowest dry matter, prepupae reared on manure had the highest moisture content while prepupae reared on visceral rejects had the lowest moisture content. These findings concurred with previous studies done (Aniebo et al., 2008; Pretorius, 2011; Ipinmoroti et al., 2019), whose results show that the moisture content of *M. domestica* maggot is inversely proportional to dry matter content (Connor, 2006). From the study, prepupae reared on visceral rejects, carcasses, crop content, blood and manure would be preferred for feed formulation since the dry matter is an important component for weight gain in animal diet as observed in diet formulated for fish (Hezron et al., 2019). There was a significant difference in crude fibre content of the prepupae whereby prepupae reared on blood had the highest crude fibre (44.50%) while prepupae reared on manure had the lowest (17.00%) which had no significant difference with prepupae reared on visceral rejects (20.00%) and crop content (18.50%). From these findings the range of crude fibre based on the five substrates is (44.5 to 17.00%) which was far much higher than the findings recorded in previous studies done using same method and procedures apart from the substrates, for instance (6.14%) by Ukanwoko and Olalekan (2015), (7.11%) by Hussein et al. (2017) and (7.20%) by Hezron et al. (2019). Studies have shown that fibre rich diets in poultry production can stimulate the secretion of digestive enzymes and it also has positive impacts on intestinal villi surface and nutrient absorption (Wenk, 2001). Such effects depends on the fibre source and the physical structure of the fibre material used. In pigs, multiple data indicates that fermentable fibre has an impact on the lower Gastro-Intestinal Tract (GIT), increased growth rate of bacteria, with cellulolytic, pectinolytic and hemicellulytic activities has been seen. There is evidence that certain types of dietary fibres can promote beneficial bacteria such as lactobacilli and bifidobacteria in the GIT microbiota of pigs (De Leeuw, 2008).

The crude fat content recorded in this study ranged from (24.00%) to (12.00%) from the five substrates; these findings are higher than those recorded by previous study where (9.3%) was recorded by Teotia and Miller (1974) while other study found fat content within the range of (16%) by St-Hilaire et al. (2007), (25.3%) by Aniebo et al. (2008), (14.39%) by Pretorius (2011), (19%) to (16%) by Ukanwoko and Olalekan (2015), 20% by Hussein et al. (2017) and (22.23%) by Ipinmoroti et al. (2019). There was no significant difference in the crude fat content of *M.*

domestica prepupae reared on all the five Broiler-by products with the following averages of prepupae detected fat; broiler blood (17.00%), broiler crop content (19.75%), carcasses (23.33%), visceral rejects (24.30%) and manure (12.00%), an indication that substrate type has no effect on prepupal fat concentration in *M. domestica.*

There was significant difference in the crude protein content of *M. domestica* prepupae whereby prepupae reared on carcasses had the highest crude protein (45.83%) while prepupae reared on manure had the lowest crude protein (30.65%) this gives the range of findings from the study. The crude protein content of the prepupae in the study was lower than those in previous studies (47.1%) by Aniebo et al. (2008), (60.38%) by Pretorius (2011), (60%) by Hussein et al. (2017). Such difference can be associated to the type of substrate used or the age of harvesting as Hezron et al. (2019) recorded that the concentration of crude protein varied with the time of harvesting whereby an extension of the harvesting day by two days resulted into a decline from (48.6%) to (46.7%) and finally to (44.6%) crude protein showing that the longer the prepupae reared on the same substrate takes to be harvested the lower the concentration of crude proteins. On the same note the crude protein content recorded among the prepupae reared on carcasses (45.83%) was not having much difference with those analyzed by Ipinmoroti et al. (2019) which was (47.45%). The crude protein content recorded in all the prepupae reared on the five broiler by-products as in Table 1, was higher compared to pig requirement which ranges between (18.5%) to (11%) (Fang et al., 2019; Zhou et al., 2019), that makes the prepupae reared on the five substrates ideal for formulating a complete pig feed, study on Genetically Improved Farmed Tilapia (GIFT), a strain of *Oreochromis niloticus* shows that it requires an optimum dietary protein of 34.53 to 38. 10% in Inland Saline Water (ISW) of 5 g/L (Singha et al., 2021) which is within the range of crude protein attained from the M. *domestica* prepupae reared on broiler by-products. In commercial poultry production, the crude protein differs depending on the class of poultry for instance peckin duck requires between (20.63%) to (23.25%) crude protein (Cho et al., 2020). For broiler, their feed is classified into two main categories broiler-starter which has a crude protein of (22-21%) and broiler-finisher with a crude protein of (19-18%) (Pretorius, 2011), all the findings are within the range of crude protein attained in the current study which is a positive sign that the prepupae are rich in protein and can be utilized in formulating a complete feed for fish, pigs and poultry. From this study, it is worth noting that the substrates used in rearing *M. domestica* had effects on the nutritional value, especially the moisture content, crude fibre, crude fat and crude proteins which are key components of any diet usually classified as macro-nutrients.

Mineral analysis

Trace minerals play a critical role in the metabolic functions of livestock. These functions help support growth and development, immune function and the reproductive performance and supporting several enzymatic systems. Prepupae reared on visceral rejects had the highest concentration of phosphorus (211.77 mg), expressed as percentage would be 2.11% which is close to 2.40% recorded by Pretorius (2011) for *M. domestica* larvae while prepupae reared on carcasses had the lowest concentration (88.38 mg) expressed in percentage as (0.88%). These figures are higher than the percentage concentration of phosphorus in soya meal (0.735%) as recorded by Hussein et al. (2017) but lower than the concentration in fishmeal (3.13%). For correct bone development of broilers, there should be a balanced concentration of calcium and phosphorus in the ratio of about (2:1), expressed in weights as 6-6.5 g/Kg and 2-2.3 g/Kg (Matuszewski et al., 2020). Prepupae reared on visceral rejects had the highest concentration (2.71 mg) expressed in percentage as (0.027%) which is far much lower than (0.41%) recorded by Pretorius (2011) while prepupae reared on carcasses had the lowest concentration (0.76 mg) expressed in percentage as (0.0076%). The concentration of calcium in prepupae reared on visceral rejects is higher than (0.33%) of Soya meal (Hussein et al., 2017), but lower than the calcium concentration of prepupae reared on visceral. According to Hussein et al. (2017), fishmeal has calcium concentration of (5.55%) which is far much higher than the concentration of all the prepupae reared on the five substrates.

Prepupae reared on crop content had the highest concentration of potassium (105.77 mg) expressed in percentage as (1.05%) which is lower than (1.27%) recorded by Pretorius (2011) while prepupae reared on carcasses had the lowest concentration of potassium (47.67 mg) expressed in percentage as 0.48%. The concentration of potassium in soya meal (2.25%) as per (Hussein et al., 2017) was far much higher than that of fishmeal (0.71%) and all the prepupae reared on the five substrates in this study.

Prepupae reared on manure had the highest concentration of magnesium (63.97 mg) expressed in percentage as (0.64%) which was lower than (1.15%) recorded by Pretorius (2011) while prepupae reared on dead broiler had the lowest concentration (15.81%) expressed as (0.15%) which was far much lower than the findings recorded by Pretorius (2011) the findings compared to both soya meal and fish meal magnesium concentration of 0.3 and 0.71% respectively as recorded by Hussein et al. (2017). Prepupae reared on manure had the highest concentration (13.46 mg) expressed in percentage as (0.13%) as compared to 275 ppm as the Pretorius (2011) findings. On the same note prepupae reared on blood had the lowest concentration (3.56 mg), as for soya meal the iron concentration was 90 ppm while fishmeal iron concentration was 478 ppm as per (Hussein et al., 2017).

CONCLUSION AND RECOMMENDATIONS

Based on nutritive value of *M. domestica* prepupae measured in terms of crude protein, crude fibre, dry matter, crude fat and mineral profile, the best performing treatment was broiler carcasses, followed by blood and visceral rejects in that order, but due to unpredictability of getting broiler carcasses cheaply, the study recommended adoption of broiler blood and intestinal rejects as the best broiler by-products for rearing *M. domestica* prepupae as a source of protein for formulating feeds for fish, pigs and poultry.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

This study was co-funded by Jaramogi Oginga Odinga University of Science and Technology (JOOUST) through the Africa Center of Excellence in Sustainable Use of Insects as Food and Feeds (INSEFOODS) in conjunction to the World Bank. Sincere gratitude to Farhan Farm and Supreme Chicks Company who gave space and conducive environment for this study.

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