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Nutritional Composition Profile of Mealworm Larvae (*Tenebrio molitor*) Reared on Different Substrate: A Potential Protein Substitute for Fishmeal

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Abstract

Mealworm (*Tenebrio molitor*) insect meal has been adjudged a protein source for human and livestock consumption. Substrates of agricultural by-product are been used to rear this insect, and it is of importance to investigate the implication of substrate on the content of the end product. This study was conducted to assess the nutrient composition of *T. molitor* cultured on three grain by-products as growth substrate media [Rice Bran (RB), Corn Bran (CB) and Wheat Bran (WB)] for 90 days. Harvested larvae were sundried, ground into powder and analysed for proximate composition as well as mineral content and the data statistical analysed. Protein was highest in CB reared larvae 51.33% with least in RB reared larvae 46.74%. Fat was high in all sampled substrate reared mealworm. Ash content in CB and WB was significantly different ($P<0.05$) from RB reared larvae. Macro mineral Mg and Na are not significantly different, while micro mineral profile showed significant variations ($P<0.05$). The nutritional composition of *T. molitor* in this study positioned it as a potential substitute for incorporation into fish feed formulation as animal protein source.

Keywords: Corn bran, Fish feed, Fishmeal, Rice bran, *Tenebrio molitor*, Wheat bran

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INTRODUCTION

The global food demand is anticipated to rise by 70% by the year 2050 and this will lead to food security and environmental issues (Shati *et al.,* 2022). There have been a significant dietary change of humans in the recent past preferring more animal-based items, particularly milk, meat, fish, and eggs, and it's expected that these preference would increase over time (FAO, 2020). Aquaculture is an important source of high-quality proteins for humans and this industry has been experiencing rapid growth to meet the escalating global demand for fish and seafood. However, this expansion has led to increased pressure on marine ecosystems and the depletion of conventional fish feed ingredients such as fishmeal and fishoil (Barragan-Fonseca *et al.,* 2019). Alternative protein sources are been explored that can effectively substitute or supplement conventional feed ingredients. One of such promising candidates is mealworms (*Tenebrio molitor*), a widespread insect species that has drawn significant interest as a

potential replacement for creating fish feeds (Alfiko *et al.,* 2022). Mealworms (*T. molitor*), a member of the order Coleoptera and the larval stage of darkling beetles, are widespread across the world. They are well known for being used as a wholesome and long-lasting source of food for several species, including fish, fowl, and reptiles. Additionally, because of their nutritional advantages and environmental advantages, they have attracted interest as a possible source of protein for human consumption. Mealworms are being investigated as a sustainable alternative protein source for human consumption and aquaculture due to their high nutritional content and capacity to be raised on organic waste materials (Barragan-Fonseca *et al.,* 2019). Mealworms can valorize agricultural byproducts, which aids in trash reduction and recycling (Derler *et al.,* 2021; Zim *et al.,* 2021).

The development of the aquaculture industry relied heavily on conventional feed ingredients especially fishmeal, but their scarcity and negative effects on the environment have sparked a growing interest in sustainable alternatives like insect meal, microalgae, and other plant- and animal-based proteins (Tacon, 2017). Understanding mealworms' usefulness as a potential replacement for fishmeal in fish diets requires knowledge of their nutritional profile, which includes their protein, fat, fiber, minerals, and vitamins (Gasco *et al.,* 2019). Protein-rich foods from alternative sources are needed to alleviate the rising price of ingredients used for food and feed. Mealworm can be used in thr nutrition of monogastric either as whole, defatted or hydrolysed (Hong *et al.,* 2020) and has been reported to improve performance of quail (Sarica *et al.,* 2020) and most importantly fish (De-Lima *et al.,* 2021). According, to scientific studies, aquaculture is the only method to meet the rising demand for animal protein, since edible insects like mealworms are a particularly rich source of nutrients to replace fishmeal in aquafeed (Bernard and Womeni, 2017). This study was conducted to investigate the nutritional composition of *T. molitor* larvae reared on three different substrates with the view of using it as substitute for fish meal/feed production.

MATERIALS AND METHODS

Study Area:

The experiment was conducted at Wabillahitaofiq Farm, Eyenkorin Ballah road, Ilorin, Kwara State.

Experimental Materials

Six transparent plastic containers of 12 mm x 24 mm were bought from grocery store at Eyenkorin Market, Ilorin. Mealworm beetle samples were collected from the group that was maintained at insectaria of division of Wabillahitaofiq Farm at Eyenkorin, Ballah road, Ilorin since 2020 and the substrates (rice bran (RB), corn bran (CB) and wheat bran (WB)) were purchased from raw food seller at Ojatuntun market, Ilorin. Watermelon peels were sourced from the fruit seller at Eyenkorin Market, Ilorin, Kwara State.

Experimental Design

The plastic containers were arranged in three groups (A_1, A_2, B_1, B_2, C_1 and C_2) for the mealworm rearing. The three upper containers (A_1, B_1, and C_1) were perforated at the bottom, filled with 2-3 inches depth of Rice bran (A_1), Corn bran (B_1) and Wheat bran (C_1) separately as their substrates (growth medium), watermelon peels was put on a flat surface, placed on the growth medium to provide supplements and moisture needed by the *T. molitor* beetles (Kotsou *et al.,* 2024) and the *T. molitor* beetles were shared into the prepared three different substrates.

Mealworm rearing/feeding

Brown paper sheet was placed on the substrate to prevent the medium from packing too solidly, to facilitate free movement of the beetles and to provide shade for the beetles then covered with net to prevent other insects in and reared in constant indoor condition (Turck *et al.,* 2021). Each group consisted of 200 mealworm beetles, reared for three months. The substrate was replaced every two weeks and replaced with fresh substrates to remove exuvia and frass. The moisture source was added every five days, dead beetles and clumps of mold were removed and discard from the growth medium to prevent molding every five days interval. All experiments were replicated.

The laid eggs and newly hatched larval were dropped inside the lower containers (A_2, B_2 and C_2), grown and matured in it. After 30 days, matured larvae were harvested by mechanical sieving from the lower rearing containers (A_2, B_2 and C_2), rinsed with water, killed by blanching (immersion for 1–5 min in boiling water), drained, sundried for 3 days and ground into powder for proximate and chemical analysis.

Proximate Analysis

Mealworm larvae was analyzed for its composition according to AOAC (2016). Crude protein was determined by Kjedahl method through digestion, distillation and titration of samples. Fat determined the Soxhlet apparatus method with the use of petroleum ether. Moisture content was determined by the oven method. Ash, crude fibre was determined by the use of muffle furnace and carbohydrate content was determined by difference from the sum of all analysed parameters. Mineral profile was determined by the AAS (Atomic Absorption Spectrophotometer machine).

Statistical Analysis

Data were analyzed using one-way analysis of variance (ANOVA) SPSS version 20.

RESULTS

The proximate composition (Table 1) revealed that protein content values ranged from 46.74% to 51.33% with highest value obtained from CB larvae reared, while the lowest was from the larvae reared on RB. The fibre content values ranged from 1.70% to 2.13%. The carbohydrate content value ranged from 10.36% to 15.24%, while other parameters varied.

Table 1: Proximate composition of *Tenebrio molitor* larvae reared on three different substrates

Substrates	Ash %	Fibre %	Lipid%	Protein%	CHO%	Dry matter%
RB	4.71±0.00 *b*	1.96±0.00 *b*	26.85±0.00 *b*	46.74±0.11 *b*	12.98±0.52 *ab*	93.05±0.15^{b}
CB	6.22±0.63 *a*	1.70±2.12 *c*	20.34±0.01 *c*	51.33±0.13 *a*	15.24±1.09^{a}	93.50±0.21^{ab}
WB	6.42±0.50 *a*	2.13±2.40 *a*	28.37±0.07 *a*	48.63±1.41 *ab*	10.36±1.01^{b}	95.19±0.02^{a}

Mean values with different superscripts on the same column are significantly different (P>0.05).
RB: Rice Bran; CB: Corn Bran; WB: Wheat Bran

The highest Ca value was from larvae reared in wheat bran substrate while the least value was from larvae reared on corn bran substrate. The value of Mg, K and Na were not significantly different (Table 2).

Table 2: Macro mineral content of *Tenebrio molitor* larvae reared on three different substrates

Substrates	Mg%	Ca%	K%	Na%
RB	5.60±0.021 ^{a}	0.84±0.0028 ^{b}	6.60±0.021 ^{a}	2.60±0.060 ^{a}
CB	5.80±0.022 ^{a}	0.57±0.017 ^{c}	6.70±0.0014 ^{a}	2.80±0.041 ^{a}
WB	5.80±0.098 ^{a}	2.60±0.048 ^{a}	6.30±0.054 ^{ab}	2.80±0.0092 ^{a}

Mean values with different superscripts on the same column are significantly different (P>0.05). RB: Rice Bran; CB: Corn Bran; WB: Wheat Bran

Noticeable significant variations were recorded in the micro mineral content of mealworm raised on different substrate (Table 3) except for Pb
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Table 3: Micro mineral content of *Tenebrio molitor* larval reared on three different substrates

Substrates	Pb (mg/kg)	Mn (mg/kg)	Fe (mg/kg)	Zn (mg/kg)	Cu (mg/kg)	Ni (mg/kg)
RB	0.068±0.003^{a}	2.5±0.030 ^{a}	6.6±0.0360 ^{b}	0.35±0.0057^{a}	0.20±0.0028 ^{b}	0.12±0.0099 ^{b}
CB	0.078±0.0028^{a}	3.3±0.031 ^{a}	7.2±0.0310 ^{a}	0.31±0.0085 ^{c}	0.25±0.0064 ^{a}	0.18±0.0140 ^{a}
WB	0.066±0.0021^{a}	0.15±0.0014 ^{b}	3.0±0.0028 ^{c}	0.33±0.0042 ^{b}	0.29±0.0049 ^{a}	0.066±0.0049 ^{c}

Mean values with different superscripts on the same column are significantly different (P>0.05). RB: Rice Bran; CB: Corn Bran; WB: Wheat Bran

DISCUSSIONS

Culturing mealworm from stage of larvae growth is an acceptable production method for its safe consumption (Liu *et al.,* 2020) and this can impact on the nutritional content of the final product of mealworm (Toviho and Bársony, 2022). Substrates from different sources also has effect on the final composition of mealworm as they serve in providing the nutrient needed during the growth process in the different medium. Results from earlier study indicated use of 44 substrates (Rumbos *et al.,* 2020), Jajić *et al.,* 2022); 11 substrates (Said and El Defrawy, 2022) and 2 substrates feed (Khanal *et al.,* 2023), 6 substrates (Langston *et al.,* 2023 and 4 substrates (Andreasson, 2023). This study aligned within the range of substrate feed diet for culturing mealworm. The choice of bran by-products was noted to multiply the number in folds for both larvae and pupae stages of development (Andreasson, 2023). The use of different substrate suggests from the aforementioned that nutrient utilization by the larvae resulted in varied digestibility.

Our findings have been corroborated by (Rumbos *et al.,* 2020; Said and El Defrawy, 2022; Khanal *et al.,* 2023) with a protein range of 41% - 68%; 36% - 45%; 40%

- 47% respectively, which is similar to this study. This high content in protein positioned *T. molitor* as a protein source ingredient to substitute fishmeal in feed (Rumbos *et al.,* 2020; Said and El Defrawy, 2022; Jajić *et al.,* 2022, Khanal *et al.,* 2023). Liu *et al.* (2020) stated that dietary supplementation of substrate with fresh plant materials improved the proximate composition and production of mealworm. But contrary to Kröncke and Benning (2023) that reported high protein yield with low fat content in larvae fed supplemented rice protein. The results are similar to that of Noyens *et al.* (2023) who noted that use of wet feed substrate resulted in concentration of carbohydrate at high levels in the composition of biomass of *T. molitor.* The variations in protein contents in this study is similar to the results of Tacon (2017) which indicated that T. *molitor* is safe as Novel food (NF). Rumbos *et al.* (2020) reported that protein content in substrate impact on the derived increase nutritional content of mealworm. High dry matter in this study conformed with the findinds of Toviho and Bársony (2022) and negates the low crude fibre in this study. Siemianowska *et al.* (2013) reported that dried mealworm contains more protein than fresh larvae (which is as a result of the reduction in the moisture content that led to increased concentration of protein. Hence percent nutrient in mealworm is specie, climate, habitation and feed substrate dependent (Siemianowska *et al.,* 2013). The difference in composition can also be attributed to life stages development strategies that require factors for growth and development which leads to increase or decrease in nutritional content (Rumbos *et al.,* 2020). The lipid content in this study was high and in line with the findings of Bragad (2017) who reported that lipid is the second highest constituent in mealworm.

Mineral content in feed ingredients are important in ameliorating deficiency in feed due to the contributory effects of ingredients used in feed formulation. Variation in mineral content is inevitable based on the embedded mineral profile of the substrate feed. Different ranges have been reported for mealworms reared on different substrates. Mineral content in wheat bran falls within the range reported by (Jajić *et al.,* 2022) for sodium, potassium and copper. However, Siemianowska *et al.* (2013) reported higher values of mineral content in dried mealworm over fresh mealworm. Furthermore, substrate feed have been reported to affect the mineral profile of mealworm (Khanal *et al.,* 2023). Mineral content reported by different authors noted iron and zinc to be high (Hong *et al.,* 2020) which was similar to our study for the same elemental nutrients. The detection of Lead (Pb) at low levels aligned with (Costa *et al.,* 2021) which makes the use of mealworm dietary friendly. From their study (Khanal *et al.,* 2023) noted that metamorphosis of mealworms from development stages may not necessarily affect its nutrient composition. Use of different substrate of agriculture which has been treated with agrochemicals of pesticide, herbicides and fungicides has given rise to the issue of safety due to bioaccumulation (Ribeiro, 2017). However, von Nicolo (2021) reported that *T. molitor* can be used to convert contaminated grains to useful product due to metabolic digestion by the insect, as no trace of artificial contaminant was detected on *T. molitor* harvested from contaminated grain. Nutritional quality of mealworm can be improved by feeding adequate dietary substrate (Khanal *et al.,* 2023) so as to aid valorization of by-products of agricultural origin into feed biomass of importance.

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CONCLUSION

This study further noted that *Tenebrio molitor* larvae has a potential as alternative for

compounding fish feed based on its nutritional and mineral content quality. It demonstrated that optimizing the substrate composition for rearing mealworm larvae, using locally sourced organic waste can encourage sustainable development.

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