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# Nutritional Potential and Microbial Status of African Palm Weevil (*Rhynchophorus phoenicis*) Larvae Raised on Alternative Feed Resources

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## Abstract

A six (6)-week long experiment was carried out to explore some feed resources for the production of African palm weevil (*Rhynchophorus phoenicis*) larvae and to ascertain the nutritional potential and microbial status of larvae raised on different diets. Four-hundred (400) two (2)-weeks old young larvae with weights ranging from 0.6 to 1.6 g were harvested and randomly grouped into 40 sets, each group comprising ten (10) larvae of similar weights and then randomly allocated to four (4) dietary treatments, using a completely randomized design (CRD) with ten (10) replications of 10 larvae in each treatment. The four diets which were administered were labeled as T1, T2, T3 and T4 containing varying levels of oil palm yolk at 100%, 50%, 50% and 25% respectively with various combinations of agro-waste materials including fruit waste of banana and pineapple and millet waste. Three (3) kilograms of each diet was fed *ad libitum* every two weeks for six weeks. After the feeding trial, it was revealed that the various diets fed did not have any significant effect on the nutrient composition except Nitrogen free extract of different groups of larvae produced indicated significant ($p<0.05$) differences among larvae. Microbial status of crushed samples of larvae also revealed the presence of bacteria species including *Klebsiella* and *Salmonella*. It was concluded that *R. phoenicis* larvae possessed great potentials for use as food and feed for humans and animals respectively and the diets used served as nutritionally suitable growth media for production of palm weevil larvae.

***Keywords:*** *ad libitum*; agro-waste materials; bacteria; food; growth.

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## 1. Introduction

“Entomophagy” which literally refers to consumption of insects and their products has been highlighted as a promising strategy to salvaging the projected concerns of global food insecurity and poverty alleviation [11, 17]. Besides their ecological importance, insects play a significant role in the nutrition of humans [16]. In most cases, insects serve as food in diverse cultures particularly as a delicacy or substitute food during periods of shortage or famine (Fasunwon and his colleagues 2011). However, consumption and utilization of insect proteins have been heckled with several challenges including disgust factor, lack of nutritional insight as well as social unacceptability [9]. There is therefore the need to educate the general populace about the potential of edible insects and their products in order to clear the misconception surrounding the acceptability of the practice [9]. All over Tropical Africa, especially where palm species are cultivated the African palm weevil (*Rhynchophorus phoenicis*) and their larvae are widespread and often recognized as pests of the palm species they feed on. In Ghana, the matured adult weevils are locally called “Asɔmrɔdwe” whiles the larva is locally called “Akokono” among most rural folks in Southern Ghana. Elsewhere in Beti communities of the central and southern regions of Cameroon, the larva is locally called “fos” [10]. Both the adult weevils and larvae are great sources of essential amino acids such as lysine, methionine and cysteine, threonine, tryptophan, leucine, isoleucine, valine etc. [13]. Study by [6] reported that the larvae contain a very good composition of essential mineral elements including iron, zinc, manganese, magnesium, calcium, copper, sodium and potassium. Based on dry weight [18] reported the nutrient content of palm weevil larvae to have contained approximately 21.06 % proteins, 66.61 % lipids and 7.63 % carbohydrates with an energy value of 714.25 kcal. Reference [13] in their quest to investigate the fatty acid profile reported as much as 30.28 % and 69.72 % of Palmitoleic and Stearic acids respectively for the larvae of raffia palm weevil. Earlier reports of vitamins in these larvae showed appreciable values of vitamin A (479.7 iu/ 100 g), riboflavin (5.9 mg/ kg), niacin (1.9 mg/ kg) and thiamine (5.1 mg/ kg) [13]. In certain instances, the medicinal value of the larvae in aiding treatments of ailments like women’s infertility, rashes and wounds, coughs and colds among others have been reported by [10].

All of these benefits further explains the need for the general populace to embrace this practice of entomophagy as insect proteins compare favorably to the other conventional protein sources (like meat and eggs) and thus complement them greatly [1]. This study is therefore a center of keen interest owing to the rising concerns over optimizing the potential of insects as alternative resources against global food insecurity as well as generating livelihood from insects farming as mini-livestock farming [10].

### *1.1 Objectives*

The objectives of the study included;

1. To ascertain the nutritional potential of African palm weevil (*R. phoenicis*) larvae raised on different diets.
2. To examine the microbial status of the different groups of larvae produced on the various diets.
3. To identify bacteria species that may be present in the larvae produced.

## 2. Materials and Methods

### *2.1 Location and duration of study*

This study was undertaken at the "*Akokono*" Farming Research Centre, Aspire Food Group at Kwame Nkrumah University of Science and Technology, Kumasi- Ghana, West Africa.

### *2.2 Experimental diets*

The ingredients used for formulating experimental diets comprised mainly of Agro-waste materials such as oil palm yolk (OPY), Millet waste (MW) and Fruit waste (FW) of both banana and pineapple. African palm weevil larvae (akokono) raised under the control treatment group (T1- 100 % OPY) were solely fed the conventional oil palm yolk (OPY). The experimental treatment groups (T2- 50 % OPY, T3- 50 % OPY and T4- 25 % OPY) were fed with varying percentages of oil palm yolk in addition to various combinations of other ingredients. The composition of the formulated diets for the different groups of larvae is shown below.

Experimental diets were designated as;

- T1= 100 % OPY= 3 kg of OPY
- T2= 50 % OPY + 50 % FW= 1.50 kg OPY + 1.50 kg FW (banana + pineapple; 1:1)
- T3= 50 % OPY + 50 % MW= 1.50 kg OPY + 1.50 kg MW
- T4= 25 % OPY + 75 % (MW+ FW)= 0.75 kg OPY + 1.125 kg FW (banana + pineapple; 1:1) + 1.125 kg MW

### *2.3 Experimental Insects, Procedure and Design*

Four-hundred (400) two (2)-weeks old young larvae with weights ranging from 0.6 to 1.6 g were harvested and randomly grouped into 40 sets, each group comprising ten (10) larvae of similar weights and then randomly allocated to four (4) dietary treatments, using a Completely randomized design (CRD) with ten (10) replications of 10 larvae in each treatment. Three (3) kilograms of each diet was fed *ad libitum* every two weeks for six weeks. After the feeding trial, matured larvae were harvested and divided randomly into 3 groups per treatment. Larvae were then sampled from treatments and collected to respective laboratories for further analyses such as proximate, free fatty acid and microbial. Experiment was terminated after 6-weeks from its commencement.

### *2.4 Parameters evaluated*

#### *2.4.1 Proximate analysis*

The proximate analysis of samples of larvae from each treatment was done using the standard procedures as defined by the Association of Official Analytical Chemists (AOAC) [3] and was undertaken at the Soil Science laboratory of the Department of Crop and Soil Sciences, Faculty of Agriculture, KNUST. Metabolisable energy values of larvae samples were calculated from the results of the proximate composition using the [15] equation;

$$M.E\ (Kcal/kg) = (37 \times \%\ CP) + (81.8 \times \%\ E.E) + (35 \times \%\ N.F.E) \quad (1)$$

#### *2.4.2 Free fatty acid content*

The Free Fatty Acid (FFA) content of larvae samples was analyzed based on standard procedure as defined by the Association of Official Analytical Chemists (AOAC) [2] at the Mycotoxin and Food Laboratories, Department of Food Science and Technology, College of Science, KNUST.

#### *2.4.3 Microbial status*

The total viable count (TVC) of microbial species was determined by the Spread plate method. Isolation, identification and enumeration of different species of bacteria present in larvae samples were done by culturing on MacConkey and Bismuth Sulfite Agar. Colony counter was used for counting the colonies of bacteria after incubation. Colonies within the range of 30-300 were used for calculating the concentration of bacteria in the original sample. Biochemical test was carried out to confirm the characteristics of the bacteria species that have been successfully isolated.

### *2.5 Statistical Analysis*

The entire sets of data collected were organized in Microsoft Excel Spreadsheet and further subjected to the analysis of variance (ANOVA) using GenStat Release 11.1 [12] at $p< 0.05$. Differences between treatment means were separated using Tukey's test at 95 % confidence intervals.

## 3. Results and Discussions

### *3.1 Nutritional Potential of larvae*

Crude fibre percentage (Table 1) seemed to show a significant ($p<0.05$) declining trend from larvae raised on T1 (8.36 %) to T3 (6.01 %) but this was not the same between T3 and T4 (7.58 %) as a significantly ($p<0.05$) higher crude fibre value was observed in the latter than T2 (7.39 %) and T3 (6.01 %). The range of mean crude fibre values (6.01 – 8.36 %) recorded in this study surpasses the mean crude fibre values of 3.35 % and 1.09 % reported by [9] and [14] respectively for *R. phoenicis* larvae. Crude protein percentage values followed a steadily significant ($p<0.05$) declining pattern from T1 (31.98 %) to T4 (24.96 %) with larvae solely fed oil palm yolk (T1) recording the highest significant ($p<0.05$) crude protein value. Study by [14] reported a crude protein value of 23.44 % for *R. phoenicis* larvae which almost equaled the minimum value recorded in this study for the range of mean crude protein values from 24.96 to 31.98 %. This observation was however supportive of the suggestion by [14] that crude protein level tends to increase as larvae age to a high value of 34.9 % for mature pupae. The mean crude protein values of 22.06 % and 21.06 % reported by [5,18] for the larvae were below the range recorded in the present study. Meanwhile, a more reflective value of 28.01 % was reported by [9] for the larvae which falls within the range of mean values recorded in this study. Results of ether extract values also showed a significantly ($p<0.05$) highest value for larvae raised on T2 (38.79 %) against a significantly ($p<0.05$) least value of 31.87 % for larvae on T3. The authors [9] and [18] reported a mean fat value of 66.61 % for palm weevil larvae which is more than about twice the minimum value recorded in the present study's range of mean ether extract values (31.87 – 38.79 %). Meanwhile, [9] reported a lower mean ether extract value of 18.91 % whiles [14] reported a higher value of 54.20 % for the larvae of *R. phoenicis*. Metabolisable energy values revealed a highest significant ($p<0.05$) value for larvae raised on T2 (4273.1 Kcal/kg) whiles their counterparts on T3 (3633.6 Kcal/kg) registered the least significant ($p<0.05$) value. The energy value recorded for larvae raised on T1 (4023.9 Kcal/kg) was however significantly ($p<0.05$) greater than T4 (3854.5 Kcal/kg) and T3 (3633.6 Kcal/kg). The set of energy values, 714.24 Kcal and 684.81 Kcal per 100 g reported on dry and wet weight basis respectively by [18] disagree with the range of energy values (3633.6 – 4273.1 Kcal/kg) obtained for this study.

**Table 1:** Nutrient composition of larvae (on dry matter basis)

| PARAMETERS (%) | TREATMENTS | | | | P-VALUE |
|---|---|---|---|---|---|
| | **T1**(100% OPY) | **T2**(50%OPY+ 50%FW) | **T3**(50%OPY+ 50%MW) | **T4**[25%OPY+75 %(MW+FW)] | |
| Ash | 2.29a | 2.08b | 1.88c | 1.08d | <0.001 |
| Crude fibre | 8.36a | 7.39b | 6.01c | 7.58b | <0.001 |
| Crude protein | 31.98a | 29.37b | 27.58c | 24.96d | <0.001 |
| Ether extract | 34.63c | 38.79a | 31.87d | 35.75b | <0.001 |
| Moisture | 22.51c | 21.98c | 32.49a | 30.45b | <0.001 |
| Free-fatty acid | 10.04c | 5.78d | 23.25a | 11.05b | <0.001 |
| Nitrogen free extract* | 0.23 | 0.39 | 0.18 | 0.19 | 0.058 |
| M.E (Kcal/kg)* | $4023.9^{b}$ | $4273.1^{a}$ | $3633.6^{d}$ | $3854.5^{c}$ | <0.001 |

*a, b, c, d- Mean values in the same row with different superscripts are significantly (p< 0.05) different, P-Probability value, OPY- Oil palm yolk, FW- Fruit waste, MW- Millet waste, M.E- Metabolisable Energy. *-Calculated based on proximate values obtained.*

Free fatty acid percentage, revealed significant ($p<0.05$) differences among larvae raised on the various treatment diets or feeds. Larvae on T3 recorded a mean value of 23.25 % which was significantly ($p<0.05$) higher than T4 (11.05 %), followed by T1 (10.04 %) and finally T2 which registered the least significant ($p<0.05$) value of 5.78 %. The range of free fatty acid values (5.78 – 23.25 %) recorded for this study surpasses the mean value of 3.65 % which comprised two fatty acids, Palmitoleic and Stearic acids, reported by [13]. Quite contrary to these observations [7] reported a range of lipid values from 25.30 – 66.61 % and its oil constituted both Oleic and Linoleic acids that make it liquid at room temperature.

### *3.2 Microbial status of larvae produced*

The microbial status of the different groups of larvae produced on the various diets have been summarized in Table 2. Evidently, crushed larvae samples from T2 ($7.4 \times 10^7$ cfu/ g) recorded numerically the least total viable counts of microbes whiles a numerical highest microbial count was observed for T4 ($11.4 \times 10^7$ cfu/ g). This range of microbial load observed for the different groups of larvae were greater than the microbial levels of 4.9 $\times 10^5$ and 9.1 $\times 10^5$ cfu/ g reported by [4] for larvae raised on oil and raffia palm substrates respectively. The range of microbial values recorded also surpasses the recommended standards of 5.0 $\times 10^5$ cfu/ g for meat products as suggested by [8].This however, does not necessarily disqualify the products for consumption because [19] confirmed that, even though *E. coli* and other microbes may be present in substantial volumes, their effects could be easily terminated or broken by sufficient heating especially for food products which demand additional cooking.

**Table 2:** Microbial status of larvae raised on various treatments

| Samples | Total viable count (TVC) | Enumeration of bacteria species identified | |
|---|---|---|---|
| | | *Klebsiella sp.* | *Salmonella sp.* |
| | Concentration (cfu/g $\times 10^7$) | Concentration (cfu/g $\times 10^6$) | Concentration (cfu/g $\times 10^5$) |
| T1 | 8.3 | 22.1 | 9.8 |
| T2 | 7.4 | 28.5 | 12.3 |
| T3 | 8.7 | 29.0 | 17.5 |
| T4 | 11.4 | 28.3 | 13.6 |

**Table 3:** Biochemical test for bacteria identification

| Samples | MacConkey Agar- *Klebsiella sp.* | | | Bismuth Sulfite Agar- *Salmonella sp.* | | |
|---|---|---|---|---|---|---|
| | Indole | Catalase | Citrate | Indole | Catalase | Citrate |
| T1 | -ve | +ve (bubbles) | Blue | +ve (Red) | +ve (bubbles) | Blue |
| T2 | -ve | +ve (bubbles) | Blue | +ve (Red) | +ve (bubbles) | Blue |
| T3 | -ve | +ve (bubbles) | Blue | +ve (Red) | +ve (bubbles) | Blue |
| T4 | -ve | +ve (bubbles) | Blue | +ve (Red) | +ve (bubbles) | Blue |

## 4. Conclusions and Recommendations

In summary, the results of this study demonstrated that;

1. The different treatment diets served as nutritionally suitable growth media for the production of palm weevil larvae.
2. Nutrient analysis of larvae produced indicated that they were nutritious products that can complement human protein needs.
3. The larvae are very wholesome for consumption with improved nutritional and health benefits.

It is therefore recommended that with regards to this practice of insect farming, further researches should be carried out to explore the potential of *R. phoenicis* and its larvae for optimum use as feed resources for production of insect proteins for both human consumption and animal industry.

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