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# Growth performance and feed conversion of *Ruspolia differens* on plant-based by-product diets

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**Abstract**

The use of food industry by-products in insect feeds has gained increasing attention recently. However, the understanding of how well the economically valuable edible insect *Ruspolia differens* (Serville) (Orthoptera: Tettigoniidae) can grow and develop with plant-based by-product feeds is currently lacking. It is important to determine the nutritional requirements, especially protein demand, of this species before developing artificial feeds for mass-rearing. We reared *R. differens* with four control diets and 12 plant-based by-product diets in which the major protein source came from food industry by-products, including potato-protein, barley mash, barley feed, turnip rape, a mix of broad bean and pea, and a mix of potato, carrot, and apple. We asked whether the performance (development time, survival, and weight), feed conversion, and fatty acid composition and content differed among diet treatments. Furthermore, the 12 experimental by-product diets were designed to reach six protein levels. We found that *R. differens* can be reared with various by-product diets, but development time, survival, and weight differed among diets. Barley feed, barley mash, and potato protein diets seem to be good options for rearing, and potato glycoalkaloids do not affect the performance of *R. differens*. Individuals fed on the various by-product diets also differed in their fatty acid composition and content. Increasing protein levels in diet up to 17% enhanced growth, development time, and survival, but no further enhancements were seen when fed diets with protein levels higher than this. The high protein levels decreased feed conversion rate. Our results can be valuable for designing feeds for insect mass-rearing technology. The use of food industry by-products in the diets for *R. differens* could increase the re-use of local resources and enhance circular economy.

## Introduction

*Ruspolia differens* Serville (Orthoptera: Tettigoniidae) – also known as 'nsenene' in Uganda, or 'African bush-cricket' – is one of the most important edible insect species for human consumption in sub-Saharan Africa (van Huis et al., 2013). It has high cultural and economic value in East Africa, for example in Uganda and Tanzania (Mmari et al., 2017; Okia et al., 2017) where it is harvested from the wild during the two annual swarming seasons (van Huis et al., 2013). *Ruspolia differens* is highly nutritious as it contains essential amino acids, fatty acids, vitamins, and minerals (Kinyuru et al., 2010; Siulapwa et al., 2014). In addition, the fatty acid composition of *R. differens* can be manipulated with its diet (Lehtovaara et al., 2017; Rutaro et al., 2018a,b), i.e., with specific feeds, it can become even more suitable for human consumption. Recently, there have been attempts to develop mass-rearing methods for this species (Lehtovaara et al., 2017, 2018; Malinga et al., 2018a,b; Rutaro et al., 2018a,b), which could prevent overharvesting of the wild populations in the long-term. *Ruspolia differens* has potential for mass-rearing in the Lake-Victoria basin, where there are long traditions for consuming this species, but also elsewhere, as the species is nutritionally valuable due to its healthy fatty acids.

The design of appropriate feeds is one of the key issues in developing mass-rearing methods for this species. In the

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wild, *R. differens* is a selective grass-feeder, preferring inflorescences over leaves (Opoke et al., 2019). In the laboratory, it accepts, for example, wheat, finger millet, rice, chicken feed, and sorghum, as well as many East-African grasses (Lehtovaara et al., 2017; Valtonen et al., 2018; Malinga et al., 2018a,b). An interesting future possibility is to design feeds for edible insects which utilize food industry by-products (Collavo et al., 2005; Oonincx et al., 2015; Miech et al., 2016). This would create possibilities for more efficient use of local resources, avoid or lessen the use of ingredients which could be consumed directly by humans, and enhance circular economy. However, the performance of *R. differens* on diets, including food industry by-products, is currently unknown. Also, the protein requirement in its feed is not well-understood, but is needed for the mass-rearing of this species.

Even though by-product feeds for insects might improve the sustainability of mass-rearing (Smetana et al., 2016), the diet also needs to fulfill the nutritional requirements of the insects. The nutritional value of the diet determines the insects' survival and development time (Cohen, 2004; Chapman, 2013), which are the typical performance variables used to determine the diet quality (Cohen, 2004). The by-product diets should also allow maximal weight gain during rearing and high feed efficiency to increase the sustainability of feed. In addition, the nutritional quality, such as fatty acid composition, of the insects could be modified by the by-product feeds. Also, the by-products can contain toxins that are harmful to humans (e.g., plant glycoalkaloids), and therefore it must be ensured that the insects fed on by-products are safe for human consumption.

The purpose of this study was to evaluate how *R. differens* grow and develop with 12 by-product diets compared to four control diets, how the protein level of the feed explains growth performance, and how the diet treatment modifies the fatty acid content and composition. Our specific study questions were: (1) do the development time, survival, weight, and feed conversion rate (FCR) differ among the diet treatments? (2) Does the protein level of the diet explain development time, survival, and weight? And (3) does the fatty acid composition and the content of total fatty acids (TFA), saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) differ among the diet treatments?

## Materials and methods

### Study insects

The *R. differens* individuals used in this experiment were derived from the laboratory population at the University of Eastern Finland that originated from grasslands surrounding the Makerere University Agricultural Research Institute Kabanyolo (MUARIK), Uganda. Prior to the experiment, i.e., during the first three instars, the nymphs were fed a standardized mixture of oatmeal, reindeer pellets (Poro-Elo, Suomen rehu, Hyvinkää, Finland), chicken feed (Milka kanatäysrehu, Biofarm, Karkkila, Finland), and mealworm meals (Suomalainen, 1999). Additionally, a piece of carrot, fresh shoots of oats, and water absorbed in cotton wool and placed in a plastic cylindrical jar were provided. Nymphs were kept in groups of 6–10 individuals in 0.75-l plastic containers (17 × 12 × 14 cm). A piece of tissue paper was placed hanging in the roof to provide hiding places and places for molting. The individuals were kept at 28–30 °C, 50–70% r.h., and L12: D12 photoperiod.

### Experimental diets

The experiments included 16 diet treatments, of which 12 included by-products (Table 1, Table S1). The four control diets included: (1) chicken feed, (2) reindeer feed, (3) mealworm diet (Suomalainen, 1999), and (4) mealworm diet (Suomalainen, 1999), with Vanderzant vitamin and Wesson mineral mixtures (Cohen, 2004). The by-product diets were based on modified Suomalainen (1999) and Patton's (1967) diet no. 16, in which the major protein source, soybeans, was partly or totally replaced with by-product protein sources. Based on their ample availability from food industries at the location where the experiments were conducted (Finland), the following by-products were selected as ingredients in the by-product diet treatments: potato-protein, barley mash, barley feed, turnip rape, a mix of broad bean and pea, and a mix of potato, carrot, and apple (Table 1). The diets were designed with WinOpti, a feed optimization software program (AgroSoft, Tørring, Denmark) to reach one of six protein levels (7.2, 10.0, 15.0, 17.4, 22.5, and 30.5%). As the by-products differ in nutritional content, and our aim was to design feeds of certain protein levels, the amount of by-products in each diet differed (Table S2).

### Experimental design

The experiment was arranged as a randomized block design to determine whether the 16 diet treatments explained the growth performance, feed conversion, or fatty acid composition and content of *R. differens*. The randomized block design allowed us to reveal the differences among diets while the environmental variation was controlled. The replicates were 3-l plastic rearing containers (11.5 × 18.5 × 18.5 cm) each having a 5-cm-diameter ventilation hole in the lid covered with mesh. Four fourth instars (± 1 day from molting) (Brits & Thornton, 1981)

**Table 1** Nutritional content and details of experimental diets. For potato protein diets, codes 2.5, 5.0, 10.0, and 20.0 describe the percentage of potato protein in the diet. The percentage values after each by-product diet name describe the protein level

| Diet name | Protein (%) | Carbohydrate (%) | Fat (%) | Base of by-product diets | % by-product in diet | By-product | Source |
|---|---|---|---|---|---|---|---|
| Control diets | | | | | | | |
| Chicken feed | 15.2 | 56.6 | 4.4 | | | | Milka Kanatäysrehu, Biofarm Oy |
| Suomalainen (1999) | 17.4 | 60.4 | 4.6 | | | | Suomalainen (1999) |
| Suomalainen + vitamins | 17.4 | 58.3 | 4.3 | | | | |
| Reindeer feed | 11.7 | Not known | Not known | | | | Poro-Elo, Hankkija Oy |
| By-product diets | | | | | | | |
| Potato-2.5, 17% | 17.4 | 56.5 | 4.7 | Suomalainen (1999) | 2.5 | Potato protein[1] | Finnamyl Oy |
| Potato-5.0, 17% | 17.4 | 60.1 | 4.0 | Suomalainen (1999) | 5 | Potato protein[1] | |
| Potato-10.0, 30% | 30.5 | 51.2 | 4.0 | Patton (1967) | 10 | Potato protein[1] | |
| Potato-20.0, 30% | 30.5 | 52.2 | 4.1 | Patton (1967) | 20 | Potato protein[1] | |
| Barley mash 22.5% | 22.5 | 58.0 | 6.5 | Patton (1967) | 41 | Barley mash[2] | Panimo Honkavuori Oy |
| Barley mash 15% | 15.0 | 66.0 | 5.4 | Patton (1967) | 20 | Barley mash[2] | |
| Barley feed 22.5% | 22.5 | 58.2 | 7.4 | Patton (1967) | 44 | Barley feed[3] | A-rehu |
| Barley feed 15% | 15.0 | 66.0 | 6.5 | Patton (1967) | 31 | Barley feed[3] | |
| Broad bean pea 15% | 15.0 | 66.0 | 4.2 | Patton (1967) | 13 | Broad bean and pea[4] | Boreal Plant Breeding, Apetit Oyj |
| Turnip rape 15% | 15.0 | 66.0 | 5.0 | Patton (1967) | 7 | Turnip rape[5] | Kankaisten Öljykasvit Oy |
| Vegetable 7.2% | 7.23 | | 7.52 | | 89 | Potato protein[1], carrot[6], apple[7] | Finnamyl Oy, anonymous apple juice producer |
| Vegetable 10% | 9.75 | | 7.54 | | 89 | Potato protein[1], carrot[6], apple[7] | |

[1–7]By-product of [1]potato flour production, [2]beer production, [3]ethanol production, [4]common protein sources in Finland (excess of production), [5]rape seed oil production, [6]vegetable industry, [7]apple juice production.

were placed randomly in each container (Figure S1). The experiment involved a total of 160 containers.

Each container was randomly subjected to one of the 16 diet treatments (Table 1), each with 10 replicates. The only exception was the Suomalainen diet with 20 replicates. The diets were applied over the course of two experimental times (Figure S1), with the Suomalainen diet applied on both experimental times. In each experimental timing, the containers were randomized in blocks so that each block included one replicate of each experimental diet. There were in total 10 blocks (on each experimental time), which represented thermally regulated growth chambers, and they were in two thermally regulated rooms.

In each container, the experimental diet was offered on a Petri dish. At the start of the experiment, 2 g of feed was offered. In addition, water was absorbed in cotton wool and placed in two cylindrical jars, and a fresh piece of carrot were offered. Two pieces of tissue paper were placed hanging in the roof to provide hiding places and places for molting. All containers were checked 3× a week and 1 g of feed was added, when needed, to allow insects to feed ad libitum. At the same time, a fresh piece of carrot and water were added. The individuals were kept at 30 ± 1.5 °C and L12:D12 throughout the experiment.

#### Measured variables

The starting weight of each insect was measured individually at the beginning of the experiment. Before weighing, individuals were stunned with $CO_2$. The development time (days) and feed left-over (g) in each container were recorded when half of the individuals reached the adult stage in each rearing container. The experiment was

terminated 1 week after half of the insects reached adult stage in each container. At the same time, the final weight, number of individuals alive (survival), and left-over feed weight was recorded. The insects were not marked individually, so we were unable to match the start weight to later measurements on an individual basis. For containers with no emerged adults by 81 days after the start of the experiment, the experiment was terminated on that day. These containers were excluded from the analyses of development time. Feed conversion rate was calculated based on a formula used by Wilkinson (2011), using the fresh matter from the beginning until the termination of the experiment as follows: feed given/weight gain. The lower the FCR index value is, the higher the feed efficiency. After the experiment was terminated, the insects were frozen and kept at −25 °C until further use.

#### Fatty acid analysis

We analysed the fatty acids of both diets and *R. differens* individuals reared on each diet. Five females from each diet were randomly selected for the fatty acid analyses. As an exception, for four treatments (potato-10.0, 30%, potato-20.0, 30%, vegetable 7.2%, and broad bean 15%), only four females were selected because not enough females survived until adulthood (n = 76). Before fatty acid analyses, samples were first freeze-dried using an Alpha 1-4 LD Plus freeze dryer (Martin Christ Gefriertrocknungsanlagen, Osterode am Harz, Germany) (main drying 24 h + final drying 6 h) and the wings were removed. Also, one composite sample of each diet treatment was freeze-dried (main drying 24 h+ final drying 6 h) for fatty acid analyses.

The fatty acid analyses were performed with the direct transmethylation method (Sukhija & Palmquist, 1988) with minor modifications as described by Lehtovaara et al. (2017) at the Bio-Competence Centre of Healthy Dairy Products (Bio–CC, Tartu, Estonia). Gas chromatography with flame ionization detection (GC-FID) was used to quantify the fatty acid methyl esters (FAME), and common fatty acids were identified by comparing sample peak retention times with FAME standards. Fatty acids were quantified based on peak areas in relation to the internal standard.

#### Glycoalkaloid analysis

Four female individuals from each diet which included potato protein were randomly selected for glycoalkaloid analyses (n = 16). Insects were first freeze-dried (main drying 22 h+final drying 1 h). The wings were removed before the analysis. The glycoalkaloid analyses were carried out at the Centre of Food and Fermentation Technologies (Tallinn, Estonia). The ultra performance liquid chromatography/mass spectrometry (UPLC-MS) internal standard method was used to determine the α-solanine and α-chaconine concentrations. The results of the glycoalkaloid analysis are shown in Table S3.

#### Statistical analysis

We fitted general linear mixed models to test whether the development time, weight at the end of the experiment, and FCR differ among the diet treatments (Table S3). For survival, we fitted generalized linear model (binary logistic model), where the dependent variable was the number of individuals that survived to the end of the experiment (events) out of individuals at the start (trials). In all models, the terms describing the structure of the experimental design (Figure S1, Table S4) were used as random factors. The male ratio (number of males/number of females at the end of the experiment) was included as a covariate for models of development time, survival, and FCR, where the response variable was measured from each container. For the model of weight (measured from each individual at the end of the experiment), the sex of the individual was included as a fixed factor. Spearman's correlation test was used to measure the degree of association between protein level and each performance variable: development time, survival, and weight. All statistical analyses for performance variables and FCR were done with IBM SPSS Statistics v.25 (IBM, Armonk, NY, USA).

We visualized the fatty acid compositions (% fatty acids) among diets and *R. differens* fed on these diets with nonmetric multidimensional scaling (NMDS). For this, we used Bray Curtis similarity matrix, where samples were individuals or composite samples of the diet. Fatty acids with levels of 0.05% and above in a sample were included in the analysis.

We used the permutational multivariate analysis of variance (PERMANOVA+routine of Primer-E) (Anderson et al., 2008) to test the differences in fatty acid composition among the diet treatments of *R. differens* (type III sums of squares and 999 permutations). We also conducted the PERMDISP routine to find out whether there were differences in degree of variability among diet treatments.

The RELATE routine was used to test the matching of the multivariate patterns in the two data sets (insects and their feed), by calculating Spearman's rank correlation between the similarity matrices (Clarke & Gorley, 2006). For this, we used similarity among centroids of *R. differens* individuals and the similarity of feed. The multivariate analyses were conducted with untransformed values, which emphasized the most common fatty acids. To emphasize the rare fatty acids, we repeated the multivariate analyses using a fourth-root transformation for the fatty acid composition. The multivariate analyses were

conducted in PRIMER v.6 (Clarke & Gorley, 2006) and PERMANOVA+ for PRIMER (Anderson, 2008). Finally, to test whether there were differences in fatty acid content (mg FA $g^{-1}$) of *R. differens* among diet treatments (i.e., TFA, SFA, MUFA, and PUFA content), one way-ANOVA-models were fitted in IBM SPSS Statistics v.25.

## Results

### Development time, survival, and weight

Development time, survival, and fresh weight differed among diet treatments (Table 2). Low protein content (7–10%) prolonged the development time, whereas a protein content of 15–22% generally led to shorter development times. The fastest development was obtained with barley feed (22% protein content); on average, *R. differens* developed from fourth instar into adulthood in 25 ± 3 days (mean ± SEM; Figure 1A). For two low-protein vegetable diets, vegetable 7.2% and vegetable 10%, the development time was approximately twice as long: 49 ± 5 and 56 ± 3 days, respectively. Additionally, none of the individuals in seven vegetable-7.2% and three vegetable-10% rearing units reached the adult stage (units excluded from this model). The development time correlated negatively with protein level. When protein levels increased, the development time was shorter (Spearman's rho = −0.74, P = 0.001) but protein level higher than 17.4% did not shorten the development time further (Figure 1D).

Overall, the survival of *R. differens* increased along with the protein content in the feed (rho = 0.43, P = 0.001), but protein levels higher than 17.4% did not show any additional increase in survival (Figure 1E). Survival rates differed among the diet treatments (Table 2) and out of the by-product diets, *R. differens* survived the best on barley feed and potato protein diets (Figure 1B). Among all the diet treatments, the highest survival (84.1 ± 5.8%) was observed for the Suomalainen diet with vitamin and mineral supplement. The lowest survival (35.6 ± 8.9%) was observed in the broad bean and pea diet.

The final fresh weights differed among the diets (Table 2). The two Suomalainen control diets and the potato-protein by-product diets resulted in the highest final weights (Figure 1C). The highest weight (mean ± SEM = 0.507 ± 0.042 g) was observed in the Suomalainen diet with vitamin and mineral supplement. The lightest individuals were observed in the vegetable 7.2% diet (0.264 ± 0.06 g) and the broad bean and pea diet (0.290 ± 0.051 g). The weight correlated with the protein level of the diet (rho = 0.38, P = 0.001). Weight increased when the protein level of the diet increased up to 17%, but protein levels higher than that did not increase the weight (Figure 1F).

In summary, the best overall performance was obtained with the control diet Suomalainen + vitamins and the by-product diets containing protein level of 17.4–22.5%. These diets led to heavier individuals, faster development, and higher survival rates than low-protein diets. However, out of the by-product diets, the broad bean and pea diet led to low survival and low individual weight compared to other by-product diets with comparable protein level. Low-protein vegetable diets led to the lowest final weight, lowest survival, and slowed development.

**Table 2** Details of the general linear models for development time, weight, and feed conversion rate (FCR) and generalized linear model explaining the survival of *Ruspolia differens*. For model of development time, survival, and FCR measured from each rearing unit, 'male ratio' was added as a covariate. For model of weight, 'sex' was added as a fixed factor

| | F | d.f. | P | Coefficient |
|---|---|---|---|---|
| Development time | | | | |
| Diet treatment | 7.23 | 15,126.4 | <0.001 | −4.928 |
| Male ratio | 3.995 | 1,139.95 | 0.048 | |
| Survival | | | | |
| Diet treatment | 2.462 | 15,145 | 0.003 | −0.896 |
| Male ratio | 10.207 | 1,145 | 0.002 | |
| Weight | | | | |
| Diet treatment | 2.756 | 15,28.17 | 0.01 | −0.178 |
| Sex | 22.161 | 1,398.01 | <0.001 | |
| FCR | | | | |
| Diet treatment | 2.625 | 15,106.99 | 0.002 | 0.678 |
| Male ratio | 0.029 | 1,118.11 | 0.87 | |

### Feed conversion rate

The FCR differed among the diet treatments (Table 2), and it correlated negatively with the protein content of the diet (Spearman's rho = −0.33, P<0.001; Figure 2). The feed efficiency improved when the protein level of the diet increased. The lowest FCR (i.e., the highest efficiency) was found in the potato-20, 30% diet (mean ± SEM = 0.63 ± 0.61) and the highest FCR (lowest efficiency) in the vegetable-10% diet (4.28 ± 0.76). The FCR of the potato-20% diet was only approximately half of the other potato protein diets.

### Fatty acid composition

The most common fatty acids found in *R. differens* were palmitic acid (C16:0), stearic acid (C18:0), palmitoleic acid (C16:1:c9), oleic acid (C18:1:c9), linoleic acid (C18:2n6), and α-linoleic acid (C18:3n3) (Figure 3). The predominant fatty acid was oleic acid (C18:1c9), ranging from 32 to 51%.

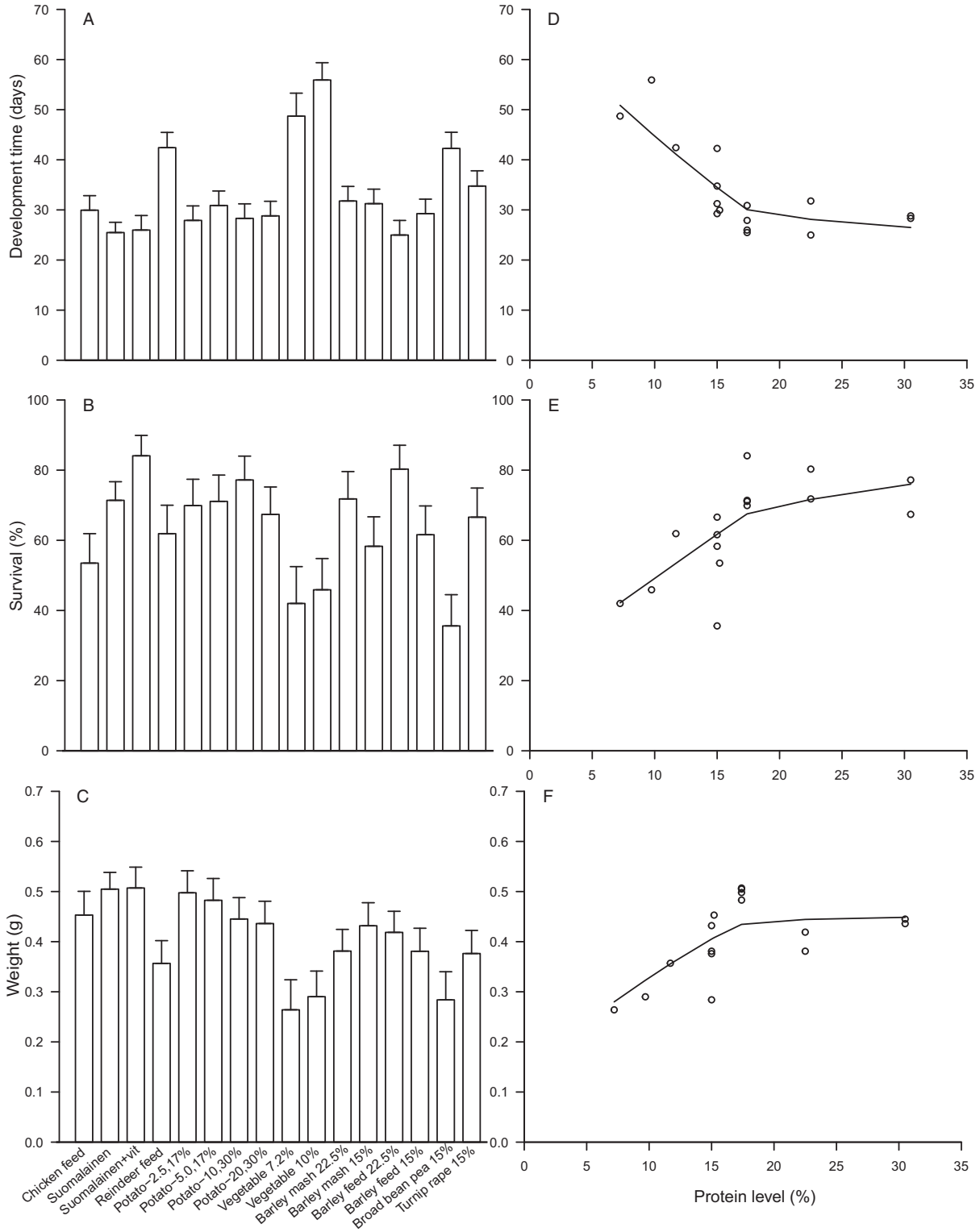


**Figure 1** Left: estimated marginal means (+ SEM) of *Ruspolia differens* (A) development time (days) from fourth instar until adult stage, (B) survival (%), and (C) weight (g) measured 1 week after half of the insects reached adult stage in one rearing unit on the 16 diet treatments. Right: estimated marginal means of (D) development time, (E) survival, and (F) weight against protein level of the diet. The lines are estimated Loess curves obtained by 'gam' function (span = 1) in *gam* package of R v.3.3.2 (R Core Team, 2014).

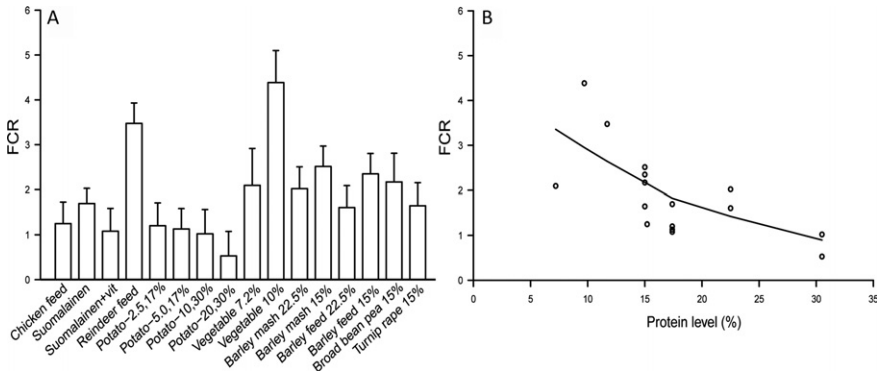


**Figure 2** Estimated marginal means (+ SEM) of *Ruspolia differens* (A) feed conversion rate (FCR; i.e., amount feed given/weight gain) on the 16 diet treatments, and (B) estimated marginal means of feed conversion rate shown against protein level of the diet. The line is an estimated Loess curve obtained by 'gam' function (span = 1) in *gam* package of R v.3.3.2 (R Core Team, 2014).

The fatty acid composition of *R. differens* differed among diet treatments (un-transformed dataset; pseudo-$F_{15,60} = 8.164$, $P<0.001$) as illustrated in NMDS ordination (Figure 4). There was also a difference in the degree of variability among diet treatments in fatty acid composition (PERMDISP: $F_{15,60} = 3.995$, $P = 0.009$). The fatty acid composition similarity matrix of the diets did not correlate with the fatty acid composition similarity matrix of *R. differens* individuals (RELATE analysis; $P>0.05$) (Figures 4 and 5).

The NMDS was repeated with the fourth-root-transformation of fatty acid compositions in order to emphasize the rare fatty acids (Figure S2), and there was a difference among the diet treatments (PERMANOVA: $P = 0.001$). Furthermore, there was no difference in the degree of variability among diet treatments (PERMDISP: $P>0.05$). The fatty acid composition similarity matrices of diet and individuals did not correlate (RELATE: $P>0.05$).

#### Fatty acid content

The TFA content (mg FA $g^{-1}$) of insects differed among diet treatments ($F_{15,60} = 7.472$, $P<0.001$). The highest TFA content was in the vegetable diets (Figure 6). On the contrary, the lowest TFA content was in the broad bean

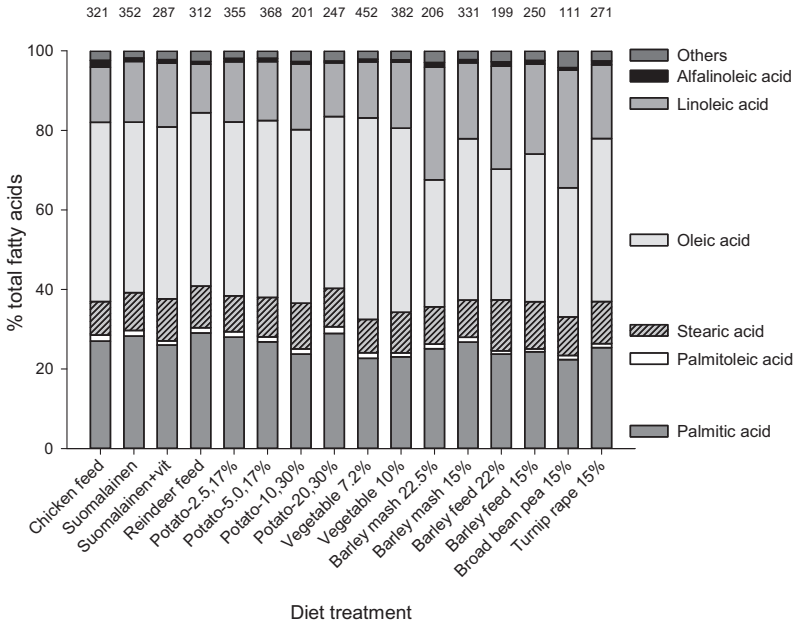


**Figure 3** Distribution of the six most common fatty acids (% of total fatty acids) of *Ruspolia differens* fed on the 16 diet treatments. Fatty acids with a mean composition of >0.5% are shown. The numbers at the top of each bar indicate the total fatty acid content (mean mg $g^{-1}$ dry weight).

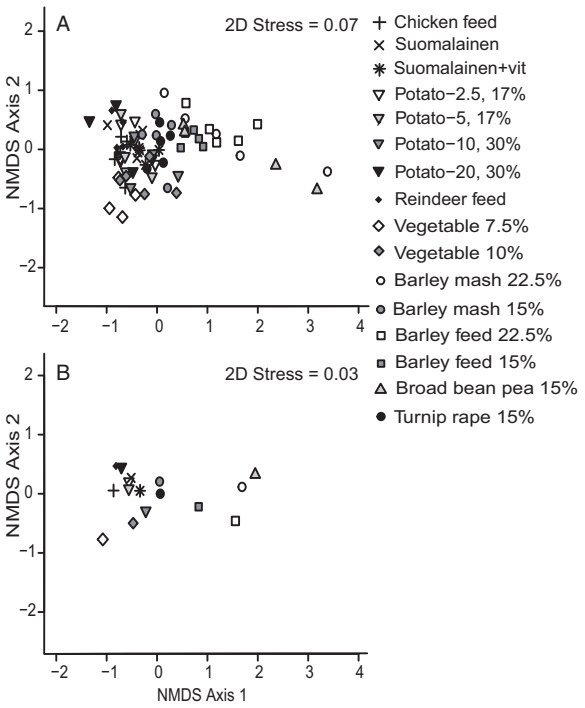


**Figure 4** Nonmetric multidimensional scaling (NMDS) ordination showing fatty acid composition of *Ruspolia differens* individuals that fed 16 experimental diets. The closer the individuals are in the ordination, the more similar is their fatty acid composition. (A) All individuals separately, and (B) the centroids of each diet treatment for clarity.

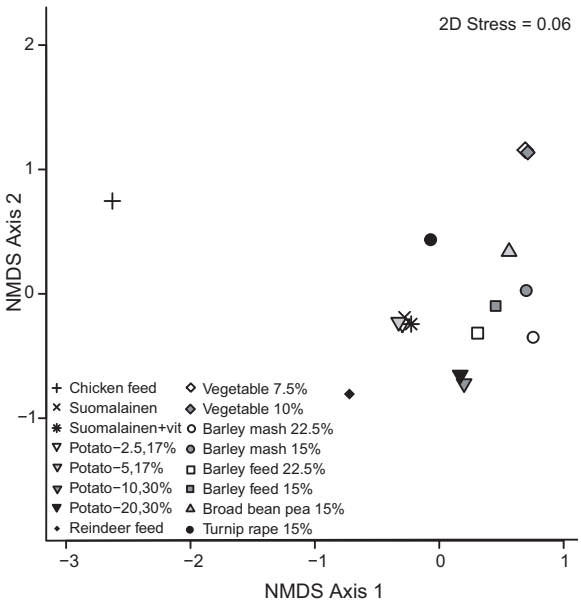


**Figure 5** Nonmetric multidimensional scaling (NMDS) ordination showing fatty acid composition of the 16 experimental diets. The closer the data points (diets) are in the ordination, the more similar is their fatty acid composition.

and pea diet (Table 3). Also, the contents of SFA ($F_{15,60}$ = 3.891), MUFA ($F_{15,60}$ = 12.947), and PUFA ($F_{15,60}$ = 7.469, all P<0.001) differed among the diets. High PUFA contents were found in barley mash, barley feed, and broad bean diets (Table 3).

## Discussion

This study showed that it is possible to rear *R. differens* on various by-product diets and to modify its fatty acid composition with the diet. *Ruspolia differens* accepted many diets of different nutritional content with varying by-products as the major protein source.

The development time and final weight of *R. differens* in this study were comparable to those found in previous rearing studies where they were fed with natural grasses (Rutaro et al., 2018c) or artificial carbohydrate-rich and protein-rich diets (Lehtovaara et al., 2017). Here, the development of *R. differens* from the fourth instar to adult stage took on average 29–59 days. In previous laboratory studies, the development from the fifth instar to adulthood took 17–23 days on diets with a high content of fatty acids, 42 days on carbohydrate-rich diets, and 18 days on protein-rich diets (Lehtovaara et al., 2017). Overall, the final weights in our study were similar to those of emerged adults reared on natural grasses (0.41–0.45 g) (Rutaro et al., 2018c) and artificial carbohydrate-rich and protein-rich diets (0.55 and 0.56 g) (Lehtovaara et al., 2017). Higher weights were reported with diets that had a high fatty acid content (0.64–0.95 g) (Lehtovaara et al., 2017).

*Ruspolia differens* rearing experiments with various by-product diets demonstrated their extensive ability to efficiently utilize by-products in their diets but, as expected, they also showed performance differences on different diets (Table S5). The most favorable diet for the growth and development of *R. differens* was based on the Suomalainen (1999) recipe. This diet is rich in carbohydrates (60.4%), with relatively low contents of protein (17.4%) and fat (4.6%). In this study, it resulted in one of the shortest development times, highest survival rates, and the highest final weights. Many by-product diets, including potato protein, barley feed, and barley mash, led to high survival and normal growth and development compared to control diets. These diets were also rich in carbohydrates (51.2–66.0%), with varying contents of protein (17.4–30.5%) and fat (4.0–7.4%). Generally, herbivorous insects require rather high carbohydrate levels in the diet. For example, grasshoppers of genus *Schistocerca* require 20% of sugar in their diet for normal growth, but insects feeding on seeds

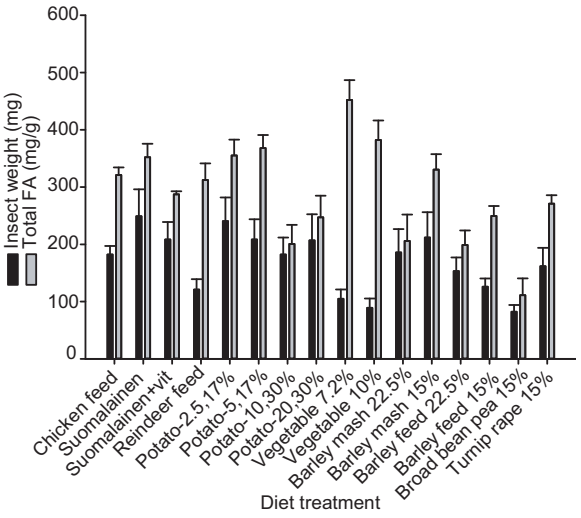


**Figure 6** Mean (+ SEM) total fatty acid content (FA; mg $g^{-1}$ dry weight) and dry weight (mg) of *Ruspolia differens* individuals that fed 16 experimental diets.

and grains require up to 70% carbohydrates (Panizzi & Parra, 2012). There are also many other factors that determine normal growth and development in insects, such as amino acid composition of diet, vitamins and minerals, sterols, and feeding stimulants and deterrents (Cohen, 2004; Klowden, 2007; Panizzi & Parra, 2012). However, findings of this study show that many by-product diets are sufficient for the performance of *R. differens* with the exception of low-protein vegetable diets, broad bean, and pea diets, and reindeer feed that seem to lack one or multiple of these components and are deficient to build a substantial fat body.

As expected, protein levels were a very important factor for all measured performance variables, but the effect of proteins saturated rather low, at approximately 17.4% protein. The higher protein levels increased weight gain, survival rate, and shortened development time of *R. differens*. Conversely, the low protein diets slowed the development, caused lower survival rates, and the insects tended to be smaller after they reached adult stage. An increase in protein in the diet favored the weight gain of the grasshopper *Ageneotettix deorum* Scudder (Joern & Behmer, 1997). In addition, the higher protein level in the diet increased the protein storage levels of *Heliothis virescens* Fabricius (Telang et al., 2002). In this study, the performance of *R. differens* increased with diets up to 17.4% protein, but higher protein levels did not drastically increase the overall performance. Amino acids are the building blocks of protein, and high enough protein content enables normal growth and development. However, in contrast with fats and carbohydrates that are easily stored, excessively consumed amino acids are generally utilized as metabolic fuel or excreted (Klowden, 2007). When rearing *R. differens*, it is important that the diet fulfils the protein and other nutritional requirements to ensure good growth and development but also minimizes the cannibalism that has been observed for this species (Lehtovaara et al.,

**Table 3** Mean fatty acid content (mg $g^{-1}$ dry weight) and dry weight (mg) of *Ruspolia differens* individuals reared on 16 diet treatments. TFA = total fatty acids, SFA = saturated fatty acids, MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids. n6/n3 describes the omega-6 and omega-3 fatty acid ratio. Five females from each diet were randomly selected for the fatty acid analyses (n), except for four treatments in which only four females were selected because not enough females survived until adulthood

| Treatment | Weight | TFA | SFA | MUFA | PUFA | n6/n3 | n |
|---|---|---|---|---|---|---|---|
| Chicken feed | 182.2 | 321.23 | 37.0592 | 47.1032 | 15.8377 | 8.07177 | 5 |
| Suomalainen (1999) | 249.2 | 352.16 | 39.0251 | 44.6497 | 16.3252 | 17.5152 | 5 |
| Suomalainen+vit | 208.6 | 287.40 | 38.1494 | 44.6857 | 17.1649 | 18.5679 | 5 |
| Reindeer feed | 120.88 | 312.44 | 41.4273 | 45.515 | 13.0577 | 18.3007 | 5 |
| Potato-2.5, 17% | 240.6 | 355.36 | 38.3159 | 45.4841 | 16.2 | 17.4021 | 5 |
| Potato-5, 17% | 208.5 | 368.06 | 38.1217 | 46.0709 | 15.8074 | 17.6564 | 5 |
| Potato-10, 30% | 182.175 | 200.78 | 36.9693 | 45.5199 | 17.5107 | 26.709 | 4 |
| Potato-20, 30% | 206.975 | 247.23 | 40.477 | 45.2606 | 14.2624 | 27.4184 | 4 |
| Vegetable 7.2% | 104.775 | 452.22 | 32.5233 | 52.5293 | 14.9475 | 21.6191 | 4 |
| Vegetable 10% | 88.98 | 382.23 | 34.9253 | 47.5546 | 17.5201 | 26.7433 | 5 |
| Barley mash 22.5% | 185.9 | 205.94 | 36.3026 | 33.7518 | 29.9456 | 21.8974 | 5 |
| Barley mash 15% | 211.98 | 330.54 | 37.537 | 42.2333 | 20.2297 | 22.6954 | 5 |
| Barley feed 22.5% | 153.2 | 199.01 | 38.3477 | 34.1652 | 27.487 | 21.232 | 5 |
| Barley feed 15% | 125.9 | 249.75 | 37.7006 | 38.2752 | 24.0241 | 23.5694 | 4 |
| Broad bean pea 15% | 82.2 | 111.11 | 35.0374 | 34.232 | 30.7306 | 39.5604 | 5 |
| Turnip rape 15% | 161.74 | 271.20 | 37.5306 | 42.5496 | 19.9198 | 18.1294 | 5 |

2019) and other Tettigoniidae (Hartley, 1967). During molting, insect individuals are vulnerable to cannibalism before the exoskeleton hardens (Fox, 1975). This could explain the low survival rates in low-protein vegetable diets. Even though cannibalism was not directly observed, the low-protein vegetable diets could be nutritionally deficient. Starvation and availability of vulnerable individuals have been shown to increase cannibalism in insects (Fox, 1975).

Of the by-product protein sources, potato, is one of the diets causing successful growth and development. The potato glycoalkaloids, present in small amounts, do not seem to impact growth and development (Table S3). Also, other diets in our experiment could have contained secondary plant metabolites which could be deterrent or harmful for *R. differens*. For example, the broad bean and pea diets caused low survival, weight gain, and long development times. The diet might be unappealing for the insects, or it did not stimulate the feeding. Broad beans naturally contain lectin, which could limit growth of *R. differens* or lectin could operate as a feeding-deterrent and inhibit feeding (Michiels et al., 2010). In relatively large field studies, *R. differens* has been found to almost entirely use grasses and sedges as their host plants (Opoke et al., 2019). Therefore, the acceptance of Fabaceae (beans and peas) and Brassicaceae (turnip rape) in this study was an unexpected finding.

The nutritional content of the diet impacted the FCR of *R. differens*. The protein level was negatively associated with FCR and clearly showed that the insects compensated for a low protein level diet by adjusting the amount of food eaten. The FCR values in this study ranged between 0.63 and 4.28 and many high-protein by-product diets caused rather efficient feed conversion whereas, for the low-protein diet (vegetable-10%), the feed conversion was less efficient (4.28). Compared to other orthopteran species, the FCR values for *Acheta domesticus* L. has been reported to be 1.3–6.1 on food waste and by-products (Oonincx et al., 2015; Lundy & Parrella, 2015).

The fatty acid composition and content of *R. differens* individuals can be modified by the given by-product diets. Different by-product diets caused different fatty acid compositions and differences in the TFA, SFA, MUFA, and PUFA content. The predominant fatty acid was oleic acid (32–51%), the content of which is comparable to that of wild-harvested individuals (26–44%) (Kinyuru et al., 2010; Opio, 2015; Fombong et al., 2017). Palmitic acid (C16:0), oleic acid (C18:1c9), and linoleic acid (C18:2n6) contributed the most to the differences in the fatty acid composition. A higher proportion of linoleic acid was found in individuals feeding on the barley feed and barley mash diets, which naturally contains linoleic acid (Table S6). The dietary intake of linoleic acid could explain the higher proportions of linoleic acid and increased PUFA content in these treatments. Some insect species, for example *A. domesticus*, can synthesize linoleic acid because it has the necessary Δ12-desaturase enzyme for synthesis (Barlow, 1964; Beenakkers et al., 1985; Cripps et al., 1986; Stanley-Samuelson et al., 1988). Most insects can synthesize palmitic, stearic, and oleic acids (Barlow, 1964; Stanley-Samuelson et al., 1988; Klowden, 2007) and depending on life stage, environmental conditions, and physiological need, insects can modify their fatty acid composition by synthesizing common saturated and MUFA from nonlipid precursors, amino acids, carbohydrates, or existing fatty acids (Gilbert, 1967; Beenakkers et al., 1985; Stanley-Samuelson et al., 1988; Klowden, 2007; Sönmez et al., 2016). It seems that *R. differens* incorporates PUFAs (linoleic and alfa-linoleic acids) without modification into its tissues, which is in line with results by Lehtovaara et al. (2017). However, it is also able to modify and synthesize saturated and MUFAs to suit the physiological needs if the given diet does not meet the nutritional demand.

The TFA content of *R. differens* can be modified with diets with nutritionally different contents. The highest TFA content was produced by low-protein vegetable diets, which had high carbohydrate levels. These insects had high FCR, developed rather slowly, and their adults were small. When insects are forced to eat high quantities of food in order to satisfy their need for protein, there is an increase in the amounts of fatty acids and carbohydrates in the digested food. However, many insects have limited capacity to store polysaccharides. Thus, the excessively ingested carbohydrates are converted to fatty acids in the fat body and stored in the form of triacylglycerols (TGA) (Beenakkers et al., 1985; Klowden, 2007; Arresse & Soulages, 2010).

Our results are valuable for designing feed for *R. differens* because they promote the re-use of resources and also enhance the targets of circular economy. For future applications, we recommend a by-product diet containing 17–22% of protein to ensure fast growth, high survival rate, and rather heavy individuals. Potato protein, barley mash, and barley feed are good by-product candidates for *R. differens*, allowing high body weight, fast development, high survival, and low FCR compared to control diets. In addition, the barley feed and barley mash by-product diets increased the healthy PUFA proportions which are essential for humans. In future, the feasibility of various East-African food-industry by-products should be studied as part of the efforts to develop mass-rearing for *R. differens* in the region where it is now widely consumed by humans and has high economic importance. Furthermore,

mixtures of by-products should be studied at different protein levels, as our previous studies have shown that *R. differens* significantly benefits from diet mixing (Malinga et al., 2018b). Finally, it is important to find out how these diets modify other nutritional components for humans (e.g., amino acids, vitamins, or minerals) and the sustainability of the by-product diets in multigenerational rearings.

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## Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Table S1.** Detailed diet ingredients (%). CF = chicken feed Milka, S = Suomalainen, S + vit = Suomalainen + vitamins, RF = reindeer feed (PoroElo)

**Table S2.** Nutrient content of by-products

**Table S3.** Mean (n = 4) glycoalkaloid content (mg $kg^{-1}$) of *Ruspolia differens* individuals fed on potato protein diet treatments

**Table S4.** Details of the statistical models

**Table S5.** Estimated marginal mean (± SEM) development time from fourth instar to adult stage, survival, weight, and feed conversion rate (FCR) of *Ruspolia differens* on 16 diet treatments

**Table S6.** Percentage of total fatty acids of diet treatments

**Figure S1.** Experimental design. We conducted experiments in two time periods (experimental time). In each period, the experiment was conducted in two thermally regulated rearing rooms, each comprising five blocks. A block was a growth chamber, in which the temperature was microregulated with heat cables. Each block included one replicate of each diet treatment in separate containers, each container with a single treatment. The control diet Suomalainen + vitamins was applied in both experimental periods. Each container had four *Ruspolia differens* individuals.

**Figure S2.** Non-metric multidimensional scaling (NMDS) ordination showing fourth root transformed fatty acid composition of (A) centroids of *Ruspolia differens* individuals and (B) diet treatments.