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Nutrient composition of the long-horned grasshopper *Ruspolia differens* Serville: effect of swarming season and sourcing geographical area

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Abstract

This research investigates the effect of swarming season and sourcing geographical area on the nutrient composition of *R. differens*. On a dry matter basis, protein (34.2 - 45.8 %) and fat (42.2 - 54.3 %) are the major macro-nutrients. Leucine (80.9 - 88.5), lysine (54.0 - 69.8) and valine (59.2 - 61.8) are the most abundant essential amino acids (EAA) (mg/g protein). Tryptophan is the most limiting EAA. Its EAA composition is like the children and adults' reference protein. Its fat is highly unsaturated (63.5- 64.7). Oleic (38.4 - 42.7), linoleic (19.0 - 23.0) and palmitic (26.6 - 27.8) fatty acids (FA) (g/100 g total FA) are the most abundant. Geographical area significantly influences its mineral content within seasons. Season significantly influences its fat, protein, ash and mineral content but not its AAs' and FAs' content. Both factors significantly influence its vitamin B12 content (0.73 - 1.35 µg/100 g).

Key words

***Ruspolia differens*, nutrient profiles, amino acid score, Vitamin B12, Dietary Requirements**

1. Introduction

The projected doubling of the sub-Saharan African population from about 1.2 to 2.4 billion people in 2050 shall definitely double the demand for food, including the need for high quality protein foods, such as meat and fish. To meet the demand, edible insects are envisioned to be a solution given their high protein content and quality in particular, and a high nutritional quality in general (Van Huis *et al.*, 2013). Because the technology and expertise for commercial mass production of native edible insects, such as *Ruspolia differens*, in sub-Saharan Africa is still limited, wild harvested insects shall continue to play a significant role in food, nutrition and income security of many people in the region in the near future. In Uganda and other parts of sub-Saharan Africa, *R. differens* is presumably the most exploited edible insect for economic and nutritional benefits (Mmari, Kinyuru, Laswai, & Okoth, 2017). With the increasing urbanization and shift from informal to formal food markets that emphasise product quality as an important factor, it is important that the nutritional quality of wild harvested *R. differens* is thoroughly characterized.

Nutrient composition is one of the intrinsic quality attributes that influences the quality of a food. This is because the nutritional components support the growth of ‘spoilage and pathogenic’ microorganisms and also support the progression of chemical reactions, such as fat oxidation (oxidative rancidity), all of which influence a food’s sensory and edible quality (colour, texture, aroma, etc.) and safety (Erkmen & Bozoğlu, 2016). *R. differens* collected from different parts of East Africa exhibit a high nutrient content with 33.3 - 44.3 % crude protein, 46.2 - 54.6 % crude fat, 3.90 - 14.9 % crude fibre, 2.60 - 5.38 % total mineral/ash and 0.01 - 2.60 % carbohydrates, expressed as Nitrogen Free Extract (NFE) (Fombong, Van Der Borgh, & Vanden Broeck, 2017; Kinyuru, Kenji, Muhoho, & Ayieko, 2011; Ssepuuya, Mukisa, & Nakimbugwe, 2016).

Additionally, *R. differens* contains several micro-nutrients, such as provitamin A (total carotenoids), other vitamins (vitamin E, C, B2, B3 and B9) and minerals, such as iron, zinc and calcium (Fombong *et al.*, 2017; Kinyuru *et al.*, 2011; Ssepuuya *et al.*, 2016). *R. differens* is thus not only an alternative source of protein but also as a source of other nutrients of public health concern, such as vitamin A, iron and zinc (Harika, Faber, Folake, Mulugeta, Kimiywe & Eilander, 2017). Vitamin B12 can only be obtained from foods of animal origin and it is therefore one of the most important vitamins from a nutritional point of view, especially in communities with a majorly plant-based diet. It has also been identified in *Tenebrio molitor* (0.47 µg per 100 g) and *Acheta domesticus* (5.4 and 8.7 µg per 100 g in adults and nymphs respectively) and efforts to identify and quantify this vitamin in other edible insects are necessary (Van Huis *et al.*, 2013). However, the presence of vitamin B12 in grasshoppers in general and *R. differens* in particular has not been cited in any published literature yet, although it would be a valuable source.

The chemical/nutritional composition of foods of animal and plant origin is affected by different factors, including season and sourcing geographical area (Romotowska, Karlsdóttir, Gudjónsdóttir, Kristinsson, & Arason, 2016). Though the above factors are cited as affecting the nutritional composition of edible insects (Ssepuuya *et al.*, 2016), there is limited information about their specific effects. This research therefore aimed to test the effect of sourcing geographical area and swarming season on the nutritional quality of *R. differens*.

2. Materials and methods

2.1. Sampling and sample preparation

For each of the seasons of April-May and November-December 2016, approximately 14 kg of wild harvested plucked (with wings, legs and antennae removed) *R. differens* were obtained from three geographical areas and major harvesting hubs (Kampala, Masaka and Fort Portal). Kampala (0° 18' 58.61" N; 32° 34' 55.88" E) is the capital city and located in the central part of Uganda; Masaka (0° 20' 1.64" N; 31° 44' 2.72" E) is 119 km south west of Kampala while Fort portal (0° 39' 42.26" N; 30° 16' 29.28" E) is 295 km west of Kampala. The April-May and November-December *R. differens*' swarming seasons coincide with the March-May and September/October-December rainy seasons. For each sourcing geographical area, 3 trading points were identified and from each point, 4 traders were randomly selected. April-May and November-December seasons correspond to the short and long rainy seasons. From each trader, 345 g of *R. differens* was collected using the quartering technique (*i.e.*, 85 g of *R. differens* picked from four quarters of the area occupied by the *R. differens* displayed for sale) to obtain a 1,380 g sample and thus 4,560 g from each trading point in a geographical area. Because of the commercial purchase on the local market, there was no control on, nor information about gut emptying or killing method. Samples from the three trading points in each geographical area were packed in zipping polyethylene bags and delivered to Makerere University on flaked ice in a cool box. They were then lumped together and mixed thoroughly to make one gross sample per sourcing geographical area, washed clean, drained, placed in a tightly self-closing polybag and stored at -18 °C until further analyses.

2.2. Nutrient analyses

2.2.1. Proximate composition

Moisture content was determined by the forced air oven (UF 110, Memmert, Schwabach, Germany) at 105 °C for 17 hours (Nielsen, 2010). Crude protein was determined by the

Kjeldahl method using acetanilide (UCB, Belgium) as the reference standard and a nitrogen to protein conversion factor of 6.25 (Sáez-Plaza, Navas, Wybraniec, Michałowski, & Asuero, 2013). Crude fat was gravimetrically determined using the Soxhlet extraction procedure. In brief, the sample was refluxed with technical grade petroleum ether (VWR International BVBA, Belgium) for 17 hours (10 to 12 drain off cycles/hour) and the extraction solvent evaporated under vacuum by a rotary evaporator (Büchi R-200) at 50 °C for 2 hours to obtain the fat (Nielsen, 2010). Ash content was determined by heating the sample in porcelain crucibles (459-0211, VWR International, Germany) in a muffle furnace (B 180, Nabertherm, Lilienthal, Germany) at 550 °C until constant weight (Nielsen, 2010). Fibre content was measured gravimetrically after deproteinization using 1 M NaOH and subsequent treatment with 1 M HCl to remove minerals and catechols (Kumaria & Rathb, 2014), and expressed as chitin (Finke, 2007). Carbohydrate content was obtained by difference as nitrogen free extract (NFE) (Nielsen, 2010). All determinations were carried out in triplicate. Proximate composition of raw broiler meat (Soriano-Santos, 2010) and right hand shoulder pork meat (Kim et al., 2008) was obtained from literature.

2.2.2. Fatty acid profile

Fatty acid methyl esters were prepared from *R. differens* oil extracts and separated and identified by gas chromatography (GC) coupled with mass spectrophotometry (Fombong *et al.*, 2017). In brief, 0.025 g of the *R. differens* oil extract was weighed to the nearest 0.0001 g. The acylglycerols in the sample were trans-esterified by 0.5 M sodium methoxide (VWR International, BVBA, Belgium) while free fatty acids were esterified by a 10 % methanolic boron trifluoride (BF₃) solution (Sigma-Aldrich, St. Louis, USA) to form fatty acid methyl esters (FAMES). Dissolved in GC grade hexane (VWR International BVBA, Belgium), 1.0 µl of the

esterified sample or fatty acid methyl ester standard (Sigma-Aldrich, St. Louis, MO, USA) was injected onto the gas chromatograph (Agilent 7820A-5977E GC-MSD, Agilent Technologies, Santa Clara, CA, USA) and carried over the stationary phase [1,12-di(tripropylphosphonium)dodecane-*bis*-(trifluoromethylsulfonyl)imid] in a SLB-IL60 capillary column (Sigma-Aldrich, St. Louis, USA) by helium (12 psi) as the carrier gas/mobile phase. After injection (1 μ l, split ratio: 10:1), the oven temperature was initially held at 40 °C for 4 min, and increased to 280 °C at 5 °C per minute during the analysis. The run time was 55 minutes. The MS was used in the SIM mode under electron impact ionization of 70 eV. Using methyl tricosanoate (Sigma Aldrich, St. Louis, MO, USA) as the internal standard, the MassHunter software (Agilent Technologies, Santa Clara, CA, USA) was used to quantify the fatty acids. For each sample, extraction, detection, identification and quantification were performed in triplicate.

2.2.3. Amino acid profile and amino acid score

The amino acid profile was determined after acid and alkaline hydrolysis of the fat-free sample and subsequent separation and quantification of the amino acids by a Waters Acquity UPLC H-class system (Iwasaki, Chavali, Wheat, LeBlanc, & Mcmillan, 2015). In brief, 0.025 g of a sample was weighed to the nearest 0.0001g into a clean dry test tube and hydrolysed with 10 ml of 6 M HCl (Puris. P.a, ACS Reagent grade, Sigma Aldrich, Germany) for acid hydrolysis or 4 ml of 4 M LiOH (Biosolve SARL, France) for alkaline hydrolysis for 22 hours at 110 °C. During the acid hydrolysis, asparagine (Asn) and glutamine (Gln) were converted into aspartic acid (Asp) and glutamic acid (Glu), respectively. The acid hydrolysed samples were flushed with helium gas before oven heating to prevent amino acid oxidation. The solutions were neutralized to pH 7 and filtered into a 25 ml volumetric flask through a filter paper (Grade 454, VWR

International, Belgium). From this 25 ml neutral solution, 1 ml was centrifuged (Micro Star 17R VWR International, Germany) for 5 minutes at 15,000 g. An aliquot of 10 μ l was taken from the sample after centrifugation and thoroughly mixed with 70 μ l (0.08 M, pH = 8.8) of a borax buffer (ACROS ORGANICS, USA). To achieve derivatization, 20 μ l of 6-aminoquinoyl-N-hydroxysuccinimidyl carbamate (AQC) reagent (Waters, U.S.A) was added to the sample, the mixture vortexed for 10 seconds and heated at 55 °C for 10 minutes. Before sample injection, the system was calibrated using analytical standards (Sigma Aldrich, St. Louis, MO, USA) at varying concentrations for each amino acid. For separation of amino acids, 1.0 μ l of sample was injected into the UPLC and carried over a Waters Cortecs® UPLC C18 1.6 μ m, 2.1 x 150 mm column maintained at 55.0 ± 1.0 °C, by mobile phases, A (ultra-pure water + 0.1 % formic acid) and B (acetonitrile + 0.1 % formic acid) during a runtime of 12 minutes. Formic acid and acetonitrile were obtained from Biosolve SARL, France. Detection was done using the Waters QDA mass detector operated in Selected Ion Recording (SIR) mode. The m/z ratios used were those corresponding to the mass of the AQC adducts of the different amino acids. The voltages of the capillary and cone were 0.8 kV and 10 V respectively. The capillary was kept at 600 °C to facilitate desolvation of the aerosol in the ESI source enclosure of the QDa. The Waters Empower™ 3 software was used to obtain calibration curves and subsequent quantification of the amino acids. For each sample, extraction, detection, identification and quantification were performed in triplicate.

The amino acid score was determined as a ratio of mg/g of the essential amino acid in the test protein, *R. differens* protein in this case, to mg/g of the same amino acid in the reference protein (Gropper, Smith, & Carr, 2016). The reference amino acid scoring pattern for infants (0 - 6

months) was the amino acid composition of human milk while for children and adults, it was the amino acid requirement of pre-school children aged 1-3 years (Gropper, Smith, & Carr, 2016).

2.2.4. Mineral profile

To determine the mineral profile, crude ash was dissolved in nitric acid and its mineral components determined using the inductively coupled plasma-optical emission spectrometry (ICP-OES) technique. In brief, to 0.5 g of ash weighed to the nearest 0.0001 g, 8 ml of 65% HNO₃ (Sigma-Aldrich, St. Louis, MO, USA) was added and heated until they dissolved. The solution was cooled and transferred to a clean 100 ml volumetric flask. The samples were then measured using the ICP-OES (Optima 4300™ DV ICP-OES, Perkin Elmer Wellesley MA, USA) procedure using WinLab32 program software. Quantification was possible by use of a calibration curve drawn using certified standard solutions (Chem-Lab NV, Zedelgem, Belgium). For each sample, extraction, detection, identification and quantification were performed in triplicate.

2.2.5. Vitamin B12

This was determined by a microbiological quantitative method using the VitaFast vitamin B12 microtiter plate procedure (IFP, 2017). In brief, to the blank and 1.0 g of a sample weighed to the nearest 0.0001 g, 20 ml of the sodium acetate buffer and 250 µl of 1% sodium cyanide were added and vortexed for 60 seconds. To both, the samples and the blank, 300 mg of α -amylase (Sigma Aldrich, St. Louis, MO, USA) was added, vortexed and incubated for 1 hour at 37 °C, after which the volume was made to 40 ml using ultra-pure water. The samples were incubated at 95 °C in a water bath with a shaker at 90 rpm for 30 minutes, cooled to 30 °C in ice and centrifuged for 20 minutes at 1,560 g (Multifuge 3s, Kendro Laboratory products, Germany).

From this solution, 5 ml of the extract was filtered using a sterile polyethersulfone syringe filter (25 mm, 0.2 μm pore size, VWR International, LLC Belgium) into a 15 ml sterile falcon tube (VWR International, LLC Belgium) and used to prepare the required dilutions for further analysis. To the selected wells of the test kit plate, 150 μl of the vitamin B₁₂ assay medium was added followed by 150 μl of either, the standard solution, sample or blank. The wells were covered with adhesive foil and incubated for 44 - 48 hours at 37 °C in the dark. After removal of the foil the developed turbidity was measured at 630 nm using Genesys 10S UV-VIS spectrophotometer (Thermo Fisher Scientific, China). For each sample, extraction, detection, identification and quantification were performed in triplicate.

2.3. Statistical analysis

SPSS for windows (Version 20, IBM Corporation, Armonk, New York) software was used to perform statistical analyses. Using descriptive statistics, means and standard deviations were obtained. Exploration of the data using box plots and the Levene's test ($P > 0.05$) showed that it was normally distributed and thereafter, ANOVA was used to determine the effect of sourcing geographical area and swarming season on the proximate composition; mineral, amino acid, fatty acid profiles; and, Vitamin B₁₂ content of *R. differens*. Mean separation was obtained by the Tukey's option of the ANOVA test. For all tests, a significance level of 0.05 was considered. Cluster analysis using non-metric multidimensional scaling (NMDS) was used to determine the extent to which swarming season and sourcing geographical area influenced *R. differens*' proximate composition, amino acid profile, fatty acid profile, mineral profile and all the parameters combined. The NMDS was composed using the R-package (R Development Core Team, 2013) "Vegan" (v2.43) in RStudio (v1.1.442).

3. Results and discussion

3.1. Proximate composition of *Ruspolia differens*

The proximate composition of *R. differens* is presented in Figure 1. On a dry matter basis, the average protein (34.2 - 45.8 %), fat (42.2 - 54.3 %) and ash (1.79 - 2.72 %) content range is similar to that observed in earlier studies (Fombong *et al.*, 2017; Kinyuru *et al.*, 2011; Ssepuuya *et al.*, 2016). The fibre content (3.93 – 5.34 %) expressed as chitin in this study is similar to that observed by Kinyuru *et al.* (2011) but lower than that observed by Fombong *et al.* (2017) and Ssepuuya *et al.* (2016). The carbohydrate (4.29 - 6.03 %) content expressed as nitrogen-free extract (NFE) is higher than that observed in earlier studies (Fombong *et al.*, 2017; Ssepuuya *et al.* 2016). Protein and fat make up between 86 and 89 % of the nutritional components and are thus the major nutrient compounds of *R. differens*.

On a dry matter basis, the protein content observed in this study (34.2 - 45.8 %) and other similar studies on *R. differens* is, on average, less than that of other edible grasshopper species (53 - 77 %) and other Orthopterans (61.3 %) (Paul *et al.*, 2016; Rumpold & Schluter, 2013). In this study and earlier studies on edible insects, the general nitrogen-to-protein conversion factor of 6.25 is used to calculate the protein content. However, Janssen *et al.*, (2017) established a factor of 5.6 for *Tenebrio molitor*, *Alphitobius diaperinus*, and *Hermetia illucens* larvae, indicating an over-estimation of the protein content of these and possibly other insects. The nitrogen-to-protein conversion factor for other insects such as *R. differens* needs to be established as well, based on the AA profile as discussed in the next section. This is, however, out of the scope of the present work. *R. differens* shows a higher fat content than other edible grasshopper species (2.5 - 16.3 %) and Orthopterans (13.4 % on average) (Paul *et al.*, 2016; Rumpold & Schluter, 2013).

Consumption of 100 g of dry *R. differens* contributes about 49 - 82 % of the daily protein requirement of 0.8 - 1.0 g/kg body weight (Whyte, Harries, & Williams, 2005). *R. differens* can therefore contribute to improving the low daily per capita protein consumption of 55 - 65 g/person/day in sub-Saharan Africa (excluding the republic of South Africa) compared to approximately 100 g/person/day in developed countries (OECD/FAO, 2016).

3.2. Amino acid composition of *Ruspolia differens*

Overall, alanine, aspartic acid (+ aspartate) and glutamic acid (+ glutamate) are the most abundant amino acids, similar to observations by Fombong *et al.* (2017) while leucine, lysine and valine are the most abundant essential amino acids (Table 1). Similar to observations by Fombong *et al.* (2017) in *R. differens* and Paul *et al.*, (2016) in other edible grasshoppers, tryptophan is the limiting amino acid in *R. differens*. Compared to other edible grasshoppers, *R. differens* has either higher or similar isoleucine, leucine and lysine contents. *R. differens* also has a similar histidine and methionine content, except for *Chrotogonus trachypterus* and *Spathosternum prasiniferum*, which have a higher histidine content of 46 and 53 mg/100 g protein respectively, and *Sphenarium purpurascens* and *Melanoplus femurrubrum*, with a higher methionine content of 25 - 42 and 29 mg/100 g of protein respectively (Paul *et al.*, 2016). *R. differens* is generally lower in phenylalanine, tyrosine, cysteine, total sulphur containing amino acids (methionine + cysteine) and total aromatic amino acids (phenylalanine + tyrosine) contents than other grasshoppers (Paul *et al.*, 2016).

Compared to the amino acid profile of common meat sources in Table 1, *R. differens* has a higher leucine, phenylalanine and valine content and similar amounts of all other essential amino acids to those in raw pork loin muscle (Cai *et al.*, 2010) and raw chicken (broiler) meat (Straková, Suchý, Vitula, & Večerek, 2006). This is similar to observations made by Rumpold &

Schluter (2013) that edible insects contain low levels of sulphur containing amino acids (cysteine and methionine). The methionine + cysteine content is rather low in *R. differens* but similar to that of chicken meat (Table 1).

Based on the human amino acid reference pattern by the FAO expert consultation committee on dietary protein quality evaluation in human nutrition, the amount of most essential amino acids in 1 g of *R. differens* meets the adult dietary requirements as evidenced by the amino acid score range that is higher than 1 except for lysine (0.95-1.22) and tryptophan (0.80-1.14) whose lower range amino acid scores are less than 1 (Table 1). For infants, *R. differens* contributes over 40 - 57 % of their tryptophan daily requirements and meets over 70 % of their daily requirement for all the other essential amino acid requirements (Table 1).

Regarding protein quality expressed as the amino acid score, the first limiting amino acid in *R. differens* is tryptophan (Table 1). In adult diets, *R. differens*' essential amino acids have a supplementary value because its essential amino acid scores are greater than 1, except for lysine and tryptophan (Table 1), implying the presence of higher quantities of these amino acids per g of *R. differens* protein than those in 1 g of the reference pattern (Lasztity & Hidvégi, 1985). This therefore implies that when consumed together with foods deficient in the essential amino acids that *R. differens* has in excess, it can potentially compensate the deficiency of other food of lower protein quality (Lasztity & Hidvégi, 1985). The ratio of essential to non-essential amino acids ranged from 0.58 to 0.66, similar to 0.6, the ratio recommended by the FAO/WHO/UNU expert consultation report on energy and protein requirements in human beings. However, more detailed measures of protein quality should be evaluated for *R. differens* protein *e.g.*, including protein digestibility as recommended by FAO (Abelilla, Liu, & Stein, 2018). In addition, also the effect of processing on the nutritional value and the sensory perception needs to be evaluated. It can be

concluded that protein quality and quantity of (raw) *R. differens* can potentially contribute to the recommended dietary allowances of individuals of all age groups.

3.3. Fatty acid composition of *Ruspolia differens*

Oleic acid (C18:1, ω -9) is the most abundant fatty acid in *R. differens*, ranging from 38.4 to 42.7 g/100 g fat (Table 2). This is followed by palmitic acid (C16:0) that ranges from 26.6 to 27.8 g/100 g fat and linoleic acid (C18:2, ω -6, LA) that ranges from 19.0 to 23.0 g/100 g of fat. These results are consistent with findings by Fombong *et al.* (2017) but contrary to the findings by Kinyuru *et al.* (2011) in *R. differens*. Kinyuru *et al.* (2011) reports palmitic acid as the most abundant (32 %) followed by linoleic acid (30 - 31 %) and oleic acid coming third, with 25 %. These differences in fatty acid content can be attributed to the differences in development phase, diet and environmental factors (Paul *et al.*, 2016). Studies by Kinyuru *et al.* (2011), Fombong *et al.* (2017) and the present study confirm that *R. differens* fat is highly unsaturated with oleic and linoleic acid being the most abundant unsaturated fatty acids and palmitic acid being the most abundant saturated fatty acid. Oleic acid, linoleic acid and palmitic acid are also the most abundant in other grasshoppers (Paul *et al.*, 2016) and many other edible insects (Rumpold & Schluter, 2013). They are also the most predominant fatty acids in common meat sources, such as pork (Cai *et al.*, 2010) and chicken (Žlender, Holcman, Stibilj, & Polak, 2000). *R. differens* has a higher total poly unsaturated fatty acids (TPUFA) content than pork and chicken meat (Table 2). It has a higher content of linoleic acid than pork and chicken meat and comparable amounts of alpha linolenic acid (C18:3, ω -3, ALA) (Table 2). It has similar amounts of eicosapentaenoic acid (C20:5, ω -3, EPA) and arachidonic acid (C20:4, ω -6, ARA) to that of pork but lower amounts of EPA and higher amounts ARA than that in chicken meat (Table 2). The amounts of ARA in *R. differens* are low, similar to observations in other foods of animal origin.

However, it can be synthesised from linoleic acid if the latter is present in sufficient amounts in the diet.

In the 1950/60's, consumption of oils and foods high in polyunsaturated fat (ω -6 and ω -3) was preferred due their serum and plasma cholesterol lowering effect that leads to reduced incidences of heart disease, contrary to diets high in saturated fat (Frankel, 2014). Recently, there is an increased awareness towards the ratio of ω -6 and ω -3 fatty acids.

Consumption of foods with high amounts of LA (ω -6) and low amounts of ω -3 fatty acids (ALA, EPA, and docosahexaenoic acid or DHA), hence a high ω -6/ ω -3 ratio in the diet, is associated with inflammatory, cardiovascular and immunological disorders (Russo, 2009). A low ω -6/ ω -3 ratio is preferred for protection against the abovementioned health risks (Russo, 2009).

In this study, the ω -6/ ω -3 ratio ranged from 12:1 to 20:1, which is similar to that observed in chicken meat (15:1) but higher than that observed in pork (7:1) (Table 2). FAO/WHO recommends an ω -6/ ω -3 ratio of 5:1 and in line with this recommendation, Canada recommends a ratio between 4:1 and 10:1, while Sweden and Britain recommend a ratio of 5:1 and 7:1, respectively, with 2.5/3:1 being the lowest ratio suggested by research for a positive impact on human health (Shils & Shike, 2006). The range of the recommended values possibly indicate that there is still some uncertainty about the appropriate low ratio of ω -6 and ω -3 essential fatty acids, however keeping the ratio as low as possible is better for a positive health outcome. Docosahexaenoic acid is not detected in *R. differens* fat. This is possibly because the feed available to wild *R. differens* during growth was deficient in DHA since the diet affects the fatty acid composition of *R. differens* (Lehtovaara *et al.*, 2017). Thus, in mass rearing/production of *R. differens*, the diets should have adequate amounts of either all the essential fatty acids or their

precursors, *i.e.*, ALA for the synthesis of EPA and DHA and LA for the synthesis of arachidonic acid (Kliegman *et al.*, 2015) when *R. differens* is ingested into the human body. The amount of essential fatty acids in 100 g of the total fatty acid content of dry *R. differens* is similar to the daily dietary requirements for ω -6 fatty acids (4.4 – 17 g/day) and ω -3 fatty acids (0.5 – 1.3 g/day) for children and adult men and women (Kliegman *et al.*, 2015). For infants, essential fatty acids are sufficiently supplied by the breast milk although their adequacy in breast milk depends on the maternal diet (Kliegman *et al.*, 2015).

3.4. The mineral profile and vitamin B12 content of *Ruspolia differens*

On a dry matter basis, potassium, ranging from 446 to 673 mg/ 100g; phosphorus, ranging from 429 to 627 mg/100 g and calcium, ranging from 34.9 to 128 mg/100 g are the most abundant mineral elements. The amount of macro-mineral elements is either similar or higher than those observed in pork and chicken meat (Table 3). Low levels of calcium have been reported in *R. differens* and other grasshoppers as well (Kinyuru *et al.*, 2011; Paul *et al.*, 2016). Though the low levels of calcium can be attributed to insects lacking a mineralized skeleton, animal sources with a mineralized skeleton, such as pork and chicken meat, have a lower calcium content (Table 3). This is possibly because the bones with calcium deposits are not part of the edible muscle tissue that is analysed. However, Fombong *et al.* (2017) reports higher amounts of calcium in *R. differens*, ranging from 977 to 1124 mg/100 g of dried *R. differens*. This large variation within the same species can be a result of the diet/feed to which the *R. differens* are exposed in the different sourcing geographical areas and swarming seasons. The sodium content is much lower than that observed in other edible grasshoppers and other edible insects (Paul *et al.*, 2016; Rumpold & Schluter, 2013). Though the magnesium content values are similar to those observed

by Kinyuru *et al.* (2011), they are lower than those observed by Fombong *et al.* (2017) in the same species. *R. differens* is thus a poor source of magnesium compared to other edible grasshoppers such as *A. fallax* (657 mg/100 g), *Melanponus spp* (498 - 902 mg/100 g) and *Sphernarium spp* (352 - 824 mg/100 g) (Paul *et al.*, 2016). The potassium and phosphorus content of *R. differens* is comparable or higher than that of other edible grasshoppers, except for *Zonocerus variegatus*, which has a higher phosphorus (> 4,500 mg/100 g) and potassium (761 - 2,030 mg/100 g) content on a dry matter basis (Paul *et al.*, 2016).

The *R. differens*' micro-element (iron, zinc, copper and manganese) content is much higher than that of most commonly consumed meats such as pork and pig edible offal (Tomovic *et al.*, 2015) and chicken meat (NIIR, 2008) as shown in Table 3. *R. differens* has a similar manganese content, lower zinc content and a higher copper content compared to other edible grasshoppers (Paul *et al.*, 2016). The iron content in *R. differens* is higher than that of other edible grasshoppers, except for *Z. variegatus*, that has a much higher content (184 - 910 mg/100 g).

Incorporation of 100 g of dry *R. differens* in the daily diet meets the daily trace mineral requirements for both children and adults, meets the phosphorus and magnesium requirements for children but not adults but fulfils only partially the dietary requirements for sodium, potassium and calcium for children and adults (Stipanuk & Caudill, 2013). Hence, *R. differens* is poor source of macro-minerals and a good source of micro-mineral elements. However, compared to pork and chicken meat, *R. differens* is a better source of dietary macro- and micro-mineral elements. Hence, like other animal foods, *R. differens* can potentially contribute to

alleviating the effects of zinc and iron micro-mineral deficiencies, which are among the most important micro-nutrients of public health concern (Harika *et al.*, 2017) especially in Africa.

The *R. differens*' vitamin B12 content ranges from 0.73 to 1.35 µg/100 g on a dry matter basis depending on the geographical region and the harvesting season (Table 3). This is comparable to the vitamin B12 content in *Tenebrio molitor* (0.47 µg/100 g) but much less than that in *Acheta domesticus* (5.4 and 8.7 µg/100 g in adults and nymphs respectively) (Van Huis *et al.*, 2013). Compared to other animal sources, the vitamin B12 content is higher than that in pork (Esteve, Farre', Frí Gola, Farre', & Pilamunga, 2001) and poultry (Damodaran & Parkin, 2017) as shown in Table 3. It is however much lower than that in beef (2.3 µg/100 g), one of the best sources of vitamin B12 so far (Damodaran & Parkin, 2017). Vitamin B12 daily dietary requirements increases from 0.4 µg/day for infants younger than 6 months to 2.4 µg/day for adults and 2.8 µg/day for lactating women (Stipanuk & Caudill, 2013). Depending on the amounts consumed or incorporated into a food product, *R. differens* can potentially contribute to meeting the dietary requirements for infants and adults. However, given that vitamin B12 is heat labile (Henry & Chapman, 2002), the effect of cooking methods used to process/prepare *R. differens* on vitamin B12 content should be investigated.

3.5. The effect of season and sourcing geographical on the nutrient composition of *Ruspolia differens*

To study the effect of season and the sourcing geographical area, the results were visualized after cluster analysis in multidimensional scaling plots (Figure 2). In these plots, clusters are formed basing on swarming seasons rather than sourcing geographical area, indicating that swarming

season has a substantial and consistent influence on the proximate composition (Figure 2A), amino acid (Figure 2B), fatty acid (Figure 2C) and mineral (Figure 2D) profiles of *R. differens*, and, on all the nutrient parameters combined (Figure 2E). In figures 2B (amino acid profile) and 2C (fatty acid profile), the two clusters (based on swarming season) are closer to each other and thus more similar in nutrient composition/content. Figures 2A (proximate) and 2D (mineral profile) indicate that the clusters generated from the proximate and mineral composition data from the two seasons are more distant from each other, indicating that the proximate and mineral composition of *R. differens* from the two seasons differs. In the mineral NMDS plot, within each season, clustering is consistently based on sourcing geographical area and the clusters are distant from each other, indicating the influence of geographical sourcing on the mineral profile. Overall, the nutrient composition (including vitamin B12) of *R. differens* from the April-May season differs from that of the November-December season (figure 2E).

For the proximate composition, *R. differens* from the April-May season exhibit a significantly higher protein and ash content and a lower fat content than that in the November-December season expressed on a dry matter basis (figures 1 & 2A). A similar observation was made by Ssepuuya *et al.* (2016) where the protein content of *R. differens* in the April-May season was slightly higher than that in the November-December season, though the difference wasn't statistically significant ($P > 0.05$). The observed seasonal significant differences in protein, ash and fat content show no significant influence on the amino and fatty acid profiles (table 1 and 2, figures 2B and 2C) but show an influence on the mineral profile of *R. differens* from each sourcing geographical area (table 3, figure 2D). Soil type influences the mineral composition of the vegetation (Joy *et al.*, 2015), including the grass on which *R. differens* feed, and feed influences the nutrient content of *R. differens* (Lehtovaara *et al.*, 2017). Its therefore possible that

the soils and hence the vegetation in the three geographical areas on which the *R. differens* feed differ in mineral composition which translates into differences in *R. differens*' mineral composition based on sourcing area. The difference in soil mineral composition is caused by differences in either the type or magnitude of the factors that influence soil nutrient composition, such as parent (rock) material, climate, soil particle size, pH, humus content, aeration, temperature, water content, root surface area and mycorrhizal development among others (Jackson, 2008).

Sourcing geographical area and swarming season influenced the vitamin B12 content of *R. differens* (Table 3). Generally, *R. differens* from Masaka shows the highest content of vitamin B12, followed by Fort Portal and then Kampala. Regarding season, *R. differens* collected in the April-May season from Kampala and Fort Portal show significantly higher amounts ($P < 0.05$) of vitamin B12 than that collected in the November-December season. On the contrary, *R. differens* collected in the same season from Masaka has a significantly lower ($P < 0.05$) vitamin B12 content than that collected in the November-December season.

Since it is known that the type of feed influences the nutrient composition of *R. differens* (Lehtovaara *et al.*, 2017), it can be assumed that the available feed sources in the two swarming seasons differ which influences the amount and profile of the nutrients. However, this may have no influence on the amino acid profile since it is genetically determined. The contribution of other factors such as insect age (maturity stage) and reproductive stage that could possibly explain such variations should be investigated. Given the absence of adequate information on the types and abundance of vegetation in the 3 harvesting areas in the two seasons as well as the differences or similarities in climate, the effects of season and geographical area cannot be thoroughly explained. For example, though the March-May and September-December seasons

were formerly regarded as the long and short rainy seasons respectively (van de Steeg, Herrero, Kinyangi, & Thornton, 2009), the March-May season has become shorter with rains being delayed by as many as 30 days, making it more unreliable than the September-December rainy season. The effect of such changes on the abundance and type feed/vegetation available for *R. differens* and thus its nutrient composition can only be stated but hardly explained.

4. Conclusions and recommendations

Ruspolia differens is highly nutritious with protein and fat being the major nutrients and has a high protein quality and quantity. *R. differens* is a good source of essential amino acids with tryptophan being the first limiting amino acid. It is also a good source of essential fatty acids but its ω -6/ ω -3 ratio is rather high, which can potentially predispose consumers to a cardiovascular disease risk according to the recent scientific insights. The presence of ω -3 fatty acids (ALA and EPA) in sufficient amounts can lower this risk. Consumption of 100 g of dry *R. differens* meets 49 - 82 % of the daily protein requirements; the dietary amino acid requirements for children and adults; contributes 40 - 57 % of infant tryptophan requirements and meets over 70 % of the infant daily requirements of other essential amino acids. It also contributes to 12 - 100 % of the daily adult essential fatty acid requirements and 100 % of the trace mineral infant and adult requirements. It also potentially contributes to meeting infants', children and adult daily dietary requirement for macro-minerals and vitamin B12. Unlike sourcing geographical area, season has a consistently significant effect on protein and ash content, and the mineral profile of *R. differens* but not the amino and fatty acid profiles. *R. differens* can supplement the quality of inferior protein foods if consumed together. *R. differens* therefore has a high potential to contribute to combatting the micro- and macro-nutrient deficiencies especially for protein and iron. To fully

evaluate the contribution of *R. differens* to food security in sub-Saharan Africa, the digestibility and bio-availability of its nutrients as well as the influence of processing on its nutritional quality should be investigated.

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Declaration of interest

None

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Figure 1: Effect of season and sourcing geographical area on the proximate composition of *Ruspolia differens* collected from three geographical areas Masaka (Msk), Kampala (Kla) and Fort Portal (FPT) in the two seasons of April-May and November-December (Nov-Dec), 2016 in comparison to right hand shoulder pork meat (Kim et al., 2008) and broiler meat (Soriano-Santos, 2010) as animal muscle tissue (AT). Bars carrying either the same capital ('ABC' for the November-December season and 'XYZ' for the March-May season to assess the effect of geographical area) or small letters ('ab' to assess the effect of season) correspond to values that are not significantly different ($p < 0.05$, $n = 3$).

composition of the samples, the smaller the distance between the points. Sample MA 1 was left out of the ordination for mineral profile (D) and combined parameters (E).

Table 1. The amino acid profile (mg/g protein) and amino acid score of *Ruspolia differens* and typical amino acid values of selected animal tissue (mg/g protein)

Amino acid	Season and sourcing geographical area						Animal tissue	
	Masaka		Kampala		Fort Portal		Pork	Broiler chicken
	Nov-Dec	April-May	Nov-Dec	April-May	Nov-Dec	April-May		
Essential amino acids (E)								
His	26.5±2.7 ^{aA}	25.9±0.4 ^{aX}	22.8±0.9 ^{aA}	24.4±0.4 ^{bY}	20.9±0.3 ^{aB}	21.6±0.7 ^{aY}	34.6-36.3	24.7
Ile	46.5±0.7 ^{aA}	48.2±0.6 ^{bX}	49.0±0.7 ^{aB}	47.5±0.3 ^{bX}	48.0±0.3 ^{aB}	48.0±0.6 ^{aX}	36.9-48.0	30.9
Leu	80.9±4.8 ^{aA}	85.5±1.0 ^{aX}	86.5±1.3 ^{aA}	88.5±0.7 ^{aY}	88.1±0.9 ^{aA}	88.5±0.7 ^{aY}	65.0-73.6	51.2
Met	16.0±4.2 ^{aA}	17.9±1.9 ^{aX}	19.7±1.4 ^{aA}	18.0±2.0 ^{aX}	19.9±0.3 ^{aA}	19.7±0.3 ^{aX}	23.6-28.6	14.0
Lys	54.0±3.6 ^{aA}	60.7±1.4 ^{bX}	63.6±4.1 ^{aB}	66.0±3.9 ^{aXY}	69.8±0.9 ^{aB}	68.8±1.7 ^{aY}	78.0-87.8	58.1
Phe	36.6±3.2 ^{aA}	36.1±0.9 ^{aX}	37.8±2.4 ^{aA}	36.2±1.5 ^{aX}	38.0±0.5 ^{aA}	37.4±1.1 ^{aX}	28.3-39.8	23.3
Trp	7.7±0.5 ^{aA}	6.8±0.6 ^{aX}	8.0±0.6 ^{aA}	8.7±0.4 ^{aY}	9.7±0.6 ^{aB}	8.7±0.3 ^{aY}	8.2-13.3	10.5
Thr	39.7±0.5 ^{aA}	40.8±0.4 ^{bX}	41.4±0.2 ^{aB}	42.3±1.0 ^{aX}	41.6±0.1 ^{aB}	43.1±1.2 ^{aX}	33.7-51.1	27.8
Val	59.2±2.9 ^{aA}	58.2±0.5 ^{aX}	58.1±1.9 ^{aA}	61.8±1.3 ^{bY}	59.8±0.14 ^{aA}	60.7±0.7 ^{aY}	33.6-36.2	33.6
Met_Cys	23.2±4.6 ^{aA}	25.3±2.5 ^{aX}	27.6±2.0 ^{aA}	24.4±2.5 ^{aX}	26.9±0.7 ^{aA}	26.1±0.5 ^{aX}	33.5-40.0 ^c	22.2
Phe_Tyr	104±20 ^{aA}	84.8±1.3 ^{aX}	86.5±1.2 ^{aA}	86.0±0.3 ^{aX}	86.3±0.2 ^{aA}	86.3±0.7 ^{aX}	46.3-58.9 ^c	42.8
Non-essential amino acids (N)								
Arg	52.1±2.1 ^{aA}	53.2±0.5 ^{aX}	52.8±1.8 ^{aA}	63.4±2.2 ^{bY}	65.9±1.7 ^{aB}	62.2±0.9 ^{bY}	46.0-66.2	37.6
Ser	43.0±2.7 ^{aA}	42.1±0.7 ^{aX}	43.1±1.0 ^{aA}	46.8±1.3 ^{bY}	47.7±1.3 ^{aB}	46.0±0.3 ^{aY}	31.1-33.0	25.8
Gly	58.2±2.1 ^{aA}	58.0±1.0 ^{aX}	56.7±1.7 ^{aA}	57.5±1.6 ^{aXY}	52.3±0.8 ^{aB}	55.0±0.2 ^{bY}	29.9-31.4	34.4
Asp+Asn	92.8±2.1 ^{aA}	94.7±2.2 ^{aX}	92.7±4.1 ^{aA}	90.3±2.8 ^{aX}	95.2±2.4 ^{aA}	96.0±2.3 ^{aX}	72.6-80.9	59.6
Glu+Gln	125±5 ^{aA}	129±2 ^{aX}	127±6 ^{aA}	130±2 ^{aX}	125±3 ^{aA}	124±3 ^{aX}	129-133	93.5
Ala	104±21 ^{aA}	110±3 ^{aX}	105±11 ^{aA}	105±8 ^{aX}	93.7±1.3 ^{aA}	98.3±6.3 ^{aX}	39.3-42.1	37.9
Pro	82.7±3.4 ^{aA}	78.7±1.7 ^{aX}	75.8±6.1 ^{aA}	68.4±3.7 ^{aY}	64.6±1.3 ^{aB}	65.8±1.9 ^{aY}	29.9-31.4	19.4
Cys	7.1±0.6 ^{aA}	7.2±0.7 ^{aX}	7.9±0.6 ^{aA}	6.3±0.5 ^{aX}	7.0±0.3 ^{aA}	6.4±0.4 ^{aX}	9.9-11.4	8.2
Tyr	67.6±1.8 ^{aA}	48.7±1.3 ^{aX}	48.7±1.3 ^{aA}	49.8±1.5 ^{bX}	48.3±0.3 ^{aA}	48.9±0.5 ^{aX}	18.0-19.1	19.5
*E/N	0.58	0.64	0.61	0.66	0.64	0.66	0.84-0.92 ^c	0.82
*E/(E+N)	0.37	0.39	0.38	0.40	0.39	0.40	0.46-0.48 ^c	0.45

For the same geographical area, figures with the same superscripts 'ab' are not significantly different ($n = 3$, $p < 0.05$), showing the effect of season; For the same season, figures carrying the same superscripts 'ABC' for the December-November season and 'XYZ' for the April-May season are not significantly different ($n = 3$, $p < 0.05$) showing the effect of sourcing geographical area. * Arginine is not considered an essential amino acid. ^c Calculated from data available. ^T Amino acid values for pork loin muscle (Cai et al., 2010) and raw chicken (broiler) meat (Straková, Suchý, Vitula, & Večerek, 2006).

Table 2. The fatty acid profile (g/100 g of total fatty acids) of *Ruspolia differens* and typical values of selected animal tissue (g/100 g of total fatty acids)

Fatty Acid	Season and Sourcing Geographical Area						Pork
	Masaka		Kampala		Fort Portal		
	Nov-Dec	April-May	Nov-Dec	April-May	Nov-Dec	April-May	
C12:0	0.04±0.01 ^{aA}	0.37±0.01 ^{aX}	0.04±0.03 ^{aA}	0.04±0.01 ^{aX}	0.06±0.01 ^{aA}	0.12±0.07 ^{aX}	0.21
C14:0	0.82±0.02 ^{aA}	0.73±0.11 ^{aX}	0.78±0.09 ^{aA}	0.66±0.02 ^{aX}	0.75±0.04 ^{aA}	0.62±0.17 ^{aX}	1.3-1.4
C15:0	0.01±0.00 ^{aA}	0.15±0.03 ^{bX}	0.04±0.04 ^{aA}	0.12±0.01 ^{bXZ}	0.11±0.01 ^{aB}	0.09±0.02 ^{aYZ}	4.1-4.7
C16:0	27.3±0.2 ^{aA}	26.6±1.1 ^{aX}	26.9±0.5 ^{aA}	26.8±0.3 ^{aX}	26.84±0.20 ^{aA}	27.8±0.4 ^{bX}	23.2-27.7
C17:0	0.18±0.01 ^{aA}	0.4±0.1 ^{bX}	0.19±0.02 ^{aA}	0.3±0.0 ^{bXZ}	0.26±0.01 ^{aB}	0.21±0.06 ^{aYZ}	0.2-0.3
C18:0	6.72±0.04 ^{aA}	7.2±0.1 ^{aX}	7.2±1.3 ^{aA}	7.5±0.0 ^{aXZ}	6.67±0.04 ^{aA}	7.71±0.30 ^{bYZ}	12.2-16.6
C16:1, ω-7	1.75±0.00 ^{aA}	1.5±0.3 ^{aX}	1.8±0.0 ^{aA}	1.2±0.0 ^{bXZ}	1.9±0.1 ^{aA}	0.89±0.32 ^{bYZ}	2.1-2.8
C18:1, ω-9	41.2±0.1 ^{aA}	38.9±0.1 ^{aX}	41.4±1.5 ^{aA}	38.4±0.2 ^{bXY}	42.7±0.3 ^{aA}	39.2±0.4 ^{bY}	32.8-43.5
C18:2, ω-6	20.8±0.1 ^{aA}	22.1±0.18 ^{aX}	20.3±0.3 ^{aA}	23.0±0.2 ^{bX}	19.0±0.1 ^{aB}	22.0±1.1 ^{bX}	10.7-14.4
C18:3, ω-3	0.96±0.01 ^{aA}	1.5±0.3 ^{bX}	1.0±0.0 ^{aA}	1.3±0.0 ^{bXY}	1.1±0.1 ^{aA}	1.09±0.08 ^{aY}	1.0-1.3
C20:4, ω-6	0.25±0.00 ^{aA}	0.43±0.08 ^{aX}	0.27±0.04 ^{aA}	0.38±0.01 ^{bX}	0.40±0.02 ^{aB}	0.29±0.06 ^{bX}	0.1-0.2
C20:5, ω-3	0.09±0.01 ^{aA}	0.4±0.09 ^{bX}	0.14±0.08 ^{aA}	0.33±0.03 ^{bXY}	0.29±0.02 ^{aB}	0.24±0.06 ^{aY}	0.2-0.4
TSFA	35.6±0.2 ^{aA}	35.6±0.8 ^{aX}	35.5±1.4 ^{aA}	35.8±0.3 ^{aX}	35.1±0.3 ^{aA}	36.8±0.3 ^{bX}	40.7
TMUFA	42.9±0.1 ^{aA}	40.4±0.3 ^{aX}	43.1±1.4 ^{aA}	39.6±0.1 ^{bX}	44.6±0.2 ^{aA}	40.1±0.5 ^{bX}	47.2
TPUFA	21.8±0.1 ^{aA}	24.0±0.5 ^{aX}	21.5±0.3 ^{aA}	24.6±0.2 ^{bX}	20.3±0.1 ^{aB}	23.4±1.0 ^{bX}	11.7
TUFA	64.7±0.2 ^{aA}	64.4±0.8 ^{aX}	64.6±1.4 ^{aA}	64.2±0.3 ^{aX}	64.9±0.3 ^{aA}	63.5±0.7 ^{bX}	-
PUFA/SFA ratio	0.62±0.01 ^{aA}	0.68±0.03 ^{aX}	0.6±0.0 ^{aA}	0.69±0.01 ^{aX}	0.58±0.00 ^{aA}	0.63±0.03 ^{bX}	0.6
Total: ω-6	22.1±0.2 ^{aA}	22.5±0.2 ^{aX}	20.6±0.3 ^{aA}	23.4±0.2 ^{bX}	19.4±0.1 ^{aB}	22.3±1.1 ^{bX}	-
Total: ω-3	1.0±0.0 ^{aA}	1.9±0.4 ^{bX}	1.1±0.0 ^{aA}	1.6±0.1 ^{bXY}	1.4±0.1 ^{a^{dB}}	1.3±0.1 ^{aY}	-
n6/n3 ratio	20.2±0.1 ^{aA}	11.8±1.8 ^{bX}	18.2±0.9 ^{aA}	14.8±0.5 ^{bXY}	14.4±0.9 ^{aB}	16.9±2.2 ^{aY}	7.2
EFA	21.8±0.1 ^{aA}	23.6±0.4 ^{aX}	21.3±0.3 ^{aA}	24.3±0.2 ^{aX}	20.0±0.1 ^{aB}	23.1±1.0 ^{bX}	-

^{ab}For the same geographical area, figures with the same superscripts are not significantly different ($n = 3$, $p < 0.05$), showing the effect of season; For the same season, figures carrying the same superscripts 'ABC' for the December-November season and 'XYZ' for the April-May season are not significantly different ($n = 3$, $p < 0.05$) showing the effect of sourcing geographical area. TSFA = Total Saturated Fatty Acids; TMUFA = Total Mono Unsaturated Fatty Acids; TPUFA = Total Poly Unsaturated Fatty Acids; TUFA = Total Unsaturated Fatty Acids; PUFA= Poly Unsaturated Fatty Acids; SFA = Saturated Fatty Acids; EFA = Essential Fatty Acids. ^TFatty acid profiles for pork (Cai et al., 2010) and chicken meat (Žlender, Holcman, Stibilj, & Polak, 2000).

Table 3. Mineral (mg/100 g dry matter) and Vitamin B 12 (µg/100 g dry matter) content of *Ruspolia differens* and typical values of selected animal tissue (mg/ 100 g dry matter)

Mineral /Vitamin	Season and Sourcing Geographical Area						Animal tissue ^T	
	Masaka		Kampala		Fort Portal		Pork	Broiler chicken
	<i>Nov-Dec</i>	<i>April-May</i>	<i>Nov-Dec</i>	<i>April-May</i>	<i>Nov-Dec</i>	<i>April-May</i>		
<i>Macro-elements</i>								
Phosphorus	440±17 ^{aA}	588±6 ^{bX}	437±12 ^{aA}	627±7 ^{bY}	429±15 ^{aA}	579±11 ^{bX}	223-320	407
Potassium	463±20 ^{aA}	582±15 ^{bX}	446±13 ^{aA}	673±4 ^{bY}	454±19 ^{aA}	610±11 ^{bZ}	370-400	248
Calcium	64.4±9.3 ^{aA}	48.6±1.0 ^{bX}	34.9±1.6 ^{aB}	106±3 ^{bY}	35.7±1.7 ^{aB}	128±4 ^{bZ}	4.3-6.0	5.8
Magnesium	45.8±1.7 ^{aA}	59.3±1.5 ^{bX}	40.8±1.3 ^{aB}	69.0±0.9 ^{bY}	41.8±1.6 ^{aB}	68.2±1.4 ^{bY}	21.0-26.1	29
Sodium	21.6±1.1 ^{aA}	29.9±0.9 ^{bX}	19.7±1.5 ^{aAB}	41.9±0.7 ^{bY}	18.6±0.8 ^{aB}	36.9±2.3 ^{bZ}	45-87	46
<i>Micro-elements</i>								
Iron	45.6±0.8 ^{aA}	57.8±0.7 ^{bX}	32.4±0.9 ^{aB}	53.6±2.0 ^{bY}	33.2±1.0 ^{aB}	69.0±1.3 ^{bZ}	1.4-21	0.4-0.7
Zinc	9.73±0.38 ^{aA}	15.0±0.2 ^{bX}	8.51±0.19 ^{aB}	12.4±0.08 ^{bY}	8.11±0.37 ^{aB}	13.7±0.2 ^{bZ}	2.4-6.9	0.7-1.3
Copper	1.76±0.06 ^{aA}	2.09±0.03 ^{bX}	1.60±0.05 ^{aB}	2.23±0.03 ^{bY}	1.68±0.06 ^{aB}	2.08±0.06 ^{bX}	0.1-2.7	0.04-0.1
Manganese ^{AI}	1.95±0.07 ^{aA}	2.68±2.31 ^{bX}	1.41±0.04 ^{aB}	4.81±0.15 ^{bX}	1.73±0.10 ^{aC}	7.23±0.22 ^{bY}	-	-
<i>Vitamin</i>								
Vitamin B12	1.35±0.02 ^{aA}	1.04±0.03 ^{bX}	0.73±0.08 ^{aB}	0.88±0.01 ^{bY}	0.98±0.04 ^{aC}	1.27±0.03 ^{bZ}	0.55	0.37

^{ab}For the same geographical area, figures with the same superscripts are not significantly different ($n = 3$, $p < 0.05$), showing the effect of season; For the same season, figures carrying the same superscripts 'ABC' for the December-November season and 'XYZ' for the April-May season are not significantly different ($n = 3$, $p < 0.05$) showing the effect of sourcing geographical area. RDA = Reference dietary allowances according to (Strain & Cashman, 2009) [†], (FAO/WHO, 2004) ^{*} and (Stipanuk & Caudill, 2013) [□]. ^TMineral profiles for pork and edible pig offal (Tomovic et al., 2015) and chicken meat (NIIR, 2008). ^{AI}Adequate Intake values provides instead of RDA.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Highlights

- ✓ Protein and fat make up 86 - 89 % of the *Ruspolia differens*' nutrient components
- ✓ A vital source of PUFAs, essential amino acids, & trace elements (Fe, Zn, Mn, Cu)
- ✓ *R. differens* has a high ω -6/ ω -3 ratio & tryptophan is its first limiting amino acid
- ✓ Unlike geographical area, season influences *R. differens*' nutrient composition
- ✓ It can contribute to alleviating protein, Zn, Fe, & vitamin B12 deficiency