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LIFE CYCLE AND NUTRITIONAL COMPOSITION OF DUNG BEETLE, *HELIOCOPRIS BUCEPHALUS* (FABRICIUS, 1775), UNDER CAPTIVE CONDITION*

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Abstract

Larvae of *Heliocopris bucephalus*, commonly known as the dung beetle, are edible insects with high market value in Myanmar. The aim of the present study was to examine the life cycle of the cow dung beetle, *Heliocopris bucephalus* (Fabricius, 1775), under captive conditions and analyze the nutritional composition of its pupal stages. The rearing of dung beetles were conducted in two parts: Experiment I focused on the developmental stages from adults to the third instar larvae, while Experiment II covered the development from natural brood balls, fourth instar larvae, to the adult stages. For the first part of the study, thirty adult beetles were randomly collected from a three-feet-height dung hill in Pobba Thiri Township, Nay Pyi Taw Division, and reared in insect boxes (15.75 x 11.75 x 9.5 inches) filled with layers of cow dung at the Applied Entomology Laboratory, Zoology Department, University of Yangon, in November 2023, with a sex ratio of one male to three females. The adults built brood balls, but they were not as tightly packed as those in natural conditions. After about two and a half months, the third instar larvae in the brood balls were killed due to infection by entomopathogenic fungi and predation by mole crickets that had contaminated the dung. The rearing boxes were renovated to prevent fungal infection and predators and Experiment II was conducted. Experiment II started with the collection of wild dung brood balls containing fourth instar larvae which were then cultivated in the laboratory. The fourth instar larvae developed into fully grown adults after a study period of three months. The entire life cycle of *Heliocopris bucephalus*, combining the results from Experiments I and II, lasted approximately six months to complete. The nutritional values of the pupal stage, which is the edible stage, were analysed for protein, fat, carbohydrate, fibre, moisture and ash content. The pupae contained 45.40% protein, 29.16% fat, 13.92% carbohydrate, 3.86% fibre, 3.44% moisture, 4.22% ash content and 497 kcal/g energy value. The nutritional content of *H. bucephalus* is higher than that of livestocks, with the protein content being nearly double that of soybeans, which have the highest protein content among plant-based foods.

Keywords : Dung beetles, under captive condition and nutritional composition

Introduction

Edible insects are seen as a key solution to the challenge of meeting the increasing global demand for animal protein, which is highly valued for its nutritional benefits (Bruinsma *et al*., 2003). Insects should therefore be considered as an alternative source of animal protein, or "micro-livestock," especially since livestock already occupies over two-thirds (68%) of all agricultural land (FAOSTAT, consulted August, 2015). This is largely due to insects' high feed conversion efficiency and their ability to consume a wide range of feed sources (Halloran *et al*., 2016).

Entomophagy, the practice of eating insects, is traditionally followed in 113 countries worldwide (MacEvilly and Ms Claire, 2000). It is estimated that insects are part of the diet for at least two billion people, and over 1,900 insect species are currently consumed as food (FAO, 2013). Worldwide, the most commonly eaten insects are those that are easily accessible, such as

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beetles (Coleoptera: 31%), caterpillars (Lepidoptera: 18%), bees, wasps, and ants (Hymenoptera: 14%), grasshoppers, locusts, and crickets (Orthoptera: 13%), cicadas, leafhoppers, planthoppers, scale insects, and true bugs (Hemiptera: 10%), termites (Isoptera: 3%), dragonflies (Odonata: 3%), flies (Diptera: 2%), and various other species (5%) (Van Huis *et al.*, 2013). Insects are most frequently consumed in Africa, Asia, and Latin America (Jongema, 2015). Most edible insects have protein levels similar to those in beef, fish, or shrimp, ranging from 27 g to 54 g of protein per 100 g of edible portion on a fresh weight basis (FAO, 2003).

Most larval stages of insects, including certain species of dung beetles such as *Heliocopris bucephalus*, have been traditionally consumed in various parts of Myanmar where they are often considered as a delicacy. As researchers have discovered that the local people consume edible forest insects not because they are environmentally-friendly or nutritious but because they are cheap compared to meat or poultry that are widely available. Rather they choose to eat insects simply because the insects are good in taste (DeFoliart, 1995).

Since Myanmar is an agricultural country, there is a lot of cattle breeding and therefore beetles that eat dung from herbivores like cows and buffaloes can find anywhere. Through manipulating faeces during their feeding process, cow dung beetles initiate a series of ecosystem functions ranging from nutrient cycling to parasite suppression (Andresen, 2002). In which, *Heliocopris bucephalus* (Coleoptera, Scarabaeidae) plays an important role in nutrient cycling by decomposing large herbivore dung (Nichols *et al*., 2008). They also act as secondary seed dispersers by burying them in the brood ball, which also protects the seeds from rodent predators (Hanski & Cambefort, 1991).

In rural areas like Rakhine and Kayin States, people harvest *Heliocopris bucephalus* from the wild, particularly from agricultural fields and forested areas where these cow dung beetles are abundant. The beetles are typically collected by hand and prepared in various ways, such as frying, roasting, or boiling. According to the local people, adult beetles are not very suitable for human consumption because of the high chitin substances content (wings, exoskeleton, legs etc.).

There is potential for developing small-scale farming of edible cow dung beetles as a sustainable food source. This would involve breeding and raising *Heliocopris bucephalus* in controlled environments, which could reduce pressure on wild population while providing a reliable source of nutritious food. The present study aims to investigate the developmental stages and the duration of the life cycle of *Heliocopris bucephalus* under captive condition and to examine the nutritional composition regarding the edible pupal stages of *Heliocopris bucephalus.*

Materials and Methods

Study area and period

This study was conducted from November 2023 to October 2024 at the Applied Entomology Laboratory of the Department of Zoology, University of Yangon. The adult beetles used in Experiment I and brood balls for Experiment II were collected from a dung hill in Pobba Thiri Township, Nay Pyi Taw Division (19°57′16″N 96°10'19"E) (Fig.1). Fresh cow dung for feeding was sourced from Hmawbi Township, Yangon Division.

Life cycle of *Heliocopris bucephalus* under captive condition

The life cycle of *Heliocopris bucephalus* was studied in two separate experiments. The first involved rearing adult beetles to the third instar larval stages, while the second focused on rearing fourth instar larvae from natural habitats to the fully grown adult stages.

Experiment I: Rearing Adults to Third Instar Larvae

Collection of breeders

A total of 30 adult *Heliocopris bucephalus* individuals were collected in November 2023. During the sampling period, the temperature and humidity of the area were 26°C and 70% respectively. The samples were collected during their breeding season. The height of the selected dung hill, which is in the forest where most of the farmers are grazing their herds, was about three feet (91 cm) (Plate 1 A). At a depth of around 30 cm and below the surface, twenty to fifty adults *Heliocopris bucephalus* were found (Plate 1 B). The beetles were collected by digging dung hill with a spade and by using hand-picking method. Collected samples were placed into plastic boxes filled with a mixture of solid cow dung and soil from their natural habitats.

Additionally, five natural brood balls were taken from burrows which about 100 to 150 meters away from their habitat cow dunghills and each of the burrows are about 100 meters apart. The places of the burrows were marked and the brood balls were carefully brought to the laboratory for further observation.

Solid and fresh cow dung from the study sites were also brought to the Applied Entomology Laboratory of Zoology Department, University of Yangon.

Preparation for rearing *Heliocopris bucephalus*

Upon arrival to the laboratory, adult *Heliocopris bucephalus* were placed into rearing plastic boxes measuring (15.75 x 11.75 x 9.5 inches) (Plate 1 C).

Before putting the collected samples, the boxes were prepared with layers. Two inches of soil layer were added at the bottom of rearing containers. Two inches of solid cow dung in the middle layer and about one inch of fresh cow dung was added at the top twice a week. The twenty collected *Heliocopris bucephalus* were maintained in the five prepared plastic boxes with a sex-ratio of 1 male and 3 females (1:3) in each box. The boxes were sealed to the top with iron filter for ventilation and to effectively prevent the escape of collected samples. The rearing plastic boxes were carefully dug twice a week and checked whether there will be oviposition or egg hatching.

After oviposition, the eggs were put back into their rearing plastic containers to get parental care and the sizes of the eggs were also recorded. After hatching of eggs, the young larvae were placed again into 2500 ml plastic containers separately. Larvae developmental periods and morphological characteristics were also observed and recorded every week.

Fresh cow dung about 180 g to 200 g were fed every three days because adult beetles absorb only the liquid nutrients of the fresh dung as the attractiveness of fresh dung lasts only 72 hours for dung beetles. Fresh cow dung was taken once a month from Hmawbi Township (17°05′53″N 96°04'13"E), Yangon Division. The boxes used for rearing adult *Heliocopris bucephalus* were maintained under their ambient temperature of (26 °C - 28 °C) and relative humidity of (50% to 80%). All these experiments were made under the fixing laboratory condition.

Experiment II: Rearing from brood balls to adult beetles

Collection of natural brood balls

In February 2024, twenty-one brood balls were dug from Pobba Thiri Township (19°57′16″N 96°10'19"E), Nay Pyi Taw Division (Fig. 1). The brood balls were collected from the four marked burrows that can be found easily as the soils are different from the soil around them (Plate 2 B). In each burrow, there were at least three brood balls and eight the most (Plate 2 C). They were brought to the laboratory to rear from the brood balls, the fourth instar stage, to the adult stage. In the laboratory, each brood ball was broken with an approximately 5 cm hole to check the larval stage development through the opening.

Preparation of rearing boxes for experiment II

To prevent fungal infection and predators, the rearing boxes were renovated by placing a layer of soil from brood balls' natural habitat at the bottom of the rearing plastic boxes. The half of the brood balls were buried in the soil and the other half with an exit hole were exposed for ventilation (Plate 2 D). The brood balls were also sprayed with water about twice a day to maintain humidity and moisture. Temperature was maintained between 25°C to 30°C by showing sunlight once a week to prevent contamination and fungal infection.

Preparation of the samples for nutritional analysis

For nutritional values analysis, another twenty-four brood balls with pupal stages were collected from the same area were used. The brood balls were cracked from their exit holes because the other parts were as hard as a stone (Plate 3 A). The edible fully grown *Heliocopris bucephalues*'s pupal stages were undergo physical termination by sun- drying and subsequent oven-drying in the oven at a specified temperature (200°C for five times – for 10 minutes each) until it became a crispy stage (Plate 3 B, C, D and E). The crispy stage larvae were grinded by a blender and measured the weight of the powder (Plate 3 F and G). The resulting powder (50g) were analysed at the "Food Industries Development Supporting Laboratory (FIDSL)" which is located in the Union of Myanmar Federation of Chambers of Commerce and Industry (UMFCCI) Yangon. Moreover, the nutritive values of commonly consumed nutrient groups such as chicken, pork, beef, soybeans and edible insects were claimed from the previous studies by different authors for comparison (Plate 3 H).

Chemical analysis

Each nutritional value was analysed chemically as a test method recommended by the Association of Official and Analytical Chemists (AOAC, 2019). The crude protein was determined by using Kjeldahl method. Determination of moisture (loss on drying) content by Air Oven method and ash content was performed by using loss of weight in ignition using temperature control oven, Muffle Furnace method. Next, fat content was determined by Buchisoxlet Extraction method and fibre by Fibre Cap method.

Data analysis

The recorded data were calculated by Mean ± Standard Deviation (SD) in Excel.

Figure. 1. Map of the study area (Source from Google Map)

A. Selected three feet height dung hill

B. Cluster of adults *Heliocopris bucephalus*

C. Measurements and Preparation design of rearing plastic box

Plate 1. Preparation for rearing *Heliocopris bucephalus*s

A. Sample collection site

B. Soil showing unusual condition of burrowing insects

C. Brood balls found in natural condition

D. Brood balls with exit holes

Plate 2. Samples collection for Experiment II and renovation of rearing boxes

A. Breaking brood balls

B. Pupal stages

C. Sun-drying

D. Oven-drying

E. Crispy stage larvae

F. Grinding

G. Measuring the weight of powder (50g)

H. Ready to analyse

Plate 3. Preparation for the examination of the nutritional values of *Heliocopris bucephalus's* pupal stages

Results

Experiment I: Rearing of adult beetles under captive condition

In Experiment I, adult *Heliocopris bucephalus* were maintained in experimental plastic boxes, and their egg-laying and brood ball-making processes were examined.

Brood ball-making process

After 2 weeks of maintaining the adult *Heliocopris bucephalus* in the experimental plastic boxes, some part of the soil in the rearing plastic boxes were seen to be dried. The brood balls which have various sizes and shapes were seen but some were found exclusively as eggs. There were about 15 to 20 eggs, averaging four eggs per rearing box. However, there was only one egg per brood ball, and the measurements of the brood balls were 5 ± 3.5 mm.

Morphologies of the life stages of Experiment I

Eggs were pearl white colour and they were about 1.83 ± 0.29 mm in length and 1.6 ± 0.15 mm in width. When the ovoid shape egg was approached to hatch, the egg became yellowish colour egg. This stage lasted from 10 to 15 days.

First instar larva which has a translucent and cylindrical body with cephalic capsule was found. The first larva was about 5 ± 1 mm in length and 1.33 ± 0.29 mm in width. This stage lasted from 5 to 10 days.

The second instar shed its exoskeleton and its body segments became distinct and jaws became well developed. Spiracles and legs were also distinct. The body was crescent shape and pale translucent in colour. The larva was about 10 ± 2 mm in length and 4.3 ± 1.53 mm in width. This stage lasted from 15 to 20 days.

The head capsule was orange in colour and yellowish body colour with C shaped. Body length was 25 ± 5 mm and width was 7.6 ± 2.52 mm. The most active stage and the larvae eat solid dung only. A small short hair occurred along the body and the larvae were stay buried when they feed and grow. A single orange spots line was present on each side of the body and this stage lasted from 40 to 60 days.

However, under laboratory conditions, the larvae were killed by entomopathogenic fungi and mole crickets (Plate 4). Therefore, the life cycle of Experiment I started from collected individuals and ended in third instar larval stage. The measurements of both length and width are increasing along with their life stages (Table 2). The duration of the life cycle of *Heliocopris bucephalus* lasted for two and a half months.

Plate 4. Recorded fungal infection and predators of *Heliocorpris bucephalus* inside the rearing boxes

Experiment II

In Experiment II, brood balls of the *Heliocopris bucephalus* were collected from their natural habitats and reared in the experimental plastic boxes. The changes of larval stages were examined through the opening.

Morphologies of the life stages of Experiment II

Fourth instar larva which is elongated and yellowish colour body was found in one of the natural brood balls. They feed intensively on solid dung. Their body size became large and robust compared to earlier instars and reached its maximum size around 5.16 ± 0.76 cm in length and 3.67 ± 0.76 cm in width. Their whole body were full with cow dung. If their brood balls were accidently broken, they will close the hole with the dung which are coming out from their mouthparts. The fourth instar stage lasted from 20 to 30 days.

The prepupa stage is the edible stage. It is a grub with C-shaped and cream white to light yellow colour body. The prepupa body is full with fat although there was no more cow dung throughout its body. The body was 4.5 ± 0.5 cm in length and 3.5 ± 0.5 cm in width. During this stage, the larva was not found eating and less active as they are undergoing significant physiological changes, setting the stage for the transition to the pupal stage. When transforming to pupal stage, there was a cocoon like skin which is protecting the transforming pupa and it was stripped off when the pupa is ready to emerge. This stage lasted from 10 to 12 days.

Prepupa was moulted its exoskeleton and the pupa was emerged. This is the critical period of metamorphosis where the larva transformed into an adult beetle. Feeding was not observed in this stage. Although the pupa stayed quiet, but sometimes the pupa shakes and twists suddenly. This stage was completely free with cow dung throughout its body. Body length was 4.5 ± 0.5 cm and width was 3 ± 0.5 cm. The size of the pupa is generally proportionate to the size of the adult beetle. Initially, the pupa was yellowish in colour, but it darkens slightly as it matures and prepares for adult emergence. Wing form, legs and head appeared in this stage. This pupal stage lasted from 15 to 20 days.

The initial adult stage was emerged from the pupal chamber with reddish colour exoskeleton. It actively fed on fresh cow dung which they used it to help their body to fully growth stage. The legs and ventrum are covered with coarse rust-red hairs. They mostly stay beneath the soil and active at night that they are trying to escape from the rearing boxes by using their unfolded wings. This stage lasted from 30 to 40 days.

Table 1. Morphology of fully grown adult stage

No	Name	Description
1.	Body 	Robust, oval, and convex, with a shiny black exoskeleton.
2.	Head 	Equipped with a broad, shovel-like structure. Males have a prominent, forward-facing horn on their head while the absence of a horn in female is a key feature of sexual dimorphism in this species.
3.	Antennae 	Antennae are clubbed at the end with a segmented, fan-like structure, used for detecting scents (especially dung).
4.	Pronotum 	Broad and shield-like, often covered in fine punctuations. In males, this area is particularly robust.
5.	Abdomen 	Deep red with sex segmented and present rust red hairs.
6.	Legs 	Clothed with erect red hair. Forelegs are modified with spines and teeth for digging. The tibiae of the hind legs are flattened and adapted to aid in rolling dung balls.

Duration of the life cycle of Experiment II

The life cycle of Experiment II started from the fourth instars which are brought from four marked natural burrows and ended in fully grown adult stage (Table 1). As the fourth instar body is large and robust, they are highest in length and largest in width among the measurements of the life stages of Experiment II. Adult is the second highest in length and the prepupa and pupa shared the same lengths. Pupa is the smallest while prepupa and adult have the same width (Table 3). The duration of this life cycle lasted for three months.

Complete life cycle of dung beetle, *Heliocopris bucephalus* (Fabricius, 1775)

The completed life cycle is the combination of the life cycles of Experiment I and Experiment II (Plate 5). Among the measurements of the life stages, the fourth instars took the highest in both length and width. Following by the prepupa, pupa and adult stage was the second highest in both length and width (Fig 3). In durations, the adult stage was the longest and the third instar was in the second place. Followed by fourth instar, the pupa, the second instar, the egg, the prepupa and the first instar was the shortest respectively (Fig 4). The durations of the whole life cycle lasted for six months to complete.

Table 2. Biomorphometrics and life stages of dung beetles in Experiment I

No	Stages	Shape & Colour	Length (mm) Mean ± SD n = 3	Width (mm) Mean ± SD n = 3	Duration (Day) Mean ± SD n = 3
1	Egg	ovoid shape yellowish colour	1.83 ± 0.29	1.6 ± 0.15	12 ± 2
2	1[st]instar larva	cylindrical translucent	5.0 ± 1.0	1.33 ± 0.29	6 ± 1
3	2[nd] instar larva	Crescent Pale translucent	10.0 ± 2.0	4.3 ± 1.53	15 ± 3
4	3[rd] instar larva	C shaped orange in color (Head) yellowish color (Body)	25.0 ± 5.0	7.6 ± 2.52	35.33 ± 7.51

Table 3. Biomorphometrics and life stages of dung beetles in Experiment II

No	Stages	Shape & Colour	Length (mm) Mean ± SD n = 3	Width (mm) Mean ± SD n = 3	Duration (Day) Mean ± SD n = 3
1	4[th] instar stage	Elongated yellowish colour	5.16 ± 0.76	3.67 ± 0.76	20.67 ± 5.13
2	Prepupa edible stage	C-shaped grub Cream white to light yellow colour	4.5 ± 0.5	3.5 ± 0.5	6.6 ± 2.0
3	Pupa edible stage	Oval shaped Yellowish in color	4.5 ± 0.5	3 ± 0.5	17 ± 2.52
4	Initial adult stage	Robust, oval & convex Black and dark brown with deep red elytra	5 ± 0.5	3.5 ± 0.5	37.67 ± 2.52

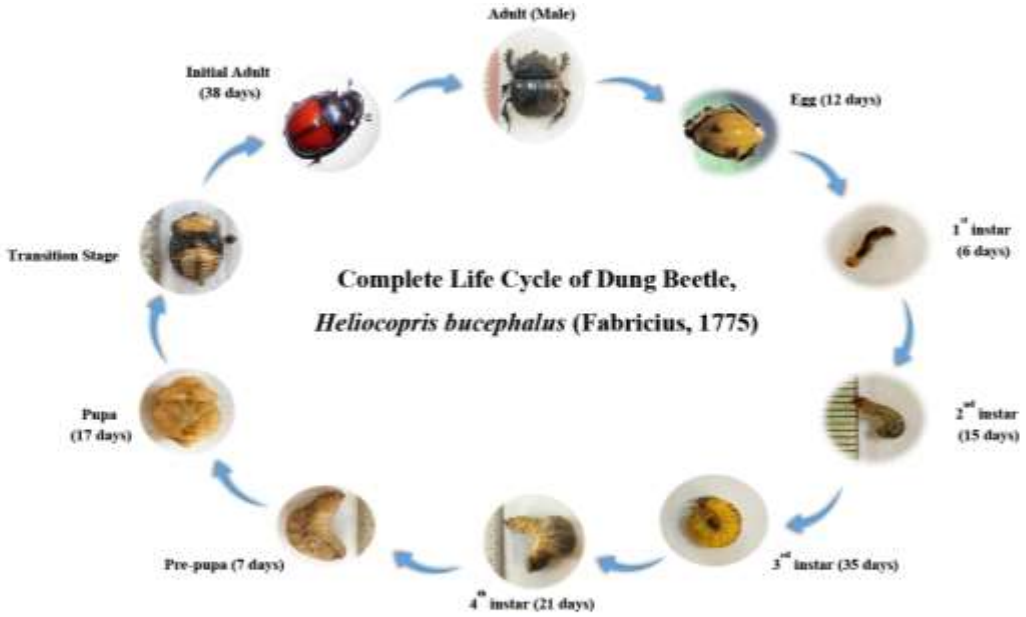

Plate 5. Complete Life Cycle of *Heliocopris bucephalus* (Fabricius, 1775)

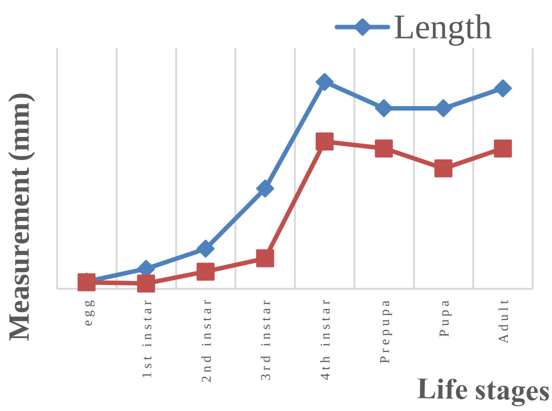

Figure. 3. Measurements of the life stages of *Heliocopris bucephalus*

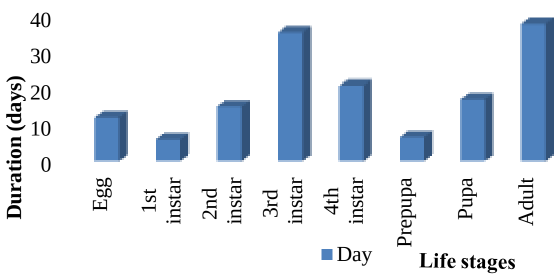

Figure. 4. Duration of the life sages of *Heliocoprris bucephalus*

Nutritional composition of *Heliocopris bucephalus*

In the analysed nutritional contents of the pupal stages of *H. bucephalus*, five kinds of nutritional values were recorded. Among them, the content of the protein has the highest with 45 g and fat is the second highest with 29 g. Carbohydrate took the last place with 14 g. The nutritional content of the analysed energy on 1 package per 100g is 497 kcal. As for fibre, the content was analysed as 3.86%. And this study also investigated the other extracts such as moisture and ash content of *Heliocopris bucephalus* with 3.44% and 4.22% respectively (Fig. 5).

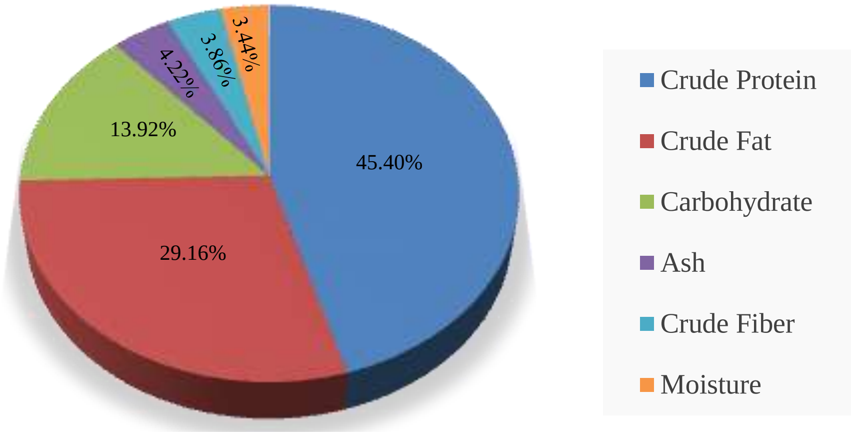

Figure. 5. Nutritional composition of *Heliocopris bucephalus* (Fabricius, 1775)

Discussion

Successful farming of edible insects, such as crickets and silkworm larvae, has been reported in previous studies (Zhou *et al*., 2007 and Fujita *et al*., 2013). However, the farming of *Heliocopris bucephalus* has not yet been fully developed. This study provided valuable insights into the reproductive behavior and growth stages of *H. bucephalus* under laboratory conditions, supporting its potential for future cultivation.

In Experiment I, *H. bucephalus* was unable to construct brood balls successfully. According to Halffter and Matthews (1966), larger brood balls are essential for larval survival, providing necessary nutrition and enhancing survival rates. In this study, *H. bucephalus* adults formed brood balls of variable sizes, likely due to the limited availability of cow dung in the rearing containers, which may have affected larval nutrition and subsequent survival rates.

High mortality among larvae was observed, likely due to entomopathogenic fungi and predation by mole crickets. Rath *et al*. (1992) and Viljoen (1997) found that *Beauveria bassiana* and *Metarhizium anisopliae* often exploit moist conditions, favoring fungal growth and spread. Mole crickets, which damaged brood balls, may also have contributed to reduced egg and larval survival. These observations indicate the necessity of regular monitoring and strict environmental management in captive conditions to reduce larval losses. Additionally, disinfection of cow dung before introducing it into the rearing boxes could help mitigate brood ball contamination.

The pupal stage of *H. bucephalus* in this study lasted between 17 and 25 days, aligning with observations by Halffter and Edmonds (1982), who noted similar durations under varying environmental conditions. Differences in temperature and humidity likely contributed to the variations between this study and others, underscoring the importance of environmental control in developmental timing. As environmental factors, particularly humidity and temperature, significantly influence the life cycle of insects (Bale *et al*., 2002, Hance *et al*., 2007 and Khaliq *et* *al*., 2014). Further research should be carried out to investigate the optimal temperature and humidity conditions for *H. bucephalus* development.

The nutritional composition analysis demonstrated *H. bucephalus* as a rich protein source, providing 45 grams of protein per 100 grams, comparable to beef (FAO, 2003). This beetle's protein content is almost twice that of soybeans, highlighting its potential as a sustainable protein source (Food Data Central, 2019). These findings align with et.al(2013), who advocated for insect proteins as viable alternatives to traditional livestock sources.

In conclusion, *H. bucephalus* shows significant promise as a sustainable protein source, especially in regions where conventional protein sources are limited. However, additional research is essential to optimize captive breeding, control fungal infections, and assess the environmental parameters that influence growth and survival rates.

Conclusion

The present study successfully examined the life cycle and nutritional composition of *Heliocopris bucephalus* under captive conditions. Rearing *Heliocopris bucephalus* involves several steps to ensure their successful growth and reproduction. It was found that the dung beetle has a life cycle of approximately six months, with distinct developmental stages from egg to adult stage. Despite challenges faced during Experiment I, including fungal infections and predation, the modifications made in Experiment II allowed for successful rearing. Maintaining stable temperature and humidity levels throughout the larval development are the most important steps of the life cycle of the studied species. Although their optimal temperature is around 26°C, they thrived better in temperature above 30°C.

The nutritional analysis revealed that the pupal stage, which is edible, offers a significant source of protein, fat, and carbohydrates. With 45.40% protein content, the dung beetle surpasses many conventional food sources, highlighting its potential as an alternative protein source. These findings not only enhance our understanding of *H. bucephalus* but also underscore its importance for food security, particularly in regions where edible insects are a cultural and economic asset. Further research is encouraged to optimize captive breeding methods and explore the commercial viability of this species as a sustainable food source.

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