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PII: S1226-8615(18)30507-7
DOI: doi:10.1016/j.aspen.2018.10.011
Reference: ASPEN 1270

To appear in: *Journal of Asia-Pacific Entomology*

Received date: 19 July 2018
Revised date: 18 October 2018
Accepted date: 22 October 2018

Please cite this article as: Karlmax Rutaro, Geoffrey M. Malinga, Robert Opoke, Vilma J. Lehtovaara, Francis Omujal, Philip Nyeko, Heikki Roininen, Anu Valtonen , Artificial diets determine fatty acid composition in edible Ruspolia differens (Orthoptera: Tettigoniidae). Aspen (2018), doi:10.1016/j.aspen.2018.10.011

Artificial diets determine fatty acid composition in edible *Ruspolia differens* (Orthoptera: Tettigoniidae)

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Abstract

There are increasing interests in rearing edible insects in Africa, but information on how the feeds modify their fatty acids is largely lacking. In this work, the influence of artificial diets on the fatty acid contents and composition in the edible *Ruspolia differens* (Serville, 1838), in Uganda was assessed. *R. differens* was reared on the mixtures of six gradually diversified diets of two, three, four, six, eight and nine feeds. The diets were formulated from rice seed head, finger millet seed head, wheat bran, superfeed chicken egg booster, sorghum seed head, germinated finger millet, simsim cake, crushed dog biscuit pellet and shea butter. Fatty acid methyl esters were prepared using direct transesterification method, and analysed using gas chromatography. The contents of saturated, monounsaturated and polyunsaturated fatty acid differed significantly among the diets. The more diverse diets resulted in increased content of the polyunsaturated fatty acids. The n6:n3 ratio differed significantly among the diets and between the sexes, with *R. differens* fed on the four-feed diet having a higher n6:n3 ratio than those fed on other diets. Also, the fatty acid composition differed significantly among the diets, and diet diversification corresponded with the proportions of polyunsaturated fatty acids, especially linoleic acid. Overall, our results demonstrate that higher levels of essential fatty acids can be achieved by rearing *R. differens* on highly diversified diets. These findings are important in informing the design of future mass-rearing program for this edible insect.

Key words: diet; edible insects; edible grasshopper; essential fatty acids; fatty acid content; nutritional composition; nsenene

Introduction

The greatest challenge of African food systems is to enhance food security by producing more nutritious foods for the growing human population (Sasson, 2012). Mass-rearing of edible insects could provide one solution to this challenge. In Africa, edible insects are commonly used to supplement the largely carbohydrate-rich diets, with fatty acids, proteins, vitamins and minerals (van Huis et al., 2013). Currently, edible insects are predominantly harvested from the wild, but there is increasing interest in rearing them to enhance production (Ramos- Elorduy, 1997; van Huis et al., 2013).

Edible insects are valued for their high fat content (Bukkens, 1997; Barker et al., 1998; Banjo et al., 2006; Chakravorty et al., 2016), with some species rich in essential fatty acids (Raksakantong et al., 2010; Alves et al., 2016). It is well known that the fatty acid content and composition in insects can be modified by their diet (Komprda et al., 2013). Studies on edible insects, such as *Locusta migratoria* (Oonincx and van der Poel, 2011) and *Tenebrio molitor* (Alves et al., 2016) have shown that artificial diets greatly influence their nutritional composition, including fatty acids. For fatty acids, particularly polyunsaturated fatty acids (PUFAs), modifications can occur either through absorption of dietary fatty acids (Finke and Oonincx, 2014), or *de novo* biosynthetic pathways (synthase enzyme system) from dietary carbohydrates and proteins, using acetyl-coenzyme A (Stanley-Samuelson et al., 1988). The strong association between fatty acid composition in insects and their diet could provide a basis to design diets from the local feeds for insect rearing, and for improving the quality of edible insects as human food.

The edible *Ruspolia differens* (Serville, 1838, Tettigoniidae), is one of the most consumed insects in the Afro-tropical region, with high potential of alleviating food insecurity and malnutrition, and providing household incomes to rural communities (Agea et al., 2008; van Huis et al., 2013). The insect is nutritionally rich and contains 47–49% fat, 44–46% proteins and 8% carbohydrates on a dry weight basis (Kinyuru et al., 2010; Siulapwa et al., 2014). Additionally, *R. differens* is rich in essential PUFAs and contain 31% linoleic acid and 4.2% α-linolenic acid of the total methyl esters (Kinyuru et al., 2010). However, the current utilisation of *R. differens* as a source of food and income is hampered by scarcity, due to its natural seasonal availability (Nyeko et al., 2014). Thus, there is a growing demand to develop mass-rearing methods, using artificial feeds to ensure sustainable production throughout the year.

It has already been established that *R. differens* can be reared on a variety of natural and artificial diets in the laboratory (Malinga et al., 2018a, b; Ssepuuya et al., 2018). They readily eat grass leaves and inflorescences, rice, millet, sorghum, maize flour and oats (Hartley, 1967; Nyeko et al., 2014; Malinga et al., 2018a, b; Valtonen et al., 2018), and many artificial feeds, such as ground dog biscuits (Brits and Thornton, 1981) and superfeed chicken egg booster (Malinga et al., 2018a). It has been shown that the fatty acid content and composition of *R. differens* can be modified using diets with manipulated contents of fatty acid, carbohydrate and protein (Lehtovaara et al., 2017). However, suitable diet mixtures for mass-rearing developed from commonly available feeds in Africa are not well understood (but see Malinga et al., 2018a, b).

In this study, we examined the influence of locally sourced artificial diets in Africa on the fatty acid content and composition of *R. differens*. We reared *R. differens* through the full life cycle, between 4–6 months from neonate nymphs to adults, on mixtures of six gradually diversified diet treatments, varying from mixtures of two, three, four, six, eight and nine feeds. Our specific questions were: i) Does the content of (a) saturated fatty acids (SFAs) (b) monounsaturated fatty acids (MUFAs) (c) PUFAs, and (d) ratio of omega-6 to omega-3 (n6:n3), differ among individuals feeding on the different diet treatments? ii) Does the compositions (i.e., proportions of fatty acids) of *R. differens* differ among individuals feeding on the different diets? iii) Do male and female *R. differens* differ in their composition of fatty acids? This knowledge is useful in designing future mass rearing programs.

Materials and Methods

Study insects

The parent population of *R. differens* was collected from the wild around the Makerere University Agricultural Research Institute, Kabanyolo (MUARIK), Uganda (0°27'03.0"N and 32°36'42.0"E). We selected equal numbers of adult males and females (50:50), and placed them into 10 plastic containers (Thermopak Limited, Nairobi; 24 cm length × 18 cm width × 12.5 cm height). Each container housed 10 males and 10 females, to increase chances of mating and oviposition (Brits and Thornton, 1981). We used four small round plastic jars (Thermopak Limited, Nairobi; 5.3 cm width × 7.1 cm height) filled with moistened cotton wool placed at the corners of the plastic container, as the egg-laying substrate. Once laid, the eggs were collected onto small round plastic jars (5.3 cm width × 7.1 cm height), containing sieved moistened sand and cotton wool (50:50), and sprayed daily with water, until hatching in about 2–3 weeks.

Diets preparation

The feeds (both processed and unprocessed) were obtained from the local markets in Kampala, Uganda. We included only the most accepted feeds based on our previous work (Malinga et al., 2018a). The unprocessed feeds included rice seed head, finger millet seed head, sorghum seed head and germinated finger millet (Table 1). The feeds were selected because they are readily available throughout the year in Uganda (Malinga et al., 2018a). Furthermore, wheat bran, superfeed chicken egg booster, simsim cake and crushed dog biscuit pellet were selected because they are readily available in local markets throughout the year. To enhance the insects' feeding and improve palatability, the seed heads of rice, finger millet and sorghum feeds were separately crushed to a coarse powder. Germinated finger millet was obtained by soaking millet seeds in a cotton net cloth, draining the water and leaving it to sprout for 3–4 days. Simsim cake was prepared as described in Malinga et al. (2018a). The resulting simsim cake and dog biscuit pellets were lightly crushed with a grinding stone to ease insect feeding.

Experimental set-up

The effect of diets on the fatty acid content and composition in *R. differens* was evaluated by randomly selecting newly hatched (1–2-day-old) nymphs into round plastic containers measuring 12.5 cm × 8 cm (one individual per container). The six diet treatments formed a gradient of gradually diversifying diet, so that the least and most diverse diets comprised two and nine feeds, respectively (Table 1). The containers were arranged in blocks to control for possible environmental variations. We used 10 blocks, each consisting of two diet replicates per treatment. For each diet treatment, an equal quantity (2 g) of diet was randomly placed in each container (i.e., the nymphs on the two-feed diet received 1 g of each constituent feed diet and so on). To minimize bias towards a particular feed, the individual feeds were placed relatively close to each other (Bernays et al., 1997). The offered 2 g of diet allowed *ad libitum* feeding for insects, with regular diet replenishments every 3–4 days, until the nymphs moulted to adults. Water was offered through a wet rolled up tissue paper. Each rearing jar had its top covered using a netting cloth. The experiment was set at 23–27°C, 50–60% relative humidity and a 12:12 h (L:D) photoperiod. Newly emerged adults were harvested, and their sex recorded based on the presence or absence of the ovipositor (Brits and Thornton, 1981). For fatty acid analysis, a total of 30 individuals i.e., five from each diet treatment were randomly selected for lyophilisation.

Fatty acid analysis

Fatty acids were determined as methyl esters using a gas chromatography, equipped with a FID detector and an auto sampler at the Bio-Competence Centre of Healthy Dairy Products (Bio–CC), Tartu, Estonia. It followed fatty acid methyl esters preparation, GC-FID analysis and fatty acid identifications.

Preparation of FAMEs**:** The preparation of the fatty acid methyl esters was based on a direct transesterification method (Sukhija and Palmquist, 1988), with minor modifications (also see, Lehtovaara et al., 2017), using crushed de-winged *R. differens* individuals. Briefly, to each of pyrex tubes containing the weighed crushed de-winged *R. differens* individuals were added 1 mL toluene and 1 mL of internal standard C17:0 (15 mg/mL, Sigma-Aldrich CAS: 506-12-7), followed by 3 mL of 5% methanolic HCl solution. The tubes were tightly capped, vortexed for 5 minutes, heated for 2 hours in an oven at 100 °C before cooling to room temperature. Then, 5 mL of 6% potassium carbonate was added, followed by 2 mL of toluene and the contents vortexed for 0.5 minutes at a medium speed followed by centrifugation at 2500 ×*g* for 5 minutes. Using a Pasteur pipette, the upper layer was transferred to a new tube. To the toluene extract, was added 1 g anhydrous sodium sulfate and 1 g activated carbon, the mixture was vortexed for 0.5 minutes and allowed to stand for 1 hour and later centrifuged at 4000 ×*g* for 5 minutes. Finally, the clear toluene (upper) layer containing methyl esters were transferred to gas chromatography (GC) vials, and kept at –20 °C until analysis.

GC-FID analysis**:** FAMEs were analysed on an Agilent 6890A GC (Agilent Technologies Inc. USA), equipped with a FID detector and an auto sampler. Fatty acids were separated using a 100 m × 0.25 mm i.d. CP-Sil 88 capillary column, with 0.20 µm film thickness, using hydrogen as a carrier gas with a flow rate of 30 mL/min and a column inlet pressure of 20 psi at a 1:60 split ratio. The injector temperature was set at 250°C and the detector temperature at 270°C. The injection volume was 1 µL. The initial oven temperature was set at 100°C and held for 1 min, then increased to 180°C at 13°C/ min and held for 40 min. The oven temperature was further increased to 225°C at 5°C min^{-1} and held for 15 min.

Identification of fatty acids**:** The fatty acids were identified by comparison of sample peak retention times with FAME standard mixtures (Supelco 37 component FAME mix, Nu-Chek Prep GLC-603 and GLC-408, bacterial acid methyl ester (BAME) mix, and linoleic acid methyl ester isomer mix) and individual FAME standards. Fatty acid peak areas were quantified using ChemStation chromatography software (Agilent Technologies). Unresolved fatty acids are reported in the text and Table 2 in the format X+Y (e.g., C12:1n9c+C13:0); they did not separate under the present conditions and were quantified together. The relative amounts of each fatty acid were expressed as a percentage of the total analysed fatty acids and as content (milligrams of the fatty acid per gram) of dry weight of *R. differens*, and presented separately for both males and females. For the comparison with the wild harvested *R. differens*, we used the fatty acid proportions (% of total fatty acids) data reported in Rutaro et al. (2018).

Statistical analyses

ANOVA models (type III sums of squares) were fitted in SPSS (IBM SPSS Statistics, version 23), to test whether the SFAs, MUFAs, PUFAs (mg/g dry weight) contents or n6:n3 ratio of *R. differens* were explained by diet, sex (fixed factors) or their interaction. Before statistical analyses, PUFAs and the n6:n3 ratio were ln-transformed, and MUFAs was square root transformed, to improve normality. Duncan's post hoc test was used for pairwise comparisons because for some variables, the more conventional pairwise test (Tukey) was too conservative to find any significant differences, even when ANOVA indicated significant differences among the diets. Permutational multivariate analysis of variance (PERMANOVA) was ran to test for differences in the fatty acid compositions (proportions of fatty acids) among the six diets, between the sexes and for the interaction between these two factors (Anderson, 2001), with Type III sums of squares and 999 permutations. Monte Carlo tests (Anderson et al., 2008) were employed to assess pairwise differences. PERMANOVA is sensitive to differences in dispersions (i.e., heterogeneity of variances) and, therefore, a permutational analysis of multivariate dispersions (PERMDISP) was conducted (Anderson et al., 2008). We carried out a similarity of percentages analysis (SIMPER) (Clarke and Gorley, 2006), to identify which fatty acids contributed most to differences in the fatty acid composition among the diets. Also, to visualise fatty acid patterns of individual *R. differens* fed on diversifying diets, we used non-metric multidimensional scaling (NMDS), with 50 restarts. In all multivariate analyses, Bray-Curtis was used as a measure of similarity. As the response dataset in the multivariate analysis, we only included the proportions of each fatty acid with levels of 0.05% and above in a sample (n = 26 out of the 44 detected fatty acids) (Table 2). Also, branched chain (iso/anteiso) fatty acids were combined, before inclusion in the analysis. All multivariate statistical analyses were performed using PRIMER version 6.0 and PERMANOVA+ add-on (Clarke and Gorley, 2006; Anderson et al., 2008).

Results

Fatty acid contents

The fatty acid content (SFA, MUFA, PUFA) and the n6:n3 ratio differed significantly among the diets (SFA: $F_{5, 18} = 3.5$, $p = 0.02$; MUFA: $F_{5, 18} = 4.4$, $p = 0.009$; PUFA: $F_{5, 18} = 16.6$, $p < 0.001$; n6:n3 ratio: $F_{5, 18} = 9.6$, $p < 0.001$). For SFA, the individuals fed on the three-feed diet treatment had a higher SFA content than in more diversified (four-, six-, eight- and nine-feed) diet treatments (Fig. 1A). Furthermore, the individuals fed with the two- and three-feed diets had a significantly higher MUFA content than in the more diversified four, six, eight and nine feed diets (Fig. 1B). Also, the PUFA content significantly increased in individuals fed the most diversified nine-feed diet than in those fed the least diversified (two-feed) diet (Fig. 1C), and the *R. differens* fed on the four-feed diet had a significantly higher n6:n3 ratio than those fed the two-, three-, six-, eight- and nine-feed diets (Fig. 1D). Additionally, the contents did not differ significantly between the sexes (SFA: $F_{1, 18} = 1.6$, $p = 0.23$; MUFA: $F_{1, 18} = 0.0$, $p = 0.99$; PUFA: $F_{1, 18} = 0.06$, $p = 0.81$), but the n6:n3 ratio differed between sexes ($F_{1, 18} = 13.5$, $p = 0.002$), with females having a lower n6:n3 ratio (mean = 18.0, SE

= 1.8) than males (mean = 26.7, SE = 3.7). However, in all cases, there was no significant diet × sex interaction (SFA: $F_{5, 18} = 1.1$, $p = 0.38$; MUFA: $F_{5, 18} = 0.8$, $p = 0.54$; PUFA: $F_{5, 18} = 1.27$, $p = 0.32$; n6:n3 ratio: $F_{5, 18} = 1.7$, $p = 0.197$).

Fatty acid composition

The proportions of fatty acids differed significantly among the diets (PERMANOVA; pseudo-$F_{5, 18} = 10.5$, $p = 0.001$), explaining 39% of the variation. Sex (pseudo-$F_{1, 18} = 4.3$, $p = 0.021$) and the interaction between diet and sex (pseudo-$F_{5, 18} = 2.2$, $p = 0.038$) explained 13 and 20% of the variation in fatty acid compositions, respectively. When the pairwise differences were assessed separately for males and females, the differences in fatty acid composition were found only among females. Among the females, the differences in fatty acid composition were found among all pairs of diet treatments ($p < 0.05$), except between the three-feed versus eight-feed, four-feed versus eight-feed, six-feed versus eight-feed and eight-feed versus nine-feed diet treatments ($p \geq 0.05$). Based on the NMDS ordinations, within either males or females, there was a distinct gradient in fatty acid compositions following the diversifying diet (Fig. 2). Three fatty acids, i.e., linoleic, oleic and palmitic acids, made the strongest contribution to the dissimilarities in the fatty acid composition across diets (SIMPER analysis). For all comparisons between pairs of diet treatments, linoleic acid contributed between 17 and 43% to the dissimilarity, oleic acid contributed between 9 and 41% to the dissimilarity, and palmitic acid contributed between 10 and 35% to the dissimilarity. We also found significant differences in the degree of variability in fatty acid composition among the diets (PERMDISP; $F_{5, 24} = 6.7$, $p = 0.007$; see NMDS ordination; Fig. 2A). The largest variability in fatty acid composition was found in *R. differens* fed on the eight-feed diet (dispersion from the centroid, mean ± SE; 6.0 ± 0.9) and the least variability was observed on the three-feed diet (1.6 ± 0.3).

The total PUFAs on average ranged from 5% in the least diversified two-feed diet to 19% in the most diversified nine-feed diet (Table 2). In all treatments, the most predominant PUFAs were linoleic acid (18:2n6) and α-linolenic acid (18:3n3), while the other four (i.e., γ-linolenic acid (18:3n6), eicosatrienoic acid (20:3n3), docosadienoic acid (22:2n6) and eicosadienoic acid (20:5n6)) were present in trace amounts (Table 2). Also, in all treatments, the proportions of linoleic acid (18:2n6) ranged from 5−18%, while α-linolenic acid (18:3n3) ranged from 0.3−0.9%. The proportion of SFAs ranged from 35% in the nine-feed diet to 42% in the three-feed diet. The predominant SFAs were palmitic acid (16:0) ranging between 24−33% of total fatty acids, followed by stearic acid (18:0) that ranged from 7% in the two-feed diet to 9% in the nine-feed diet (Table 2). The proportion of MUFAs ranged from 46% in the nine-feed diet to 55% in the two-feed diet. The predominant MUFA was oleic acid, ranging between 44−52% (Table 2).

Discussion

Our study demonstrated that when fed over the full life cycle (neonate nymph to adult), the diversifying gradient of artificial diets strongly modified the content and composition of fatty acids in *R. differens*, one of the most important edible insects in the Afro-tropical region. Notably, the content of PUFAs was about 3.5-fold higher in *R. differens* that received the most diversified diet compared to those that received the least diversified diet. Artificial diets have also been shown to modify fatty acid compositions of edible insects in other studies (Dreassi et al., 2017; Lehtovaara et al., 2017). *R. differens* could have selected the favourable food particles from the diversified diet treatments (also see, Waldbauer et al., 1984), which might explain the high PUFA content in the most diversified eight- and nine-feed diets compared to the least diversified two-feed diet. Furthermore, diets with eight- and nine-feed mixtures contained shea butter and simsim seed cake that are generally rich in PUFA content (Shea butter, 6-8%; simsim cake, 22-46% of the total fatty acid content; Okullo et al., 2010; Honfo et al., 2014; USD, 2016; Gharby et al., 2017). Therefore, it is possible that *R. differens* absorbed and incorporated such PUFAs from PUFA-rich diets, to produce the observed high PUFA levels, relative to other diets where dietary PUFA sources were minimal or lacking. In diets containing shea butter and simsim cake, the PUFA levels were five times higher than those without, and the PUFA levels in the most diversified (nine feed) diet was almost similar to the wild harvested individuals (Table 2). Though in trace amounts, *R. differens* has also demonstrated the capacity to synthesise or absorb higher chain PUFAs, such as eicosapentaenoic acid (EPA, C20:5n3), further highlighting its nutritional importance to humans. The total SFA, MUFA and PUFA contents observed in this study compare well with those reported for wild insect species, such as *L. migratoria* (Mohamed, 2015), June beetles, termites, cicadas, dung beetles and short-tailed crickets (Raksakantong et al., 2010), and the melon bug, *Aspongubus viduatus* and the sorghum bug, *Agonoscelis pubescens* (Mariod et al., 2011).

The *R. differens* produced in this experiment had relatively high n6:n3 ratio (Fig. 1D), compared to the nutritionally recommended ratio of less than five (Wood et al., 2003; Kouba and Mourot, 2011). In this study, we fed *R. differens* mostly on a cereal-based diet, which, according to Weihrauch and Matthews, (1977), contains higher levels of linoleic acid, an n6 fatty acid, than α-linolenic acid, an n3, which could explain the high and unfavourable n6:n3 PUFA ratio. Therefore, to overcome this imbalance, n3 PUFA-rich feed sources, such as *Salvia hispanica* (chia) and linseeds, previously used to increase the n3 in livestock, chicken meat, quail eggs (Kouba and Mourot, 2011; Komprda et al., 2013), and some edible insect species (Komprda et al., 2013) could be included in diet formulations of *R. differens*.

The observed fatty acid compositions in this study concur with previous studies that analysed composite samples of *R. differens* harvested from the wild (Kinyuru et al., 2010; Nyeko et al., 2014). In Kinyuru et al. (2010) and Nyeko et al. (2014), the dominant fatty acids were palmitic, oleic and linoleic acids. In this study, oleic acid was the most predominant fatty acid, and its proportions were considerably higher than in the wild harvested *R. differens* (Kinyuru et al., 2010; Nyeko et al., 2014). This could be attributed to oleic acid-rich cereal feeds, for example, rice and wheat (Weihrauch and Matthews, 1977) used in this study, as well as the elongation and desaturation of the SFAs, such as palmitic and stearic acid, by the insects' fatty acid synthase system (Stanley-Samuelson et al., 1988).

Finally, the differences observed between the fatty acid proportions among male and female *R. differens* could be a result of differing physiological functional roles, such as reproduction. For example, female insects require certain fatty acids, like oleic acid, in greater proportions during egg formation (Lease and Wolf, 2011; Sönmez et al., 2016). It could be the need to satisfy such requirements that the different sexes could have consumed different amounts of feeds, which ultimately modify the overall fatty acid proportions in their tissues. Therefore, this could be the reason why in this study, there were proportional differences in fatty acids of female and not male *R. differens*, although they were offered similar diets.

Conclusion

Overall, the study has shown that the diversifying gradient of local feeds strongly modified the content and composition of fatty acids in the edible *R. differens*. Furthermore, the study suggests that diversified sources of feeds can increase the content of PUFAs, possibly because of the ability of *R. differens* to select the favourable food particles in the diet. The diet offered to the *R. differens* were rich in n6 PUFA relative to n3 PUFA, which caused a high n6:n3 ratio, suggesting that n3-rich feeds should be included in the diet to balance n6 and n3 fatty acids, in future rearing. Our results demonstrate that artificial feeds can support growth and development of *R. differens* in rearing conditions and ultimately modify their fatty acids. For improved food safety and improved food quality in Africa, it is important to plan the future mass-rearing of *R. differens*, to produce nutritious foods that are rich in essential fatty acids for humans.

Author contribution

KR, HR, PN, AV, FO, GMM designed the study, KR conducted the laboratory studies in Uganda, statistical analyses and drafted the manuscript. All authors (KR, HR, PN, AV, FO, GMM, VJL and RO) contributed to the interpretation of the data, writing and review of the manuscript.

Competing interests

None declared.

Funding

This work was supported by the Academy of Finland grant (Project no 14956 to HR) and Bugbox Limited (Estonia).

Acknowledgements

We are grateful to the Uganda National Council of Science and Technology for permitting the study and the Makerere University Agricultural Research Institute, Kabanyolo, Uganda for hosting the project. We thank I. Mwesige for assistance during rearing of insects. The authors would like to thank the anonymous reviewers for their helpful and constructive comments that greatly improved the final version of the paper.

Table 1. Energy (Kcal/100g) and the amounts (g/100g dry weight) of protein, fat, and carbohydrate of feeds used in rearing *R. differens*. (Nutritional content of the feeds extracted from Malinga et al., 2018a). The composition of the feeds (g) in the diets are also included (summing to 2 grams).

						Treatment levels					
Common or trade name	**Scientific name**	**Energy**	**Protein**	**Fat**	**Carbohydrate**	**Two feed**	**Three feed**	**Four feed**	**Six feed**	**Eight feed**	**Nine feed**
Rice seed head*	*Oryza sativa*	349.0	6.9	0.6	78.3	1.0	0.67	0.5	0.33	0.25	0.22
Finger millet seed head ‡†	*Eleusine coracana*	336.0	7.7	1.5	72.6	1.0	0.67	0.5	0.33	0.25	0.22

Wheat bran*	*Triticum aestivum* L	282.0	15.9	4.8	23.2	0.67	0.5	0.33	0.25	0.22
Chicken egg booster§		12.5	3.4	-	-		0.5	0.33	0.25	0.22
Sorghum seed head*	*Sorghum bicolor*	354.0	9.3	3.9	65.5			0.33	0.25	0.22
Germinated millet#¢	*Eleusine coracana*	303.2	8.6	0.6	80.9			0.33	0.25	0.22
Simsim cake¥ɸ	*Sesamum indicum* L.	2753.0	44.4	13.1	35.4				0.25	0.22
Crushed dog biscuit pellet§		341	22.0	9.0	47.5				0.25	0.22
Shea butter oil‡		884.0	0.0	100.0	0.0					0.22

§Nutritional facts provided by the manufacturer, ‡U. S. Department of Agriculture, 2016, *FAO, 2016, #Muyanja et al., 2003, ¢Ocheme and Chinma, 2008, ¥Babiker, 2012, ɸBukya and Vijayakumar, 2013, †Kumar et al., 2016.

272 **Table 2.** Total fat content (mg/1g), and the fatty acid proportions (mg individual fatty acid/100 mg of total fatty acids) of *R. differens* feeding on the six gradually
273 diversifying diets compared to those harvested from the wild.

	Diet treatments												
	Two feed		**Three feed**		**Four feed**		**Six feed**		**Eight feed**		**Nine feed**		**Wild samp**
Fatty acid	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**
C12:0	0.05±0.01	0.06±0.01	0.06±0.00	0.09±0.00	0.07±0.02	0.14±0.01	0.10±0.01	0.07±0.02	0.07±0.02	0.10±0.01	0.08±0.01	0.11±0.02	
C14:0	0.71±0.03	0.71±0.07	0.82±0.02	0.91±0.02	0.87±0.03	0.99±0.03	1.01±0.06	0.75±0.08	0.86±0.12	0.83±0.05	0.85±0.03	0.76±0.07	3.90±1.25
C15:0	0.05±0.02	0.04±0.01	0.04±0.01	0.05±0.01	0.10±0.03	0.05±0.01	0.05±0.01	0.06±0.01	0.06±0.02	0.05±0.01	0.05±0.00	0.09±0.02	
C16:0	31.13±1.43	31.77±0.86	32.91±0.14	32.18±0.57	31.30±1.58	31.37±1.12	33.16±1.71	26.61±0.44	28.36±2.63	26.35±2.22	28.50±2.77	21.72±0.18	19.95±2.51
C18:0	7.07±0.98	7.27±0.25	7.95±0.30	8.67±0.52	9.24±0.35	7.43±0.04	8.26±0.14	6.90±0.73	9.30±0.31	6.37±0.53	9.38±0.38	8.93±0.54	6.87±0.90
C20:0	0.26±0.03	0.26±0.02	0.23±0.00	0.27±0.01	0.34±0.00	0.24±0.01	0.28±0.05	0.31±0.02	0.31±0.04	0.46±0.21	0.24±0.03	0.31±0.03	0.64±0.25
C22:0	0.04±0.01	0.04±0.01	0.03±0.01	0.04±0.00	0.07±0.00	0.05±0.00	0.06±0.02	0.06±0.01	0.06±0.02	0.07±0.03	0.04±0.01	0.06±0.01	
C24:0	0.06±0.01	0.05±0.00	0.05±0.00	0.05±0.00	0.06±0.00	0.05±0.00	0.05±0.01	0.05±0.00	0.05±0.01	0.04±0.00	0.03±0.00	0.05±0.01	
C26:0	0.03±0.00	0.05±0.02	0.02±0.00	0.05±0.01	0.04±0.01	0.05±0.01	0.03±0.02	0.04±0.02	0.04±0.01	0.09±0.06	0.01±0.00	0.07±0.05	
∑SFA	**39.40±2.31**	**40.25±0.69**	**42.13±0.44**	**42.30±0.78**	**42.09±1.23**	**40.38±1.13**	**43.00±1.67**	**34.86±0.94**	**39.12±2.08**	**34.38±2.44**	**39.19±3.14**	**32.10±0.55**	31.36±2.97
C14:1n5t	0.00±0.00	0.02±0.01	0.02±0.01	0.02±0.00	0.00±0.00	0.01±0.00	0.06±0.02	0.00±0.00	0.04±0.02	0.04±0.03	0.00±0.01	0.05±0.02	
C14:1n5	0.00±0.00	0.01±0.00	0.01±0.00	0.01±0.00	0.04±0.04	0.03±0.02	0.02±0.01	0.01±0.00	0.02±0.01	0.01±0.01	0.01±0.00	0.00±0.00	2.95±0.94
C16:1n9	0.06±0.00	0.06±0.01	0.06±0.00	0.08±0.01	0.06±0.01	0.08±0.00	0.05±0.00	0.08±0.01	0.05±0.01	0.07±0.01	0.07±0.00	0.10±0.01	
C16:1n7	2.65±0.44	2.64±0.13	2.37±0.17	2.27±0.18	1.55±0.36	2.26±0.14	1.97±0.23	2.01±0.19	1.25±0.29	1.76±0.13	1.33±0.32	1.04±0.09	22.24±0.98
C16:1n3	0.00±0.00	0.00±0.00	0.04±0.04	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	
C17:1n8	0.05±0.01	0.04±0.01	0.05±0.00	0.06±0.01	0.10±0.02	0.06±0.00	0.07±0.02	0.07±0.01	0.07±0.02	0.08±0.03	0.05±0.01	0.09±0.02	
C18:1n9t	0.07±0.01	0.06±0.01	0.05±0.00	0.07±0.01	0.08±0.00	0.07±0.01	0.08±0.01	0.09±0.00	0.12±0.01	0.09±0.00	0.11±0.00	0.16±0.04	
C18:1n9	52.36±1.29	51.94±0.12	49.20±0.73	47.79±0.48	44.99±1.32	46.45±0.77	44.76±0.01	52.95±0.81	43.12±2.08	48.65±1.07	44.06±0.94	44.61±0.34	21.68±0.49
C24:1n9	0.00±0.00	0.00±0.00	0.01±0.00	0.00±0.00	0.00±0.00	0.01±0.01	0.01±0.00	0.02±0.02	0.00±0.00	0.01±0.01	0.02±0.01	0.00±0.00	
∑MUFA	**55.19±1.75**	**54.77±0.10**	**51.81±0.84**	**50.29±0.65**	**46.82±1.60**	**48.97±0.78**	**47.03±0.18**	**55.23±0.59**	**44.67±2.30**	**50.72±1.12**	**45.65±1.25**	**46.04±0.49**	**46.87±1.76**
C18:2n6	4.78±0.49	4.33±0.72	5.41±0.35	6.67±0.30	10.37±2.90	9.83±0.68	9.11±1.72	8.59±1.21	15.42±4.24	13.84±3.35	14.27±4.33	20.32±1.26	20.84±4.21
C18:3n6	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.03±0.01	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.01±0.01	0.02±0.02	
C18:3n3	0.31±0.01	0.32±0.00	0.35±0.01	0.39±0.02	0.24±0.05	0.36±0.02	0.46±0.01	0.92±0.20	0.37±0.05	0.67±0.09	0.56±0.02	1.07±0.19	0.93±0.19
C20:2n6	0.05±0.01	0.04±0.00	0.05±0.00	0.04±0.00	0.00±0.00	0.03±0.01	0.02±0.02	0.05±0.01	0.02±0.02	0.03±0.02	0.03±0.00	0.03±0.02	
20:3n3	0.00±0.00	0.00±0.00	0.00±0.00	0.01±0.01	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.02±0.02	
C22:2n6	0.01±0.01	0.05±0.04	0.03±0.01	0.03±0.02	0.01±0.02	0.04±0.03	0.08±0.06	0.01±0.01	0.03±0.03	0.04±0.03	0.01±0.01	0.02±0.02	
∑PUFA	**5.15±0.48**	**4.74±0.76**	**5.84±0.36**	**7.13±0.30**	**10.62±2.94**	**10.30±0.66**	**9.67±1.76**	**9.56±1.42**	**15.84±4.24**	**14.58±3.44**	**14.88±4.31**	**21.49±1.06**	**21.77±4.34**
∑n6	4.84±0.49	4.42±0.75	5.49±0.35	6.73±0.29	10.38±2.89	9.94±0.65	9.21±1.75	8.65±1.22	15.47±4.20	13.91±3.37	14.32±4.33	20.39±1.26	20.84±4.21
∑n3	0.31±0.01	0.32±0.00	0.35±0.01	0.40±0.02	0.24±0.05	0.36±0.02	0.46±0.01	0.92±0.20	0.37±0.05	0.67±0.09	0.56±0.02	1.09±0.20	0.93±0.19
n6/n3	**15.53±1.96**	**13.80±2.14**	**15.59±0.56**	**16.81±0.53**	**42.61±2.88**	**27.52±1.81**	**19.97±3.34**	**9.79±0.83**	**40.72±6.07**	**20.71±4.01**	**25.68±8.59**	**20.84±6.02**	**24.65±5.95**
iso/anteiso	0.02±0.01	0.02±0.00	0.01±0.00	0.04±0.01	0.09±0.03	0.03±0.02	0.01±0.00	0.04±0.01	0.00±0.00	0.01±0.00	0.03±0.00	0.05±0.02	

UR1	0.03±0.01	0.03±0.02	0.04±0.03	0.04±0.01	0.10±0.05	0.05±0.03	0.09±0.05	0.04±0.01	0.14±0.09	0.06±0.04	0.04±0.04	0.01±0.00
UR2	0.16±0.04	0.15±0.02	0.14±0.01	0.15±0.01	0.26±0.04	0.18±0.02	0.17±0.03	0.19±0.02	0.18±0.04	0.20±0.06	0.14±0.03	0.22±0.05
TF/mg/g	490.70±37.23	463.52±52.22	511.22±53.52	493.28±47.44	**280.10±8.31**	392.99±20.16	**342.10±66.76**	**311.60±31.20**	368.56±74.69	337.42±83.17	499.95±71.41	388.38±59.63

Data are expressed as mean±SE; n=5: SFA= saturated fatty acids; MUFA= monounsaturated fatty acids; PUFA= polyunsaturated fatty acids; n6/n3= ratio of omega–6 to omega–3 acids; C=number of carbon atoms in the fatty acid structure; c=*cis*; t= *trans* fatty acid; UR= fatty acid not separated and quantified together; UR-1= C12:1n3c+C13:0 and UR- 2= C18:1n3c+C19:0; TF=Total fat content; Wild harvested =*R. differens* collected from the field (Fatty acid data reproduced from Rutaro et al, 2018; M, F=Male and Female *differens* respectively.

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Figure legends

Fig. 1. The contents of (A) SFAs, (B) MUFAs, (C) PUFAs, and (D) the n6:n3 ratio of *Ruspolia differens* on the six gradually diversifying diets. The values represent the marginal means (± SE) (for SFA) and back-transformed marginal means (± SE) (for MUFA, PUFA and the n6:n3 ratio) from two-way ANOVAs. Treatments with different letters indicate significant ($p < 0.05$) differences in pairwise tests (Duncan).

Fig. 2. (A) Similarity of fatty acid compositions of *Ruspolia differens* individuals under the six gradually diversifying diets based on non-metric multidimensional scaling (NMDS) ordination. (B) and (C) show the similarity in fatty acid compositions among individual male and female *R. differens*, respectively, extracted from panel A. Numbers 2, 3, 4, 6, 8 and 9 represent the number of feeds per diet on which individual insects were fed.

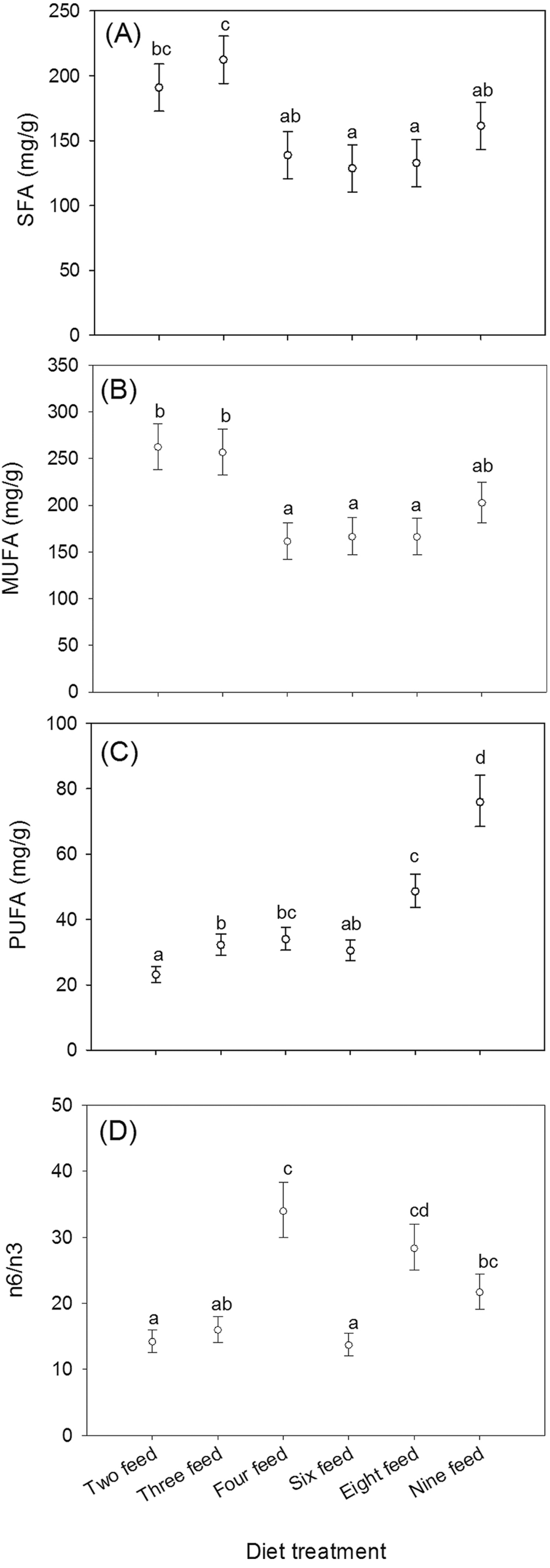
(A)
SFA (mg/g)
250
200
150
100
50
0
bc
c
ab
a
a
ab
(B)
MUFA (mg/g)
350
300
250
200
150
100
50
0
b
b
a
a
a
ab
(C)
PUFA (mg/g)
100
80
60
40
20
0
a
b
bc
ab
c
d
(D)
n6/n3
50
40
30
20
10
0
a
ab
c
a
cd
bc
Two feed
Three feed
Four feed
Six feed
Eight feed
Nine feed
Diet treatment

Figure 1

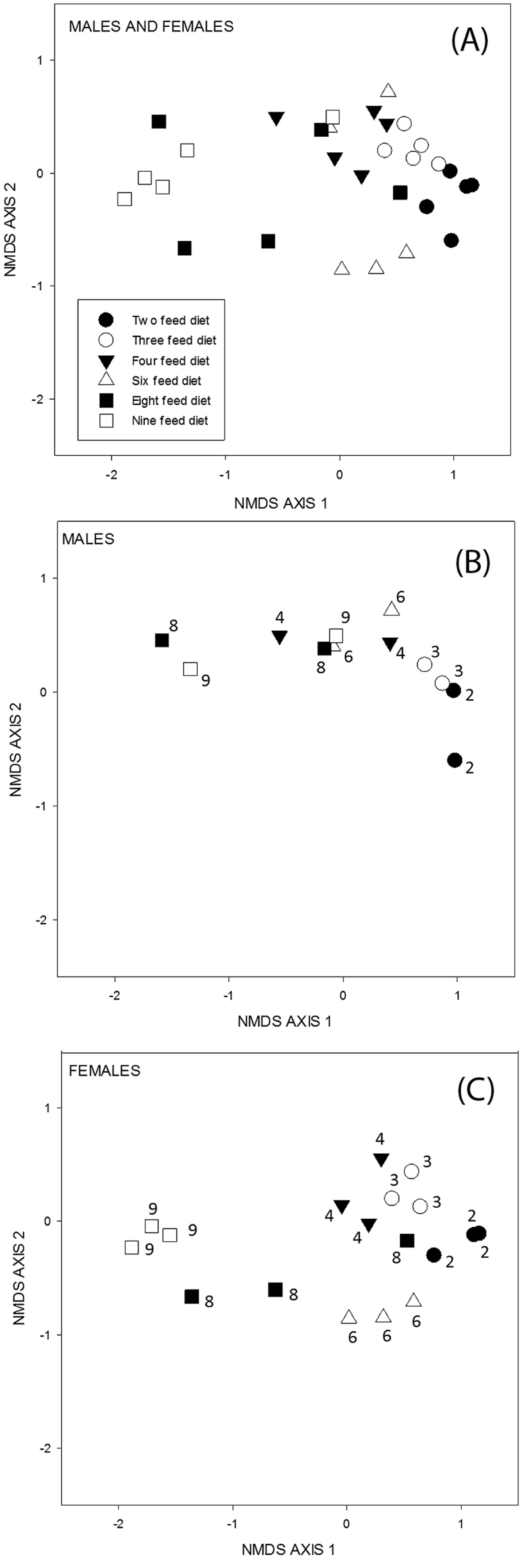
MALES AND FEMALES
(A)
NMDS AXIS 1
NMDS AXIS 2
● Two feed diet
○ Three feed diet
▼ Four feed diet
△ Six feed diet
■ Eight feed diet
□ Nine feed diet
MALES
(B)
FEMALES
(C)
1
0
-1
-2
2
3
4
6
8
9

Figure 2