



THE NUTRITIONAL POTENCY OF SOUTHERN AFRICAN *SCHISTOCERCA GREGARIA*, *ENCOSTERNUM DELEGORGUEI* AND *MACROTERMES* SPP. FOR FOOD AND FEED

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Abstract

The current study examined the chemical composition of four (4) African adult edible insects: *Schistocerca gregaria* (desert locust), *Encosternum delegorguei* (green stinkbug), *Macrotermes falciger* (soldier termite) and *Macrotermes natalensis* (winged termite). Fresh insects (1 000 g of each) were acquired, euthanised, processed and packaged into triplicates. Only *M. natalensis* were further exposed to oil extraction to produce by-products: winged termite meal (WTM) and winged termite oil (WTO). Hence, a total of six (6) insect samples were chemically and statistically evaluated for proximate, amino acid and fatty acid contents. *S. gregaria* (66.11%) and WTM (53.64%) had higher ($P<0.05$) crude protein than the other insect samples, respectively. Fats, crude fibre and NDF were more abundant ($P<0.05$) in *E. delegorguei* than other insect samples, whereas only WTM and *M. natalensis* had elevated ash content. A total of 16 amino acids (9 essential and 7 non-essential) and 30 fatty acids (15 SFA, 6 MUFA and 9 PUFA) were detected in selected insect samples. Although, *S. gregaria* and *M. natalensis* had more ($P<0.05$) essential amino acids (EAAs) than other insect meals, EAAs from all insect samples met the human protein requirements recommended by the WHO (2007). Leucine (2.32 to 5.67 g/100 g) was the most predominant ($P<0.05$) while methionine (0.38 to 1.03 g/100 g) was the least abundant ($P<0.05$) EEA across all insect meals. MUFA were predominantly higher ($P<0.05$) in *E. delegorguei* (59.53 g/100 g) and WTO (52.05 g/100 g), respectively, while PUFA (29.11 g/100 g) and omega 6 (n-6) fatty acids (26.92 g/100 g) concentration were higher ($P<0.05$) in *M. falciger*, but omega 3 (n-3) fatty acids (12.41 g/100 g) dominated in *S. gregaria*. In conclusion, the selected African insects could be utilised in human and livestock diets to supply protein, essential amino acids and fatty acids. Hence, their mass production could help improve the overall availability of conventional edible insects in the market.

Key words: nutritional value, edible insects, insect powder, oil extraction, entomography

The utilisation of insects as food and feed has long been practised in about 113 countries around the globe (Kouřimská and Adámková, 2016). Out of 1.4 to 1.8 million of known insects (Scudder, 2017), about 1611 species were detected as suitable for consumption by humans, livestock and pets worldwide (Van Itterbeeck and Pelozuelo, 2022; Ummara et al., 2023). In sub-Saharan Africa insects such as locusts, stinkbugs and termites are part of the main food source, especially in rural areas (Dzerefos et al., 2013; Kinyuru, 2014, doctoral dissertation). They are highly documented to be rich in protein, fats and minerals, which play a significant role in the growth and proper functioning of the body (Rumpold and Schlüter, 2013; Musundire et al., 2016; Kuntadi et al., 2018; Straub et al., 2019; Nogales-Merida et al., 2019; Mousavi et al., 2020; Mulazzani et al., 2021). The sustainable farming of the above-mentioned edible insects will not only help create employment but will, most importantly, be useful as an alternative to expensive conventional protein sources such as soybean and fishmeal in diets (Tanga et al., 2021). Subsequently, it can reduce food and feed costs and address other issues such as malnutrition, hunger and poverty, which are currently the main targets of Sustainable Development Goals by 2030 (Kewuyemi et al., 2020; Sithole et al., 2021). Hence, knowledge of the nutri-

tional value and safe consumption of edible insects found in South Africa is crucial (van Huis, 2020).

The chemical composition of most edible insects, particularly crude protein (fresh or dry matter), has been reported to be superior to that of soybeans and highly comparable to that of meat, fish and chicken eggs (Mlček et al., 2014; Kim et al., 2019; Kuntadi et al., 2018). Although high chitin from insects has been reported as the main anti-nutritional factor inhibiting the accessibility of insect protein, processing techniques such as drying and oil extraction have been reported to alter and improve protein availability in edible insects (Meyer-Rochow et al., 2021). For instance, oven drying has been reported to elevate the protein content of insects (Ssepunya et al., 2017; Matandirotya et al., 2022). Moreover, the removal of exoskeletons during processing has been reported to significantly improve insect digestibility (Rumpold and Schlüter, 2013). However, less work has been done on the nutritional composition of South African edible insects such as *Schistocerca gregaria*, *Encosternum delegorguei*, *Macrotermes falciger* and *Macrotermes natalensis* compared to soybean and fishmeal.

A thorough understanding of the chemical characteristics of southern African edible insects will aid in determining their quality and sustainability as food and feed

(Freccia et al., 2020). Thus, the present study seeks to summarise the proximate, amino acid and fatty acid composition of *S. gregaria*, *E. delegorguei*, *M. falciger* and *M. natalensis* for potential rearing to address food insecurity. The alternative hypothesis of the present study stated that there will be significant differences in the nutrient composition of *Schistocerca gregaria*, *Encosternum delegorguei*, *Macrotermes falciger* and *Macrotermes natalensis* as a potential protein source for human and livestock.

Material and methods

Insect acquisition and preparation

Fresh live adult *Schistocerca gregaria*, *Encosternum delegorguei*, and *Macrotermes falciger* and *Macrotermes natalensis* (each at about 1 000 g) were harvested (Figure 1) in Mopani woodlands of Limpopo province, South Africa in separate containers by insect harvesters from Maskiri Food Suppliers situated in Sibasa, Tohoyandou, Venda, Limpopo. Prior to sun drying for 6 hours, the acquired live insects were humanely euthanised by freezing a few hours after collection as described by Klunder et al. (2012) and Egan et al. (2021). The samples were then de-winged, de-legged and air dried for 24 hours at 40°C using a Labotec Eco Therm Digital Oven (Labotec, Gauteng, South Africa) until they were completely dry at a constant weight and thereafter, cooled at room temperature (25°C) for 30 minutes. Each insect sample was ground into approximately 500 g of meal/powder using a Jug Blender 550W Optima model (Mellerware, Durban, South Africa) prior to analysis. About 250 g of dried *Macrotermes natalensis* (winged termites) sample was further defatted using an oil press machine (Ulimac Machine, Ankara, Turkey) to produce insect cake/meal (WTC=WTM) and oil (WTO) as byproducts in which the ratio of meal to oil was 3:1. Thus, a total of six (6) composite samples including *S. gregaria*, *E. delegorguei*, *M. falciger*, *M. natalensis*, WTM and WTO were prepared

for analysis. Each sample was divided and packaged into three subsamples of sealable air-tight ziplock bags. However, WTO was stored in lid-closing plastic containers. All samples were then sent to Animal NutriLab, University of Pretoria, South Africa proximate and amino acid analysis. Whereas, fats and fatty acid profiling was done at Central Analytical Facilities, Stellenbosch University, South Africa.

Proximate analysis of insects

The percentage dry matter (DM), protein (CP), crude fat (EE), crude fibre (CF), neutral detergent fibre (NDF) and ash were analysed using methods of the Association of Official Analytical Chemists (AOAC, 2000). Five (5) g of insect powder from *S. gregaria*, *E. delegorguei*, *M. natalensis* and WTM were prepared and weighed into pre-heated, cooled silica dishes, then oven-dried at 105°C for 24 hours until a constant weight was obtained. Protein determination was done following the procedure described by Han and Heinonen (2021). Insect protein was extracted and separated from chitin using the alkaline hydrolysis method. Each insect powder was mixed with 0.5 M NaOH and then stirred at 25°C for 4 hours until the protein was removed to minimal levels. Eventually, chitin pellets were produced after centrifuging the samples as outlined by Han and Heinonen (2021). EE was determined using an ANKOM XT 20 extractor (ANKOM Technology, New York, USA). CF and NDF were determined by refluxing 0.45 g of samples with a neutral detergent solution for one (1) hour using the ANKOM 2000 fibre analyser (ANKOM Technology, New York, USA). The ash content was determined by ashing dried samples at 550°C for about six (6) hours in a muffle furnace and the remaining residue was the total ash, however, the dish and ash contents were allowed to cool in a desiccator before weighing. The available carbohydrate was determined using the formula: Available CHO = 100 – (% lipid + % ash + % moisture + % protein + % dietary fibre). The gross energy (GE) value was determined using a bomb calorimeter (AOAC, 2010).

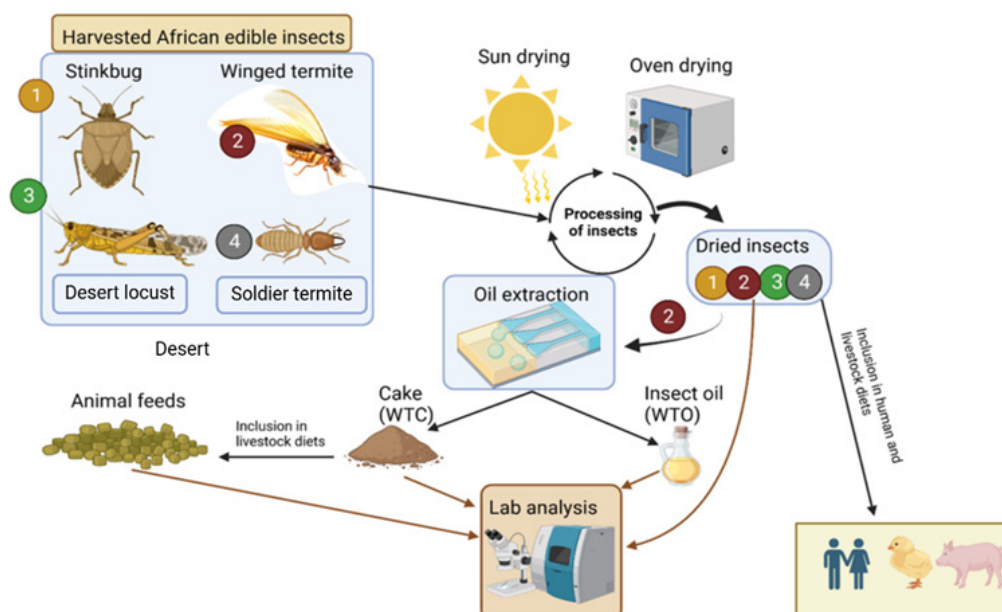


Figure 1. Collection procedure and processing of harvested edible insects for use as food and feed

Analysis of insect amino acids (AA)

Free amino acids were analysed using phenyl isothiocyanate (PITC) pre-column derivatisation and high-pressure liquid chromatographic (HPLC) combined with solid phase extraction (SPE) (Weichun et al., 2019). To achieve that, protein from insect material was extracted and further precipitated with tungstate, and the concentration of different carbohydrates in the filtrate was determined by HPLC with refractive index detection.

Analysis of insect fatty acids (FA)

The total fats and fatty acid profile was done following the procedure by Pieterse et al. (2019). Two (2) g of insect samples including *E. delegorguei*, *M. falciger*, *M. natalensis*, WTM, and WTO were weighed into an extraction tube, and 20 mL of 2:1 (v/v) chloroform/methanol solution (containing 0.01% butylated hydroxytoluene as antioxidant) and 500 µL internal standard (C17:0) were added to each tube. The mixture was homogenised for 10 s using a polytron mixer (Kinematica AG homogenizer, PT-500 E, Serial No. PF-799-0004-02-19) and then transferred to an extraction funnel covered with glass microfibre filter paper (Whatman, GF/A, 47 mm diameter). The funnel led into a 50 mL volumetric flask, and the final volume was 50 mL. A 250 µL aliquot of the filtered phase was transferred to a Klimax tube and dried under nitrogen in a water bath with an electronically controlled temperature of 45°C and water circulation. A transmethylating agent (19:1, v/v, methanol-sulfuric acid solution) was added (2 mL) to each sample and placed in a water bath at 70°C for 2 hours. The mixture was cooled to room temperature and the fatty acid methyl esters (FAME) were extracted with distilled water (1 mL) and hexane (2 mL) by transferring the top hexane phase to a clean Klimax tube and then drying it under nitrogen in a water bath at 45°C. For chromatographic analysis, 50 µL hexane was added to the dried FAME sample and transferred to an autosampler vial with 0.1 mL insert and closed with a screw cap (PTFE/red silicone septa) (Supelco™, Bellefonte, Pennsylvania, USA).

A Thermo Finnigan Focus gas chromatograph instrument (Thermo Electron Corp., Milan, Italy) coupled with a flame ionisation detector and split injector port, set at 260°C and 220°C, respectively, was used for determining FAME. Chromatographic separation of FAME was performed on a BPX70 column (60 m × 0.25 mm; ID 0.25 µm; SGE International Pty Ltd, Ringwood, Victoria, Australia). The split flow of the gas chromatograph was 20:120 with hydrogen as carrier gas (20 mL min⁻¹ flow rate). Each sample volume injected was 1 µL and the run time was approximately 45 minutes. Fatty acids were identified by comparing their retention times to those found in a standard FAME mixture (Supelco 37 Component FAME mix, C₄–C₂₄, 10 mL min⁻¹ in CH₂Cl₂; Bellefonte, PA, USA). The results were recorded as g/100 g of the total fatty acids.

Statistical analysis

The data for chemical analysis was statistically processed and analysed using one-way ANOVA from Proc

GLM of SAS version 9.4.1 (SAS, 2010). The following linear statistical model was applied:

$$Y_{ij} = \mu + d_i + E_{ij}$$

where Y_{ij} = response variable, μ = population mean, d_i = different insect composition, E_{ij} = random error associated with observation ij assumed to be normally and independently distributed. For all statistical test, the significance level was declared at $P < 0.05$. The least square means was compared using Turkey's HSD test.

Results

Proximate composition

As shown in Table 1, macronutrients (DM, CP, EE, CF, NDF, ash, carbohydrates, and GE) analysed in the present study varied significantly ($P < 0.05$) across all insect meals including *Schistocerca gregaria*, *Encosternum delegorguei*, *Macrotermes natalensis* (winged termite) and winged termite meal (WTM). CP (39.23 to 66.11%), EE (11.29 to 48.95%) and NDF (18.02 to 28.55%) were the most abundant macronutrients across all insect meals. *S. gregaria* had the lowest ($P < 0.05$) DM content compared to other insect meals which had similar ($P > 0.05$) DM. Higher ($P < 0.05$) CP was observed in *S. gregaria* followed by WTM, *M. natalensis* and *E. delegorguei*. The respective insects *E. delegorguei*, *M. natalensis*, and WTM had elevated ($P < 0.05$) EE whilst *S. gregaria* contained lowest ($P < 0.05$) EE levels. CF content was the highest ($P < 0.05$) in *E. delegorguei* followed by *S. gregaria* and WTM, and lowest ($P < 0.05$) in *M. natalensis*. Again, *E. delegorguei* and WTM had higher ($P < 0.05$) NDF than both *M. natalensis* and *S. gregaria*. However, ash content was the highest ($P < 0.05$) in WTM followed by *M. natalensis*, *E. delegorguei*, and *S. gregaria*. Again, *M. natalensis* had higher ($P < 0.05$) carbohydrate contents than *E. delegorguei* and *S. gregaria*. GE levels were higher ($P < 0.05$) in *E. delegorguei* than in *S. gregaria* and *M. natalensis*. However, neither carbohydrates nor GE were detected in WTM.

Amino acid content

The essential amino acid (EAA) (g/100 g) composition of *S. gregaria*, *E. delegorguei*, *M. natalensis*, and WTM presented in Table 2 varied significantly ($P < 0.05$) across all selected insect meals in which leucine and methionine were the most and least predominant EAA, respectively. The lowest ($P < 0.05$) arginine concentration was observed in *E. delegorguei* while *S. gregaria*, WTM and *M. natalensis*, respectively had the highest ($P < 0.05$) arginine levels. However, histidine was higher ($P < 0.05$) in *S. gregaria* and *M. natalensis* than in WTM and *E. delegorguei*. Both isoleucine and leucine levels were higher ($P < 0.05$) in *S. gregaria* followed by WTM, *M. natalensis* and *E. delegorguei*. Nonetheless, lysine was significantly higher

in WTM than *M. natalensis* and *S. gregaria* while *E. delegorguei* had the lowest ($P<0.05$) lysine content. *M. natalensis* had higher ($P<0.05$) methionine levels than *E. delegorguei*, WTM and *S. gregaria*, respectively. The lowest ($P<0.05$) threonine levels were observed in *E. delegorguei*, whereas it was the highest ($P<0.05$) in WTM followed by *S. gregaria* and *M. natalensis*. However, valine levels were higher in *S. gregaria* than in WTM, *M. natalensis* and *E. delegorguei*. Similarly, the total EAA and non-essential amino acids (NEAA) were the highest in *S. gregaria* followed by WTM, *M. natalensis* and *E. delegorguei*. However, a better EAA to NEAA ratio was observed in *M. natalensis* followed by WTM, *E. delegorguei*, and *S. gregaria*.

All NEAA (g/100 g) varied significantly ($P<0.05$) across all insect meals, including *S. gregaria*, *E. delegorguei*, *M. natalensis*, and WTM (Table 3). Glutamine and serine were

the most and least predominant NEAA, respectively in all insect meals. Higher ($P<0.05$) alanine levels were observed in *S. gregaria* than WTM and *E. delegorguei*, respectively, while *M. natalensis* had the lowest ($P<0.05$) alanine contents. Aspartic acid was the highest ($P<0.05$) in WTM followed by *S. gregaria* and *M. natalensis*, while it was the lowest ($P<0.05$) in *E. delegorguei*. Elevated ($P<0.05$) glycine content was found in *S. gregaria* followed by WTM, *E. delegorguei* and *M. natalensis*. However, *E. delegorguei* had the lowest ($P<0.05$) glutamine content, while the highest ($P<0.05$) concentration was observed in WTM, followed by *S. gregaria* and *M. natalensis*. Proline on the other hand, was only detected in WTM, while serine content was highest ($P<0.05$) in *S. gregaria* compared to WTM, *M. natalensis* and *E. delegorguei*, respectively. Again, tyrosine concentration was higher in *S. gregaria* followed by *M. natalensis*, WTM and *E. delegorguei*.

Table 1. Proximate composition (%; unless stated otherwise) of *S. gregaria*, *E. delegorguei*, *M. natalensis*, winged termite meal (WTM) and conventional foods

Parameters ¹	Insect meals				SEM ³	P value	Conventional foods ⁴	
	<i>S. gregaria</i>	<i>E. delegorguei</i>	<i>M. natalensis</i>	WTM ²			fish	soybean
DM	91.31 b	96.56 a	95.47 a	95.60 a	0.270	<.0001	91.10	92.00
CP	66.11 a	39.23 c	40.56 c	53.64 b	0.415	<.0001	71.50	37.80
EE	11.29 d	48.95 a	43.47 b	22.51 c	0.409	<.0001	8.00	18.38
CF	9.89 b	15.16 a	4.45 d	6.83 c	0.247	<.0001	1.20	5.12
NDF	18.02 c	28.55 a	23.68 b	26.50 ab	0.767	<.0001	NR	12.98
Ash	4.56 c	4.51 c	6.36 b	9.67 a	0.130	<.0001	7.30	4.86
Carbohydrates	0.79 b	0.37 c	2.42 a	–	0.095	<.0001	NR	4.66
GE (kcal/kg)	1557.77 b	1688.59 a	1562.34 b	–	0.264	<.0001	497.13	466.00–469.80

¹Parameters: DM = dry matter; CP = crude protein; EE = crude fat; CF = crude fibre; NDF = neutral detergent fibre; GE = gross energy.

²WTM: Winged termite meal, a by-product of *M. natalensis*.

³SEM = standard error of mean.

⁴Conventional foods: Regost et al., 2003; Najafi et al., 2012; Chen et al., 2012; Etiosa et al., 2018; Cho and Kim, 2011; NR = not reported; “–” = not detected.

a, b, c, d = means with different letters in the same column indicate significant differences between treatments ($P<0.05$).

Table 2. Essential amino acid composition (g/100 g) of *S. gregaria*, *E. delegorguei*, *M. natalensis*, winged termite meal (WTM) and conventional foods

EAA ¹	Insect meals				SEM ³	P value	Conventional foods ⁴	
	<i>S. gregaria</i>	<i>E. delegorguei</i>	<i>M. natalensis</i>	WTM ²			fish	soybean
Arginine	3.72 a	1.88 b	2.89ab	2.96 ab	0.249	<.0001	5.34	3.15
Histidine	1.72 a	1.14 b	1.77 a	1.32 b	0.046	<.0001	4.19	1.10
Isoleucine	2.96 a	1.28 d	1.78 c	2.40 b	0.078	<.0001	2.62	1.97
Leucine	5.67 a	2.32 d	3.44 c	4.43 b	0.062	<.0001	8.31	3.31
Lysine	2.63 b	1.52 c	2.13 b	4.14 a	0.111	<.0001	10.96	2.71
Methionine	0.38 c	0.66 b	1.03 a	0.48 bc	0.052	<.0001	2.26	0.55
Phenylalanine	2.61 b	1.59 c	2.84 a	2.83 a	0.069	<.0001	5.52	2.12
Threonine	2.21 b	1.15 c	1.88 b	2.77 a	0.079	<.0001	5.28	1.77
Valine	4.21 a	2.18 c	2.12 c	3.16 b	0.075	<.0001	5.88	2.03
Σ EAA	26.11	13.72	19.88	24.49			50.36	18.71
Σ NEAA	31.93	15.39	17.73	26.13			NR	23.07
EAA/NEAA ratio	0.82	0.89	1.12	0.94			NR	0.81

¹EAA = essential amino acids; ΣEAA = sum of essential amino acids; ΣNEAA = sum of non-essential amino acids.

²WTM: winged termite meal, a by-product of *M. natalensis*.

³SEM = standard error of mean.

⁴Conventional foods: Regost et al., 2003; Najafi et al., 2012; Chen et al., 2012; Cho and Kim, 2010; “–” = not detected.

a, b, c, d = means with different letters in the same row indicate significant differences between treatments ($P<0.05$).

Table 3. Non-essential amino acid composition (g/100 g) of *S. gregaria*, *E. delegorguei*, *M. natalensis* and winged termite meal (WTM) and conventional foods

NEAA ¹	Insect meals ²			WTM	SEM ³	P value	Conventional foods ⁴	
	<i>S. gregaria</i>	<i>E. delegorguei</i>	<i>M. natalensis</i>				fish	soybean
Alanine	9.85 a	2.93 c	2.16 d	3.48 b	0.057	<.0001	3.76	1.92
Aspartic acid	3.81 b	2.76 c	3.63 b	5.19 a	0.102	<.0001	5.33	5.12
Glycine	4.40 a	1.97 bc	1.87 c	2.44 b	0.105	<.0001	4.56	1.88
Glutamine	6.09 b	3.29 d	4.32 c	6.65 a	0.072	<.0001	7.62	7.87
Proline	–	–	–	2.21	0.026	<.0001	2.85	2.38
Serine	2.49 a	1.67 c	1.94 bc	2.11 ab	0.092	<.0001	2.98	2.36
Tyrosine	5.29 a	2.77 c	3.81 b	4.05 b	0.066	<.0001	2.07	1.54

¹NEAA = non-essential amino acids; Σ NEAA: sum of non-essential amino acids.²Insect meals: WTM = winged termite meal, a by-product of *M. natalensis*; “–” = not detected³SEM = standard error of mean.⁴Conventional foods: Chen et al., 2012; Ween et al., 2017; NR = not reported.

a, b, c, d = means with different letters in the same row indicate significant differences between treatments (P<0.05).

Table 4. Fatty acid composition (g/100 g, on a dry matter basis) of *S. gregaria*, *E. delegorguei*, *M. falciger* and *M. natalensis*, winged termite by-products (WTM and WTO) and common edible oils

Fatty acid profile ¹	Insect meals ²				By-products ³		SEM ⁴	P value	CEO ^{5*}	
	<i>S. gregaria</i>	<i>E. delegorguei</i>	<i>M. falciger</i>	<i>M. natalensis</i>	WTM	WTO			fish	soybean
1	2	3	4	5	6	7	8	9	10	11
Saturated fatty acids										
Caproic acid (C6:0)	–	–	–	#	–	–	0.005	0.003	NR	NR
Caprylic acid (C8:0)	–	–	–	0.43	–	–	0.016	<.0001	NR	NR
Hendecanoic acid (C11:0)	0.71 a	0.29 ab	0.60 a	–	–	–	0.172	0.034	NR	NR
Lauric acid (C12:0)	–	#	#	#	#	#	0.010	0.101	NR	NR
Tridecylic acid (C13:0)	–	#	–	–	–	#	0.057	0.008	NR	0.28
Myristic acid (C14:0)	1.47 a	0.80 b	#	0.79 b	0.16 c	0.78 b	0.058	<.0001	6.20–9.90	0.05–0.70
Pentadecylic acid (C15:0)	–	–	#	0.25 a	0.10 b	0.24 a	0.024	<.0001	NR	NR
Palmitic acid (C16:0)	29.32 b	34.78 ab	10.84 c	34.90 ab	10.81 c	35.20 a	1.608	<.0001	10.70–22.50	3.07–7.54
Margaric acid (C17:0)	0.66 b	–	1.49 a	0.50 b	0.16 c	0.54 b	0.050	<.0001	NR	NR
Stearic acid (C18:0)	10.04 a	1.57 c	9.79 a	11.00 a	3.17 b	10.05 a	0.360	<.0001	1.11–4.80	2.34–5.15
Nonadecanoic acid (19:0)	–	–	2.23	–	–	–	0.057	<.0001	NR	NR
Arachidic acid (C20:0)	0.77 b	0.08 d	1.78 a	0.27 c	0.12 d	0.21 cd	0.046	<.0001	NR	0.20–1.40
Heneicosanoic acid (C21:0)	–	–	–	0.34 a	0.08 b	0.30 a	0.021	<.0001	NR	1.76
Behenic acid (C22:0)	0.18 b	–	0.94 a	–	–	–	0.027	<.0001	NR	0.17–1.64
Lignoceric acid (C24:0)	–	–	#	#	–	–	0.006	0.007	NR	NR
Monounsaturated fatty acids										
Myristoleic acid (C14:1)	0.41 b	0.57 a	–	–	–	–	0.022	<.0001	NR	1.19
CisPentadecylic acid (C15:1)	–	–	0.36 a	0.06 b	#	–	0.014	<.0001	NR	NR
Palmitoleic acid (C16:1)	1.60 bc	34.26 a	–	1.64 bc	1.32 c	4.14 b	0.900	<.0001	7.50–12.50	9.32

Table 4 – contd.

1	2	3	4	5	6	7	8	9	10	11
Vaccenic acid (C18:1) (trans)	1.18 a	0.35 b	#	#	#	#	0.030	<.0001	NR	NR
Oleic acid (C18:1) (cis)	35.65 c	24.35 d	42.46 bc	45.45 b	39.04 c	47.85 a	2.147	<.0001	12.00–25.80	8.79–23.10
Eicosenoic acid (C20:1)	0.24	–	–	–	–	–	0.008	<.0001	1.90–19.80	0.18–4.28
Polyunsaturated fatty acids										
Linoleic acid (C18:2n-6)	5.29 b	2.85 c	26.92 a	1.75 d	–	–	0.228	<.0001	1.10–5.10	27.60–68.02
α -Linolenic acid (C18:3n-3)	12.41 a	–	0.78 b	0.05 c	0.06 c	0.62 bc	0.146	<.0001	0.70–1.30	4.60–12.11
Conjugated linoleic acid (C18:3n-6)	–	–	–	0.41	–	–	0.013	<.0001	0.20	0.10
Stearidonic acid (C18:4n-3)	–	–	1.41	–	–	–	0.024	<.0001	1.40–2.90	0.90
Eicosadienoic acid (C20:2n-6)	–	–	#	#	–	0.06	0.008	0.037	0.30	0.20–3.91
Eicosatrienoic acid (C20:3n-3)	–	–	–	#	–	–	0.007	0.018	0.20–0.40	0.10–3.77
Arachidonic acid (C20:4n-6)	–	–	#	–	–	#	0.008	0.038	0.10–1.60	0.30
Eicosapentaenoic acid (C20:5n-3)	–	–	–	–	–	#	0.005	0.002	3.70–18.30	2.35–3.70
Docosadienoic acid (C22:2n-6)	–	–	–	#	–	–	0.008	0.045	NR	2.21
Totals										
Total FA (%)	99.93 a	99.90 a	99.60 a	97.84 a	55.02 b	99.99 a	4.338	<.0001	NR	NR
Σ SFA	43.16 bc	37.52 c	27.67 d	48.48 ab	14.60 e	47.32 a	1.651	<.0001	22.50	18.50
Σ MUFA	39.08 c	59.53 b	42.82 c	47.15 bc	40.36 c	52.05 a	2.669	<.0001	47.10	29.80
Σ PUFA	17.70 b	2.85 c	29.11 a	2.21 c	0.06 d	0.62 d	0.306	<.0001	NR	NR
Σ PUFA/ Σ SFA ratio	0.41 b	0.08 c	1.05 a	0.05 c	#	0.01 c	0.019	<.0001	NR	NR
Σ PUFA/ Σ MUFA ratio	0.45 b	0.05 c	0.68 a	0.05 c	#	0.01 c	0.013	<.0001	NR	NR
Σ n-6	5.29b	2.85 c	26.92 a	2.16 c	–	0.06	0.225	<.0001	6.30	36.20
Σ n-3	12.41 a	–	2.19 b	0.05 d	0.06 d	0.62 c	0.511	<.0001	24.10	15.50
n-6/n-3 ratio	0.43 b	–	12.29 ab	43.20 a	–	0.11 b	4.716	0.002	0.26	2.34

¹Fatty acids: values obtained based on the percentage of g/100 g of the lipids extracted from the dry matter Σ SFA = saturated fatty acids; Σ MUFA = monounsaturated fatty acids; Σ PUFA = sum of polyunsaturated fatty acids; n-3 = omega-3 fatty acids; n-6 = omega-6 fatty acids

²# = detected at low concentration (<0.01 g/100 g); “–” = not detected.

³By-products: WTM = winged termite meal; WTO = winged termite oil; “–” = not detected.

⁴SEM = standard error of mean.

⁵CEO = conventional edible oils; NR = not reported: Regost et al., 2003; Najafi et al., 2012; Ivanov et al., 2010; Budran et al., 2023.

a, b, c, d, e = means with different letters in the same row indicate significant differences between treatments (P<0.05).

Fatty acid concentrations

The fatty acid profile (g/100 g), including saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) of *S. gregaria*, *E. delegorguei*, *M. falciger*, *M. natalensis*, WTM and WTO are depicted in Table 4. From the current study, eleven (11) fatty acids including caproic, caprylic, lauric, nono-decanoic, eicosenoic, conjugated linoleic, stearidonic, eicosadienoic, eicosatrienoic, eicosapentaenoic and docosadienoic acids did not vary significantly (P>0.05) across all insect samples. However, nineteen (19) fatty acids namely hendecanoic, tridecylc, myristic, pentade-

cylic, palmitic, margaric, stearic, arachidic, heneicosa-noic, behenic, lignoceric, myristoleic, cisPentadecylic, palmitoleic, vaccenic, oleic, linoleic, α -linolenic and arachidonic acids varied (P<0.05) across insect samples. Palmitic and oleic acids ranging from 10.84 to 35.20 g/100 g and 24.35 to 47.91 g/100 g, respectively, were the most predominant fatty acids across all insect samples.

In terms of SFAs, hendecanoic acid was the highest (P<0.05) in *S. gregaria*, *M. falciger* and *E. delegorguei*, respectively. WTM had the lowest myristic acid concentration of all insect samples. Pentadecylic acid (only

detected in few samples), was higher ($P<0.05$) in *M. natalensis* and WTO than WTM. Palmitic acid was the lowest ($P<0.05$) in *M. falciger* and WTM, respectively, while it was the highest ($P<0.05$) in WTO followed by *M. natalensis*, *E. delegorguei* and *S. gregaria*. However, higher ($P<0.05$) levels of margaric acid were found in *M. falciger* than in *S. gregaria*, *M. natalensis* and WTO, respectively, while WTM had lowest ($P<0.05$) proportion. The WTO, *M. natalensis*, *S. gregaria* and *M. falciger* had higher stearic acid levels than WTM and *E. delegorguei*. Moreover, the lowest ($P<0.05$) arachidic acid levels were found in WTM and *E. delegorguei*, respectively, while *M. falciger* followed by *S. gregaria*, *M. natalensis* and WTO had the highest ($P<0.05$) levels. Both *M. natalensis* and WTO had higher ($P<0.05$) heneicosanoic acid levels than WTM. However, only *M. falciger* had higher behenic acid levels than *S. gregaria*.

For MUFAs, myristoleic acid was higher ($P<0.05$) in *E. delegorguei* than in *S. gregaria* while cis-pentadecylic acid contents were higher in *M. falciger* than in *M. natalensis*. Higher ($P<0.05$) palmitoleic acid levels were observed in *E. delegorguei* than in WTO, *M. natalensis*, *S. gregaria* and WTM, whereas the vaccenic acid concentration was higher ($P<0.05$) in *S. gregaria* than *E. delegorguei*. The highest ($P<0.05$) oleic acid levels were found in WTO followed by *M. natalensis*, *M. falciger*, WTM, *S. gregaria* and *E. delegorguei*. In terms of PUFAs, linoleic acid levels were the lowest ($P<0.05$) in *M. natalensis* while it was higher ($P<0.05$) in *M. falciger* than in *S. gregaria* and *E. delegorguei*, respectively. However, highest ($P<0.05$) α -linolenic acid contents were observed in *S. gregaria* followed by *M. falciger*, WTO, WTM and *M. natalensis*.

The total fatty acids (TFA), SFA and MUFA were higher ($P<0.05$) in WTO than in *M. falciger*, *E. delegorguei*, *M. natalensis* and *S. gregaria*, respectively, while WTM had the lowest ($P<0.05$) TFA concentration. PUFA levels on the other hand, were lower ($P<0.05$) in WTO and WTM, whereas both PUFA and omega 6 (n-6) were the highest ($P<0.05$) in *M. falciger* followed by *S. gregaria*, *E. delegorguei* and *M. natalensis*. Again, the best ($P<0.05$) PUFA/SFA and PUFA/MUFA ratios were also observed in *M. falciger* followed by *S. gregaria*, *E. delegorguei*, *M. natalensis* and WTO. However, omega 3 (n-3) levels were higher ($P<0.05$) in *S. gregaria* than in *M. falciger*, WTO, *M. natalensis* and WTM, respectively. Moreover, poor ($P<0.05$) n-6/n-3 ratios were found in *S. gregaria* and WTO while *M. natalensis* and *M. falciger* had better ($P<0.05$) n-6/n-3 ratios, respectively.

Discussion

Evaluating the nutrient quality of *Schistocerca gregaria*, *Encosternum delegorguei* and *Macrotermes natalensis* commonly consumed in southern Africa remains a vital source of nutrients for both humans and livestock, especially in rural communities (Nyangena et al., 2020;

Egan et al., 2021; Freccia et al., 2020). All insect samples varied significantly ($P<0.05$) in proximate, amino acid and fatty acid proportions. This variation may be due to the insects' species type, physiological state or processing of samples prior to analysis. This agrees with findings by Meyer-Rochow et al. (2021). Again, the evaluated insect meals seem to be more comparable or superior to soybean (SBM) and fishmeal (FM) in overall nutritional value. The current findings are supported by Kim et al. (2019).

Proximate composition

The protein (CP) followed by fats (EE) and neutral detergent fibre (NDF) were the most abundant macronutrients constituting above 80% of the total dry matter across all insect meals. Several articles reported similar findings (Musundire et al., 2016; Fombong et al., 2021; Hlongwane et al., 2020). Higher CP content in all insect meals, especially *S. gregaria* and WTM makes them a good dietary protein source that could be vital in poultry diets. This is aligned with findings made by (Fombong et al., 2021; Hlongwane et al., 2021). Furthermore, the selected insects (39.23 to 66.11%) outcompeted SBM (37.80%) but were lower than FM (71.50%) in terms of CP. Emphasising their potential as alternative replacement for SBM in both humans and livestock diets. However, Zielińska et al. (2015) reported higher CP content (76%) in *S. gregaria* than that observed (66.11%) in the current study. This may be due to factors such as foliage type and environmental conditions in different countries. Nonetheless, the CP of all insect samples fell within the range (31.06 to 76.69%) reported by few authors (Kewuyemi et al., 2020; Kuntadi et al., 2018; Mlček et al., 2014).

Excessive EE accumulation observed in *E. delegorguei* and *M. natalensis*, respectively could adversely cause cardiovascular related complications when incorporated in diets (Sales-Campos et al., 2013). Moreover, the inclusion of insects with high fat levels in poultry diets could also prevent proper mixing of the diet and ultimately result in lipid oxidation that reduces meat shelf-life (Mutungi et al., 2019; Mishyna et al., 2020). Thus, there is a need to defat insects through oil extraction prior to inclusion in livestock diets. Nonetheless, the fat composition of selected insects in the current study fell within the range (22.51 to 48.95%) reported by Musundire et al. (2016) and Dobermann et al. (2017). Additionally, high dietary supplementation of fats/lipids from edible insects could aid in improving food palatability and flavour (Igwe et al., 2011). Again, lower EE in *S. gregaria* (11.29%) observed in the present study in comparison with that (27.95%) reported by Khalil (2018, doctoral dissertation) could be the exposure to different foliage and environment as the insects were harvested in different countries.

Dietary insect fibre particularly comprises chitin and its derivatives responsible for modifying gut microbiota (Stull and Wier, 2023), antioxidative, antimicrobial, im-

munostimulatory and anti-inflammatory effects in humans and livestock (Mousavi et al., 2020; Son et al., 2021). Excessive consumption of fibre rich tree foliage by *E. delegorguei* could be the cause of elevated CF compared to other insects observed in the current study. According to Rumpold and Schlüter (2013), *E. delegorguei* mainly feed on leaves from tanniniferous savanna plants such as *Combretum* spp., *Peltophorum africanum*, *Uapaca kirkiana*, *Dodonaea viscosa* and *Diospyros mespiliformis* known to modify the fibre content of insects. The elevated NDF contents in *E. delegorguei* on the other hand, could be due to the presence of undigested feed substrate material remaining in the insect gut, which subsequently alters the nutritional composition of the whole insect. Although the CF of *M. natalensis* was comparable to SBM and higher than FM, all other insects had higher crude fibre than both SBM and FM. This agrees with reports by Egan (2013, doctoral dissertation) and Meyer-Rochow et al. (2021). Similarly, NDF levels in all insect meals were higher than SBM. Meyer-Rochow et al. (2021) observed similar findings.

Ash content of all selected insects was slightly better than that reported by Kuntadi et al. (2018). However, ash content of *M. natalensis* fell within the same range, while *E. delegorguei* had higher ash levels compared to that reported by Kolobe et al. (2023 a). Moreover, *S. gregaria* on the other hand, had ash content similar to that obtained by Khalil (2018, doctoral dissertation) but lower than that reported by several other authors (Dobermann et al., 2017; Khalil, 2018, doctoral dissertation; Zielińska et al., 2017; Egonyu et al., 2021; Fombong et al., 2021). Nevertheless, ash contents in *S. gregaria* and *E. delegorguei* were comparable to SBM but lower than FM. On the other hand, WTM had higher ash levels than both SBM and FM. Elevated ash is a good indication of high mineral contents. Hence, the selected insect meals could be used as sources of minerals in human and livestock diets (Kim et al., 2019; Kuntadi et al., 2018; Elahi et al., 2022).

The level of carbohydrate in *S. gregaria* (0.79%) was lower than that (1.7%) reported by Zielińska et al. (2015) while *M. natalensis* had significantly higher (2.42%) carbohydrates than those (0.017%) reported by Khalil (2018, doctoral dissertation). However, carbohydrate content in *E. delegorguei* was lower than that reported by Kolobe et al. (2023 a). Nonetheless, all selected insects seem to have lower concentrations of carbohydrate than SBM. The GE levels obtained in all insect meals were higher than those cited by Kolobe et al. (2023 a) but fell within the ranges reported by vast literature (Teffo, 2007, doctoral dissertation; Kuntadi et al., 2018; Kewuyemi et al., 2020; Mlček et al., 2014). Again, the GE proportion of selected insect was higher than SBM and FM. The notable carbohydrate and energy concentration in selected insects could be essential when included in humans and livestock.

Essential (EAA) and non-essential (NEAA) amino acid contents

Significant variation in EAA levels between the observed insect meals may be due to chitin which adversely

influences the availability and accessibility of protein and amino acid as reported by (Oonincx and Finke, 2021). Nonetheless, leucine and methionine were the most predominant and limiting EEA, respectively across all insect meals. Indicating that insect meals are rich in limiting AAs specifically methionine required for muscle development. Similar findings were obtained by Köhler et al. (2019). Moreover, lysine, methionine well as methionine present in all insect meals, particularly in WTM and *S. gregaria* were higher than SBM or comparable to FM. Signalling the potential of observed insects as a substitute for SBM to provide not only %CP but also help elevate the EAA in human and livestock diets. These outcomes agree with vast literature (Kinyuru et al., 2015; Nogales-Mérida et al., 2019; Straub et al., 2019; Adepoju, 2020; Awobusuyi et al., 2020; Hlongwane et al., 2020). Hence, all insects met the EAA requirements for humans and livestock, especially poultry species as cited by FAO/WHO/UNU (2007), Kinyuru et al. (2009) and Kolobe et al. (2023 a). In addition, increased total EAA concentrations from observed insect samples, particularly WTM, *M. natalensis* and *S. gregaria*, is also a good indication that they are considerable as food and feed. This is in line with recent publications (Hermans et al., 2021; Adli, 2021; Hlongwane et al., 2021; Elahi et al., 2022).

Nevertheless, NEAA concentrations were only higher in WTM and *S. gregaria*, in which alanine and glutamine were the most predominant NEAAs while serine on the other hand, was the least abundant NEAA, particularly in *S. gregaria*. These findings are supported by Ghosh et al. (2017). The significant variation in NEAA across all insect meals may be due to comparison of different insect species. The NEAAs such as alanine, glycine and serine in *S. gregaria* were comparable to those in FM. Whereas NEAAs including aspartic acid, glutamine and proline were higher in WTM compared to those in both SBM and FM. Nevertheless, *M. natalensis* followed by WTM had a better EAA to NEAA ratio than other insect samples and SBM. Kinyuru (2014) reported similar findings. Nonetheless, all insect samples met the FAO/WHO requirements in terms of EAA (40%) and EAA to NEAA ratio (0.6) (WHO, 2007; Köhler et al., 2019).

Fatty acid contents

Although most insect samples contained higher SFA and MUFA than SBM and FM, only WTO had elevated SFA and MUFA contents. Which was expected as WTO is crude oil extracted from *M. natalensis*. This is in line with the opinion of vast authors (Kinyuru et al., 2013; Fombong and Kinyuru, 2018; Tang et al., 2019; Meyer-Rochow et al., 2021; Anankware et al., 2021). MUFA levels in WTM, *M. falciger* and *S. gregaria* were higher than SBM but lower than FM. The most predominant SFA, MUFA and PUFA across all insect samples were palmitic, oleic, and linoleic acids, respectively. The results are in line with the vast literature (Igwe et al., 2011; Kinyuru et al., 2013; Zielińska et al. 2015; Akullo et al., 2018; Monter-Miranda et al., 2018; Mariod, 2020;

Fombong et al., 2021). Elevated MUFA mainly oleic acid across all insect samples could be useful in lowering cholesterol levels when inoculated in diets whereas higher dietary SFA levels such as palmitic acid could adversely cause heart related diseases by elevating bad cholesterol levels in livestock meat consumed by humans. Similar findings were reported by (Kinyuru et al., 2013; Zielińska et al., 2015). Moreover, PUFA types including linoleic acid, α -linolenic and eicosapentaenoic acid in *M. falciger* and *S. gregaria* species reported by Khalil (2018, doctoral dissertation) and Zielińska et al. (2015) were also observed in the present study. Although linoleic acids in all insect samples were lower than those in SBM but comparable to FM, only *S. gregaria* had a higher α -linolenic acid content than both SBM and FM. Indicating that the observed insects could generally provide insufficient essential fatty acids when included in diets as a replacement for conventional protein sources. Nevertheless, *M. falciger*, *S. gregaria* and *E. delegorguei* contained elevated PUFA, n-3 and n-6 with better PUFA: SFA and PUFA:MUFA ratios of ≥ 0.40 , hence meeting the recommended levels required for human consumption (Osimani et al., 2017). The current findings aligned with reports by Tang et al. (2019). According to Riekkinen et al. (2022) this PUFA types are vital for proper growth and health of children and infants, especially since humans cannot synthesise them when supplemented in diets. Notably n-6 was only higher in *M. falciger* than SBM but lower than FM. Again, *M. natalensis* and *M. falciger* had a better n-6:n-3 ratio than both SBM and FM. However, Kinyuru (2014) found that higher n-6: n-3 ratio ≥ 10 could have negative effects when incorporated in humans and livestock since it can lead to atherosclerosis and heart diseases. Nonetheless, the overall variation in fatty acid types such as PUFA levels, ratio between n-3 and n-6 fatty acids, the concentration of n-3 among selected insects may be due to physiological nature of the samples, developmental stage and/or different processing techniques, including freeze, sun and oven drying and oil extraction applied in the current study as supported by (Tiencheu et al., 2013; Melgar-Lalanne et al., 2019; Indriani et al., 2020; Nyangena, 2021, doctoral dissertation; El Hajj et al., 2023; Keil et al., 2022).

The total fatty acids in *E. delegorguei*, *S. gregaria*, *M. falciger*, *M. natalensis*, WTM and WTO ranged from 55.02 to 99.99 g/100 g, with WTO and WTM containing highest and lowest fatty acid concentrations, respectively. Thus, suggesting that oil extraction process was effective in reducing insect oil content from *M. natalensis* to produce WTM. Hence, elevated total fatty acids in WTO could be vital in providing essential fatty acids such as MUFA and PUFA when supplemented in human and animal diets. Nonetheless, the total SFA, MUFA and oleic acid concentration of *S. gregaria*, *M. falciger* and *M. natalensis* fell within the range while total PUFA, n-6 and linoleic contents were slightly lower than those reported by Kolobe et al. (2023 b). Igwe et al. (2011), further emphasizes the potential of insects rich in MUFA and PUFA

but low SFA to reduce the risk of cardiovascular-related diseases. This is because edible insect oils have lower cholesterol levels relative to oleic acid (Akullo et al., 2018; Kuntadi et al., 2018), with other benefits including immune response and anti-inflammatory properties (Babarinde et al., 2021), hence suggesting that observed insects, especially *S. gregaria*, *M. falciger* and *M. natalensis* could potentially provide vital and health-beneficial fats and fatty acids when included in diets.

Conclusion

There is a clear variation in the selected southern African edible insects *Schistocerca gregaria*, *Encosternum delegorguei*, *M. natalensis* and *M. falciger* and the by-products of *M. natalensis* (WTM and WTO) in terms of proximate composition, amino acid, and fatty acid contents. *S. gregaria*, *M. natalensis* and WTM appear to be rich in crude protein, ash, and essential amino acid (lysine, methionine, phenylalanine, and threonine). Whereas *M. falciger* seems to be a promising source of essential fatty acids (PUFA and n-6), especially α -linolenic. In overall, the selected insects prove to be comparable to SBM and FM with both *M. natalensis* and *M. falciger* had a better n-6: n-3 ratio than the above-mentioned conventional proteins sources. Additionally, WTM (defatted) had improved crude protein and ash as well as reduced total crude fats and SFA particularly palmitic acid compared to *M. natalensis* (un-defatted raw form). Thus, signifying the importance of insect oil extraction prior to utilization in diets since excessive accumulation of fats oils mainly with high proportion of SFA in diets causes health complications. Based on their nutritional qualities, the selected insects especially *S. gregaria* and WTM could potentially be considered as a source of protein and essential amino acids whilst *M. falciger* could be a promising candidate for providing essential fats and fatty acids when included in human and livestock diets. Thus, sustainable, and strategic mass rearing of selected edible insects should be considered to improve their availability, production, and supply for consumption by human and livestock. This could help in addressing problematic societal issues mainly food insecurity, job creation, high feed costs and food-feed competition in Africa and worldwide.

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