



Nutritional composition of house fly larvae (*Musca domestica*) reared on different mixture ratio of cattle blood with organic wastes

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Abstract

Cattle blood is an animal byproduct enriched with protein and minerals. However, the improper management of cattle blood has a bad impact on the environment and human health. This study was aimed to analyze the nutritional content of housefly larvae including proximate, mineral and fatty acid compositions reared on different mixture ratios of cattle blood with cattle manure and vegetable wastes. The experimental diets of housefly larvae were: T₁ (1:3:1), T₂(2:2:1) and T₃(1:1:3) mixture of cattle blood, cattle manure and vegetable wastes respectively. The results showed that the moisture content of larvae varied ranges 85% to 90% among treatments. The crude protein (56.27±1.87%) and ash content (11.17±1.13%) were highest in maggots or larvae of T₂, but maggots of T₃ were highest in crude fat (29.17±2.95) and crude fiber (9.25±1.12). Differences in the fatty acid profile of maggots were small. Larval fatty acid profiles were characterized by high levels of palmitic acid, palmitoleic acid and oleic acid in all treatments. On the other hand, the mineral contents differed substantially. Larvae reared on T₂ were high in Ca, P, K, Fe and Zn exception Mn and Cu compared to other treatments.

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Introduction

Waste management is a major challenge globally in both developed and developing countries. Inappropriate treatment of waste creates a negative impact on the health, environment, social and economic life of the community (Sumantri, 2010). In Bangladesh, a total of 7.690 kton waste is produced daily in the six-city corporations (Huda *et al.*, 2014). It was accounted that nearly 68–81% of produced from organic matters, 7–11% from paper, 3–4% from plastic and 9–16% from textile, wood, leather, rubber, metal, glass and others (Alamgir and Ahsan, 2010). The organic matter is generally higher than any other waste source caused by the use of animal by-products, fresh vegetables and foods and lack of food processing industries. One source of organic waste, cattle blood which is available in most abattoirs in Bangladesh. Cattle blood is currently not efficiently used and spoiled in sewerage from abattoirs in Bangladesh. Waste is considered as a resource rather than a problem, additionally, the McKinsey Global Institute declared that food waste placed third of fifteen identified resources with productive opportunities (Dobbs, 2011). Consequently, bioconversion has got attention for its environmentally friendly, sustainable and renewable properties to address organic waste by insect rearing.

The house fly (*Musca domestica*, Linnaeus, 1758; Diptera) is widely known as a pest and a key vector of diseases both larvae (maggots) and adult flies. Housefly maggots can grow on a wide range of decaying organic wastes, including animal manure and feed (Hogsette and Farkas, 2000). The maggots are a potential supply of aquaculture feeds for more than 50% crude proteins (in dry weight) which are higher than those in soybean, meat and bone scrap (Akpodiete *et al.*, 1997; Iniguez *et al.*, 1994) and a promising source of limiting amino acids, such as lysine, methionine and phenylalanine (Ocio and Vinaras, 1979). Additionally, maggots contain a variety of biologically active substances, including antimicrobial peptides, lectins and chitins (Fu *et al.*, 2009; Hou *et al.*, 2007). It was observed that maggot proteins may stimulate the animal appetite when it

adding to animal feed (Zhu *et al.*, 2012). Nutritional value of housefly larvae and pupae reared in manure were similar to that of fish meal or animal proteins (Akpodiete *et al.*, 1997; Miller *et al.*, 1974; Ocio and Vinaras, 1979). Recently, chitosan from maggots was even used in cosmetics and medicines (Ai *et al.*, 2008; Jing *et al.*, 2007). Previous literature mentioned that house fly maggots can develop some substrates for example pig manure (Viroje and Malin, 1988; Zhu *et al.*, 2012), cattle blood and wheat bran (Aniebo *et al.*, 2008), cattle blood and gut contents (Dankwa *et al.*, 2002), cattle gut and rumen contents (Ekoue and Hadzi, 2000), fish guts (Ossey *et al.*, 2012) and a mixture of egg content, hatchery waste and wheat bran (Ebenso and Udo, 2003).

Presently, there are no data that explains house fly growth and nutritional content in cattle blood mixture with manure and vegetable media. In the present study, we have examined the impact of different mixture ratios of cattle blood with cattle manure and vegetable wastes on the *M. domestica* larval proximate, mineral and fatty acid compositions.

Materials and methods

Rearing of the adult flies

The *M. domestica* was captured from the wild in Dhaka, Bangladesh. The experimental culture of *M. domestica* was conducted in the insectary of the Biological Research Division, BCSIR from March to June 2019. Geographically, our study area lies in the co-ordination of 23°44'30" N and 90°22'49" E. Every 500 adults were cultured in one 50 *50*50 cm mesh cage (0.2 mm pore size). *M. domestica* colonies were maintained on a diet of 2:2:1 sugar powdered: milk: hen egg at 27°C with a photoperiod of 14:10 (L:D) h cycle. Small white bags filled with moist wheat bran and brown sugars (4:1) were kept in the colony cages as oviposition medium. Every other 12 h, the concentrated eggs were collected and inoculated into different culture mediums.

Experimental feeding of maggots of M. domestica

Three experimental diets were made by cattle blood, cattle manure and vegetables in different ratios (Table

1). The vegetable waste composed of spoiled gourds, rotten carrots, peel of green papaya, bottle gourd and potato wastes cut into small pieces. After four-six days of cultivating, the maggots and culture medium were separated and dried in the oven (105°C).

Proximate analysis

Harvested maggots were processed by oven drying at a temperature of 105°C. Crude protein (AOAC, 1990), crude fat (Folch, 1957), and crude fiber of dried samples were estimated using standard procedures (AOAC, 1980) and all were replicated thrice in a completely randomized design. Crude protein content was determined using the Kjeldahl method and calculated by multiplying total nitrogen with a conversion factor of 6.25. A soxhlet apparatus was used for the determination of fat content. The crude fiber was estimated by using a total 5 g defatted sample boiling with 1.25% dilute H₂SO₄ and 1.25% dilute NaOH solution respectively. The boiling sample was transferred in the crucible and dried overnight at 80°C. The crucible was heated in a muffle furnace at 600°C for 1 hour.

Minerals

At first, 10g dried maggots powder in the beaker were placed in a muffle furnace covered by a watch glass remaining a slight gap at 150°C for 1 h and then the samples were ashed with 550°C keeping for 4-5 h. The ash was white and free from carbon. After ashing, 1-3 mL of concentrated nitric acid and distilled water (1:1) were added in the cooled beakers to wet the sample and for the complete removal of unwanted carbon particles which was then heated on a hot plate at about 150°C under a fume hood chamber until all the fumes removed or almost to dryness. Then the beakers were returned to the furnace setting the temperature directly at 550°C for 2-3 h and cooled. The ash was then dissolved in 10 mL of concentrated nitric acid warming on a hot plate at 180-200 °C for 5-10 min to aid in solution by keeping a watch glass on each beaker and heated until boiling. After the complete dissolved of ash, the beakers containing the sample were taken out from the hot plate and cooled at room temperature. Then the samples were taken in

50 mL calibrated volumetric flasks rinsing several times with deionized water and made up to the mark. The flasks were shaken well to uniform mixing the samples and then filtered to previously cleaned and labeled 100 mL non-transparent plastic bottles with Whatman™ qualitative 1 filter paper (125 mm dia.*100 circles) and preserved in the laboratory for metal analysis. Three replicates were made for each sample preparation in both of the above processes.

The analysis of metals was conducted following the previous report (Hasan *et al.*, 2020). The minerals such as Zn, Ca, Cu, Fe, Mn, and Ni were analyzed using atomic absorption spectrometer (AAS) (Model: AA240FS, Varian, Australia), Na and K were determined by a flame photometer (Model: PFP7, Jenway, UK), P was estimated with UV-Visible Spectrophotometer (Model: UV-1650PC, SHIMADZU, Japan). The details of the analytical procedure are described elsewhere (APHA, 1998). The results of the minerals were expressed as dry mass (DM) of BSF larvae.

Fatty acid

The fatty acid composition lipid from BSF prepupae was extracted using the chloroform: methanol (2:1, v/v; containing BHT 0.1 mg/100 g) (Floch *et al.*, 1957). The fatty acid composition was estimated by preparing methyl esters and analyzing them by gas chromatography (AOCS, 1992).

The gas chromatography (Model 14B SHIMADZU, Japan) with a flame ionization detector (GC-FID) was loaded with software class GC-10 (Version-2.0). The GC was prepared with a flame ionization detector (FID) and capillary column, with dimension 15 m length and 0.25 mm ID. The functional condition was automated at oven temperature 150°C (hold time 5 min), 8°C /min-190°C (hold time 0 min), 2°C/min-200°C (hold time 10 min), injection port temperature 250°C and detector temperature 250°C. Fatty acid peaks were identified from standard fatty acid mixtures. Nitrogen was used as a carrier gas, flow rate 20 ml/min and aliquots of 1 µl FAME (formed by esterification of fish oil samples) were injected and

the peaks of fatty acids were documented for their particular holding time and areas by the data processor unit of GC.

Data analysis

The recorded data were analyzed using Statistical software, SPSS of version 22.0. Descriptive statistics, mean and standard deviation (SD) were calculated first. Then to test the equality of these parameters, an analysis of variance (ANOVA) was performed. A few parameters varied significantly ($p < 0.05$). Finally, the

Duncan Multiple Rank Test (DMRT) of Post Hoc series of tests were performed.

Result and discussion

Proximate analysis showed (Fig. 1-6) that the moisture content of maggots was comparable among the treatments, ranging between 85.91 ± 1.41 % and 90.57 ± 1.28 % (Fig.1). These results are in close agreement with (Odesanya *et al.*, 2011; Sogbesan *et al.*, 2005) ensuring the high water content of maggots.

Table 1. Composition of artificial diets for *M. domestica*

Ingredients	T1	T2	T3
Cattle blood	200g	400g	200g
Cattle manure	600g	400g	200g
Vegetables	200g	200g	600g

In contrast, crude protein, crude fat, crude fiber and ash contents were significantly affected by the rearing substrate (Fig. 1-6). Maggots reared on treatment three (T3) was low in crude protein (39.59 ± 2.51 % on dry weight), compared to those reared on the other treatments. Crude protein of maggots (Fig.2) reared

on T2 media was highest among the crude protein of other treatments which was higher than that of previously reported by Aniebo *et al.*, 2008 and Odesanya *et al.*, 2011 but slightly lower with Hussein *et al.*, 2017. However, it is far from Calvert *et al.* (1971) who accounted for 63%.

Table 2. Fatty acid compositions of housefly maggot percentage (dry weight basis).

Name of fatty acids	T1	T2	T3
Lauric Acid (C12:0)	1.54	0.49	0.69
Myristic Acid (C14:0)	6.97	3.89	9.23
Palmitic Acid (C16:0)	27.14	35.68	20.28
Stearic Acid (C18:0)	7.23	4.32	11.78
Total Saturated Fatty Acids	42.88	44.38	41.98
Palmitoleic Acid (C16:1)	24.12	28.59	25.56
Oleic Acid (C18:1)	21.34	18.54	20.02
Monounsaturated Fatty Acids	45.46	47.13	45.58
Linoleic Acid (C18:2) (Omega-6)	9.68	7.59	11.43
Linolenic Acid (C18:3) (Omega-3)	1.98	0.9	1.01
Polyunsaturated Fatty Acids	11.66	8.49	12.44
Total Unsaturated Fatty Acids	57.12	55.62	58.02

The crude protein of maggots depends on rearing substrate for example 47.1% on cattle blood and wheat bran (Aniebo *et al.*, 2008), 48% on poultry manure (Odesanya *et al.*, 2011) and 59% on cattle manure (Hussein *et al.*, 2017). So, previous studies support our results in this study that the range of

crude protein between 39-63% (Calvert *et al.*, 1971; Fasakin *et al.*, 2003; Gado *et al.*, 1982; Atteh and Ologbenla, 1993). The fat content of maggots reared on T2 (Fig 3) was lowest among those of other treatments. The results of fat contents in this study were largely supported by previous investigations that

the range of fat content of maggots was within 14 - 37% (Atteh and Ologbenla, 1993; Adeniji, 2007; Ogunji., *et al.* 2015; Hwangbo., *et al.* 2009; Aniebo and Owen, 2007; Pretorius, 2011; Arong and Eyo, 2017). The ash content in maggots of T2 (Fig.4) was highest but lowest in crude fiber among the result of other treatments.

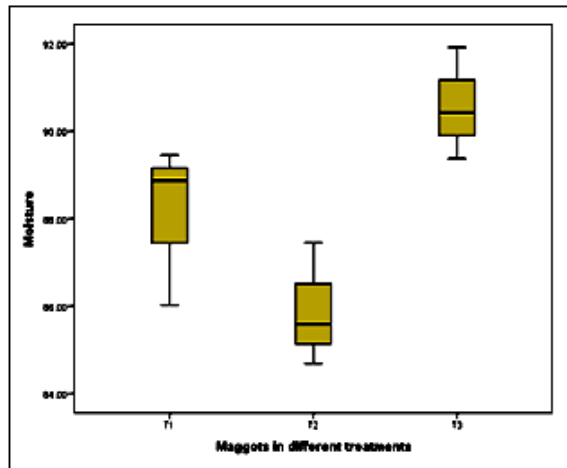


Fig. 1. Moisture (%) of maggots among treatments.

In early studies observed, the ranges of ash content and crude fiber were 4.50-10.68% and 3.14-9.95% respectively (Atteh and Ologbenla, 1993; Adeniji, 2007; Ogunji., *et al.* 2015; Hwangbo., *et al.* 2009; Aniebo and Owen, 2007; Pretorius, 2011; Arong and Eyo, 2017).

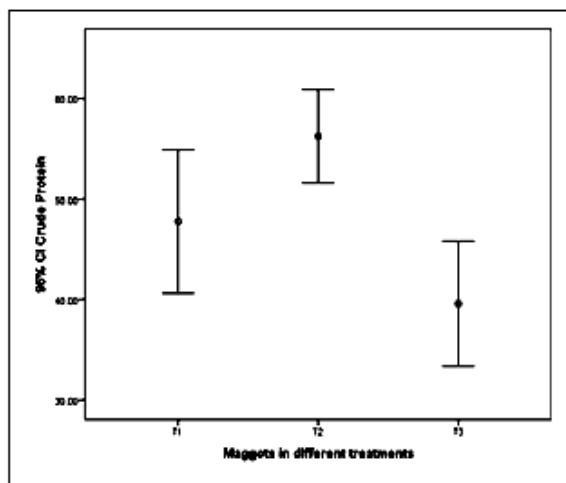


Fig. 2. Crude Protein (%) among treatments.

The current findings in this study strongly agreed with former investigations (Atteh and Ologbenla, 1993; Adeniji, 2007; Ogunji., *et al.* 2015; Hwangbo., *et al.* 2009; Aniebo and Owen, 2007; Pretorius, 2011; Arong and Eyo, 2017).

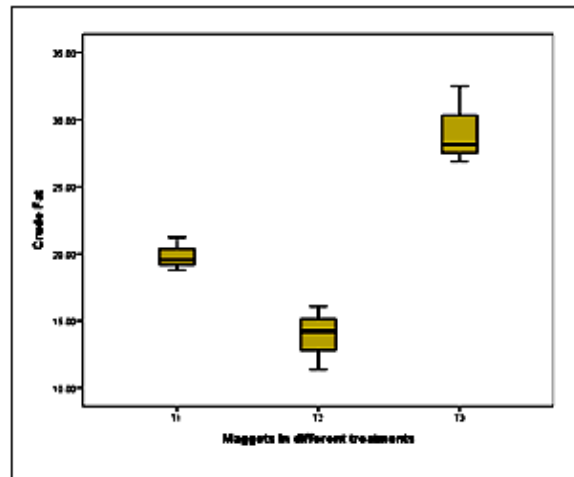


Fig. 3. Crude fat (%) of maggots among treatments.

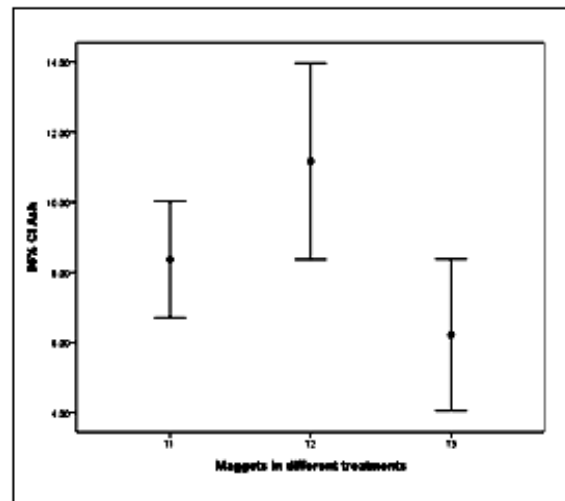


Fig. 4. Ash (%) of maggots among treatments.

It showed from the test results that all proximate compositions moisture, crude protein, crude fat, ash and crude fiber are significantly different in treatments ($p < 0.05$) at a 5% level of significance.

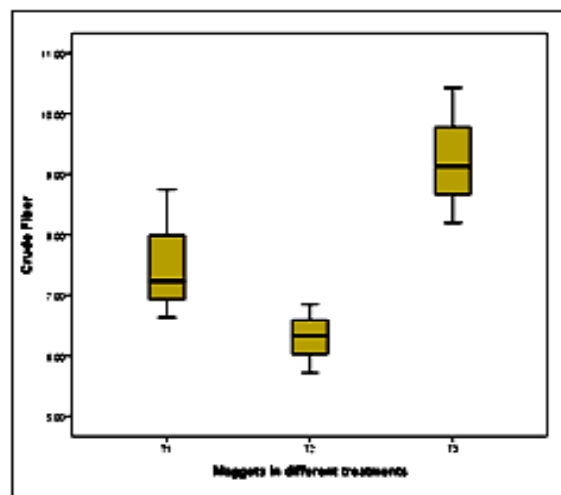


Fig. 5. Crude fiber (%) of maggots among treatments.

DMRT has been presented in Fig. 1 as alphabetical letters beside the means. Here, the means containing the same letter do not differ at a 5% level of significance. Consequently, the variation in proximate composition results between current findings and earlier findings may be due to rearing food sources, applied methodology, species and geographical location. The estimated mineral elements in this investigation were displayed in Fig. 7-13.

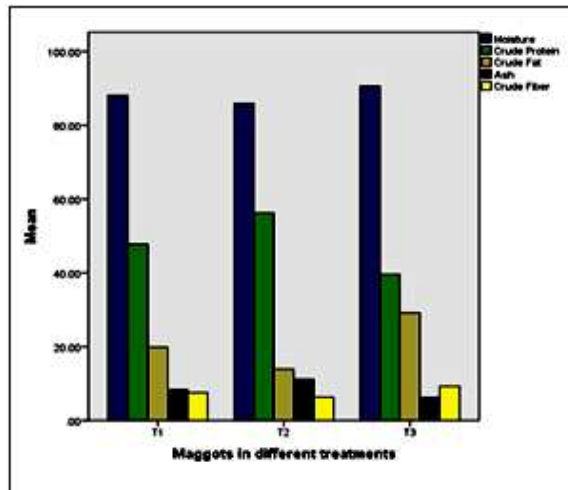


Fig. 6. Proximate composition of maggots treatments.

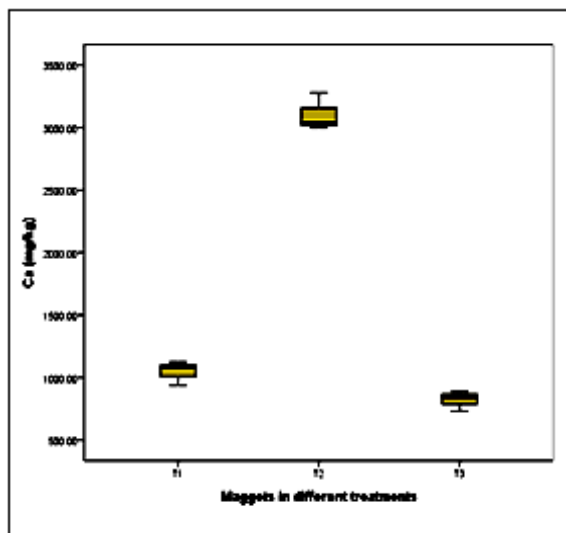


Fig. 7. Ca of housefly maggot mg/ kg (dry weight basis).

The levels ranged from Ca (822-3104 mg kg⁻¹ on dry weight), P (9165-18400 mg kg⁻¹ on dry weight), K (1016-1538 mg kg⁻¹ on dry weight) and Fe (1528-2274 mg kg⁻¹ on dry weight) were satisfactory while of the other minerals were all within a small range (Table 3).

The fluctuation of Calcium levels among treatments (Fig. 7) was strongly prominent, in contrast to other minerals. For example, the level of Ca in maggots of T2 was three folds than T1 but about four folds with T3.

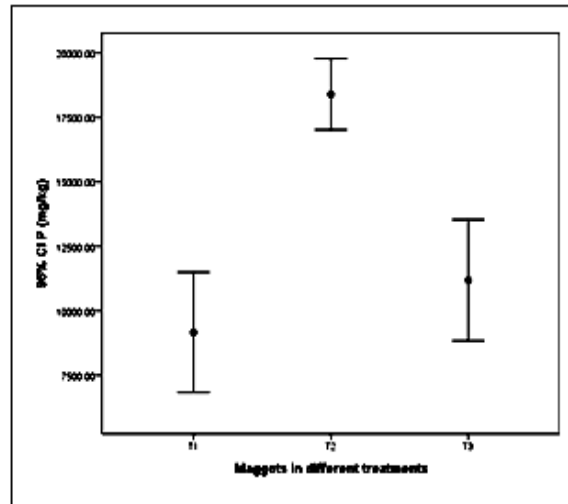


Fig. 8. P content of housefly maggot mg/ Kg (dry weight basis).

The mineral contents in maggots of T3 were higher among those results of other treatments except Cu and Zn. The level of minerals in this study was lower than the findings of Hussein *et al.* (2017) while similar to results of early reports that house fly maggots reared on different substrates (Makker *et al.*, 2014; Cadag *et al.* 1981; Fasakin *et al.*, 2003; Göhl ,1982; Hwangbo *et al.*, 2009; Odesanya *et al.*, 2011; Pretorius, 2011).

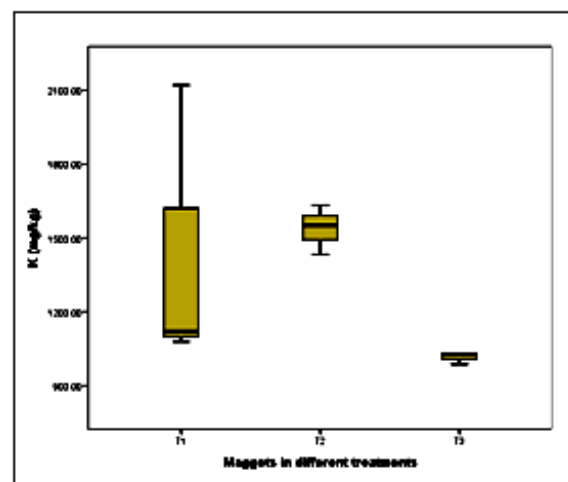


Fig. 9. K content of housefly maggot mg/ Kg (dry weight basis).

ANOVA tests show that except K and Mn ($p > 0.05$), all

other mineral content of housefly maggot mg/ kg (dry weight basis) Ca, P, Cu, Fe and Zn are significantly different in different waste compositions ($p < 0.05$) at 5% level of significance. DMRT has been presented in Fig. 3 to 7 as alphabetical letters beside the means. Here, the means containing the same letter do not differ at a 5% level of significance.

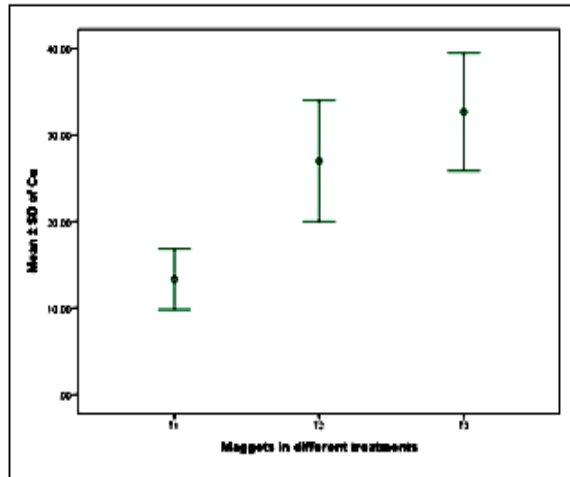


Fig. 10. Cu content of housefly maggot mg/ kg (dry weight basis).

The fatty acid profile is presented in Table 2, total of 8 types of fatty acid were recorded in all maggots among different treatments.

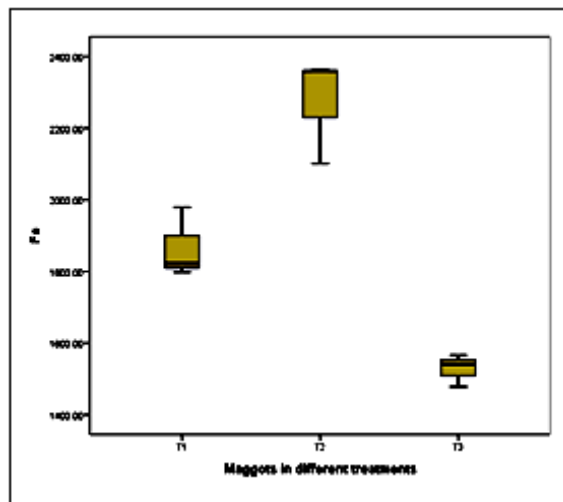


Fig. 11. Fe content of maggot mg/ kg (dry weight basis).

The investigated 8 fatty acids in maggots among treatments contained four Saturated Fatty Acids (SFA), 4 Unsaturated Fatty Acids (UFA) in which 2 were monounsaturated fatty acids (MUFA) and 2 were polyunsaturated fatty acids (PUFA). The fatty

acid composition of the maggots was largely composed of saturated fatty MUFA which was 45.46-47.13% of the total lipid in all maggots among different treatments. All groups were enriched with linoleic acid (7.59-11.43%).

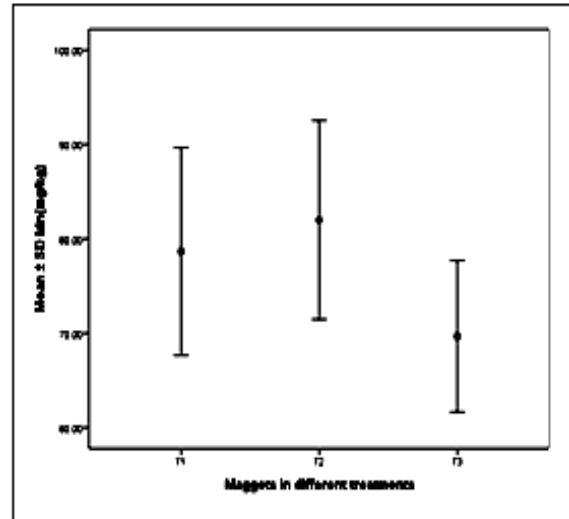


Fig. 12. Mn content of maggot mg/ kg (dry weight basis).

It was observed in these investigations that single fatty acid was largely influenced by substrate composition but the ranges of fatty acids (total SFA and total UFA) of maggots (Table 4) among treatments were not significantly varied. When comparing between the current ranges of fatty acid compositions with previous studies that maggots reared on different media were similar with our current results (Hwangbo *et al.*, 2009; Odesanya *et al.* 2011; Pretorius 2011; Hussein *et al.* 2017).

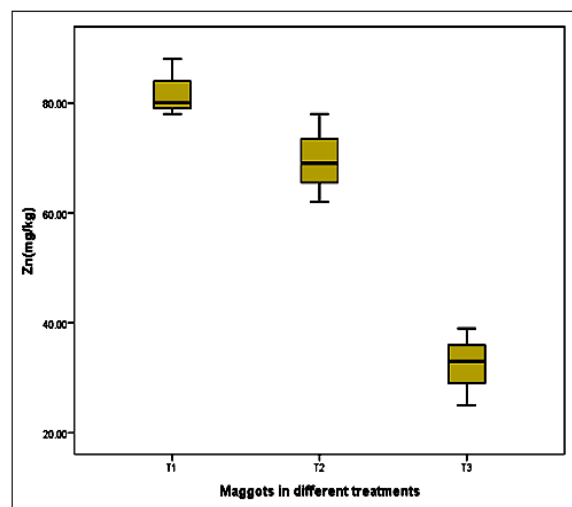


Fig. 13. Zn content of housefly maggot mg/ kg (dry weight basis).

Conclusion

Our findings indicate that the three experimental treatments gave different effects on housefly maggot nutritional value. The proximate compositions appear to be dependent on the rearing substrate. Furthermore, the highest protein content of maggots was produced by T₂ > T₁ > T₃, the highest fat by T₃ > T₁ > T₂, the ash content by T₂ > T₁ > T₃ and the highest crude fiber content by T₃ > T₁ > T₂. Moreover, the mineral element of maggots reared on T₂ media was highest among other treatments. The fatty acid compositions were not significantly different but all maggots from different treatments enriched with linolenic acid. Finally rearing system of *M. domestica* larvae on the mixture of cattle blood with cattle manure and vegetable wastes could deliver a high-quality insect resource with potential for being adopted in animal feed.

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