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The fatty acid composition of edible grasshopper *Ruspolia differens* (Serville) (Orthoptera: Tettigoniidae) feeding on diversifying diets of host plants

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Abstract

Ruspolia differens (Serville) (Orthoptera: Tettigoniidae) is a highly valued edible grasshopper species in Africa. However, the effects of plant diets on lipid content and fatty acid composition of *R. differens* are not well understood. We tested the effects of four diets on the total lipid content and fatty acid composition of *R. differens*. Sixth instar nymphs of *R. differens* were reared on one, and mixtures of two, three, and six natural plant inflorescences. Individuals collected from the field constituted a control treatment. We extracted lipids and analyzed the fatty acid methyl esters using gas chromatography–mass spectrometry. We analyzed if the total lipid content, body weight, and fatty acid composition differed among diets and between the sexes using two-way ANOVAs and a PERMANOVA model, respectively. The total lipid content and weight of *R. differens* did not differ among the diets. The nine common fatty acids were palmitic (mean across treatments, 26%), oleic (22%), palmitoleic (18%), linoleic (13%), stearic (7%), myristic (6%), myristoleic (4%), α-linolenic (2%) and arachidic acid (1%). The composition of fatty acids and the proportion of essential fatty acids significantly differed among the diets. The proportion of essential fatty acids was highest in the control treatment (21%) but low in less diversified (one to three feed) diets (12–13%). This study demonstrates that the fatty acid composition in *R. differens* can be influenced through diet. Thus, with dietary manipulations, using local plants in Africa, it is possible to produce *R. differens* with preferred high quality essential fatty acids for human consumption.

Key words: edible insect, essential fatty acids, fat content, insect rearing, plant diet.

Introduction

Fatty acids are biologically important in key life processes such as metabolic energy storage, cell structure and membrane function, temperature acclimation, cell signaling, and immune system function (Bikshapathy *et al.* 2011; Hixson *et al.* 2016; Stanley-Samuelson *et al.* 1988). In insects, the fatty acid compositions are dependent on species, developmental stage, rearing conditions, sex, metabolic activity, and, most importantly, diet (Bozkus 2003; Dadd 1973; Lehtovaara *et al.* 2017; Oonincx *et al.* 2015; Sönmez *et al.* 2016; van Broekhoven *et al.* 2015). Most insects synthesize certain fatty acids such as palmitic acid *de novo*, from plant sugars and amino acids (Stanley-Samuelson *et al.* 1988). This is made possible with the help of the multi-enzyme complex, fatty acid synthase which utilises acetyl-CoA and malonyl-CoA as

precursors and integrates the fatty acids into the insect's body tissues (Canavoso *et al*. 2001). However, certain insect species lack the multi-enzyme fatty acid synthase (Stanley-Samuelson *et al*. 1988) for synthesizing *de novo* polyunsaturated fatty acids, particularly linoleic and α-linolenic acids, yet these fatty acids are essential for their survival, growth and reproduction. Therefore, they have to rely wholly on diet to offer those fatty acids (Dadd 1973; Hixson *et al*. 2016; Stanley-Samuelson *et al*. 1988). Thus, changes in diet could directly modify the fatty acid composition of insects. The few previous studies using *Gryllus assimillis*, *Zophobas atratus*, and *Harmonia axyridis* (Komprda *et al*. 2013; Sighinolfi *et al*. 2013) have shown that diet can modify the fatty acid composition of edible insects. However, Howard and Stanley-Samuelson (1996) found the opposite trend in the tenebrionid beetle, *Zophobas atratus*.

The edible grasshopper, *Ruspolia differens* (Serville) (Orthoptera, Tettigoniidae; Local (Luganda) name 'Nsenene') is a highly valued edible insect considered a delicacy in many parts of Eastern and Southern Africa (Agea *et al*. 2008; Bailey & McCrae 1978; Kinyuru *et al*. 2010; Nonaka 2009). It is one of the most economically important edible insects in the world (van Huis *et al*. 2013). For example, in Uganda, *R. differens* is prized for its taste aroma and eaten as a fried breakfast snack. Currently, *R. differens* is harvested wild from its natural swarming populations, which are seasonal and unpredictable (Bailey & McCrae 1978), but there are now attempts to develop mass rearing programs for local communities. Swarming *R. differens* have a high nutritional value and on average contain 48% fat, 45% proteins and 8% carbohydrates on dry weight basis (Kinyuru *et al*. 2010; Siulapwa *et al*. 2014). Furthermore, *R. differens* is rich in essential polyunsaturated fatty acids (PUFA, i.e., linoleic acid (31%) and α-linolenic acid (4.2% of the total fatty acid content) (Kinyuru *et al*. 2010). Thus, the selected feeds for rearing *R. differens*, should be optimized to improve the nutritional content, especially for the PUFAs. In our previous study, we found that rearing *R. differens* on various artificial diets with manipulated fatty acid, carbohydrate and protein content significantly altered the content and composition of fatty acids in *R. differens*, including the essential linoleic (omega 6) and α-linolenic (omega 3) fatty acids (Lehtovaara *et al*. 2017). However, the effect of plant diet diversification on total lipid content and composition of fatty acids in *R. differens* has not been explored. In Africa, sustainable mass rearing of *R. differens* will to a large extent depend on the use of inexpensive wild plant feed. Therefore, an understanding of the effect of plant diversification on the nutritional composition of *R. differens* is critical before full scale mass rearing programs can be rolled out for African communities.

In the present study, our aim is to evaluate how natural feed plants modify the lipid content and composition of fatty acids in individuals of *R. differens* collected from the local population in Uganda. We studied how increasing diversity in diet (ranging from a mono- to multi-species diet (i.e., one grass species vs. mixtures of two, three, and six grass species)) affect the total lipid content and composition of fatty acids in *R. differens*. Individuals collected from the field constituted a control treatment. Specifically, we asked; (i) Does the total lipid content and the body weight in *R. differens* differ among individuals in the diet treatments or between sexes, and is there an interaction between diet and sex? (ii) Does the fatty acid composition of *R. differens* differ among the diet treatments or between sexes, and is there an interaction between diet and sex? (iii) Does the proportion of essential fatty acids differ among the diet treatments or between sexes, and is there an interaction between diet and sex? We predicted that, if *R. differens* are fed on multi-species diet, the total lipid content and body weight would be higher than when fed on less-diverse diets. We further hypothesized that the fatty acid composition would differ among levels of dietary treatments, because on the multi-species diet, the insects can eat variable food with more variable sources of nutrients than on the mono-species diets (Hahn 2005; Unsicker *et al*. 2008). Furthermore, we predicted that the total lipid content and fatty acid composition would differ between the sexes because sex hormones in insects affect lipid and fatty acid metabolism (Guan-Wang *et al*. 2014; Irwin *et al*. 2008).

Materials and methods

Study insects and experimental site

We randomly collected 64 healthy individuals (32 males, 32 females) belonging to the sixth instar (Brits & Thornton 1981), of the local population of *R. differens* from fields around the Makerere University Agricultural Research Institute (MUARIK), Uganda. MUARIK is located about 20 km north of Kampala, lying at 0°27′03.0"N and 32°36'42.0"E with an average altitude of 1,200 m above sea level. The climatic conditions are those of a tropical wet and dry region with the mean annual rainfall at 1,160 mm distributed bi-modally (the two rain seasons lasting from March to June and from September to November) and a mean monthly temperature of 24.5°C. The rearing experiment was conducted at the Animal Science Laboratory, MUARIK.

Experimental set-up

To test if increasing diversity in natural diet has an effect on the total lipid content and fatty acid composition in *R. differens,* we used the freshly opened inflorescences of six known host plants of *R. differens* (Table 1). *R. differens* were assigned to four treatments ranging from a mono to multi-species diet (i.e., one grass species vs. mixtures of two, three,

Table 1 Feeding treatments of diversified diets assigned to *Ruspolia differens*. Treatment 1 (one grass species), Treatment 2 (two grass species), Treatment 3 (three grass species) and Treatment 4 (six grass species) and Treatment 5 control (fresh samples collected from the wild)

Treatment 1	Treatment 2	Treatment 3	Treatment 4
Ribbon bristle grass, *Setaria megaphylla* (Steud.) Dur. & Schinz	Ribbon bristle grass, *Setaria megaphylla* (Steud.) Dur. & Schinz	Ribbon bristle grass, *Setaria megaphylla* (Steud.) Dur. & Schinz	Ribbon bristle grass *Setaria megaphylla* (Steud.) Dur. & Schinz
	Pyramid grass *Sporobolus pyramidalis* (Beauv.)	Pyramid grass, *Sporobolus pyramidalis* (Beauv.)	Pyramid grass *Sporobolus pyramidalis* (Beauv.)
		Elephant grass, *Pennisetum purpureum* (Schumach. 1827)	Elephant grass, *Pennisetum purpureum* (Schumach. 1827)
			Couch grass, *Digitaria scalarum* (Schweinf.)
			Signal grass, *Brachiaria ruziziensis* (R. Germ. and C.M. Evrard)
			Rhodes grass, *Chloris gayana* (Kunth)

and six grass species, Table 1). The selection of grass species was based on those accepted by *R. differens* in preliminary feeding tests (P. Junes, unpubl. data).

The insects were reared in transparent flat bottomed plastic jars (1,000 mL, Thermopak Limited, Nairobi), measuring 12.5 cm diameter by 8 cm height, under laboratory conditions (temperature 22–28°C, photoperiod 12:12 L:D, and relative humidity 50–60%). Each jar contained one male and one female *R. differens*. We placed the jars atop a table in eight blocks in order to account for microclimatic conditions. Each block contained all four treatments (i.e., four jars; each with one male and one female *R. differens*) giving a total of 64 insects in 32 rearing jars. The position of the jars within each block was routinely shuffled during each inspection after every 12 hours. Moistened tissue paper was used as a source of water. To ease inspection and allow airflow, jars were covered with a transparent nylon net. The insects were given *ad libitum* access to food plants, which were routinely replenished every 48 hours, after thorough cleaning of the rearing jars. After 14 days, the insects were harvested and frozen at −80°C. For lipid extraction and further analysis, 30 individuals (four male and four female *R. differens* from each diet treatment; except in treatments one and two where three males and three females were used, respectively) were randomly selected. Furthermore, eight fresh samples of *R. differens* (four males and four females) were collected from the wild and used as control (Treatment five).

Lipid extraction

Before extraction, individual *R. differens* were removed from the deep freezer (−80°C) and allowed to thaw for one hour under ordinary laboratory conditions. The wings and legs were then plucked off, and the insect weighed. Lipid extraction followed a method by Folch *et al*. (1957). Briefly, approximately 0.2–0.5 g of each well ground and homogenized individual *R. differens* was extracted with chloroform–methanol mixture (2:1, v/v). Each sample was filtered and transferred into a separating funnel and added to a 0.2 volume of 0.9% sodium chloride. The samples were shaken to allow the two distinct layers to separate; the lower chloroform phase was transferred into a pre-weighed tube, then evaporated using a stream of nitrogen gas until a constant weight of the lipid was achieved.

Fatty acid profiling

The fatty acid composition was determined using gas chromatography–mass spectrometry analysis. About 10 mg of the lipid extract was transferred into a reaction vial containing an internal standard, nanodecanoic acid (C19:0) and 1 mL of acidified methanol. The vials were securely closed with teflon-lined screw caps and placed in an oven for two hours at 90°C to allow for complete methanolysis. After cooling at room temperature, methanol was evaporated down to half its original volume by a stream of nitrogen to make methyl esters less soluble in the methanol phase. Then, 0.5 mL distilled water, followed by 1 mL hexane, were added to the methanolised lipid fraction. The tube was capped tightly and mixed by shaking for 3 minutes, followed by centrifugation to separate the phases. Using a pipette, the upper hexane layer containing fatty acid methyl esters (FAMEs) was carefully transferred to the vial. The water–methanol phase was extracted twice using 1 mL n- hexane. The extracts were then pooled and stored under refrigeration until GC–MS analysis. Samples were then quantitatively analyzed using a GC–MS equipment (Agilent 6890-version N.05.05, GC-System, 5301 Stevens Creek Blvd. Santa Clara, CA 95051, USA) fitted with an electronic pressure control and mass selective detection (ionising energy, 70 eV; source

temperature, 250°C). One microliter of the mixed hexane extracts was injected splitless (the split would open after 4 min) and chromatographed on a 25 m × 0.25 mm (i.d.) fused silica column with polyethylene-glycol (PEG) as stationary phase, with a thickness of 0.2 μm (CP-WAX 52CB Chrompack, Middleburg, Netherlands) with helium as a carrier gas. The injector temperature was set at 260°C; the detector was set at 330°C. The oven was programmed as follows: 90°C for 4 min, 30°C/min to 165°C, then 3°C/min to 225°C where it was left to isothermal for 10.5 minutes before cooling for the next run. The quantitatively most common fatty acids were identified in the samples, by means of the standard mixture and by mass spectrometry and quantified using internal standard C19:0 also running the same method (Yang *et al*. 2006). The peaks were further integrated using Chemstation software (Thermo LabSystems) and the contribution of each of the fatty acids was calculated based on their relative retention times and peak areas. The relative amount of each common fatty acid is expressed as a percentage of the total fatty acids.

Data analysis

We tested for differences in the total lipid content and body weight among the five treatments, sexes and their interaction, by fitting two-way ANOVAs using type III sum of squares.

We also tested for differences in the proportions of fatty acids among the diet treatments and between sexes, and for their interaction, using a permutational multivariate analysis of variance (PERMANOVA) in PRIMER-E, version 6 (Anderson *et al*. 2008). PERMANOVA partitions the sources of variation in distance matrix using permutation tests with pseudo-F ratios. The method is robust as it makes no explicit assumptions of the distributions of the original variables and takes into account only the ranks of dissimilarities (Anderson 2001). It has become widely used in studies analyzing the fatty acid compositions (e.g., Galloway *et al*. 2012; Xu *et al*. 2016). To take into account the variation due to differences in body weight of individual *R. differens*, we included body weight as a covariate in the PERMANOVA design. Multivariate PERMANOVA analysis was conducted based on the Bray–Curtis similarity matrix between samples (individuals), computed from untransformed fatty acid proportions data. In the analysis, we included only the most common fatty acids used also in Kinyuru *et al*. (2010) and Nyeko *et al*. (2014). PERMANOVA was carried out using type I sums of squares (with 999 permutations), first including the covariate (body weight), then sex, followed by the diet treatment, and finally diet treatment–sex interaction. When significant differences were found, pairwise PERMANOVA tests were used to determine differences between levels of the diet factor. To ensure that our results were not confounded by the inclusion of ambient individuals as control (which did not experience the experimental rearing conditions), we re-ran the PERMANOVA model by including only treatments 1–4.

We visualized the patterns in fatty acid compositions among the diet treatments using the non-metric multidimensional scaling (NMDS). For clarity, also distances among centroids of dietary treatments are shown. To find out which fatty acids contributed most to the observed dissimilarities among the pairs of diet treatments, we used a similarity percentages routine (SIMPER) in PRIMER-E, version 6 (Clarke & Warwick 2001).

We also assessed the degree of variability in the relative proportions of fatty acids among samples in each treatment using permutational multivariate dispersion test (PERMDISP, Anderson *et al*. 2008). PERMDISP tests the homogeneity of multivariate dispersions within factor groups based on deviations from the group centroid.

To examine whether the proportions (pooled) of essential fatty acids were modified by dietary treatments, we performed univariate PERMANOVA (using Euclidian distance as a similarity measure, type III sum of squares), with treatment and sex (and their interaction) as fixed factors. When significant differences were found, pairwise PERMANOVA tests were used to determine differences between levels of each factor.

Results

Total lipids and body weight of individual *R. differens*

The total lipid content in g/100 g fresh weight (Table 2) did not show significant differences among the five treatments (ANOVA, $F_{4, 28} = 1.39$, $P = 0.26$) and between the sexes ($F_{1, 28} = 0.56$, $P = 0.46$). Also, there was no significant interaction between sex and treatment ($F_{4, 28} = 0.44$, $P = 0.78$).

There was also no significant difference in body weight (Table 2) among the five treatments (ANOVA, $F_{4, 28} = 1.96$, $P = 0.13$). However, there was a significant difference in body

Table 2 Body weight and total lipid (wet weight basis) content (marginal mean ± SE from two-way ANOVAs after sex has been taken into account) in *Ruspolia differens* in the five treatments (for treatments 1 and 2, n = 7 and for treatments 3, 4 and 5, n = 8)

Treatment	Body weight (g)	Lipid content (g/100 g)
Treatment 1: one host	0.33 ± 0.03	7.35 ± 1.52
Treatment 2: two hosts	0.33 ± 0.03	9.23 ± 1.52
Treatment 3: three hosts	0.34 ± 0.03	12.23 ± 1.41
Treatment 4: six hosts	0.40 ± 0.03	7.79 ± 1.41
Treatment 5: control (wild)	0.45 ± 0.03	10.29 ± 1.41

weight between the sexes ($F_{1,\ 28}$ = 4.71, P = 0.04) but no significant interaction between sex and treatment ($F_{4,\ 28}$ = 1.23, P = 0.32). The females (marginal mean = 0.41, SE = 0.02) were heavier than males (marginal mean = 0.33, SE = 0.02).

Fatty acid composition

The lipid fraction contained a total of nine common fatty acids across all treatments. These included four saturated fatty acids (SFAs; i.e., myristic, palmitic, stearic and arachidic acids), three mono-unsaturated fatty acids (MUFAs; i.e., mystoleic, palmitoleic and oleic acids) as well as two polyunsaturated fatty acids (PUFAs; i.e., linoleic and α-linolenic acids) (see Table 3). The predominant fatty acids were the SFA, palmitic acid with percentage composition ranging from 21 to 33% and MUFA, oleic acid with the percentage composition ranging between 15 and 26%.

The composition of fatty acids differed among the five diet treatments (Table 4) as illustrated by the NMDS ordination (Fig. 1). The composition of fatty acids in the multi-species diet (mixture of six grass species) was more similar to that of the wild individuals (treatment five), compared to the composition of fatty acids in individuals feeding on a mono-species diet (Fig. 1). In addition, there was a significant treatment–sex interaction (Table 4, Fig. 2), implying that diet diversification causes different changes in the composition of fatty acids in males compared to females. The largest source of variation in the composition of fatty acids of *R. differens* was explained by dietary treatment (26.7%), followed by treatment–sex (18.4%), sex (10.0%) and weight (9.6%; Table 4). According to the PERMANOVA pairwise tests, all treatments differed from each other significantly in the composition of fatty acids ($P < 0.05$) except between the diet with six grass species vs. control individuals collected from the wild (P = 0.19). When PERMANOVA was re-run with only treatments 1–4 (the reared individuals), the interpretation of the results did not change, except that weight (the covariate) no longer explained the fatty acid compositions significantly (Pseudo-$F_{1,\ 21}$ = 1.73, P = 0.17). However, the sex (Pseudo-$F_{1,\ 21}$ = 3.29, P = 0.022), diet treatment (Pseudo-$F_{3,\ 21}$ = 5.55, P = 0.001) and their interaction (Pseudo-$F_{3,\ 21}$ = 3.33, P = 0.001) did explain the composition of fatty acids significantly.

We also found a significant difference in degree of variability among treatments in relative proportions of fatty acids (Permdisp, $F_{4,\ 33}$ = 6.258, P = 0.003). The largest variability in similarities within treatments was observed in

Table 4 PERMANOVA model testing for the differences in fatty acid composition of *Ruspolia differens* among the five treatments, sexes and for interaction between sex and treatment. Analysis was done using type I sum of squares, fixed effects and used 999 restricted permutations of residuals under a reduced model

Source	df	SS	MS	Pseudo-F	P (perm)	Variation explained (%)
Weight	1	564.3	564.3	3.84	0.013*	9.6
Sex	1	348.9	348.9	2.38	0.067	10.0
Treatment	4	3028.7	757.2	5.15	0.001*	26.7
Treatment × sex	4	1169.9	292.5	1.99	0.029*	18.4
Residual	27	3966.8	146.9	-	-	35.2
Total	37	9078.7	-	-	-	

*$P < 0.05$.

Abbreviations: df, degrees of freedom; MS, Mean squares; SS, Sum of squares.

Table 3 Proportions (%) of fatty acids, total SFA, MUFA, PUFA and n6:n3 in *Ruspolia differens* under the five feeding treatments (mean ± SE; for treatments 1 and 2, n = 7 and for treatments 3, 4 and 5, n = 8)

Fatty acid	Treatment 1: one host	Treatment 2: two hosts	Treatment 3 three hosts	Treatment 4: six hosts	Treatment 5: control (wild)
Myristic acid (C14:0)	5.0 ± 0.8	7.6 ± 0.8	11.9 ± 2.5	3.5 ± 0.3	2.8 ± 0.7
Myristoleic acid (C14:1n5)	3.8 ± 0.6	4.6 ± 0.4	7.7 ± 1.6	2.7 ± 0.2	2.1 ± 0.5
Palmitic acid (C16:0)	32.7 ± 3.3	24.7 ± 1.2	28.2 ± 3.6	24.2 ± 1.6	20.9 ± 3.9
Palmitoleic acid (C16:1n7)	16.2 ± 1.4	17.9 ± 1.1	15.8 ± 2.5	17.9 ± 1.9	21.3 ± 1.5
Stearic acid (C18:0)	5.7 ± 0.6	7.9 ± 0.6	6.0 ± 0.9	7.7 ± 0.6	6.7 ± 0.6
Oleic acid (C18:1n9)	22.3 ± 1.9	23.9 ± 0.6	15.1 ± 1.5	25.7 ± 1.3	25.0 ± 2.8
Linoleic acid (C18:2n6)	11.5 ± 1.2	9.0 ± 0.5	10.7 ± 1.1	14.2 ± 1.0	19.6 ± 3.4
Arachidic acid (C20:0)	0.8 ± 0.2	1.8 ± 0.3	2.3 ± 0.5	1.6 ± 0.3	0.5 ± 0.1
α-linolenic acid (C18:3n3)	1.8 ± 0.2	2.6 ± 0.4	2.3 ± 0.3	2.5 ± 0.4	0.9 ± 0.1
∑SFA	44.3 ± 3.5	42.0 ± 1.3	48.4 ± 2.5	37.0 ± 1.7	31.0 ± 3.8
∑MUFA	42.4 ± 2.8	46.4 ± 1.4	38.6 ± 2.0	46.3 ± 1.4	48.4 ± 3.9
∑PUFA	13.3 ± 1.4	11.5 ± 0.8	13.0 ± 1.2	16.7 ± 1.3	20.6 ± 3.4
n6:n3	6.6 ± 0.7	4.1 ± 0.8	5.1 ± 0.7	6.6 ± 0.9	22.8 ± 1.5

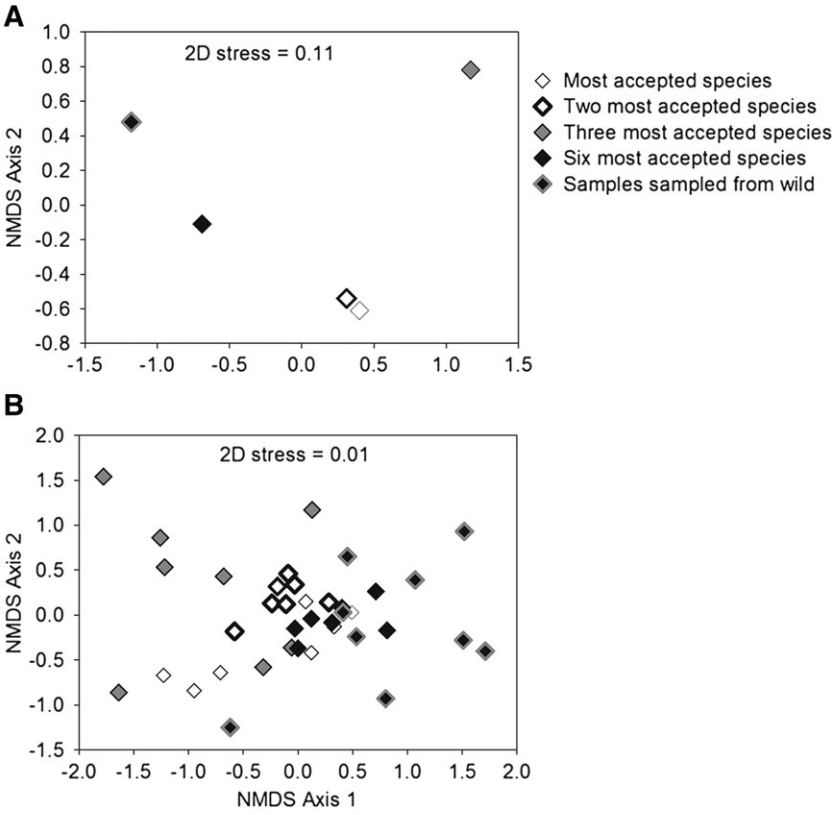

Figure 1 (a) Patterns in the fatty acid composition of *Ruspolia differens* under the five treatments based on non-metric multidimensional scaling (NMDS) ordination. The centroids within each treatment are shown. (b) NMDS ordination, but showing the patterns of fatty acid compositions among individuals. The closer the replicates are in the ordination space, the more similar was their fatty acid composition.

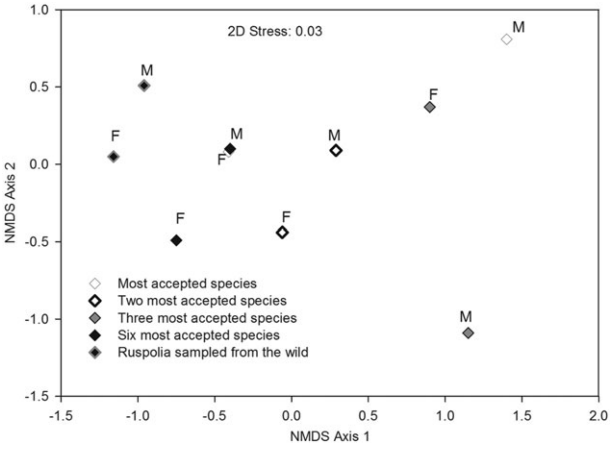

Figure 2 Patterns in the fatty acid compositions of *Ruspolia differens* under the five treatments, the sexes shown separately (based on non-metric multidimensional scaling NMDS in Fig. 1b). Letters M and F represent male and female, respectively.

treatment consisting of mixtures of the three grass species (mean ± SE; 15.67 ± 1.79) and the least variability was observed within treatment with the two grass species (mean ± SE; 5.84 ± 0.80; Fig. 1b).

Three fatty acids (palmitic, oleic and palmitoleic acids) made the strongest contributions to differences in fatty acid composition between the mono- and multi-species dietary treatments (SIMPER analysis; Table 5).

The proportion of essential fatty acids (when pooled) differed significantly among the treatments (PERMANOVA Pseudo-$F_{4,\ 28}$ = 3.44, P = 0.01), but did not differ between the sexes (Pseudo-$F_{1,\ 28}$ = 0.85, P = 0.39) and the interaction between treatment and sex was not significant (Pseudo-$F_{4,\ 28}$ = 0.68, P = 0.63). According to pairwise tests, the proportions of essential fatty acids differed significantly only between treatments 1 vs. 4, 2 vs. 4, 3 vs. 4, and 2 vs. 5 ($P < 0.05$). The proportion of essential fatty acids was highest in control treatment (21%) but low in simple (one to three feed) diets (12–13%).

Discussion

Our study shows that it is possible to rear *R. differens* harvested in the wild and modify their fatty acid composition based on their natural diet. Increasing dietary diversification from one to six feed diets alters the fatty acid composition of *R. differens* towards that resembling the fatty acid composition of wild *R. differens*. The proportions of the two main essential fatty acids (i.e., linoleic and α-linolenic acids) increase with increasingly diverse diet. The presence of substantial amounts of linoleic and α-linolenic acids suggest that local (non-

Table 5 Fatty acids in *Ruspolia differens* that contributed to the dissimilarity between different pairs of treatments (SIMPER analysis). Treatment 1 (one grass species), Treatment 2 (two grass species), Treatment 3 (three grass species) and Treatment 4 (six grass species) and Treatment 5 (Control-fresh samples collected from the wild). T = Treatment

Percentage contribution to treatment dissimilarities										
Fatty acids	T1&T2	T1&T3	T1&T4	T1&T5	T2&T3	T2&T4	T2&T5	T3&T4	T3&T5	T4&T5
Palmitic	**30.35**	**23.90**	**30.60**	**33.21**	17.78	15.63	22.04	16.52	**20.54**	**26.14**
Oleic	13.98	17.67	16.79	14.84	**21.56**	12.51	12.52	**21.73**	16.56	17.37
Palmitoleic	12.90	13.19	15.70	13.54	15.21	15.22	11.46	15.20	13.81	14.84
Myristic	10.97	16.79	6.04	6.25	15.93	15.62	11.33	17.22	15.09	4.69
Linoleic	10.65	7.96	12.45	20.03	7.42	**19.29**	**24.94**	8.70	15.56	20.52
Stearic	8.24	4.98	7.42	–	6.54	6.35	4.66	5.44	–	5.47
Myristoleic	5.58	10.07	4.90	4.79	9.73	7.26	6.25	10.22	9.34	–

Those that contributed the most are shown in bold.

swarming) populations of *R. differens* are nutritionally valuable to humans, who are unable to synthesize these fatty acids, and can only obtain them from the diet (Bjerve *et al.* 1993; Stanley-Samuelson *et al.* 1988).

Obviously, generalist feeding habits of Orthoptera, just as in *R. differens*, are beneficial in building up their fat body. Here, our results show that a multi-species rather than a mono-species diet generally enhance the proportions of essential fatty acids (linoleic and α-linolenic acids) in *R. differens*. This could be due to the fact that multi-species diets provide a large variety of fatty acids to the insect. In feeding, the insects and generally all animals aim at their 'nutrient intake target' (Fielding & Defoliart 2008), in order to allow them to fulfill their physiological functions such as reproduction. Several studies have shown that fatty acid composition in insects varies due to age, stage of development, reproductive activities, insects' state of health and, most importantly, diet (Finke 2013; Lehtovaara *et al.* 2017; Perez-Mendoza *et al.* 1999; Sihamala *et al.* 2010).

Furthermore, our results indicate that the fatty acid compositions of swarming and local (i.e., non-swarming) *R. differens* are similar. Here, the most common fatty acids of the non-swarming individuals were found in similar proportions as in previous works done with swarming *R. differens* (see, Kinyuru *et al.* 2010; Nyeko *et al.* 2014; Opio 2015) and in other insect orders (Bozkus 2003; Finke 2013). In line with previous studies (e.g., Kinyuru *et al.* 2010; Nyeko *et al.* 2014), fatty acids with carbon atoms beyond 20 as well as odd chain fatty acids such as C15, C17 and C21 were not among the most common fatty acids identified. This apparent lack of odd numbered fatty acids could be due to the fact that these fatty acids usually occur in trace proportions (Kinyuru *et al.* 2010). Palmitic and oleic acids were the most predominant fatty acids as also found in several previous studies in *R. differens* (Fombong *et al.* 2017; Kinyuru *et al.* 2010) and in other insect species (e.g., Nevin 1988; Pino Moreno & Ganguly 2015). This could be explained by the insects *de novo* system that synthesizes certain fatty acids such as palmitic acid from other sources (e.g., carbohydrates and proteins, Stanley-Samuelson *et al.* 1988). The proportion of palmitic acid was generally comparable to that reported for wild *R. differens* (32%) by Kinyuru *et al.* (2010).

In this study, the total lipid content of *R. differens* did not differ among diet treatments and between the sexes. This shows that the needs for total lipids in *R. differens* can be fulfilled even with simple diets in both sexes. However, this lack of differences in the total lipid content could be attributed to the developmental stage of the experimental insects (i.e., sixth instar nymphs but reared for two weeks). Therefore, there is a possibility that sex specific lipid content differences were yet to show, but if allowed a longer maturation period, the difference in lipid content might evidently become clear. Bukkens (1997), found that grasshoppers and generally orthopteran species possess low lipid contents (i.e., 3.8–5.3 g/100 g fresh weight) which is low compared to the *R. differens* analyzed in this study (i.e., 7.4–12.2 g/100 g fresh weight). In comparison to other orthopteran species (see, Bukkens 1997), our experimental insects had a high lipid content. The high lipid content could be attributed to the study insects being premature and possibly had not yet fully utilised the lipid reserves accumulated during other nymphal stages.

To conclude, this study shows that it is possible to rear *R. differens* harvested in the wild and modify their fatty acid composition based on mixtures of its natural diet. Diversifying diet composition from a mono- to a multi-species diet modifies the fatty acid composition towards that resembling the fatty acid composition of wild *R. differens*. Thus, with diet diversification, it might be possible to produce *R. differens* with preferred high quality essential fatty acids for humans.

Author contribution

Experiments were designed by KR, HR, PN, AV and GMM, and fieldwork, laboratory studies, statistical analysis and drafting of the manuscript was conducted by KR. JK helped in fatty acid analysis. All authors contributed in the analysis and interpretation of the data, writing and review of the manuscript.

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