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RESEARCH ARTICLE

Nutritional composition of three Aotearoa New Zealand caterpillar species: Impact of diet and life stage on amino acid and minerals

C.T. O'Connor[1,2,3*], *M.R. McNeill*[4], *M.H. Bowie*[1], *J.L. Gathercole*[2] *and J.D. Morton*[1]

[1]Faculty of Agriculture and Life Sciences, Lincoln University, Lincoln 7647, Canterbury, New Zealand; [2]Proteins and Metabolites Team, Bioeconomy Science Institute Tuhiraki, Lincoln, Canterbury 8140, New Zealand; [3]Joint Postgraduate School: Food Transitions 2050, Lincoln 7647, Canterbury, New Zealand; [4]Weeds, Pests and Biosecurity, Bioeconomy Science Institute Tuhiraki, Lincoln, Canterbury 8140, New Zealand; *chrystal.oconnor@lincoln.ac.nz

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Abstract

Entomophagy has been practised for centuries and is rooted in many cultures, including among Māori in Aotearoa New Zealand. However, limited nutritional data exists on native edible insect species. This study investigates the amino acid and mineral composition of three endemic caterpillar species: *Cleora scriptaria*, *Epalxiphora axenana*, and *Ctenopseustis obliquana*. Caterpillars were reared on native kawakawa (*Macropiper excelsum*). *Ct. obliquana* was also reared on mānuka (*Leptospermum scoparium*) and a laboratory diet to examine the effects of insect species, diet, and lifecycle stage. Amino acid analysis using high-performance-liquid-chromatography (HPLC) showed that *C. scriptaria* and *E. axenana* had high essential amino acid content, exceeding FAO/WHO recommendations for quality protein. Amino acid composition did not differ significantly between the life stages late larvae and early pupae within the same diet, but differences were observed across diets. These results suggest that in this study a change in diet, rather than within two life stages, is a key factor influencing amino acid profiles. Mineral analysis using inductively coupled plasma mass-spectrometry (ICP-MS) showed that *Ct. obliquana* reared on native plants had elevated levels of K, P, and Zn, compared to the laboratory diet, while *E. axenana* was a good source of Fe. However, Na levels were higher in plant-fed caterpillars. Trace heavy metals were detected within safe limits within *Ct. obliquana*. These findings demonstrate the nutritional potential of native caterpillars as sustainable food sources but highlight the importance of diet formulation to optimise nutritional value. This study contributes to the revitalisation of traditional Māori food practices and the development of edible insect farming in Aotearoa New Zealand, with potential for domestic consumption and export markets.

Keywords

amino acid analysis – edible insects – Kawakawa – Lepidoptera – mineral composition – nutritional analysis

1 Introduction

Overview of nutritional composition of insects

Although entomophagy has been practised for centuries, research into the nutritional value and potential of edible insects as a sustainable protein source to help address global food security challenges has only gained significant attention in the last decade (van Huis *et al.*, 2013). Insects can be highly nutritious, providing proteins, essential amino acids, minerals, lipids, and vitamins, with a lower environmental impact compared to conventional livestock (Numbi *et al.*, 2022; Ordoñez-Araque *et al.*, 2022; Payne *et al.*, 2015). Entomophagy has deep roots in many cultures worldwide (van Huis *et al.*, 2013), including among Māori in Aotearoa New Zealand, where insects have historically played a role as food, rongoā (traditional medicine), and have cultural significance (O'Connor *et al.*, 2023). However, there is limited research on the nutritional composition of native edible insect species in Aotearoa New Zealand, creating a gap in understanding their potential as food sources and product development.

Among the 2000 species of insects consumed worldwide, caterpillars (Lepidoptera) are among the most commonly consumed insects globally with good nutritional profiles (DeFoliart, 1999; Jongema, 2017; Raheem *et al.*, 2019; Raubenheimer and Rothman, 2013). For example, they are the most consumed group of insects in Sub-Saharan Africa, accounting for 31% of insect consumption (Mariod, 2020), and approx. 47% in the Democratic Republic of Congo (Kelemu *et al.*, 2015), and are commonly eaten in Thailand (Hanboonsong *et al.*, 2013). They offer an essential amino-acid profile comparable to fish and soy meal (Rumpold and Schlüter, 2013) and some species meet iron and zinc mineral requirements for humans (Numbi *et al.*, 2022; Ordoñez-Araque *et al.*, 2022; Payne *et al.*, 2015). The inclusion of caterpillars in the human diet has been proposed as a strategy to address mineral deficiencies in populations facing malnutrition (Nsevolo Miankeba *et al.*, 2022; Payne *et al.*, 2015).

The protein content of edible insects on a dry weight (DW) basis range from 20 to 77% (Bukkens, 1997; Ramos-Elorduy *et al.*, 1997; van Huis, 2013; Zielińska *et al.*, 2015) with some species meeting the FAO/WHO requirements for essential amino acids (Köhler *et al.*, 2019; Ordoñez-Araque *et al.*, 2022). An important measure of the nutritional protein quality is the essential amino acid content which play a crucial role in human physiology and can't be synthesised by metabolic pathways in the body therefore, they must be provided by food sources (Kohlmeier, 2015). Caterpillar species such as *Cirina forda* (Westwood 1849) and *Imbrasia ertli* (Rebel, 1904) have been found to contain high values of essential amino acids (Nsevolo Miankeba *et al.*, 2022; Numbi *et al.*, 2022). The lack of data for most edible caterpillar species limits efforts to determine their economic and nutritional potential (Mariod, 2020; Numbi *et al.*, 2024). Investigating the nutritional composition of other caterpillar species and understanding the impact of species, diet and life stage could provide valuable information that supports the revitalisation of traditional food practices, broadens alternative protein options, and informs optimised rearing systems.

Effect of species and life stage on the nutritional composition of insects

The nutritional composition of insects, including their amino acid profile and mineral content, can vary depending on several factors. These include species, life stage, sex, diet, and processing methods (Meyer-Rochow *et al.*, 2021; Oonincx and Finke, 2020). For example, Payne *et al.* (2016) found protein levels of ten edible insect species ranged from 10.8 g to 35.2 per 100 g fresh in the weaver ant (*Oecophylla smaragdina* (Fabricius, 1775)) and mopane caterpillar (*Gonimbrasia belina* Westwood, 1849), respectively. However, less variation is observed within insect orders, with nine Lepidoptera caterpillar species reportedly containing between 40-50 g protein (DW) (Numbi *et al.*, 2022). Another survey of twelve species of caterpillars also revealed crude protein content between 40 and 50% DW (Nsevolo Miankeba *et al.*, 2022; Numbi *et al.*, 2024). Similarly, Landry *et al.* (1986) assessed larval protein quality across six Lepidoptera species, highlighting interspecies variation of 49.4-58.1% crude protein (DW). However, each species has distinct nutritional qualities, highlighting the importance of species-specific assessments when evaluating their food potential.

Life stage also impacts nutritional composition, particularly in holometabolous insects, which undergo complete metamorphosis (egg, larva, pupa, adult). Morphological and metabolic changes between larvae and pupae often result in variations in amino acid and mineral profiles (Finke, 2002; Oonincx and Finke, 2020; Pieterse and Pretorius, 2014). For hemimetabolous insects (egg, larva, adult), like the house cricket (*Acheta domesticus*, (Linnaeus, 1758)) the amino acid composition remains constant across life stages (Finke, 2015; Köhler *et al.*, 2019; Yi *et al.*, 2013). For example, differences were reported in the amino acid composition of processed larval and pupal meal of the house fly (*Musca*

domestica L.) reared on the same diet (Pieterse and Pretorius, 2014) and honeybees (*Apis mellifera ligustica* L.) (Ghosh *et al*., 2016; Ghosh *et al*., 2020). These variations in nutritional composition across species and life stages emphasise the importance of careful selection when considering insects for human food.

Effect of diet on the nutritonal composition of insects

Diet is another factor which significantly influences the nutritional composition of insects (Meyer-Rochow *et al*., 2021; Oonincx and Finke, 2020). For instance, mineral composition in silkworms varies between larval stages and pupae when fed mulberry leaves (Pongworn *et al*., 2024). Similarly, dietary variations can affect amino acid composition (Ibarra-Herrera *et al*., 2020), although others suggest minimal impact (van Broekhoven *et al*., 2015). Cookman *et al*. (1984) showed that artificial diets increased fat content in *Anticarsia gemmatalis* Hübner, 1818 larvae, which can inversely affect protein levels. Despite the growing global body of research, there is limited data on the nutritional profiles of native insects in Aotearoa New Zealand and how diet or life stage influences their profiles.

To the best of our knowledge, the larval stage of the huhu beetle (*Prionoplus reticularis*, White, 1843) (Coleoptera: Cerambycidae) is the only native Aotearoa New Zealand insect with documented amino acid and mineral composition (Kavle *et al*., 2022). Huhu larvae are rich in protein (26.2-30.5%) (Kavle *et al*., 2022), essential amino acids (Kavle *et al*., 2023), and minerals such as iron, copper, zinc, manganese, magnesium, and phosphorus (Kavle *et al*., 2022). The wider lack of knowledge on the nutritional composition of Aotearoa New Zealand insect species represents a significant gap in our understanding of their potential nutritional benefits for human health. This study investigates the amino acid and mineral composition of three endemic caterpillar species. The selection of these three species was informed by previous work examining Māori perspectives on edible insects (O'Connor, 2025; O'Connor *et al*., 2023), which identified culturally relevant species with favourable ecological characteristics, short life cycles, and strong host-plant associations (O'Connor, 2025). Further, both *Ct. obliquana* and *E. axenana* have been reared successfully on readily available artificial diets (Clare and Singh, 1988; Singh, 1983), highlighting their potential suitability for scalable production. Specifically, we aim to (1) Compare the amino acid and mineral composition of caterpillars reared on the endemic plant kawakawa (*Macropiper excelsum* (G.Forst.) Miq. Piperales: Piperaceae, (2) Examine the impact of two lifecycle stages (larvae and pupae) on their nutritional profiles, and (3) Assess how diet influences the nutritional composition of *Ct. obliquana*.

2 Materials and methods

Study species and experimental design

Larvae of *Cleora scriptaria* (Walker, 1860) and *Epalxiphora axenana* Meyrick, 1881 were field collected. *Cleora scriptaria* (called whangawhanga by Māori) were collected in January (summer) from Hay Scenic Reserve, Banks Peninsula, Canterbury, Aotearoa New Zealand (43°42′14.7″S, 172°53′49.8″E); *E. axenana* larvae were collected from Punakaiki, West Coast, Aotearoa New Zealand (43°31.290′S, 172°34.320′E) (spring). *Ctenopseustis obliquana* (Walker, 1863) (*Ct. obliquana*) larvae (generation 193) were supplied as eggs by Plant and Food Research, Auckland, Aotearoa New Zealand. Overall, the size and weight of the caterpillars is species specific with *C. scriptaria* the largest of the species.

Ct. obliquana were initially reared on an antibiotic free artificial diet modified from Singh (1983) (A. Barrington, Plant and Food Research, unpublished data) for seven days before being randomly assigned to dietary treatments. The study involved two experiments.

(1) Nutritional composition of all three species reared on kawakawa
(2) Effects of three diets, kawakawa, mānuka and laboratory diet, on *Ct. obliquana*

Further details on the insect collection, rearing, and tikanga (Māori protocols) followed during the study can be found in (O'Connor, 2025).

Three caterpillar species reared on kawakawa

All three caterpillar species were reared in a temperature-controlled glasshouse at Lincoln University, on whole kawakawa plants. Kawakawa plants were eco-sourced (Banks Peninsula, Canterbury) from a commercial nursery (Otumatua Nursery, Christchurch) as seedlings and grown in pots in the glasshouse. At the start of the experiment, plants were 50-70 cm in height with approximately 50-70 leaves/plant. Each cage contained three plants with their leaves overlapping to ensure free movement and *ad libitum* feeding by larvae amongst plants (Figure 1E). Plants were watered every second day throughout the experiment with between 300 and 600 ml per pot were added to the potting soil surface. Around 50 early instar larvae (1-3 mm) were introduced to each group using an artist's paint brush and allowed to wander freely. The plants were randomly

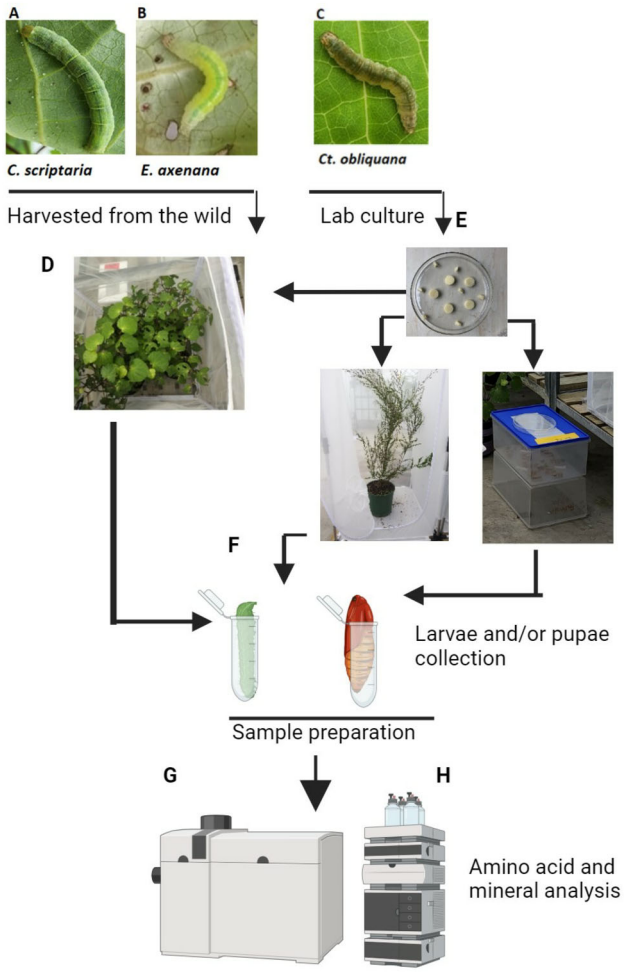

FIGURE 1 Field collected *C. scriptaria* (A), *E. axenana* (B) and *Ct. obliquana* (C) supplied from a laboratory culture. Both *C. scriptaria and E. axenana* were placed directly onto kawakawa plants as neonates and *Ct. obliquana* reared on laboratory diet for 7 days and then transferred to either kawakawa, mānuka or laboratory diets. Larvae and pupae collected for mineral via ICP (G) and amino acid analysis via HPLC (H).

placed in either medium (42 × 42 × 77 cm) or large (60 × 60 × 180 cm) cages supported by an aluminium frame and covered in a fine nylon mesh (Figure 1D). Access to the plants was via zippers. More details on replicates and rearing conditions can be found in (O'Connor, 2025).

Temperature and humidity inside and outside the cages were measured using Tiny Tag loggers (Tiny Tag Ultra 2, Gemini Data Loggers, UK). Larvae were monitored twice daily and gently repositioned onto leaves using an artist's paintbrush if found off-plant. Specimens were collected individually when they reached the target developmental stage (late larvae and early pupae) to ensure consistency across treatments. Larvae were fasted for 24 h at 20 °C before weighing to ensure gut clearance (Table 1), after which they were stored at −40 °C for further analysis.

Ct. obliquana reared on different diets

Ct. obliquana larvae were reared under similar conditions to those described in experiment one and rearing conditions followed protocols established in (O'Connor, 2025). The caterpillars were reared on either kawakawa and mānuka plants within cages, or on an antibiotic free laboratory diet modified from Singh (1983) within 7.8 litre plastic containers lined with mesh to allow ventilation (Figure 1E). Containers were placed on raised stands between the cages in the same glasshouse to ensure they experienced the same temperature and light conditions. The laboratory diet was replaced with fresh portions as needed. Diet materials were stored at 4 °C. Late larvae and early stage pupae were collected individually when they reached their target developmental stage (Figure 1F), (see Table 1 for average weights). Each treatment group was replicated three times from February (summer) to May (autumn) 2022.

Insect sample collection

Late larvae and pupae were collected, weighed, fasted for 24 hours, and reweighed. Samples were pooled into aliquots of at least 50 mg and killed by freezing and storing at −80 °C. Additionally, all samples were stored with labels that identify them as a taonga species to adhere to Māori tikanga storage protocols, in line with whakapapa (genealogy).

Protein hydrolysis for amino acid analysis

Aliquots (50 mg) of fresh sample were freeze-dried and analysed for total amino acid analysis using high-performance liquid chromatography (HPLC) at a Lincoln University laboratory, following Fountoulakis and Lahm (1998). Acid hydrolysis was performed using an EZ2 Vacuum Centrifuge. Each sample was treated with 5.0 µl of an internal standard (0.5 M amino butyric acid) and 2.5 ml of 6 M HCl. The insect samples were then heated at 110 °C for 24 hours. The amino acid content was analysed using an ACE column (150 × 4.6 mm, C18, 3 µm, ACE-111-1546, Winlab, UK) at 40 °C on HPLC. The amino acids measured included alanine (Ala), arginine (Arg), aspartic acid (Asp), asparagine (Asn), cystine (Cys), glutamic acid (Glu), glycine (Gly), histidine (His), isoleucine (Ile), leucine (Leu), lysine (Lys), methionine (Met), phenylalanine (Phe), proline (Pro), serine (Ser), threonine (Thr), tyrosine (Tyr), taurine (Tau), and valine (Val). Amino acid values are expressed as mg per g dry weight. Three biological replicates were analysed per

TABLE 1 Mean larval and pupal weight (mg) and standard deviation for *C. scriptaria* and *E. axenana* reared on kawakawa diet, and *Ct. obliquana* reared on either kawakawa, mānuka, or laboratory diet

Species	Diet	Mean larvae pre fasted weight (mg)	Mean larvae post fasted weight (mg)	Mean pupal weight (mg)
C. scriptaria	Kawakawa	180.89 ± 51.6 (38)	151.69 ± 33.0 (38)	150.49 ± 34.19 (17)
E. axenana	Kawakawa	58.97 ± 10.51 (23)	48.92 ± 7.70 (23)	42.00 ± 5.33 (11)
Ct. obliquana	Kawakawa	–	30.74 ± 23.33 (23)	30.32 ± 24.23 (17)
Ct. obliquana	Mānuka	–	20.30 ± 4.60 (27)	20.34 ± 4.62 (22)
Ct. obliquana	Laboratory diet	–	38.26 ± 11.55 (21)	39.26 ± 9.44 (22)

Number of individuals weighed for each species and life stage shown in parentheses. –, not measured

treatment group, each consisting of three pooled specimens.

Sample preparation for mineral analysis

Aliquots (50 mg) of freeze-dried ((Labconco unit (Labconco, United States)) samples were analysed for minerals using inductively coupled plasma mass spectrometry (ICP-MS) at the University of Canterbury Analytics Laboratory. Samples were digested in 5 mL of concentrated nitric acid (Milestone UltraWAVE digest unit). Digestate were diluted 100 times and analysed using an Aglient 8900 QQQ ICP-MS. For quality control, a certified reference material (CRM 2976 muscle tissue) was used. The minerals measured in the samples included macrominerals; sodium (Na), magnesium (Mg), potassium (K), phosphorus (P), calcium (Ca), microminerals; boron (B), sulphur (S), chromium (Cr), manganese (Mn), iron (Fe), copper (Cu), zinc (Zn), selenium (Se)) and heavy metals; arsenic (As), cadmium (Cd), and lead (Pb). Seven to twelve biological replicates were analysed per treatment group. Mineral values are expressed as mg per 100 g DW. The pupal stage of both *C. scriptaria* and *E. axenana* were not tested due to limited sample availability.

Statistical analysis

Amino acid and mineral data were expressed as mean ± SD (mg/g dry weight and mg/100 grams of dry weight for amino acids and minerals, respectively) using Microsoft Excel (version 2112). Analysis of variance (ANOVA) was conducted using Minitab 21 to compare the three caterpillar species on the kawakawa diet and the dietary treatments for *Ct. obliquana*. This was followed by Tukey's test for comparison of means, with significance set at $P < 0.05$.

3 Results and Discussion

This study provides the first comprehensive analysis of amino acid and mineral composition of three Aotearoa New Zealand caterpillar species (*E. axenana*, *C. scriptaria*, and *Ct. obliquana*) as potential food options for inclusion into the human diet. Additionally, we explored the impact of two caterpillar life stages (larvae and pupae) and diet on the nutritional profile of the *Ct. obliquana*. Our findings provide valuable nutritional insights into the potential of native and endemic insects in Aotearoa as sustainable protein sources. Table 1 presents average larvae and pupal weights of the three species.

Amino acid composition of three caterpillar reared on kawakawa diet

The concentrations of essential amino acids (EAAs) and non-essential amino acids (NEAAs) in the *E. axenana*, *C. scriptaria*, and *Ct. obliquana* larvae and pupae reared on kawakawa are presented in Table 2 (mg/g DW). Nineteen amino acids were identified across all caterpillar species; however, cysteine was below detection levels, and tryptophan was not measured in this study. The amino acid profiles of the three caterpillar species reared on kawakawa show good nutritional qualities in comparison to other insects (*P. reticularis*, *T. molitor*, *I. ertli*, *C. forda*), conventional protein sources (beef, soy, and egg extracts), and the UNFAO/WHO recommended values for high-quality protein (Table 3). A notable finding in our study was the overall consistency of EAAs composition maintained across all species and life stages (larvae and pupae), with no significant differences detected ($P < 0.05$). This contrasts previous literature reporting nutritional variation across insect species and developmental stages (Meyer-Rochow *et al.*, 2021; Oonincx and Finke, 2020). The lack of variation observed in certain EAAs may be attributed to the rel-

TABLE 2 Amino acid composition for larval and pupae of three Lepidopteran species fed kawakawa

	E. axenana larvae	*E. axenana* pupae	*C. scriptaria* larvae	*C. scriptaria* pupae	*Ct. obliquana* larvae	*Ct. obliquana* pupae
Essential amino acids						
His	22.9^{a} ± 4.1	19.2^{a} ± 2.3	23.4^{a} ± 4.9	22.1^{a} ± 1.9	18.8^{a} ± 3.7	18.3^{a} ± 3.6
Ile	21.4^{a} ± 1.7	21.8^{a} ± 1.8	25.9^{a} ± 6.6	22.0^{a} ± 0.03	21.5^{a} ± 8.5	19.4^{a} ± 6.1
Leu	34.6^{a} ± 2.1	38.2^{a} ± 3.0	41.4^{a} ± 11.9	36.6^{a} ± 0.2	33.3^{a} ± 4.5	33.4^{a} ± 1.3
Lys	41.6^{a} ± 10.3	37.4^{a} ± 4.1	39.8^{a} ± 8.4	38.0^{a} ± 0.4	45.8^{a} ± 18.2	39.1^{a} ± 4.7
Met	8.8^{a} ± 1.2	11.5^{a} ± 0.8	12.2^{a} ± 1.9	11.6^{a} ± 0.5	8.8^{a} ± 0.1	9.1^{a} ± 8.1
Phe	24.3^{a} ± 3.5	26.6^{a} ± 2.5	27.7^{a} ± 8.6	25.7^{a} ± 1.2	26.0^{a} ± 1.1	23.4^{a} ± 8.4
Thr	22.4^{a} ± 1.6	22.5^{a} ± 1.5	27.7^{a} ± 6.8	23.3^{a} ± 4.7	21.7^{a} ± 6.4	19.7^{a} ± 3.8
Val	27.2^{a} ± 1.0	27.1^{a} ± 2.4	35.9^{a} ± 7.7	29.9^{a} ± 0.6	23.5^{a} ± 1.6	24.3^{a} ± 1.4
Total EAAs	203.1	204.3	234.0	209.3	199.6	186.9
Non-essential amino acids						
Ala	30.3^{a} ± 4.3	34.8^{a} ± 2.4	35.4^{a} ± 8.4	31.0^{a} ± 3.8	28.9^{a} ± 2.5	30.6^{a} ± 1.8
Arg	35.0^{a} ± 4.8	37.1^{a} ± 1.2	37.8^{a} ± 8.6	35.3^{a} ± 4.8	31.3^{a} ± 4.5	31.5^{a} ± 1.2
Asn	2.2^{a} ± 0.5	2.2^{a} ± 0.1	1.8^{a} ± 2.2	1.8^{a} ± 0.2	1.9^{a} ± 9.3	1.4^{a} ± 4.5
Asp	30.4^{a} ± 2.1	29.4^{a} ± 2.6	35.6^{a} ± 10.9	29.1^{a} ± 1.5	28.3^{a} ± 2.5	24.3^{a} ± 2.4
Gln	0.1^{a} ± 0.03	0.1^{a} ± 0.11	0.1^{a} ± 0.08	0.0^{a} ± 0.07	0.1^{a} ± 0.09	0.1^{a} ± 0.05
Glu	61.5^{a} ± 5.1	44.9^{a} ± 6.3	68.2^{a} ± 18.1	49.5^{a} ± 1.2	60.1^{a} ± 4.6	41.7^{a} ± 6.6
Gly	26.1^{a} ± 3.8	23.8^{a} ± 1.5	28.4^{a} ± 7.1	22.9^{a} ± 2.2	25.2^{a} ± 1.5	18.7^{a} ± 1.4
Pro	34.5^{ab} ± 4.5	33.7^{ab} ± 2.9	43.5^{a} ± 8.4	32.0^{ab} ± 1.6	32.2^{ab} ± 5.3	26.8^{b} ± 1.9
Ser	24.5^{a} ± 2.5	22.6^{a} ± 0.9	27.8^{a} ± 7.5	26.4^{a} ± 0.6	23.5^{a} ± 0.7	19.8^{a} ± 2.2
Tau	6.8^{ab} ± 1.1	6.1^{b} ± 1.1	11.1^{a} ± 2.5	5.7^{b} ± 0.8	4.4^{b} ± 1.1	5.9^{b} ± 2.8
Tyr	33.7^{a} ± 0.2	39.6^{a} ± 1.0	45.5^{a} ± 11.6	36.1^{a} ± 1.9	32.3^{a} ± 2.4	32.2^{a} ± 1.7
Total NEAAs	285.2	274.2	335.2	269.9	268.5	2331.1
Sum of total AAs	488.3	478.5	569.2	479.2	468.1	420.1
E/T%	41.6%	42.7%	41.1%	43.7%	42.7%	44.5%

EAAs, essential amino acids; NEAAs, non-essential amino acids; E/T% = essential amino acids divided by total amino acids as a proportion. Tryptophan was not determined in this study and cysteine was below detection levels. All expressed in mg per g dry weight. Mean ± SD from three biological replicates per treatment. Means with the same letter across a row are not significantly different ($P < 0.05$). His, histidine; Ile, isoleucine; Lys, lysine; Leu, leucine; Met, methionine; Phe, phenylalanine; Thr, threonine; Val, valine; Asp, aspartic acid; Gly, glycine; Arg, arginine; Ala, alanine; Ser, serine; Tyr, tyrosine; Glu, glutamic acid; Pro, proline; Tau, taurine; Asn, asparagine; Gln, glutamine.

atively high standard deviations observed in some of our data. While most standard deviations were below the mean, suggesting normal distribution (Livingston, 2004) a few values stood out as potential outliers. For example, lysine content in the *Ct. obliquana* larvae was reported as 45.8 ± 18.2 mg/g. This variability could partially explain the lack of significant differences in EAA composition between species. These patterns may also reflect that our comparisons were between late instar larvae and early pupae, whereas greater shifts might occur between more distinct stages such as larvae and adults (Cookman *et al.*, 1984; Finke, 2002). Nonetheless, the consistency observed across all species and life stages may also indicate a broader pattern worthy of further investigation.

Total essential amino acids ranged from 186.9 mg/g in the *Ct. obliquana* pupae to 234.0 mg/g in the *C. scriptaria* larvae (Table 2), meeting the FAO/WHO requirements of 40% EAAs (ranging from 41.1% in the *C. scriptaria* larvae to 44.5% in the *Ct. obliquana* pupae). This is contrary to previous literature reporting nutritional variation across insect species and developmental stages (Meyer-Rochow *et al.*, 2021; Oonincx and Finke, 2020). Our findings fall within the higher range of the 26-54% DW protein content reported for various insect species by Ramos-Elorduy *et al.* (1997). Notably, our results are on the higher end of the 40-

TABLE 3 Essential amino acid composition (mg/g protein) of larvae of four insect species, beef, soy, egg extracts, as well as the protein value reported by the UNFAO/WHO as indicative of good nutritional quality protein for an adult human obtained from the literature

	Prionoplus reticularis larvae	*Tenebrio molitor* larvae	*Imbrasia ertli* larvae	*Cirina forda* larvae	Beef chunks	Soy isolate	Egg isolate	Good quality protein value FAO/WHO
His	20.8	22.4	21.9	22.4	29.4	15	9	15
Ile	50.9	19.7	30.0	26.9	38.4	19	16	30
Leu	81.7	29.7	43.0	38.5	61.8	50	36	59
Lys	76.9	32.3	49.1	43.9	66.6	34	27	45
Met	17.5	5.9	11.5	9.5	23.7	3	14	22 (Met + Cys)
Phe	17.5	18.8	31.7	27.5	30.9	32	23	38 (Phe + Tyr)
Thr	42.2	21.5	34.2	30.5	34.3	23	20	23
Val	53.9	34.4	41.2	36.1	44.8	22	20	39
Total	386.7	184.7	272.7	242.4	329.9	198	165	271.0

All expressed in mean mg per g dry weight. Data for *Prionoplus reticularis* larvae protein extract were sourced from Kavle *et al.* (2023); *Tenebrio molitor* (*T. molitor*) late larvae, Yu *et al.* (2021); *Imbrasia ertli* and *Cirina forda* larvae, Nsevolo Miankeba *et al.* (2022); beef chunks, Wu *et al.* (2016); soy and egg isolated protein powder, Gorissen *et al.* (2018); protein value reported for a good nutritional quality of protein for an adult human, WHO/FAO/UNU (2007). His, histidine; Ile, isoleucine; Lys, lysine; Leu, leucine; Met, methionine; Phe, phenylalanine; Thr, threonine; Val, valine.

60% and 40-50% DW protein range for ten Lepidoptera species reported by Ramos-Elorduy *et al.* (1997) and nine reported by (Numbi *et al.*, 2022), respectively. The total EAA content of these caterpillars (ranging from 186.9 to 234.0 mg/g protein) exceeds that of egg (165 mg/g protein) and mealworm (184.7 mg/g protein), and is comparable to soy (198 mg/g protein), and the *C. forda* larvae (242.4 mg/g protein) (Gorissen *et al.*, 2018; Nsevolo Miankeba *et al.*, 2022; Yu *et al.*, 2021). However, these are lower than levels reported in *I. ertli* larvae (272.7 mg/g protein), huhu grub (386.7 mg/g protein), and beef (329.9 mg/g protein) (Kavle *et al.*, 2023; Nsevolo Miankeba *et al.*, 2022; Wu *et al.*, 2016). While the EAA content of our studied caterpillars doesn't quite reach the 263 mg/g protein value reported by the UNFAO/WHO as indicative of good nutritional quality protein (WHO/FAO/UNU, 2007), these caterpillars could still contribute to a balanced diet, especially in combination with other protein sources.

The highest EAA content was observed in *C. scriptaria* caterpillars, while the lowest was in *Ct. obliquana* pupae, though differences were not statistically significant. This suggests that species may slightly influence protein composition, but within a narrow range under the same diet. All three species are comparable or met the protein value reported for good nutritional quality for the EAAs histidine, lysine, and threonine (Table 2); which are key for the synthesis of several hormones required for metabolic function; aiding in calcium absorption and collagen formation; and aiding in lipid metabolism and protein synthesis, respectively (Kohlmeier, 2015). Notably, their lysine content meets FAO/WHO recommendations and may help complement lysine-poor diets, such as those based on cereals (Temba *et al.*, 2016).

Other EAAs showed promising results, with phenylalanine (synthesis of proteins, catecholamines, and melanin), valine (used as an energy fuel), and isoleucine (signal transduction and protein synthesis) generally meeting 70-90% of the WHO/FAO reference value (Kohlmeier, 2015). Specifically, isoleucine levels (19.5 ± 0.1 mg/g to 25.9 ± 6.6 mg/g) were comparable to mealworm (19.7 mg/g) (Yu *et al.*, 2021), *I. ertli* and *C. forda* larvae (30.0 and 26.9 mg/g, respectively) (Nsevolo Miankeba *et al.*, 2022), egg (16 mg/g) and soy (19 mg/g) (Gorissen *et al.*, 2018), though lower than beef (38.8 mg/g) (Wu *et al.*, 2016) and the recommended protein value (30 mg/g) (WHO/FAO/UNU, 2007). Similarly, valine concentrations in *C. scriptaria* (35.9 ± 7.7 mg/g) were comparable to egg (20 mg/g) and soy (22 mg/g) and almost matched the recommended WHO/FAO protein value (39 mg/g). Therefore, these caterpillar species would be a good nutritional source of these EAAs.

Cysteine and methionine were identified as limiting amino acids, as previously identified in a range of other edible insect species (Landry *et al.*, 1986; Rumpold and

Schlüter, 2013). Previous rodent-feeding studies have also demonstrated methionine to be the first limiting amino acid in insect-based diets (Finke *et al.*, 1987; Goulet *et al.*, 1978), reinforcing the need to consider methionine content when evaluating insect protein quality. Cysteine was below detection levels in all our analysed caterpillars, while methionine concentrations ranged from 8.8 ± 0.1 mg/g to 12.2 ± 1.9 mg/g protein, reaching only 40-50% of the reference protein value (22 mg/g) (WHO/FAO/UNU, 2007). These values are consistent to those of *I. ertli* and *C. forda* larvae (11.5 and 9.5 mg/g, respectively), and egg (14 mg/g), but should be paired carefully with lower methionine sources such as soy (3 mg/g) (Gorissen *et al.*, 2018; Nsevolo Miankeba *et al.*, 2022) (Table 2). While these caterpillars may not serve as a sole protein dietary source, they can be effectively combined with foods high in methionine and cysteine. Leucine concentrations also fell short of the reference value (59 mg/g), ranging from 33.3 ± 0.5 mg/g in the *Ct. obliquana* larvae to 41.4 ± 10.9 mg/g in *C. scriptaria* larvae. This aligns with findings from Nsevolo Miankeba *et al.* (2022), who reported leucine deficiency in five species of Lepidoptera. Despite this, the overall amino acid profile of the species in our study supports their potential contribution to balanced diets when used alongside complementary protein sources.

There were no significant differences among the three species across both life stages (larvae and pupae) for all NEAA's except for proline and taurine. As NEAAs are less critical for protein quality scoring, therefore results are not discussed in detail here.

Mineral composition of caterpillars reared on kawakawa diet

The second research question investigated the mineral content of *E. axenana*, *C. scriptaria*, and *Ct. obliquana* reared on kawakawa (Table 4). Our findings show significant variation in both macro and micromineral larvae content despite the larvae being reared on the same diet (Table 4). The variation observed is consistent with other studies reporting variation in insect mineral composition within and between species (Kavle *et al.*, 2022; Payne *et al.*, 2015; Pongworn *et al.*, 2024).

E. axenana larvae had notably higher levels of sodium, magnesium, potassium, and calcium ($P < 0.05$). Potassium levels were unexpectedly high in all species, exceeding the 2800 mg/day New Zealand recommended daily intake (NZ RDI) in just 100 g of sample ranging from 2765.0 ± 662.0 to 4353.0 ± 1112.0 mg/100 g DW for *C. scriptaria* and *E. axenana*, respectively. Potassium is required to maintain electrolyte balance and function, and electrical and cellular functions (Soetan *et al.*, 2010). Our values surpass those reported for other edible caterpillars *I. belina* (1864 mg/100 g DW) and *G. maia* (1942 mg/100 g DW) (Payne *et al.*, 2015). Similarly, Pongworn *et al.* (2024) reported a high potassium content in silkworm larvae (3,810 mg/100 g DW). Across 22 different insect species, caterpillars generally have a higher potassium level than other insect orders including Orthoptera, Isoptera, Hymenoptera, and Diptera (1024-3259 mg/100 g DW). We also report large intraspecific variation of potassium within each of our species. This inconsistency warrants further investigation into the effects of host plant composition and diet formulation, particularly to ensure standardisation of potassium levels in future insect farming systems.

Magnesium concentrations in *E. axenana* and *Ct. obliquana* (282.7 ± 55.7, 276.5 ± 81.3 mg/100 g DW, respectively) were significantly higher than in *C. scriptaria* (160.6 ± 31.9 mg/100 g DW, $P < 0.05$). All were below the 320 NZD mg/day. Magnesium is important for cellular and physiological functions, including being the active component of several enzyme systems (Soetan *et al.*, 2010). Our magnesium levels exceed that for the five species reported in Payne *et al.* (2015) ranging from *Macrotermes* spp. (81 mg/100 g DW) to *Gynanisa maia* (167 mg/100 g DW), as well as the huhu grub (130 mg/100 g DW) (Kavle *et al.*, 2022). Sodium levels varied widely, *C. scriptaria* contained 368.4 ± 138.2 mg/100 g DW, falling within the NZ RDI range (460-920 mg/day), while *Ct. obliquana* and *E. axenana* exceeded it (1469.0 ± 654.0, and 1871.0 ± 653.0 mg/100 g DW, respectively ($P < 0.05$) (Table 4). While sodium is necessary for maintaining osmotic pressure of body fluids, and carbon dioxide transport (Soetan *et al.*, 2010), only *C. scriptaria* falls within the NZ RDI of 460-920 mg/day. This suggests that caution of sodium intake should be taken when consuming the other two caterpillars as excess of sodium can lead to hypertension (Soetan *et al.*, 2010). However, given that typical serving sizes of insects are likely to be smaller than 100 g, the practical risk may be low. Further investigation into effects of processing and cooking methods on nutritional profiles are also needed and could result in lower levels of minerals.

Calcium (important for the structural strength of bones and teeth) (Soetan *et al.*, 2010) levels were notably low across all three species, with the highest amount detected in *E. axenana* 409.0 ± 515.0 mg/day, although a 100 g serving would provide 40% of the NZ RDI of 1000 mg/day. In contrast Pongworn *et al.* (2024) reported relatively high calcium levels in silkworm larvae of 764.41-1097 mg/g DW (Pongworn *et al.*, 2024). However, our

TABLE 4 Mineral content of three Lepidopteran larval species fed kawakawa: macrominerals, microminerals and heavy metal content, all expressed in mg per 100 g dry weight

	E. axenana (10)	*C. scriptaria* (8)	*Ct. obliquana* (10)	NZ RDI[1] mg/day	NZ maximum allowable limit[2] (molluscs; mg/kg FW)
Macrominerals					
Na	1871.0^{a} ± 653.0	368.4^{b} ± 138.2	1469.0^{a} ± 654.0	460-920	
Mg	282.7^{a} ± 55.7	160.6^{b} ± 31.9	276.5^{a} ± 81.3	320	
K	4353.0^{a} ± 1112.0	2765.0^{b} ± 662.0	3579.0^{ab} ± 1486.	2800	
Ca	409.0^{a} ± 515.0	169.9^{ab} ± 28.4	43.0^{b} ± 48.9	1000	
Microminerals					
Cr	2.2^{a} ± 1.0	0.5^{b} ± 0.5	0.5^{b} ± 0.7	0.025	
Mn	1.8^{a} ± 0.4	3.1^{b} ± 0.7	0.7^{c} ± 0.4	5.0	
Fe	21.4^{a} ± 9.6	8.6^{b} ± 1.9	12.1^{b} ± 5.0	18	
Cu	1.1^{a} ± 0.6	0.8^{a} ± 0.2	1.4^{a} ± 0.7	1.2	
Zn	19.0^{a} ± 6.7	13.7^{a} ± 2.4	17.2^{a} ± 8.0	8	
Se	0.08^{a} ± 0.07	0.01^{b} ± 0.01	0.00^{b} ± 0.01	0.05	
B	421.1^{a} ± 188.8	59.7^{b} ± 26.5	287.6^{a} ± 112.8		
S	4280.0^{a} ± 1684.0	1108.9^{b} ± 278.0	1635.0^{b} ± 542.0		
Heavy metal content					
As	0.05^{b} ± 0.03	0.00^{a} ± 0.00	0.12^{b} ± 0.06		1
Cd	0.00^{a} ± 0.00	0.00^{a} ± 0.00	0.01^{b} ± 0.01		2
Pb	0.00^{a} ± 0.00	0.00^{a} ± 0.00	0.05^{b} ± 0.03		2

1 NZ RDI represents New Zealand and Australia Recommended Daily Intakes (National Health & Medical Research Council, 2006). Values are those recommended for women aged 19-70.

2 Regulatory maximum levels are from Schedule 19, Australia New Zealand Food Standards Code (Te Kāwanatanga o Aotearoa New Zealand Government, 1991). Values are expressed on a fresh-weight basis; insect concentrations are reported on a dry-weight basis.

Values are expressed as mean ± SD. Number of biological replicates for each species shown in parentheses. – samples not measured. Superscript of different letters across each column indicate significant difference ($P < 0.05$) between mean values of the mineral, as determined by post hoc Tukey's Honestly Significant Difference (HSD) test.

results align more closely with Numbi *et al.* (2022), who found low calcium levels (32-225 mg/100 g DW) across seven Sub-Saharan Lepidoptera species. In the present study, larvae were fasted prior to mineral analysis, whereas fasting procedures were not specified in the aforementioned studies. This may contribute to some of the variation in calcium concentrations reported across studies, as gut-contents can contribute greatly to the mineral content of insects (Finke, 2003). Although our caterpillars would not be suitable as a sole source of calcium as part of the RDI, paired effectively with other diet sources would complement as a good nutritional source of food.

Microminerals levels were consistently higher in *E. axenana* than the other two species. *E. axenana* has the highest iron (crucial mineral for oxygen transport and enzymatic functions; Soetan *et al.*, 2010) (21.4 ± 9.6 mg/100 g DW), surpassing the NZ RDI (18 mg/day). *Ct. obliquana* and *C. scriptaria* contained significantly lower amounts (12.1 ± 5.0 and 8.6 ± 1.9 mg/100 g DW, respectively, $P < 0.05$) (Table 4), though still nutritionally meaningful. This positions the *E. axenana* as a potential source for addressing iron deficiency aligning with previous studies highlighting Lepidoptera as potential iron-rich food sources (Payne *et al.*, 2015). All species meet or exceeded the NZ RDI for chromium (0.025 mg/day), copper (1.2 mg/day), and zinc (8 mg/day) in a 100 g serving (Table 4). Zinc levels across our three caterpillars (13.7 ± 2.4 to 19.0 ± 6.7 mg/100 g DW) are comparable to commercial edible insects such as locust, scarab beetle, house crickets and mulberry silkworm ranging from 8.22 to 21.80 mg/100 g DW (Köhler *et al.*, 2019). This positions our caterpillars, particularly the *E. axenana*, as viable dietary zinc sources.

Boron and sulphur were especially high in *E. axenana* (421.1 ± 188.8 and 280.0 ± 1684.0 mg/100 g DW, respectively), suggesting species-specific uptake or bioaccumulation of these elements. Both the *E. axenana* and *Ct. obliquana* contained a significantly higher amount of boron (421.1 ± 188.8 and 287.6 ± 112.8 mg/100 g, respectively) than the *C. scriptaria* (59.7 ± 26.5) ($P < 0.05$). Selenium levels, important for immune function and as a component of antioxidant enzymes (Soetan *et al.*, 2010), were low overall. *E. axenana* was the only species reaching the NZ RDI (0.05 mg/day) at 0.08 ± 0.07 mg/100 g. However, our levels reported for all three species were higher than those for huhu larvae (0.003-0.007 mg/100 g DW) (Kavle *et al.*, 2022), but comparable to *B. mori* larvae (0.08 mg/100 g DW) and *T. molitor* larvae (0.07 mg/100 g DW) (Rumpold and Schlüter, 2013). Manganese content fell below the NZ RDI (5 mg/day) in all three species, with *C. scriptaria* containing the highest at 3.1 ± 0.7 mg/100 g DW. This aligns with reported values for the mulberry silkworm (1.26 mg/100 g DW), house cricket (1.60 mg/100 g DW), locust (1.07 mg/100 g DW), but is lower than the scarab beetle (7.85 mg/100 g DW) (Köhler *et al.*, 2019). Differences in the variability of mineral profiles across different insect taxa emphasises the need for species-specific nutritional assessment.

Heavy metal concentrations were low across all samples. *C. scriptaria* had no detectable arsenic, cadmium, or lead, while trace amounts were observed in *Ct. obliquana* (0.12 ± 0.06, 0.01 ± 0.01 and 0.05 ± 0.03 mg/g, respectively) (Table 4). New Zealand maximum levels for inorganic arsenic (1 mg/kg FW), cadmium (2 mg/kg FW), and lead (2 mg/kg FW) in molluscs provide a relevant regulatory benchmark (Schedule 19, Australia New Zealand Food Standards Code). Although our measurements are reported on a dry-weight basis, even after accounting for typical insect moisture content these concentrations remain well below the established maximum levels, indicating a low risk for human consumption (Te Kāwanatanga o Aotearoa New Zealand Government, 1991). These values also fall within the range considered safe for human consumption in the grasshopper (*Oxya chinensis formosana*) (0.07-0.17, 0.02-0.05, and 0.12-0.29 mg/100 g DW for arsenic, cadmium, and lead, respectively) (Hyun *et al.*, 2012).

Overall, the three Lepidoptera species had favourable mineral profiles. One hundred grams of *E. axenana* would meet NZ RDI for iron (18 mg/day) and selenium (0.05 mg/day). In contrast, *C. scriptaria* and *Ct. obliquana* fall below some mineral targets but would still have value as complementary protein sources (Table 4).

Amino acid composition of the Ct. obliquana reared on three different diets

Our third research question explored how diet (kawakawa, mānuka and laboratory diet) impacts the amino acid and mineral composition of *Ct. obliquana*. We also examined the impact of life stage (larvae and pupae) on amino acid and mineral composition. A key finding was the significant variation in amino acid composition across different diets, while the two life stages (late instar larvae and early pupae) had a lesser impact. Although our focus was limited to these stages, more distinct nutritional differences may be observed across broader life stages, such as larvae versus adults, as reported in fatty acid composition of *A. gemmatalis* H., and other insects (Cookman *et al.*, 1984; Finke, 2002). The concentrations of EAAs and NEAAs in *Ct. obliquana* larvae and pupae reared on kawakawa, mānuka, and laboratory are presented in Table 5. Our finding of significant differences in EAA concentrations between larvae and pupae across different diets aligns with studies, highlighting the complex relationship between diet and insect life stage in shaping insect nutritional profiles (Ibarra-Herrera *et al.*, 2020; Kannan *et al.*, 2024; Oonincx and Finke, 2020).

Total essential amino acids in *Ct. obliquana* reared on three diets ranged from 166.6 mg/g in pupae fed a laboratory diet to 223.2 mg/g in larvae fed mānuka. To better understand why pupae fed a laboratory diet had lower total EAA, it would be useful to measure fat content, as protein and fat are typically inversely related on a dry matter basis. A similar pattern was observed in two Lepidoptera species, where larvae reared on artificial diets accumulated significantly more lipids than those fed fresh plant-based diets (Landry *et al.*, 1986). Total EAA values in *Ct. obliquana* are higher than egg (165 mg/g), comparable to mealworm (184.7 mg/g), and soy (198 mg/g), but lower than *I. ertli* and *C. forda* larvae (272.7 and 242.4 mg/g, respectively), huhu (386.7 mg/g), beef (329.9 mg/g) and the recommended protein value (271.0 mg/g) (Gorissen *et al.*, 2018; Kavle *et al.*, 2023; Nsevolo Miankeba *et al.*, 2022; Wu *et al.*, 2016). These comparisons highlight the potential of the *Ct. obliquana* as a nutritious amino acid source, particularly when reared on mānuka.

Larvae reared on mānuka consistently showed higher concentrations of histidine, isoleucine, threonine, and valine ($P < 0.05$) (Table 5). For example, histidine was significantly higher in larvae feeding on mānuka (19.3 ± 1.3 mg/g) than pupae on the laboratory diet (15.0 ± 2.1 mg/g) ($P < 0.05$). No significant differences were observed between larvae and pupae reared on

TABLE 5 Amino acid composition of *Ct. obliquana* larvae and pupae fed three different diets (kawakawa, mānuka, laboratory diet)

	Kawakawa		Mānuka		Laboratory diet	
	Larvae	Pupae	Larvae	Pupae	Larvae	Pupae
Essential amino acids						
His	$18.8^{ab} \pm 0.7$	$18.3^{ab} \pm 0.6$	$24.8^{a} \pm 1.8$	$19.3^{ab} \pm 1.3$	$19.2^{ab} \pm 3.1$	$15.0^{b} \pm 2.1$
Ile	$21.6^{ab} \pm 0.5$	$19.5^{ab} \pm 0.1$	$22.9^{a} \pm 1.2$	$20.2^{ab} \pm 1.2$	$20.7^{ab} \pm 1.7$	$18.0^{b} \pm 2.0$
Leu	$33.3^{a} \pm 4.5$	$33.4^{a} \pm 1.3$	$38.3^{a} \pm 2.8$	$35.7^{a} \pm 1.2$	$34.5^{a} \pm 2.2$	$30.6^{a} \pm 3.5$
Lys	$45.8^{a} \pm 18.0$	$39.1^{a} \pm 4.7$	$42.3^{a} \pm 2.4$	$25.8^{a} \pm 15.8$	$38.0^{a} \pm 2.1$	$31.6^{a} \pm 4.2$
Met	$8.8^{b} \pm 0.1$	$9.2^{ab} \pm 0.1$	$12.5^{a} \pm 4.2$	$10.6^{ab} \pm 0.9$	$11.4^{ab} \pm 1.4$	$10.0^{ab} \pm 1.3$
Phe	$26.0^{a} \pm 1.1$	$23.4^{a} \pm 8.4$	$27.5^{a} \pm 2.1$	$23.9^{a} \pm 1.6$	$25.4^{a} \pm 2.5$	$21.6^{a} \pm 2.4$
Thr	$21.7^{ab} \pm 6.4$	$19.7^{ab} \pm 3.8$	$24.8^{a} \pm 1.2$	$20.3^{ab} \pm 1.9$	$22.2^{ab} \pm 1.4$	$18.6^{b} \pm 2.2$
Val	$23.5^{cb} \pm 1.6$	$24.3^{abc} \pm 1.4$	$29.9^{a} \pm 7.6$	$26.7^{ab} \pm 2.1$	$23.9^{bc} \pm 1.3$	$21.3^{c} \pm 2.3$
Total EAAs	199.6	186.9	223.2	182.7	195.2	166.6
Non-essential amino acids						
Ala	$28.9^{a} \pm 2.5$	$30.6^{a} \pm 1.8$	$31.2^{a} \pm 2.5$	$31.3^{a} \pm 3.9$	$25.7^{a} \pm 1.8$	$26.1^{a} \pm 2.5$
Arg	$31.3^{a} \pm 4.5$	$31.5^{a} \pm 1.2$	$34.9^{a} \pm 2.5$	$33.7^{a} \pm 5.3$	$30.7^{a} \pm 2.4$	$29.6^{a} \pm 3.5$
Asn	$2.0^{a} \pm 0.3$	$1.4^{a} \pm 0.5$	$1.6^{a} \pm 0.2$	$1.7^{a} \pm 2.6$	$0.9^{a} \pm 4.8$	$1.5^{a} \pm 0.1$
Asp	$28.3^{ab} \pm 2.5$	$24.3^{b} \pm 2.4$	$33.0^{a} \pm 1.9$	$27.1^{ab} \pm 3.2$	$30.2^{ab} \pm 1.8$	$24.3^{b} \pm 2.8$
Gln	$0.1^{ab} \pm 0.1$	$0.1^{ab} \pm 0.0$	$0.2^{a} \pm 0.0$	$0.0^{ab} \pm 0.1$	$0.0^{b} \pm 0.0$	$0.0^{b} \pm 0.0$
Glu	$60.1^{a} \pm 4.6$	$41.7^{ab} \pm 6.6$	$60.0^{a} \pm 4.8$	$47.2^{ab} \pm 1.9$	$50.9^{ab} \pm 6.8$	$36.3^{b} \pm 4.7$
Gly	$25.2^{a} \pm 1.5$	$18.7^{a} \pm 1.4$	$14.5^{a} \pm 16.1$	$21.4^{a} \pm 0.5$	$23.8^{a} \pm 0.6$	$19.3^{a} \pm 2.6$
Pro	$32.2^{a} \pm 0.3$	$26.8^{a} \pm 1.9$	$34.7^{a} \pm 1.4$	$27.7^{a} \pm 0.3$	$44.2^{a} \pm 12.7$	$32.2^{a} \pm 3.6$
Ser	$23.5^{a} \pm 5.7$	$19.8^{ab} \pm 2.2$	$23.9^{a} \pm 2.1$	$20.2^{ab} \pm 1.1$	$22.8^{ab} \pm 7.7$	$19.1^{b} \pm 1.8$
Tau	$4.4^{ab} \pm 1.1$	$5.9^{ab} \pm 0.8$	$4.8^{ab} \pm 1.2$	$6.1^{a} \pm 3.6$	$3.9^{b} \pm 0.3$	$4.4^{ab} \pm 0.3$
Tyr	$32.3^{ab} \pm 2.4$	$32.2^{ab} \pm 1.7$	$41.3^{a} \pm 3.4$	$34.2^{ab} \pm 1.5$	$38.7^{ab} \pm 1.9$	$31.3^{b} \pm 3.8$
Total NEAAs	268.8	233.1	279.9	250.8	271.9	224.2
Sum of total AAs	468.1	420.1	503.1	433.5	467.2	390.8
E/T%	42.7%	44.5%	44.4%	42.1%	41.8%	42.6%

Essential and non-essential amino acids. All expressed in mg per g dry weight. Mean ± SD from three biological replicates per treatment. Tryptophan was not determined in this study and cysteine was below detection levels. Means with a different letter across a row are significantly different ($P < 0.05$). EAAs, essential amino acids; NEAAs, non-essential amino acids; E/T%, essential amino acids divided by total amino acids as a percentage; His, histidine; Ile, isoleucine; Lys, lysine; Leu, leucine; Met, methionine; Phe, phenylalanine; Thr, threonine; Val, valine; Asp, aspartic acid; Gly, glycine; Arg, arginine; Ala, alanine; Ser, serine; Tyr, tyrosine; Glu, glutamic acid; Pro, proline; Tau, taurine; Asn, asparagine; Gln, glutamine.

kawakawa (18.8 ± 0.7 and 18.3 ± 0.6 mg/g, respectively), pupae reared on mānuka (19.2 ± 3.1 mg/g), and larvae reared on the laboratory diet (19.2 ± 3.1 mg/g) (Table 5). All treatments met the histidine value reported for a good nutritional quality protein for human adults (15 mg/g) (Table 3).

Isoleucine concentrations were highest in larvae feeding on mānuka (22.9 ± 1.2 mg/g) and lowest in pupae that arose from larvae fed on laboratory diet (18.0 ± 2.0 mg/g) ($P < 0.05$) (Table 5). However, all values fell below the RDI reference value (30 mg/g) (Table 3). A similar pattern was observed for threonine, with larvae reared on mānuka (20.3 ± 1.9 mg/g) significantly higher than pupae that arose from larvae fed on laboratory diet (18.6 ± 2.2 mg/g) ($P < 0.05$). Only kawakawa and mānuka diets met the good quality protein value for threonine (23 mg/g) (Table 3). Valine was also significantly higher in larvae feeding on mānuka (29.9 ± 7.6 mg/g) followed by pupae from the mānuka diet (26.7 ± 2.1 mg/g). Pupae fed on laboratory diet had the lowest concentration (21.3 ± 2.3 mg/g) ($P < 0.05$). Although all fall short of the valine good quality protein value (39 mg/g).

Our findings align with other research showing that plant-based diets can enhance insect nutrition. Grasshopper (*Sphenarium purpurascens*) fed alfalfa (*Medicago sativa* L.) were enriched 10% in essential amino acid index and biological value compared to their coun-

terparts fed with maize green fodder (*Zea mays* L.) (Ibarra-Herrera *et al.*, 2020). Black soldier fly (*Hermetia illucens* L.) larvae fed rosemary showed an upregulation of essential amino acids like L-histidine, L-phenylalanine, L-tyrosine and L-valine, crucial for protein synthesis and growth (Kannan *et al.*, 2024), but their saprophagous nature limits direct comparison with our results. Overall, these findings support the idea that specific plant diets, including medicinal plants, can optimise insect nutrition for human and animal food applications.

The patterns for methionine, lysine, leucine, and phenylalanine closely mirror those observed in Experiment 1 and were below the good-quality protein values, therefore they are not discussed further here. For NEAAs there were significant differences in the concentrations of aspartic acid, serine, tyrosine, glutamic acid, taurine, and glutamine, between different life stages and diets ($P < 0.05$). Glutamic acid was the most abundant ranging from 36.3 ± 4.7 mg/g in pupae reared on the laboratory diet to 60.0 ± 4.8 mg/g in larvae reared on the mānuka diet ($P < 0.05$). Mid-range concentrations were detected for arginine, proline, tyrosine, aspartic acid, alanine, glycine, and serine (19.1 ± 1.8 mg/g to 34.9 ± 2.5 mg/g). While taurine, asparagine, and glutamine had lower concentrations (0.0 ± 0.1 mg/g to 6.1 ± 3.6 mg/g) (Table 5).

The EAA to total amino acid ratio ranged from 41.8% (lab-fed larvae) to 44.5% (kawakawa-fed pupae), all exceeding the FAO/WHO threshold of 40% (Table 5). Overall, *Ct. obliquana* reared on mānuka and kawakawa diets had superior amino acid profiles to those reared on the laboratory diet, reinforcing the potential of native plant diets to enhance nutritional quality.

Mineral composition of Ct. obliquana reared on three different diets

The mineral composition of the *Ct. obliquana* larvae and pupae reared on three different diets (kawakawa, mānuka, and laboratory diet) are presented in Table 6. In some instances, the mineral content in pupae was reduced by half compared to the larvae stage. For example, potassium levels dropped from 3579.0 ± 1486.0 in kawakawa reared larvae to 1514.0 ± 632.0 mg/100 g DW kawakawa reared pupae, $P < 0.05$. Sodium levels were significantly higher in larvae reared on mānuka (1970.0 ± 1056.0 mg/100 g) and kawakawa (1469.0 ± 654.0 mg/100 g) diets than larvae (641.4 ± 137.0 mg/100 g) and pupae (573.1 ± 203.1 mg/100 g) reared on laboratory diets, which did not differ significantly. All values fall within or above the NZ RDI for salt (460-920 mg/day), suggesting the need to monitor sodium intake when incorporating these insects into the diet.

Magnesium content showed little variation across diets and life stages, although larvae and pupae from the kawakawa diet, and larvae from the mānuka diet had significantly higher levels compared to other groups ($P < 0.05$). Potassium levels were significantly elevated in larvae reared on kawakawa and mānuka (3579.0 ± 1486.0 and 3247.0 ± 1810.0 mg/100 g, respectively), exceeding the NZ RDI for potassium (2800 mg/day), indicating their potential as a rich potassium source. Phosphorus levels varied significantly, ranging from 609.4 ± 393.0 mg/100 g DW in pupae fed laboratory diet to 1174.0 ± 427.0 mg/100 g DW in larvae fed kawakawa ($P < 0.05$). Notably, even the lowest phosphorus levels detected in our study (609.4 ± 393.0 pupae reared on laboratory diet) exceed those reported for huhu larvae (501.0 mg/100 g DW) (Kavle *et al.*, 2022) and similar to mealworms (748.3 mg/100 g DW) (Rumpold and Schlüter, 2013). Importantly, these levels approach the NZ RDI of 1000 mg/day, highlighting the potential of *Ct. obliquana*s as a good source of dietary phosphorus. Given phosphorus's crucial role in bone and teeth formation (Soetan *et al.*, 2010), this reinforces the nutritional value of *Ct. obliquana*. Calcium levels, however, remained consistently low across all diets and life stages, with no significant differences observed. This suggests that *Ct. obliquana*s may not be a reliable sole source of calcium, regardless of their diet or life stage.

Among microminerals, iron, zinc, chromium, and selenium showed minimal variation between diets. Iron levels were highest in larvae fed mānuka diet (39.1 ± 86.9 mg/100 g) and lowest in pupae from larvae fed laboratory diet (4.8 ± 1.7 mg/100 g). Selenium was highest in larvae reared on laboratory diet (0.02 ± 0.02 mg/100 g). Sulphur and boron levels varied significantly, with the highest levels in larvae fed mānuka (2262.0 ± 1158.0 mg/100 g and 362.5 ± 205.1 mg/100 g, respectively) and lowest detected in pupae fed laboratory diet (710.2 ± 228.0 mg/100 g and 109.2 ± 19.8 mg/100 g, respectively) ($P < 0.05$). There was less variation in manganese and copper levels between the treatment groups, but the highest amounts were detected in larvae reared on mānuka (1.3 ± 1.3 and 1.7 ± 0.5 mg/100 g, for Mn and Cu, respectively). Manganese levels were lowest for pupae reared on laboratory diet (0.4 ± 0.1 mg/100 g) and copper lowest for pupae reared on kawakawa (0.9 ± 0.5 mg/100 g).

When comparing 100 g of *Ct. obliquana* to the NZ RDI, all treatments exceeded requirements for copper (1.5 mg/day) zinc (8 mg/day) and chromium (0.025) NZ

TABLE 6 Mineral content (mg per 100 g dry weight) of *Ct. obliquana* larvae and pupae reared on three different diets: macrominerals, microminerals and heavy metal content expressed in mg per 100 g dry weight

	Kawakawa		Mānuka		Laboratory diet (12)		NZ RDI[1] mg/day	NZ maximum allowable limit[2] (molluscs; mg/kg FW)
	Larvae (10)	Pupae (10)	Larvae (14)	Pupae (7)	Larvae (12)	Pupae (12)		
Macrominerals								
Na	1469.0^{ab} ± 654.0	858.0^{bc} ± 370.0	1970.0^{a} ± 1056.0	867.0^{bc} ± 537.0	641.4^{c} ± 137.0	573.1^{c} ± 203.1	460-920	
Mg	276.5^{a} ± 81.3	193.2^{abc} ± 86.9	248.4^{ab} ± 84.3	144.5^{c} ± 90.2	186.1^{bc} ± 34.1	149.2^{c} ± 40.4	320	
K	3579.0^{a} ± 1486.0	1514.0^{b} ± 632.0	3247.0^{a} ± 1810.0	1317.0^{b} ± 833.0	1678.0^{b} ± 510.0	1229.0^{b} ± 359.0	2800	
P	1174.0^{a} ± 427.0	708.0^{b} ± 164.2	929.0^{ab} ± 402.0	622.0^{b} ± 350.0	751.6^{b} ± 209.1	609.4^{b} ± 393.0	1000	
Ca	43.0^{a} ± 48.9	65.6^{a} ± 42.2	44.1^{a} ± 56.5	37.3^{a} ± 33.9	80.7^{a} ± 58.9	66.1^{a} ± 25.6	1000	
Microminerals								
Cr	0.5^{a} ± 0.7	0.4^{a} ± 0.7	1.1^{a} ± 1.7	0.2^{a} ± 0.2	0.2^{a} ± 0.3	0.2^{a} ± 0.2	0.025	
Mn	0.7^{ab} ± 0.4	0.4^{ab} ± 0.2	1.3^{a} ± 1.3	0.6^{ab} ± 1.0	0.8^{ab} ± 0.6	0.4^{b} ± 0.1	5.0	
Fe	12.1^{a} ± 5.0	7.8^{a} ± 2.4	39.1^{a} ± 86.9	6.9^{a} ± 4.4	10.1^{a} ± 13.6	4.8^{a} ± 1.7	18	
Cu	1.4^{ab} ± 0.7	0.9^{b} ± 0.5	1.7^{a} ± 0.5	1.1^{ab} ± 0.8	1.2^{ab} ± 0.3	1.1^{ab} ± 0.4	1.2	
Zn	17.2^{a} ± 8.0	12.6^{a} ± 7.0	25.5^{a} ± 39.5	9.2^{a} ± 6.5	20.7^{a} ± 9.9	17.7^{a} ± 4.3	8	
Se	0.00^{a} ± 0.01	0.01^{a} ± 0.02	0.00^{a} ± 0.01	0.01^{a} ± 0.02	0.02^{a} ± 0.02	0.02^{a} ± 0.02	0.05	
B	287.6^{ab} ± 112.8	168.0^{bc} ± 69.8	362.5^{a} ± 205.1	169.6^{bc} ± 103.7	109.2^{c} ± 19.8	111.6^{c} ± 43.5		
S	1635.0^{ab} ± 542.0	970.0^{bc} ± 370.0	2262.0^{a} ± 1158.0	1148.0^{bc} ± 640.0	752.1^{c} ± 78.4	710.2^{c} ± 228.0		
Heavy metal content								
As	0.12^{a} ± 0.06	0.10^{a} ± 0.07	0.11^{a} ± 0.09	0.12^{a} ± 0.17	0.07^{a} ± 0.03	0.07^{a} ± 0.03		1
Cd	0.01^{a} ± 0.01	0.00^{a} ± 0.00	0.01^{a} ± 0.01	0.01^{a} ± 0.03	0.01^{a} ± 0.01	0.01^{a} ± 0.01		2
Pb	0.05^{ab} ± 0.03	0.03^{ab} ± 0.02	0.07^{a} ± 0.05	0.05^{ab} ± 0.07	0.03^{b} ± 0.01	0.02^{b} ± 0.01		2

1 NZ RDI represents New Zealand and Australia Recommended Daily Intakes (National Health & Medical Research Council, 2006). Values are those recommended for women aged 19-70.

2 Regulatory maximum levels are from Schedule 19, Australia New Zealand Food Standards Code (Te Kāwanatanga o Aotearoa New Zealand Government, 1991). Values are expressed on a fresh-weight basis; insect concentrations are reported on a dry-weight basis.

Values are expressed as mean ± SD. Number of biological replicates for each species shown in parentheses. – samples not measured. Superscript of different letters across each column indicate significant difference ($P < 0.05$) between mean values of the mineral, as determined by post hoc Tukey's Honestly Significant Difference (HSD) test.

RDI. Conversely, levels for manganese (5.0 mg/day), iron (18 mg/day), and selenium (0.05 mg/day) were below NZ RDI values (Table 4). This indicates that certain diets may enhance the accumulation of specific microminerals. which aligns with the growing body of research promoting edible insects as sustainable and nutritious food alternatives (van Huis *et al.*, 2013).

The elevated levels of macrominerals observed in insects fed kawakawa and mānuka diets could be attributed to higher mineral content or improved bioavailability in these plants. In a study looking at the effects of long-term mass rearing of the gypsy moth (*Lymantria dispar*), caterpillars reared on plant diets had a higher survival and faster development compared to laboratory diets, likely due the phytochemical and nutritional complexity associated with natural foliage (Grayson *et al.*, 2015). This observation aligns with previous studies demonstrating the impact of diet on insect mineral composition (Pongworn *et al.*, 2024; Spranghers *et al.*, 2017). Interestingly, mineral content was more consistent across life stages on the laboratory diet, suggesting that artificial diets could support standardisation in insect farming. Future formulations might incorporate kawakawa or mānuka to improve mineral content while maintaining consistency.

Heavy metal concentrations (arsenic, cadmium, and lead) remained low across all treatments, similar to levels observed in kawakawa fed caterpillars in Experiment 1. This is encouraging from a food safety perspective, indicating a low risk of heavy metal contamination.

Limitations and future directions

This study successfully determined the amino acid and mineral composition of three caterpillar species. However, further research is needed to assess the nutrient bioavailability and absorption in human diets. The absence of fatty acid and vitamin analysis also leaves a gap in the overall nutritional assessment, particularly given that diet has a strong influence on fatty acid composition (van Broekhoven *et al.*, 2015).

Further, given the variation observed in both amino acid and mineral composition across life stages and diets, further investigation is needed to understand the causes of these variations and to investigate the pupae stage of the *C. scriptaria* and *E. axenana*. Investigating the mechanisms by which diet influences nutritional composition could also help optimise insect rearing for enhanced food value. Moreover, comparing the nutritional content of kawakawa and mānuka could offer insights into the efficiency of mineral transfer from plant to insects. Lastly, overexploitation and habitat change has led to decreasing numbers of some caterpillar species in Africa (van Huis and Oonincx, 2017) stressing the importance of sustainable farming practices for native insects in Aotearoa New Zealand.

4 Conclusions

This study highlights the nutritional potential of Aotearoa New Zealand caterpillars as a sustainable protein source. *E. axenana* and *C. scriptaria* reared on kawakawa had high essential amino acid content, exceeding the FAO/WHO requirements of 40% EAAs indicating they can serve as a good source of quality proteins. *Ct. obliquana* reared on mānuka and kawakawa contained elevated levels of potassium, phosphorus, and zinc, while *E. axenana* was a good source of iron. However, higher sodium levels in plant fed caterpillars and trace heavy metals in *Ct. obliquana* highlight the need for careful diet formulation and consumption guidelines.

Overall, *C. scriptaria* and *E. axenana* appear to offer superior nutritional options, though all three species demonstrate potential as viable food sources. Future research should focus on bioavailability, diet optimisation, and further nutritional profiling to maximise the potential of native Aotearoa caterpillars as an alternative sustainable food source. Finally, to ensure long term viability, research should explore how to scale insect farming using culturally and ecologically responsible practices.

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Rūnanga o Koukourarata, and whānau (family) consultation was carried out with the lead author (Chrystal Te Ohorere O'Connor, Ngāti Hauā, Ngāti Paoā) prior to collection. In accordance with tikanga, a karakia was performed before the euthanasia of insects and harvesting of kawakawa. We would also like to thank Waipaia Teriaki, Tasha Teriaki, and Caitlin Hyde for their assistance with caterpillar collection. We gratefully acknowledge the funding provided from the Strategic Science Investment Fund by New Zealand Ministry of Business Innovation and Employment to AgResearch and the Joint Postgraduate Award from Food Transitions 2050, New Zealand.

Conflict of interest

The authors declare no conflict of interest.

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