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**Original Paper**

# Chitin and Carbohydrate Composition of Eight Insect Species Used as Feed and Food

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Edible insects represent a sustainable source of protein and bioactive compounds, however their carbohydrate composition, particularly structural polysaccharides like chitin, substantially affects nutritional quality. This study provides a comparative analysis of chitin and carbohydrate composition of eight insect species across larval (*Tenebrio molitor*, *Alphitobius diaperinus*, *Hermetia illucens*, *Musca domestica*), pupal (*Bombyx mori*), and adult (*Gryllodes sigillatus*, *Acheta domesticus*, *Gryllus assimilis*) stages. Neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), and chitin content varied significantly with species and developmental stage, reflecting differences in cuticular structure and physiological adaptations. Neutral detergent fiber (NDF) content varied considerably across species, with the lowest value in *Bombyx mori* pupae (92.02 ±2.44 g.kg$^{-1}$ DM) and the highest in *Tenebrio molitor* larvae (290.40 ±6.14 g.kg$^{-1}$ DM). Acid detergent fiber (ADF) showed a minimum of 72.90 ±8.68 g.kg$^{-1}$ DM (*B. mori* pupae) and a maximum of 133.16 ±0.97 g.kg$^{-1}$ DM (*Gryllus assimilis* adults). Chitin content (ADF-ADL) was lowest in *B. mori* pupae (64.32 ±9.65 g.kg$^{-1}$ DM) and highest in *G. assimilis* adults (123.45 ±1.28 g.kg$^{-1}$ DM); alternative estimation via ADF-CP$^{ADF}$ ranged from 37.95 ±4.52 to 78.78 ±2.00 g.kg$^{-1}$ DM, highlighting methodological sensitivity. Soluble sugars were highest in larvae, whereas polyols, accumulated predominantly in pupae (glycerol in *B. mori* 11.43 ±0.43 g.kg$^{-1}$ DM) and adults (glycerol in *A. domesticus* 7.20 ±0.04 g.kg$^{-1}$ DM). These results emphasize the importance of standardized chitin quantification and targeted processing strategies for optimizing the nutritional value of insect-derived feed and food. This integrative approach advances the understanding of insect carbohydrate composition and supports efforts to valorize edible insects in sustainable nutrition systems.

**Keywords:** structural carbohydrates, soluble sugars, beetle larvae, fly larvae, crickets, silkworm pupae

## 1 Introduction

Insects have emerged as a promising alternative source of protein and bioactive compounds for both food and feed, leading to growing scientific and industrial interest (van Huis, 2013; Rumpold & Schlüter, 2013; EFSA, 2022). Beyond their high protein content, edible insects are also recognized as carriers of diverse bioactive molecules, and their long tradition of consumption highlights their growing potential for modern food and feed applications (Ivanišová et al., 2022). In addition to proteins, insects provide lipids, minerals, vitamins, and other compounds that can enhance the sustainability of livestock production systems (Makkar et al., 2014; Bovera et al., 2016). The successful incorporation of insect powders into cereal-based products, such as crackers, demonstrates their ability to enrich nutritional quality and antioxidant potential while maintaining acceptable sensory properties (Ivanišová et al., 2023). Nevertheless, their utilization is limited by structural polysaccharides such as chitin and by the variability of non-structural carbohydrate fractions, which influence digestibility and functional properties (Finke, 2007; Janssen et al., 2017; Onsongo et al., 2018). Moreover, species such as the black soldier fly not only provide valuable nutrients

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but also play a role in waste biodegradation, linking insect production with circular economy strategies (Mašková et al., 2025).

Insects' carbohydrates include structural forms such as chitin, which is bound in cuticle-protein complexes, and soluble forms like mono- and disaccharides and polyols (Hu et al., 2017; Lee et al., 2019; Son et al., 2021; Shah et al., 2022). These soluble compounds function as energy reserves and are involved in stress tolerance, osmoregulation, and antioxidant defense (Lee et al., 2019; Shah et al., 2022; Abenaim & Conti, 2025). Earlier studies have consistently shown elevated concentrations of soluble sugars in larval stages compared to pupae and adults (Hu et al., 2017; Son et al., 2021).

Although there is an increasing amount of research on insect proteins and lipids, there is still a lack of systematic characterization of carbohydrate fractions across species and life stages. Furthermore, various techniques for chitin analysis (chemical, enzymatic, gravimetric) and carbohydrate measurement (colorimetric assays, chromatography) produce inconsistent results, hindering cross-study comparisons (Sharbidre et al., 2021; Psarianos et al., 2025). Consequently, a systematic assessment of structural (NDF, ADF, ADL, chitin) and soluble carbohydrate fractions (TS, individual sugars, polyols) across various species and developmental stages is crucial for clarifying their nutritional potential and functional roles in animal nutrition.

The current study aimed to compare eight insect species, including beetle larvae (*Tenebrio molitor*, *Alphitobius diaperinus*), fly larvae (*Hermetia illucens*, *Musca domestica*), silkworm pupae (*Bombyx mori*), and crickets (*Gryllodes sigillatus*, *Acheta domesticus*, *Gryllus assimilis*). Fiber fractions, chitin, total sugars, individual carbohydrates, and polyols were measured, and the differences in development between larvae, pupae, and adults were examined. The objective of the study was to provide a comprehensive dataset that clarifies the interconnections among chitin, $CP^{ADF}$ (crude protein in acid detergent fiber), and soluble carbohydrates, which helps in the formulation of insect-based diets for monogastric and ruminant species.

## 2 Material and Methods

Eight insect species were analyzed, each in triplicate ($n = 3$). The tested species included larvae of the black soldier fly (*Hermetia illucens*, Linnaeus 1758), yellow mealworm (*Tenebrio molitor*, Linnaeus 1758), lesser mealworm (*Alphitobius diaperinus*, Panzer 1797), and housefly (*Musca domestica*, Linnaeus 1758); adults of three cricket species: house cricket (*Acheta domesticus*, Linnaeus 1758), tropical house cricket (*Gryllodes sigillatus*, Walker 1869), and Jamaican field cricket (*Gryllus assimilis*, Fabricius 1775); and silkworm pupae (*Bombyx mori*, Linnaeus 1758). All insects were purchased as whole, dried, full-fat specimens from commercial suppliers, with the drying method not specified by the manufacturers. Samples were stored in sealed plastic bags at 3–5 °C and analyzed within five days. Before an analysis, insects were homogenized using a laboratory mill (FRITSCH, Idar-Oberstein, Germany) equipped with 1 mm sieves. All analyzed species are authorized for use in animal feed under European Union legislation (Regulation 2017/893/ EU; Regulation 2021/1925/EU), while *T. molitor*, *A. domesticus*, and *A. diaperinus* are also approved as novel food in the EU (Regulation 2015/2283/EU; Regulation 2017/2470/EU).

All results are expressed on a dry matter (DM) basis. Before determination of crude fiber (CF), acid detergent fiber (ADF), neutral detergent fiber (NDF), acid detergent lignin (ADL), chitin, and crude protein in ADF ($CP^{ADF}$), samples were defatted by extraction in petroleum ether for 24 h at a ratio of 10 ml solvent per 1 g sample, with continuous agitation on a pulse shaker (LT 3, NEDFORM Ltd., Benešov, Czech Republic) at laboratory temperature. The residues were subsequently filtered and dried at 105 °C for a minimum of 4 h. Crude protein (CP) was determined by the Kjeldahl method (N × 6.25) using a KJELTEC system (Tecator, Höganäs, Sweden). Fiber fractions were determined using an ANKOM 200 Fiber Analyzer (ANKOM Technology, Macedon, NY, USA), where NDF was measured with sodium lauryl sulfate, ADF with cetyltrimethylammonium bromide, ADL with 72% sulfuric acid, and CF by sequential hydrolysis with sulfuric acid and potassium hydroxide. Chitin was estimated using two approaches: the ADF-ADL method (ADF minus ADL) as described by Hahn et al. (2018), and the ADF-$CP^{ADF}$ method according to Finke (2007), where $CP^{ADF}$ represents crude protein bound within the ADF fraction (nitrogen × 6.25 measured in the ADF residue). These analyses were performed in the Laboratory of Feed Quality and Nutritional Value, Department of Animal Nutrition, Institute of Nutrition and Genomics, Faculty of Agrobiology and Food Resources, Slovak University of Agriculture in Nitra.

Total sugars (TS) were quantified by UHPLC (Thermo Scientific Dionex UltiMate 3000, Waltham, MA, USA) as the cumulative concentration of all mono- and disaccharides detected. Individual carbohydrates (glucose, fructose, sucrose, xylose, mannose, ribose) as well as polyols (glycerol, mannitol) were quantified separately. The sum of individually listed sugars may not exactly match TS values because TS also includes minor carbohydrates not reported separately. NSC were estimated by difference as NSC = 100–(NDF + CP + EE + Ash), following standard proximate analysis conventions,

*Bombyx mori* *Gryllus assimilis* *Acheta domesticus* *Gryllodes sigillatus*

*Hermetia illucens* *Musca domestica* *Tenebrio molitor* *Alphitobius diaperinus*

**Figure 1** Edible insect species evaluated in this study
Photo: Džima, 2025

where NDF-neutral detergent fiber, CP-crude protein, EE-ether extract. This indirect method may introduce cumulative error and should be interpreted with caution, particularly when comparing across insect species with highly variable composition. Total soluble sugars and individual carbohydrates were determined in collaboration with FEED LAB Ltd. (Spišská Nová Ves, Slovakia).

Statistical analyses were carried out using SPSS 26.0 (IBM Corp., Armonk, NY, USA). One-way ANOVA was applied to test for differences among species and developmental stages, followed by Tukey's post hoc test for pairwise comparisons. Independent samples *t*-tests were used where appropriate, and Pearson's correlation coefficients were calculated to assess relationships between variables. Differences were considered significant at $p < 0.05$.

## 3 Results and Discussion

Notable interspecific and developmental variations were detected in fiber fractions and chitin levels (Tables 1–2). Neutral detergent fiber (NDF) varied from 92.02 ±2.44 g.kg$^{-1}$ DM in *B. mori* pupae to 290.40 ±6.14 g.kg$^{-1}$ DM in *T. molitor* larvae. Acid detergent fiber (ADF) displayed a narrower range, with the lowest value observed in *B. mori* pupae (72.90 ±8.68 g.kg$^{-1}$ DM) and the maximum in *G. assimilis* adults (133.16 ±0.97 g.kg$^{-1}$ DM; $p < 0.05$). These patterns indicate species- and stage-specific variations in cuticular development, aligned with the gradual sclerotization and accumulation of structural carbohydrates throughout metamorphosis (Hahn et al., 2018; Psarianos et al., 2025). Similar alterations have been seen in *H. illucens*, with chitin content rising from 2.7% DM in early larvae to 8.7% DM in puparia, thereafter, decreasing to 5.8% DM in adults (Wang et al., 2020).

The estimated chitin I. content obtained through ADF-ADL (acid detergent fiber minus acid detergent lignin) was consistently greater than the chitin II. values derived from ADF-CP$^{ADF}$ (ADF minus nitrogen bound to ADF expressed as crude protein). Chitin I. content determined by the ADF-ADL method ranged from 64.32 ±9.65 g.kg$^{-1}$ DM in *B. mori* pupae to 123.45 ±1.28 g.kg$^{-1}$ DM in *G. assimilis* adults. When calculated as ADF-CP$^{ADF}$, values were considerably lower (37.95 ±4.52 to 78.78 ±2.00 g.kg$^{-1}$ DM), confirming that the choice of analytical method strongly affects chitin estimates. This discrepancy supports earlier findings that ADF-based approaches may overestimate chitin due to interference of other insoluble polysaccharides (Hahn et al., 2018; Psarianos et al., 2025). Although there are variations in absolute values, both methods demonstrated interspecific ranking, validating their appropriateness for comparative analysis.

**Table 1** Chitin and fiber fractions in eight edible insect species across developmental stages

| | *Bombyx mori* | *Gryllus assimilis* | *Acheta domesticus* | *Gryllodes sigillatus* |
|---|---|---|---|---|
| | pupae | adult | adult | adult |
| g.kg$^{-1}$ | | | | |
| DM | 930.85$^{a}$ ±0.52 | 934.15$^{b}$ ±2.72 | 950.78$^{c}$ ±1.39 | 979.08$^{d}$ ±2.83 |
| g.kg$^{-1}$ DM | | | | |
| NDF | 92.02$^{a}$ ±2.44 | 249.60$^{b}$ ±20.84 | 204.89$^{c}$ ±4.99 | 152.09$^{d}$ ±7.57 |
| ADF | 72.90$^{a}$ ±8.68 | 133.16$^{b}$ ±0.97 | 112.89$^{c}$ ±5.00 | 99.17$^{d}$ ±2.83 |
| ADL | 8.58$^{a}$ ±1.32 | 9.71$^{ac}$ ±0.33 | 4.89$^{b}$ ±1.56 | 2.33$^{b}$ ±1.21 |
| CP$^{ADF}$ | 34.95$^{a}$ ±4.16 | 54.78$^{b}$ ±0.40 | 49.87$^{bc}$ ±3.85 | 44.89$^{cf}$ ±1.28 |
| Chitin I. | 64.32$^{a}$ ±9.65 | 123.45$^{b}$ ±1.28 | 108.01$^{c}$ ±3.63 | 96.83$^{de}$ ±2.43 |
| Chitin II. | 37.95$^{a}$ ±4.52 | 78.38$^{b}$ ±0.57 | 63.02$^{c}$ ±1.71 | 54.27$^{d}$ ±1.55 |

| | *Hermetia illucens* | *Musca domestica* | *Tenebrio molitor* | *Alphitobius diaperinus* |
|---|---|---|---|---|
| | larvae | larvae | larvae | larvae |
| g.kg$^{-1}$ | | | | |
| DM | 960.25$^{e}$ ±0.96 | 937.75$^{f}$ ±0.17 | 952.55$^{c}$ ±1.12 | 964.45$^{g}$ ±0.53 |
| g.kg$^{-1}$ DM | | | | |
| NDF | 265.45$^{be}$ ±32.96 | 207.27$^{c}$ ±9.29 | 290.40$^{e}$ ±6.14 | 118.98$^{ad}$ ±2.43 |
| ADF | 109.87$^{c}$ ±2.06 | 98.87$^{de}$ ±2.50 | 89.48$^{e}$ ±2.04 | 96.66$^{de}$ ±3.19 |
| ADL | 12.00$^{c}$ ±1.28 | 5.20$^{b}$ ±1.82 | 4.36$^{b}$ ±0.51 | 9.57$^{ac}$ ±1.14 |
| CP$^{ADF}$ | 39.42$^{ad}$ ±0.74 | 20.09$^{e}$ ±0.51 | 36.10$^{a}$ ±0.82 | 43.08$^{df}$ ±1.42 |
| Chitin I. | 97.87$^{ce}$ ±2.55 | 93.67$^{def}$ ±2.90 | 85.12$^{f}$ ±2.48 | 87.09$^{df}$ ±4.10 |
| Chitin II. | 70.45$^{e}$ ±1.32 | 78.78$^{b}$ ±2.00 | 53.39$^{d}$ ±1.21 | 53.58$^{d}$ ±1.77 |

DM – dry matter; NDF – neutral detergent fiber; ADF – acid detergent fiber; ADL – acid detergent lignin; CP$^{ADF}$ – crude protein in ADF; chitin I. – estimated as ADF-ADL; chitin II. – estimated as ADF-CP$^{ADF}$; statistically significant differences were observed with different indexes in rows ($p < 0.05$)

Chitin values reported in the literature exhibit significant variability related to biological and methodological influences. Our findings are consistent with the reported levels of 40–50 g.kg$^{-1}$ DM in *T. molitor* larvae (Son et al., 2021) and 4–9% DM in *A. diaperinus* larvae (EFSA, 2022; Luparelli et al., 2023). In adult crickets (*A. domesticus*, *Gryllus bimaculatus*), chitin values ranging from 7–13% dry matter have been reported (Yi et al., 2013; Udomsil et al., 2019; Pilco-Romero et al., 2023), which align with the elevated levels noted in *A. domesticus*, *G. assimilis*, and *G. sigillatus*. In *H. illucens*, chitin content changes with developmental stage: ~3.6% in larvae, 3.1% in prepupae, 14.1% in puparia, and 2.9% in adults (Wang et al., 2020). In *H. illucens*, heavily sclerotized puparial and exuvial cuticles contain markedly more chitin than larval or adult stages, typically ~20–31% DM (Soetemans 2020 et al.; Triunfo 2022). Da Silva Lucas et al. (2021) reported that the chitin obtained from *T. molitor* cuticles represented 70.9% of the dry matter. *B. mori* pupae typically exhibit a chitin content of only 3–4% (Makkar, 2014; Abenaim & Conti, 2025), aligning with the low values observed in this study. The observed differences highlight the combined impact of developmental stage and methodology on chitin assessment.

Protein associated with ADF, recognized as CP$^{ADF}$, corresponds with chitin variation among species and developmental stages, indicating a significant

**Table 2** Comparison of fiber and chitin content by developmental stage in tested insects (g.kg$^{-1}$ DM)

| DS | NDF | ADF | ADL | CP$^{ADF}$ | Chitin I. | Chitin II. |
|---|---|---|---|---|---|---|
| Pupae | 92.02$^{a}$ ±2.44 | 72.90$^{a}$ ±8.68 | 8.58$^{a}$ ±1.32 | 34.95$^{a}$ ±4.16 | 64.32$^{a}$ ±9.65 | 37.95$^{a}$ ±4.52 |
| Adults | 202.20$^{b}$ ±43.28 | 115.07$^{b}$ ±14.90 | 5.64$^{a}$ ±3.36 | 49.85$^{b}$ ±4.72 | 109.43$^{b}$ ±11.64 | 65.22$^{b}$ ±10.48 |
| Larvae | 220.53$^{b}$ ±69.85 | 98.72$^{c}$ ±7.87 | 7.78$^{a}$ ±3.43 | 34.67$^{a}$ ±9.10 | 90.94$^{c}$ ±5.95 | 64.05$^{b}$ ±11.42 |

DS – developmental stage; DM – dry matter; CF – crude fiber; NDF – neutral detergent fiber; ADF – acid detergent fiber; ADL – acid detergent lignin; CP$^{ADF}$ – crude protein in ADF; Chitin I. – estimated as ADF-ADL; Chitin II. – estimated as ADF-CP$^{ADF}$; statistically significant differences were observed with different indexes in columns ($p < 0.05$)

relationship between structural polysaccharides and cuticular proteins. CP$^{ADF}$ was observed to be lowest in *M. domestica* larvae at 20.09 ±0.51 g.kg$^{-1}$ DM and highest in *G. assimilis* adults at 54.78 ±0.40 g.kg$^{-1}$ DM ($p$ <0.05). Janssen et al. (2017) reported that approximately 9% of nitrogen in *T. molitor* is found in the ADF fraction. These complexes exhibit biochemical recalcitrance (Hu et al., 2017) and may reduce the digestibility of lipids and minerals due to their binding capacity (Onsongo et al., 2018). Their presence is likely to account for the decreased overall digestibility frequently noted in insect meals *in vivo*. The carbohydrate composition exhibited notable ontogenetic shifts (Tables 3–4). Total sugars were lowest in larvae, with *M. domestica* at 6.19 ±0.15 g.kg$^{-1}$ DM. Sugars increased in pupae, shown by *B. mori* at 19.01 ±0.52 g.kg$^{-1}$ DM, and reached their highest levels in adults, with *A. domesticus* at 23.34 ±0.08 g.kg$^{-1}$ DM ($p$ <0.05; Tables 3-4). Stage-dependent shifts were observed, indicating a reallocation of carbohydrate pools throughout metamorphosis; UHPLC-quantified mono- and disaccharide totals were found to be lowest in larvae and increased subsequently, while it was suggested that larvae may rely more on storage polysaccharides not included in the targeted sugar spectrum (Hu et al., 2017; Son et al., 2021). Comparable pupal values have been documented for *B. mori* and *H. illucens*, frequently below 15 g.kg$^{-1}$ DM (Adámková et al., 2016; Wang et al., 2020). Soluble sugars are influenced by diet: *H. illucens* larvae exhibited approximately 40 g.kg$^{-1}$ DM on carbohydrate-rich substrates compared to around 20 g.kg$^{-1}$ DM on protein-rich diets (Onsongo et al., 2018; Shaphan et al., 2020).

Glycerol and mannitol, classified as polyols (sugar alcohols), are reported separately from total sugars (TS). Polyols exhibited a contrasting ontogenetic trajectory. Polyol concentrations were minimal in larvae, increased in subsequent stages, with pupae displaying the highest ($p$ <0.05) glycerol levels, while adults maintained elevated levels. Polyols were predominantly present beyond the larval stage, with pupae exhibiting the highest glycerol concentrations (*B. mori* 11.43 ±0.43 g.kg$^{-1}$ DM), while adults also maintained elevated levels (e.g., *A. domesticus* 7.20 ±0.04, *G. assimilis* 5.96 ±0.77 g.kg$^{-1}$ DM). Mannitol reached the highest levels in *G. assimilis* 2.43 ±0.21 g.kg$^{-1}$ DM; $p$ <0.05). These changes suggest that as insects develop, they shift from utilizing sugars for growth to accumulating polyols that help them cope with environmental stress (Hu et al., 2017; Son et al., 2021). Polyols function as cryoprotectants and osmolytes, stabilizing proteins and membranes under stress (Lee et al., 2019; Shah et al., 2022), also contribute to oxidative balance (Abenaim & Conti, 2025). Their accumulation in pupae and adults reflects an ecophysiological strategy aimed at enhancing survival, although they are only partially digestible from a nutritional standpoint.

The inversion between sugars and polyols at different developmental stages likely indicates a metabolic transition from growth in larvae to maintenance and stress tolerance in pupae and adults, with polyols serving as osmoprotectants and cryoprotectants. This pattern aligns with studies that associate the accumulation of glycerol and mannitol with resistance to cold and oxidative stress (Hu et al., 2017; Lee et al., 2019; Son et al., 2021; Shah et al., 2022). The relatively low UHPLC-

**Table 3** Non-structural carbohydrates and individual sugar and polyol composition in tested insects (g.kg$^{-1}$ DM)

| | *Bombyx mori* | *Gryllus assimilis* | *Acheta domesticus* | *Gryllodes sigillatus* | *Hermetia illucens* | *Musca domestica* | *Tenebrio molitor* | *Alphitobius diaperinus* |
|---|---|---|---|---|---|---|---|---|
| | pupae | adult | adult | adult | larvae | larvae | larvae | larvae |
| NSC | 268.07$^{a}$ ±1.33 | 223.69$^{b}$ ±7.60 | 213.31$^{be}$ ±2.83 | 130.84$^{c}$ ±10.16 | 192.72$^{d}$ ±3.06 | 260.63$^{a}$ ±1.32 | 199.40$^{de}$ ±10.38 | 171.03$^{f}$ ±8.04 |
| TS | 19.01$^{ac}$ ±0.52 | 18.45$^{a}$ ±0.72 | 23.34$^{b}$ ±0.08 | 20.10$^{c}$ ±0.62 | 11.36$^{d}$ ±0.08 | 6.19$^{e}$ ±0.15 | 9.25$^{f}$ ±0.05 | 10.14$^{f}$ ±0.43 |
| GLC | 0.37$^{ac}$ ±0.10 | 0.17$^{a}$ ±0.01 | 1.46$^{b}$ ±0.00 | 0.29$^{ac}$ ±0.01 | 0.08$^{a}$ ±0.01 | 0.19$^{a}$ ±0.01 | ND | ND |
| FRU | 1.82$^{a}$ ±0.06 | 0.89$^{a}$ ±0.13 | 2.00$^{b}$ ±0.16 | ND | ND | ND | ND | ND |
| SUC | 1.28$^{a}$ ±0.04 | 4.91$^{b}$ ±0.14 | 3.86$^{c}$ ±0.10 | ND | 10.91$^{d}$ ±0.05 | 3.84$^{b}$ ±0.08 | 8.11$^{e}$ ±0.06 | 6.98$^{f}$ ±0.01 |
| XYL | 0.10$^{a}$ ±0.02 | 0.16$^{a}$ ±0.01 | ND | ND | ND | ND | ND | ND |
| MAN | 2.52$^{a}$ ±0.03 | 3.64$^{b}$ ±0.10 | 7.55$^{c}$ ±0.00 | 8.01$^{c}$ ±0.53 | 0.37$^{d}$ ±0.12 | 1.98$^{a}$ ±0.04 | 0.21$^{d}$ ±0.03 | 0.65$^{d}$ ±0.10 |
| RIB | ND | 0.27$^{a}$ ±0.05 | 0.15$^{a}$ ±0.03 | 2.07$^{b}$ ±0.17 | ND | ND | ND | ND |
| GRO | 11.43$^{a}$ ±0.43 | 5.96$^{b}$ ±0.77 | 7.20$^{b}$ ±0.04 | 9.24$^{c}$ ±0.09 | ND | 0.18$^{d}$ ±0.02 | 0.61$^{d}$ ±0.01 | 1.58$^{d}$ ±0.08 |
| MTL | 1.49$^{ac}$ ±0.05 | 2.43$^{b}$ ±0.21 | 1.11$^{c}$ ±0.12 | 0.49$^{c}$ ±0.01 | ND | ND | 0.32$^{c}$ ±0.00 | 0.90$^{c}$ ±0.26 |

DM – dry matter; NSC – non-structural carbohydrates; TS – total sugars (sum of mono- and disaccharides detected by UHPLC; may include minor compounds not listed individually); GLC – glucose; FRU – fructose; SUC – sucrose; XYL – xylose; MAN – mannose; RIB – ribose; GRO – glycerol; MTL – mannitol; ND – not detected, statistically significant differences were observed with different indexes in rows ($p$ <0.05)

**Table 4** Summary of carbohydrate and polyol profile by developmental stage grouping in tested insects (g.kg$^{-1}$ DM)

| DS | NSC | TS | GLC | FRU | SUC |
|---|---|---|---|---|---|
| Pupae | $268.07^{a}$ ±1.33 | $19.01^{b}$ ±0.52 | $0.37^{a}$ ±0.10 | $1.82^{a}$ ±0.06 | $1.28^{a}$ ±0.04 |
| Adults | $189.28^{b}$ ±43.91 | $20.63^{b}$ ±2.21 | $0.54^{a}$ ±0.57 | $1.34^{a}$ ±0.62 | $4.49^{ab}$ ±0.58 |
| Larvae | $205.94^{b}$ ±34.89 | $9.23^{a}$ ±2.00 | $0.14^{a}$ ±0.06 | ND | $7.46^{b}$ ±2.64 |

| DS | XYL | MAN | RIB | GRO | MTL |
|---|---|---|---|---|---|
| Pupae | $0.10^{a}$ ±0.02 | $2.52^{a}$ ±0.03 | ND | $11.43^{a}$ ±0.43 | $1.49^{a}$ ±0.05 |
| Adults | $0.16^{a}$ ±0.01 | $6.25^{b}$ ±2.19 | 0.92 ±0.96 | $7.50^{b}$ ±1.58 | $1.37^{a}$ ±0.92 |
| Larvae | ND | $0.80^{a}$ ±0.73 | ND | $0.79^{c}$ ±0.63 | $0.61^{a}$ ±0.36 |

DM – dry matter; NSC – non-structural carbohydrates; TS – total sugars (sum of mono- and disaccharides detected by UHPLC; may include minor compounds not listed individually); GLC – glucose; FRU – fructose; SUC – sucrose; XYL – xylose; MAN – mannose; RIB – ribose; GRO – glycerol; MTL – mannitol; ND – not detected statistically significant differences were observed with different indexes in columns (*p* <0.05)

sugar totals in larvae may suggest a greater dependence on storage polysaccharides that are not included in the targeted sugar panel.

Correlation analysis reinforced these ontogenetic metabolic shifts. Chitin content (ADF-ADL) was negatively correlated with soluble sugars ($r$ = -0.68, $p$ <0.05), indicating that structural carbohydrate deposition occurs at the expense of soluble reserves. A strong positive correlation was found between chitin (ADF-ADL) and nitrogen bound in the ADF fraction ($CP^{ADF}$, $r$ = 0.65, $p$ <0.01), underscoring the tight linkage between structural polysaccharides and cuticular proteins. $CP^{ADF}$ was also positively correlated with glycerol ($r$ = 0.53, $p$ <0.05), while mannitol showed a weaker but positive association ($r$ = 0.30, n.s.). In contrast, soluble sugars such as fructose were strongly and negatively associated with polyols, for instance with mannitol ($r$ = -0.97, $p$ <0.001), reflecting the metabolic reallocation from readily available carbohydrates toward stress-related osmolytes. These relationships highlight the ontogenetic metabolic shift from nutrient storage in larvae to structural reinforcement and stress adaptation in adults, consistent with trends reported in *T. molitor* and *H. illucens* (Wang et al., 2019; Son et al., 2021).

From a nutritional standpoint, larvae offer the most advantageous balance of usable nutrients, characterized by reduced chitin and moderate $CP^{ADF}$ levels, despite low UHPLC-quantified mono- and disaccharide content. *T. molitor* larvae exhibited $CP^{ADF}$ levels of 36.10 ±0.82 and TS of 9.25 ±0.05 g.kg$^{-1}$ DM, while *G. assimilis* adults showed $CP^{ADF}$ levels of 54.78 ±0.40 and TS of 18.45 ±0.72 g.kg$^{-1}$ DM. Pupae exhibited intermediate profiles, reflecting their transitional metabolic state. While adults may be less advantageous in terms of digestible macronutrients, they provide functional compounds. Chitin, while reducing nutrient digestibility, may function as dietary fiber with prebiotic and immunomodulatory properties (Onsongo et al., 2018; da Silva Lucas et al., 2021). Additionally, polyols serve as protective osmolytes and cryoprotectants (Abenaim & Conti, 2025). Enzymatic hydrolysis of chitin enhances protein digestibility and modulates gut microbiota (Yang et al., 2025), suggesting that the nutritional role of adults transcends macronutrient content. From an applied perspective, larvae exhibiting reduced chitin levels and moderate $CP^{ADF}$ are more appropriate for monogastric animals, including poultry and pigs, where nutrient accessibility is essential (Hu et al., 2017; Son et al., 2021). Conversely, adult crickets exhibiting elevated chitin levels may offer protein that is less digestible, while supplying functional fiber-like compounds that could possess prebiotic properties (Finke, 2007; Janssen et al., 2017; Onsongo et al., 2018). The role of chitin in ruminants remains ambiguous, as rumen microbiota may only partially degrade this polymer, highlighting the necessity for targeted *in vivo* trials (Finke, 2007; Yi et al., 2013).

From a processing perspective, strategies that disrupt chitin-protein complexes, including mild deproteinization, targeted enzymatic hydrolysis, or fine grinding, may enhance protein accessibility in adults while preserving fiber-like functionality. Prior research indicates that chitin-protein complexes diminish protein digestibility (Finke, 2007; Janssen et al., 2017), while enzymatic hydrolysis or processing can improve nutrient availability and influence gut microbiota (da Silva Lucas et al., 2021; Yang et al., 2025). On the other hand, larval materials might require simpler processing to attain elevated digestibility. Larval meals of *Tenebrio molitor* and *Alphitobius diaperinus*, which exhibit moderate $CP^{ADF}$ and reduced chitin content, are suitable for monogastric formulations. In contrast, adult crickets can serve as functional co-ingredients, providing insoluble fiber. The applications align with recent EU approvals for *T. molitor* and *A. diaperinus* as novel food (EFSA, 2022) and findings indicating reduced digestibility alongside functional benefits of cricket chitin and polyols (Yi et al., 2013; Onsongo et al., 2018; Udomsil et al., 2019; Abenaim & Conti, 2025).

Nutritional composition of insects is influenced by species, developmental stage, and the methods of analysis employed. Chitin and $CP^{ADF}$ decrease protein availability but provide functional benefits, whereas soluble sugars and polyols exhibit differing ontogenetic patterns, indicating adaptation to growth and stress.

Larvae serve as the optimal stage for nutrient intake, while adults, despite having reduced levels of available nutrients, offer bioactive compounds that are useful for dietary purposes. Future research should concentrate on standardizing chitin assays, conducting *in vivo* digestibility trials for $CP^{ADF}$ fractions, and developing enzymatic or processing strategies to reduce the impact of chitin-protein complexes in feed and food applications.

## 4 Conclusions

Species and developmental stages fundamentally influence the carbohydrate composition and chitin content of edible insects. Larvae provides the most accessible nutrient profile due to lower chitin and moderate $CP^{ADF}$, despite low UHPLC-quantified mono-/disaccharide totals. In contrast, adults, although higher in TS and polyols, carry more chitin-bound protein, which limits digestibility but adds functional fiber-like effects. Although adults contain more structural carbohydrates and polyols that reduce digestibility, these components may offer health-related benefits such as improved gut function or stress tolerance. Methodological differences in chitin quantification highlight the need for standardized analytical protocols. Based on these findings, larval stages of *Tenebrio molitor* and *Alphitobius diaperinus* represent promising feed ingredients for poultry and fish diets due to their favorable balance of soluble carbohydrates and moderate chitin levels. Adult crickets, although less suitable as primary protein sources, can serve as functional ingredients that deliver fiber-like effects and bioactive polyols. Future research should focus on enzymatic treatments and processing interventions to enhance the digestibility and bioavailability of insect proteins, particularly addressing chitin-protein complexes. Moreover, *in vivo* digestibility trials and gut microbiome studies are crucial for fully assessing the nutritional and functional potential of edible insects. Addressing these aspects will support the development of optimized insect-based feed and food products, contributing to sustainable protein systems.

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## Conflict of Interest

The authors declare that there is no conflict of interest.

## Authors Contribution

Matúš Džima – results and discussion, laboratory analysis, Miroslav Juráček – data collection, manuscript conceptualization, Daniel Bíro – methodic supervisor, Milan Šimko – final manuscript preparation, Branislav Gálik – technical support, Michal Rolinec – data processing, Ondrej Hanušovský – statistical evaluation, Mária Kapusniaková – sample preparation, Kristína Kolbaská – laboratory analysis, Ester Vargová – laboratory analysis

## AI and AI-Assisted Technologies Use Declaration

No generative AI tools/AI-assisted technologies were used during the preparation of the manuscript.

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