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Research article

Black soldier fly larvae (*Hermetia Illucens*) as a sustainable and concentrated source of bioavailable lutein for feed.

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Abstract

Black soldier fly larvae (BSFL) are increasingly used to recycle and convert food waste into feed. We attempted to assess whether they can bioaccumulate lutein, a xanthophyll used as a food coloring, and whether it is then sufficiently bioavailable for an economically relevant incorporation of BSFL into feed. Vegetables and larvae lutein concentrations were measured by HPLC. Lutein bioaccessibility was estimated by *in vitro* digestion and lutein absorption efficiency by Caco-2 cells. BSFL were at least as rich, and sometimes richer ($p < 0.05$), in lutein than the vegetables they were reared on. For example, the larvae reared on kale contained 160.2 ± 3.4 mg/kg vs 23.0 ± 3.5 mg/kg of lutein, on a fresh weight basis, for the kale substrate. For the same substrate, lutein bioaccessibility was not statistically different between BSFL and the substrate (respectively 14.8 ± 1.2 % and 16.2 ± 2.8 %; $p = 0.7$). Finally, by considering the lutein concentration in BSFL enriched in lutein and in lutein-rich substrates, as well as the bioaccessibility and intestinal absorption efficiency of lutein contained in these matrices, it was estimated that consumption of lutein-enriched larvae would lead to a theoretical amount of absorbed lutein about 2 to 13 times higher compared to that following the consumption of an equal quantity of lutein-rich vegetables. Thus, BSFL can be used as a sustainable and concentrated source of bioavailable lutein for feed and, indirectly, for food.

Keywords: edible insects, circular economy, nutritional quality, xanthophyll, bioaccessibility.

Conflicts of interest: BC and DS work in the BioMiMetiC company. This company conducts research and development activities aimed at enhancing the value of entomo-conversion on a wide variety of organic materials generated in the area at all levels of the food value chain.

Abbreviation:
BSFL (black soldier fly larvae).

1) Introduction

Lutein is a xanthophyll, i.e. an oxygenated carotenoid, which is found in the human diet mainly from green leafy vegetables such as parsley, kale or spinach (Perry *et al.*, 2009) but this phytochemical can also be found in eggs, algae or marigold extracts. It exerts very varied biological effects, whether in photosynthetic plants, where it is involved in photosynthesis (Dall'Osto *et al.*, 2006; Demmig-Adams *et al.*, 2022), in the animal kingdom, where it can participate in the coloring of certain animals (Langi *et al.*, 2018; Nabi *et al.*, 2020), or even in humans, where it seems to be involved in visual function (Feng *et al.*, 2019; Johnson, 2014). Lutein-rich feed ingredients are used to improve egg yolk and broiler meat color (Langi *et al.*, 2018). Lutein is therefore a molecule of interest for the feed and food industry and as a consequence, strategies to obtain it at lower costs are sought after.

Nowadays, products used to feed farm animals, such as soy, whose cultivation is one of the main causes of deforestation in the world, or fishmeal produced from wild fish, which further exacerbate overfishing, are contributing to an unsustainable food system. In the context of climate change and the need to find more sustainable nutrient sources, the incorporation of insects into livestock and human diets is recommended by FAO and EFSA (EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA) *et al.*, 2021; FAO, 2021). Indeed, insect farming has many advantages over traditional animal husbandry, e.g. lower greenhouse gas production and water consumption per kg protein produced (Allegretti *et al.*, 2018; FAO, 2021). Moreover, some insects, such as black soldier fly larvae (BSFL), i.e. *Hermetia Illucens*, can also be fed a wide variety of organic wastes (Amrul *et al.*, 2022; Nguyen *et al.*, 2015). Consequently, black soldier flies (BSF) are increasingly used to recycle and valorize farm waste and/or co-products from feed and food industries (Ites *et al.*, 2020; Siddiqui *et al.*, 2022). The larvae obtained are either used as is or transformed into a defatted meal and oil before being incorporated into the feed of various production animals (Sánchez-Muros *et al.*, 2014; Secci *et al.*, 2018). They are very good sources of protein and fat, but they also contain many micronutrients and phytochemicals, including carotenoids (Borel *et al.*, 2021; Finke, 2013; Van Huis *et al.*, 2013).

Although insects, like other animals, generally cannot synthesize carotenoids, they can concentrate selected carotenoids from their diet into specific tissues with the help of carotenoid binding proteins and active transport mechanisms (Heath *et al.*, 2013). Consequently, several studies, e.g. hemipteran insects, dragonflies or silk moth, have reported accumulation of xanthophylls, such as lutein, astaxanthin or zeaxanthin (Maoka *et al.*, 2020, 2021; Yuasa *et al.*, 2014). Concerning the BSFL, they do not contain provitamin A carotenoids if they are raised on substrates that do not contain any (Borel *et al.*, 2021), and therefore they are not a priori capable of synthesizing them. However, they can bioaccumulate carotenoids when they are raised on substrates that contain them (Borel *et al.*, 2021; Leni *et al.*, 2022).

The aim of this study was first to investigate whether BSFL can be enriched with significant amounts of lutein and potentially later with lutein from plant co-products of the food industry, promoting the circular economy. The second objective of this study was to assess whether this sustainable source of lutein was bioavailable enough in order to provide animals, when incorporated into their feed, with a quantity of lutein adapted to their production needs, e.g. allowing satisfactory coloring of the egg yolk of laying hens. To answer this question, we first measured the bioaccumulation of this pigment in larvae reared on vegetables among the richest in lutein. Secondly, we compared the bioaccessibility of lutein in these larvae with that of lutein in the rearing vegetables with an *in vitro* digestion model. Finally, we compared the intestinal uptake efficiency of lutein in the micelles from the digestion of either the larvae or the rearing substrates using Caco-2 cells. Indeed, the

combination of the measurement of bioaccessibility and intestinal uptake efficiency constitute a good estimate of the relative bioavailability of carotenoids in different food matrices.

2) Material and Methods

Chemicals

Solvents used for HPLC (ethanol, *n*-hexane, methanol, dichloromethane, water and methyl tert-butyl ether) were purchased from Carlo Erba reagents (Peypin, France). Lutein and echinenone (HPLC purity > 95%) were from CaroteNature GmbH (Münsingen, Switzerland). Other chemicals and enzymes were purchased from Sigma-Aldrich (Saint-Quentin-Fallavier, France). Dulbecco's modified Eagle's medium (DMEM) containing 4.5 g/L glucose, trypsin-ethylenediaminetetraacetic acid (EDTA), penicillin/streptomycin, non-essential amino acids and phosphate-buffered saline (PBS) were purchased from Life Technologies (Villebon-sur-Yvette, France). Fetal bovine serum (FBS) was from PAA (Vélizy-Villacoublay, France).

Experimental foods

Lutein-rich vegetables (de Azevedo-Meleiro and Rodriguez-Amaya, 2005; Humphries and Khachik, 2003) were chosen as substrates for BSFL rearing : parsley (*Petroselinum sativum*), kale (*Brassica oleracea var. acephala*), endive (*Cichorium intybus var. foliosum*) and broccoli (*Brassica oleracea var. asparagoides*). White mushrooms (*Agaricus bisporus*), which do not contain detectable amounts of lutein (based on literature and verified by HPLC), were used as control substrate. These vegetables and the foods used for the *in vitro* digestion experiments, i.e. potatoes, minced beef meat with 5% fat and olive oil, were bought from a local supermarket.

BSFL farming

BSFL were reared on the above-mentioned substrates, which were ground using a kitchen chopper. The rearing protocol was performed by BioMiMetiC in Avignon, France. The rearing procedure was conducted as previously described (Borel *et al.*, 2021).

Measurement of dry matter

Larva dry matter was determined by measuring water loss after 72 h of freeze-drying. For the substrates, dry matter values were taken from the Ciqual database (https://ciqual.anses.fr/ Accessed 20.06.22).

In vitro digestions to assess lutein bioaccessibility

The in vitro digestion experiments used to measure lutein bioaccessibility were carried out in quadruplicate as previously described (Borel *et al.*, 2021). Before the experiment, 1 g of substrate or larva sample was ground with liquid nitrogen in a mortar grinder (Pulverisette 2, FRITSCH GmbH, Idar-Oberstein, Germany) and mixed with 6.7 g mashed potato, 1.2 g minced meat and 200 mg olive oil. The mixture was homogenized for 10 min at 37 °C in a rotary incubator (190 rpm). Then, 2.5 mL of artificial saliva were added and the mixture was once again incubated 10 min at 37 °C. After adjusting the pH to 4 ± 0.02, 2 mL pepsin were added to the mixture, which was incubated for 30 min at 37 °C. Then, pH was adjusted to 6 ± 0.02 prior to the addition of 9 mL pancreatin-bile extract solution and 4 mL bile solution. The mixture was then incubated for 30 minutes at 37 °C and the digestate aliquots were collected. The remaining mixture was centrifuged at 1,860 × g for 1 h 12 min at 10 °C. Aliquots of filtered supernatant, containing the mixed micelles were stored at - 80 °C until lipid extraction and quantification of lutein by HPLC.

149
150 *Micelle size and zeta potential analysis*
151 The size, measured by dynamic light scattering, and charge (ζ-potential) of particles in
152 the micellar fraction obtained at the end of *in vitro* digestions were measured using a Zetasizer
153 (NanoZS, Malvern Instruments Ltd, Worcestershire, UK). Just before the measurements, the
154 micellar fractions were filtered with a 0.22 µm filter, to eliminate possible agglomerates.
155 Measurements were conducted at 25 °C with a wavelength of 633 nm.
156
157 *Caco-2 cell culture and lutein uptake efficiency*
158 Caco-2 cells (clone TC7) were a kind gift from Dr. M. Rousset (U178 INSERM,
159 Villejuif, France). Cell culture was adapted from (Desmarchelier *et al.*, 2017). Briefly, 1 mL
160 of complete medium (i.e., DMEM supplemented with 16% FBS, 1% non-essential amino
161 acids and 1% antibiotics) was added on the cells every day during the first 7 days and every
162 other day during the next 7 days to obtain confluent differentiated cell monolayers. The day
163 before the experiment, the medium was replaced by a serum-free complete medium. To
164 measure lutein uptake efficiency, cell monolayers were washed twice with 1 mL of PBS.
165 Then, the micellar fractions obtained at the end of the *in vitro* digestion were diluted 5 times
166 with DMEM and 1.5 mL thereof were added on the cells. The micellar solutions were left in
167 contact with the cells for 4 hours (Liu *et al.*, 2004) at 37 °C. After incubation, the medium
168 was recovered. The cells were then washed twice with PBS and were finally collected in 500
169 µL PBS and stored at -80 °C prior to lutein extraction and HPLC analysis.
170
171 *Lutein extraction*
172 Lutein was extracted as described previously for other carotenoids (Borel *et al.*, 2021),
173 using a sample volume of 500 µL. For food substrate and BSFL samples, 1 g was finely
174 crushed with liquid nitrogen and the powders thus obtained were diluted and homogenized in
175 20 mL distilled water.
176
177 *Lutein quantification*
178 The lutein-containing extracts, which were previously evaporated and solubilized in
179 methanol/dichloromethane (65/35, v/v), were injected into the HPLC system. Injection
180 volumes were determined after an initial injection and adjusted to obtain signals within the
181 calibration range. Thus, 5 to 50 µL BSFL and substrate samples, 100 to 150 µL micellar
182 fractions, 25 to 150 µL digestate samples and 180 µL cell samples were injected into the
183 HPLC system. The apparatus and method used, i.e. column, mobile phase, flow rate and
184 detection method, were the same as previously described (Borel *et al.*, 2021).
185
186 *Statistics*
187 Results are expressed as means ± SEM. The homogeneity of variances ($p > 0.05$) was
188 assessed by Levene's test. If the variances were inhomogeneous, data were log-transformed.
189 To assess the normality of the data, Q-Q plots of standardized residuals were used.
190 Differences between groups were tested using several two-way ANOVA. When a significant
191 effect of the matrix in which the lutein was incorporated, i.e. plant or insect, was highlighted
192 by the ANOVA, the comparison of the means between the plant matrix and the insect matrix
193 was carried out by pairwise comparisons using bilateral t-tests, with a Bonferroni adjustment.
194 Values of $p < 0.05$ were considered significant. All statistical analyses were performed using
195 R version 4.1.1 for Windows (R Core Team, 2021).
196
197 **3) Results**
198

199 *Lutein concentration in larvae reared on lutein-rich substrates and in the corresponding*
200 *substrates.*
201 **Figure 1** represents the lutein concentration in larvae reared on lutein-rich substrates
202 and in these substrates. Neither mushrooms nor the larvae reared on these mushrooms
203 contained any detectable amount of lutein while larvae reared on other substrates all contained
204 lutein. What is most striking in **Figure 1A** (results expressed on a fresh weight basis) is that
205 lutein concentration in larvae was always higher than lutein concentration in the
206 corresponding rearing substrate. Moreover, larvae reared on kale contained 160.2 ± 3.4 mg/kg
207 of lutein while kale contained 23.0 ± 3.5 mg/kg of lutein. Thus, lutein concentration in these
208 larvae was about 7 times higher ($p < 0.001$) than in the corresponding substrate. Note that
209 lutein concentration was still more than 3 times higher ($p < 0.001$) in these larvae than in kale
210 on a dry weight basis (**Figure 1B**). The larvae reared on parsley contained more than twice as
211 much lutein as the substrate itself on a fresh weight basis (69.8 ± 5.7 mg/kg vs 29.2 ± 1.4
212 mg/kg; $p < 0.001$). On a dry weight basis however, there was no significant difference ($p =$
213 0.75). Lutein concentration in endive was 0.2 ± 0.02 mg/kg and the larvae fed with endives
214 contained 15 times more lutein (3.0 ± 0.2 mg/kg) on a fresh weight basis ($p < 0.001$), and still
215 about 3 times more on a dry weight basis ($p < 0.001$). There was a significant difference in
216 lutein concentration in broccoli and in larvae reared on broccoli (1.3 ± 0.07 and 2.4 ± 0.3
217 mg/kg on a fresh weight basis ($p < 0.05$) and 11.4 ± 0.6 and 7.6 ± 1.1 mg/kg on a dry weight
218 basis ($p < 0.05$), for broccoli and larvae respectively).
219
220 **Figure 1. Lutein concentration (mg/kg) in rearing substrates and in larvae.**
221
222 *Lutein bioaccessibility in larvae reared on lutein-rich substrates and in the corresponding*
223 *substrates.*
224 Bioaccessibility values are shown in **Table 1**. For kale and broccoli, lutein
225 bioaccessibility in the substrates and in the larvae was not significantly different. Conversely,

	Rearing substrate	Larvae	Substrate vs larvae comparison **p-value
Broccoli	12.1 ± 0.5[a]	13.7 ± 1.2[a]	0.26
Endive	16.9 ± 1.3[a]	8.1 ± 0.9[b]	<0.01
Kale	16.2 ± 2.8[a]	14.8 ± 1.2[a]	0.67
Parsley	14.5 ± 1.1[a]	7.8 ± 0.5[b]	<0.01

226 lutein bioaccessibility was about 2 times lower ($p < 0.01$) in larvae reared on endive and
227 parsley as compared to the corresponding substrates. Lutein bioaccessibility was not
228 significantly different between the substrates while lutein bioaccessibility in the larvae
229 exhibited some variability according to the substrates, namely it was approximately twice as
230 high in larvae reared on broccoli and kale compared to larvae reared on endive and parsley
231 (means around 14% for the former and 8% for the latter).
232
233 **Table 1. Bioaccessibility of lutein* from larvae and their corresponding rearing**
234 **substrates.**
235 *Percent of lutein recovered in the micelle fraction relative to that found in the digestate at the
236 end of the *in vitro* digestion. Values are means ± SEM (n=4). ***p*-value of a two-way
237 ANOVA, followed by pairwise comparisons using bilateral t-tests.
238 [a,b] In each column, means that bear different superscript letters are
239 significantly different ($p < 0.05$; ANOVA followed by Tukey's HSD test).
240

241 *Lutein amount in mixed micelles from either digestion of larvae fed with lutein-rich substrates*
242 *or from digestion of their corresponding substrates.*
243
244 **Figure 2** shows the lutein amount in the micelle-rich fractions obtained after *in vitro*
245 digestion of either larvae fed lutein-rich substrates or their corresponding substrates. This
246 amount depends both on lutein concentration in the matrix (larvae or substrates) and on lutein
247 bioaccessibility in the matrix. The amount of lutein in micelles corresponds to the quantity of
248 lutein that is presented to the intestinal cell for its absorption. On a fresh weight basis (**Figure**
249 **2A**), the mixed micelles obtained after digestion of larvae fed on kale contained about 4 times
250 more lutein than those obtained after digestion of kale (21.9 ± 2.5 and 5.4 ± 1.0 mg/kg,
251 respectively; $p < 0.001$). On a dry weight basis (**Figure 2B**), they still contained more than
252 twice as much lutein as those obtained after kale digestion (72.9 ± 8.5 and 31.7 ± 5.8 mg/kg,
253 respectively; $p < 0.01$). For the other substrates, on a fresh weight basis, no significant
254 difference was observed between larvae and their corresponding substrates. Conversely, on a
255 dry weight basis, the quantity of lutein in micelles that came from the digestion of larvae
256 compared to those that came from the digestion of their corresponding substrates differed
257 significantly for parsley ($p < 0.001$), endive ($p < 0.01$) and broccoli ($p < 0.01$).
258
259 **Figure 2. Lutein quantity in mixed micelles obtained after *in vitro* digestions of rearing**
260 **substrates and of larvae**.
261
262 *Zeta potential of mixed micelles from in vitro digestions*
263 **Table 2** shows the zeta potential of the mixed micelles obtained after *in vitro* digestion
264 of larvae fed on lutein-rich substrates and on their corresponding substrates. The zeta potential
265 of micelles from larva digestion was approximately 2 to 4 times higher than that of their
266 substrates ($p < 0.05$). Note that only one population of particles was detected in the mixed
267 micelle rich fractions. Its size was around 9.0 ± 0.4 nm and there was no significant difference
268 between the size of mixed micelles obtained after digestion of larvae and the size of those
269 obtained after digestion of substrates (data not shown).
270 .
271 **Table 2. Zeta potential of micelles from *in vitro* digestion of larvae and substrates.**

	Zeta potential (mV)		**Substrate vs larvae comparison *p-value**
	Micellar fraction from rearing substrate digestion	**Micellar fraction from larva digestion**	
Broccoli	-8.4 ± 0.8	-4.6 ± 1.0	0.039
Endive	-21.0 ± 1.3	-5.6 ± 2.3	<0.01
Kale	-15.4 ± 1.6	-7.0 ± 1.2	<0.01
Parsley	-14.2 ± 1.3	-5.6 ± 0.1	<0.01

272 Values are means ± SEM (n=4). *p-value of a two-way ANOVA followed by pairwise
273 comparisons using bilateral t-tests.
274
275 *Uptake efficiency of micellarized lutein by Caco-2 cells*
276 **Table 3** shows lutein uptake efficiency from the micellar fraction of digestions of
277 larvae reared on kale and parsley compared to that from the micellar fraction of digestions of
278 the substrates themselves. Note that the results with the other substrates are not shown
279 because the amounts of lutein in the micelles were too low to be able to be correctly measured
280 in the cells (below the quantification limit of 0.02 µg/mL). Lutein in the mixed micelle-rich
281 fraction obtained after digestion of larvae reared on kale was approximately twice as

efficiently taken up compared to lutein from the fraction obtained after digestion of kale ($p < 0.001$). For parsley, cellular uptake was approximately 2 times greater when lutein was supplied by the micellar fraction from larva digestion than by the micellar fraction from substrate digestion ($p < 0.001$).

Table 3. Lutein uptake efficiency by Caco-2 cells. [*]

	Micellar fraction from rearing substrate digestion	Micellar fraction from larva digestion	Substrate vs larvae comparison **p-value
Kale	9.1 ± 0.3	23.6 ± 0.9	<0.001
Parsley	8.4 ± 0.8	21.0 ± 0.8	<0.001

[*]Percentage of lutein that was recovered in the Caco-2 cells relative to the quantity of lutein in the medium before incubation of the cell monolayers with mixed micelle-rich fractions for 4 hours at 37 °C. Values are means ± SEM (n=4). [**]*p*-value of a two-way ANOVA followed by pairwise comparisons using bilateral t-tests.

Figure 3 shows the amount of lutein taken up by Caco-2 cells. This value is very interesting because it depends both on the concentration of lutein in the matrix (plant substrate or larvae), on the bioaccessibility of lutein in this matrix, and on the efficiency of absorption of micellarized lutein by the intestinal cell. It therefore makes it possible to compare the quantity of lutein absorbed by the cells, i.e. bioavailable, for the same quantity of digested matrix. The cells incubated with micelles from the digestion of larvae reared on kale contained around 7 (when normalized to dry weight) to around 13 (when normalized to fresh weight) more lutein than the cells incubated with micelles from the digestion of kale, i.e. 40.2 ± 3.6 vs 5.4 ± 1.1 mg/kg respectively (dry weight; $p < 0.001$), and 12.1 ± 1.1 vs 0.9 ± 0.2 mg/kg respectively (fresh weight; $p < 0.001$). Regarding parsley, there was a significant difference ($p = 0.012$) between the cells incubated with micelles from the digestion of larvae reared on parsley (1.9 ± 0.3 mg/kg) and those incubated with micelles from the digestion of parsley (0.9 ± 0.1 mg/kg) on a fresh weight basis, but not on a dry weight basis ($p = 0.3$)

Figure 3. Lutein quantity taken up by Caco-2 cells incubated with micelles obtained after *in vitro* digestions of rearing substrates or larvae.

4) Discussion

First of all, the results obtained with the white mushrooms (control substrate that does not contain any detectable amount of lutein) allow us to conclude that BSFL do not naturally contain lutein, just as they do not contain provitamin carotenoids (Borel *et al.*, 2021). Secondly, BSFL bioaccumulate lutein to concentrations more or less proportional to those present in the vegetables on which they were reared. Normalizing the results to dry matter weight attenuated the difference in concentration between larvae and vegetables likely because substrates have a higher water content compared to larvae, but nonetheless, larvae reared on kale still displayed a much higher lutein concentration compared to kale. It is therefore evident that the larvae bioaccumulate lutein. This is probably due to the fact that, unlike vegetables, they are very rich in lipids, which can solubilize lutein, a fat-soluble molecule. Whether this bioaccumulation is due to a specific mechanism allowing the larva to store this carotenoid for future use, for example in adulthood, or whether it is simply due to a “reservoir effect” in which lutein is deposited, remains to be determined. However, the fact that BSFL can also bioaccumulate another xanthophyll, zeaxanthin (data not shown), which was present in the studied vegetables but at much lower concentration than lutein, as well as

327 provitamin A carotenoids (Borel *et al.*, 2021; Leni *et al.*, 2022) and vitamin E (Shumo *et al.*, 2019) suggests that they act as a fat tank having the ability to store various fat-soluble molecules by solubilizing them (Borel *et al.*, 1996; Goupy *et al.*, 2020).

330 After having shown that BSFL can bioaccumulate high concentrations of lutein, we compared the bioaccessibility of lutein in larvae and in their corresponding rearing substrates. It could not be ruled out that certain molecules, known or unknown, and present in the insect matrix, could not interfere with bioaccessibility. The results obtained show that either there was no difference in lutein bioaccessibility between the larvae and the substrates (broccoli and kale), or the bioaccessibility of lutein from larvae was half as much as that measured from the substrates (endive and parsley). In our previous study (Borel *et al.*, 2021), we found that the bioaccessibility of another xanthophyll, β-cryptoxanthin, was dramatically lower in clementine-fed larvae (6.4 %) and in pumpkin-fed larvae (13.1 %) as compared to their corresponding substrates (respectively 67.8 and 82.4 %). The synthesis of these results allows us to suggest that the bioaccessibility of xanthophylls is generally lower in larvae than in plant substrates. We hypothesize that this is due to xanthophyll complexing with insect matrix compounds that inhibit their release from this matrix and their transfer to mixed micelles. These compounds could be chitin, or proteins with a particular affinity for xanthophylls. Nevertheless, to explain why the bioaccessibility of lutein from the larvae reared on kale and broccoli was not lower to that observed for the corresponding substrates we hypothesize that kale and broccoli contain one, or several, compound(s) which is/are specific to these vegetables and which improve the bioaccessibility of xanthophylls from larvae. These compounds could be isothiocyanates. Indeed, it has been shown that they cause partial Z-isomerization of carotenoids (Honda et al., 2020a,2020b), and it has also been shown that xanthophyll Z-isomers are more bioaccessible than corresponding E-isomers (Coral-Hinostroza *et al.*, 2004; Yang *et al.*, 2017). However, this hypothesis remains to be verified in a future study.

353 Bioaccessibility measures the transfer efficiency of lipid phytochemicals from food matrices to micelles during digestion, which is a key step for bioavailability. Nevertheless, what is important in terms of animal or human nutrition is the absolute quantity of the compound of interest, here lutein, that reaches the intestinal cells in an absorbable form, i.e. micellarized lutein. This quantity depends both on the concentration of lutein in the food matrix and on its bioaccessibility in this matrix, i.e. quantity in the micelles = quantity provided by the matrix multiplied by bioaccessibility. The micellarized lutein quantity measured (**Figure 2A**) show that the larvae reared on parsley, endive, and broccoli did not provide intestinal cells with significantly different lutein quantities compared with their respective substrates. In other words, consuming 100 g of these substrates or 100 g of larvae having consumed these substrates does not bring different lutein quantity to intestinal cells. However, this figure shows that consuming 100 g of larvae reared on kale provided intestinal cells with about 4 times more lutein than 100 g of kale (**Figure 2A**). Note that when these data are expressed in dry weight (**Figure 2AB**), the larvae reared on parsley, endive and broccoli provide significantly less micellarized lutein than their respective substrates. On the other hand, the larvae reared on kale still provide significantly more micellarized lutein than their substrate. And it is also these kale larvae which provide more micellarized lutein than all the other substrates or larvae studied.

371 Together with bioaccessibility, another key step of lutein bioavailability is its intestinal uptake efficiency. Indeed, whether BSFL digestion releases one or more compounds that could modulate lutein uptake efficiency has never been studied. First of all, it is important to mention that the uptake efficiency of lutein from the micellar fraction of the digestion of the two plant matrices studied (parsley and kale), which was approximately 9%, was quite comparable to what was measured previously, i.e. 10 (Garrett *et al.*, 2000) and 15%

377 (Chitchumroonchokchai *et al.*, 2004), which supports the validity of our measurements.
378 Secondly, we notice that micellarized lutein was taken up more efficiently, about 2 to 3 times,
379 when it came from larvae than when it came from the corresponding substrates. This suggests
380 that either the larvae do not contain one or more compounds present in the plant substrates
381 which inhibit absorption, e.g. plant fibers (Mamatha and Baskaran, 2011), or, on the contrary,
382 they contain one or several compounds that allow a better uptake of lutein by intestinal cells.
383 One of these compounds could be the lipids brought by the larvae. Indeed, BSFL are very rich
384 in lipids, triglycerides in particular, and it is likely that their hydrolysis by pancreatic lipase
385 during *in vitro* digestion generated fatty acids and monoglycerides that changed the
386 composition of mixed micelles. This enrichment of the micelles with triglyceride hydrolysis
387 products can explain a better uptake of the micelles by the enterocyte by two mechanisms.
388 The first is that these compounds, in particular fatty acids, allow a better interaction with the
389 domains of the lipid rafts where SR-BI and CD36, which have been involved in carotenoid
390 uptake (Reboul and Borel, 2011), are located (Goncalves *et al.*, 2013, 2015). The second is
391 that this enrichment in hydrolysis products has reduced the negative charge of the micelles,
392 which is what we observed, and this reduction has in turn reduced the electrostatic repulsion
393 between the micelles and the apical membrane, which is also negatively charged.
394 Knowing the absolute quantity of micellarized lutein following the digestion of larvae
395 relative to that following the digestion of the corresponding substrates, and knowing the
396 cellular uptake efficiency of micellar lutein coming from larvae relative to that of micellar
397 lutein coming from the substrates, it can be estimated that larva consumption would lead to a
398 theoretical amount of absorbed lutein about 2 times (case of parsley) to about 13 times (case
399 of kale) higher compared to that following the consumption of an equal quantity of their
400 corresponding rearing substrate. This of course remains to be verified in an in vivo study but
401 nevertheless, these promising results suggest that BSFL could constitute a nutritionally
402 relevant source of lutein, what is more by recycling and valorizing lutein-rich vegetable
403 waste.
404 To show the relevance of using BSFL enriched with lutein in animal nutrition, or even
405 in human nutrition if they were to be authorized, we calculated the quantities of lutein that
406 could be provided to different animal species if we included in their diet the quantity of larvae
407 recommended for each species (**Table S1**). These theoretical calculations show in particular
408 that lutein-rich larvae incorporated in the diet could provide one-third of the maximum
409 recommended lutein intake in broiler chicken. More interestingly, their use could provide
410 laying hens with up to 86% of the lutein amount that is usually added to the diet to obtain
411 adequate yolk color.
412 In conclusion, this study shows that BSFL can be enriched in lutein at higher
413 concentrations than some of the most lutein-rich vegetables, such as parsley and kale, when
414 the concentrations are expressed in fresh weight. Moreover, the results of the *in vitro*
415 digestion model coupled with Caco-2 cells suggest that that consumption of lutein-enriched
416 larva would lead to higher amount of absorbed lutein compared to the consumption of an
417 equal quantity of lutein-rich vegetables. Finally, our calculations show that the incorporation
418 of lutein-enriched larvae in the feed of several species of farm animals would provide them
419 with quantities of lutein which could sometimes be equivalent to the quantities necessary to
420 satisfy the organoleptic quality criteria of their products. Furthermore, our study shows that
421 the recycling of lutein from food waste containing lutein-rich plants by BSFL is theoretically
422 possible. Indeed, the proteins and oils contained in BSFL are slowly being valorized by the
423 food industry, but this study also shows that micronutrients such as lutein could also be
424 valued from food waste streams, contributing to a circular economy and cleaner production.
425 Finally, these results and those obtained in our previous study (Borel *et al.*, 2021) confirm that
426 the enrichment of BSFL with vitamins or phytochemicals of nutritional and/or organoleptic

interest can lead to the production of BSFL with higher nutritional quality, thus improving most likely their market value.

Supporting information:
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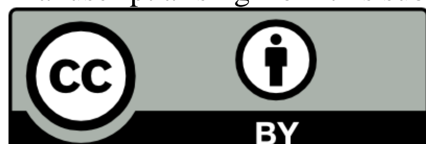

5) References

Adeoye, A.A., Akegbejo-Samsons, Y., Fawole, F.J. and Davies, S.J., 2020. Preliminary assessment of black soldier fly (*Hermetia illucens*) larval meal in the diet of African catfish (*Clarias gariepinus*): Impact on growth, body index, and hematological parameters. Journal of the World Aquaculture Society 51: 1024–1033. https://doi.org/10.1111/jwas.12691.

Allegretti, G., Talamini, E., Schmidt, V., Bogorni, P.C. and Ortega, E., 2018. Insect as feed: An emergy assessment of insect meal as a sustainable protein source for the Brazilian poultry industry. Journal of Cleaner Production 171: 403–412. https://doi.org/10.1016/j.jclepro.2017.09.244.

Amrul, N.F., Kabir Ahmad, I., Ahmad Basri, N.E., Suja, F., Abdul Jalil, N.A. and Azman, N.A., 2022. A Review of Organic Waste Treatment Using Black Soldier Fly (*Hermetia illucens*). Sustainability Multidisciplinary Digital Publishing Institute, 14: 4565. https://doi.org/10.3390/su14084565.

Borel, P., Grolier, P., Armand, M., Partier, A., Lafont, H., Lairon, D. and Azais-Braesco, V., 1996. Carotenoids in biological emulsions: solubility, surface-to-core distribution, and release from lipid droplets. Journal of Lipid Research 37: 250–261. https://doi.org/10.1016/S0022-2275(20)37613-6.

Borel, P., Hammaz, F., Morand-Laffargue, L., Creton, B., Halimi, C., Sabatier, D. and Desmarchelier, C., 2021. Using black soldier fly larvae reared on fruits and vegetables waste as a sustainable dietary source of provitamin a carotenoids. Food Chemistry 359: 129911. https://doi.org/10.1016/j.foodchem.2021.129911.

Chitchumroonchokchai, C., Schwartz, S.J. and Failla, M.L., 2004. Assessment of Lutein Bioavailability from Meals and a Supplement Using Simulated Digestion and Caco-2 Human Intestinal Cells. The Journal of Nutrition 134: 2280–2286. https://doi.org/10.1093/jn/134.9.2280.

Coral-Hinostroza, G.N., Ytrestøyl, T., Ruyter, B. and Bjerkeng, B., 2004. Plasma appearance of unesterified astaxanthin geometrical E/Z and optical R/S isomers in men given single doses of a mixture of optical 3 and 3′R/S isomers of astaxanthin fatty acyl diesters. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology 139: 99–110. https://doi.org/10.1016/j.cca.2004.09.011.

470 Dall'Osto, L., Lico, C., Alric, J., Giuliano, G., Havaux, M. and Bassi, R., 2006. Lutein is needed for
471 efficient chlorophyll triplet quenching in the major LHCII antenna complex of higher plants
472 and effective photoprotection in vivo under strong light. BMC Plant Biology BioMed Central,
473 6: 32. https://doi.org/10.1186/1471-2229-6-32.

474 de Azevedo-Meleiro, C.H. and Rodriguez-Amaya, D.B., 2005. Carotenoids of endive and New
475 Zealand spinach as affected by maturity, season and minimal processing. Journal of Food
476 Composition and Analysis 18: 845–855. https://doi.org/10.1016/j.jfca.2004.10.006.

477 Demmig-Adams, B., Polutchko, S. and Adams, W., 2022. Structure-Function-Environment
478 Relationship of the Isomers Zeaxanthin and Lutein. Photochem 2: 308–325.
479 https://doi.org/10.3390/photochem2020022.

480 Desmarchelier, C., Margier, M., Prévéraud, D.P., Nowicki, M., Rosilio, V., Borel, P. and Reboul, E.,
481 2017. Comparison of the Micellar Incorporation and the Intestinal Cell Uptake of
482 Cholecalciferol, 25-Hydroxycholecalciferol and 1-α-Hydroxycholecalciferol. Nutrients 9:
483 E1152. https://doi.org/10.3390/nu9101152.

484 EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA), Turck, D., Bohn, T., Castenmiller,
485 J., De Henauw, S., Hirsch-Ernst, K.I., Maciuk, A., Mangelsdorf, I., McArdle, H.J., Naska, A.,
486 Pelaez, C., Pentieva, K., Siani, A., Thies, F., Tsabouri, S., Vinceti, M., Cubadda, F., Frenzel,
487 T., Heinonen, M., Marchelli, R., Neuhäuser-Berthold, M., Poulsen, M., Prieto Maradona, M.,
488 Schlatter, J.R., van Loveren, H., Ververis, E. and Knutsen, H.K., 2021. Safety of frozen and
489 dried formulations from whole yellow mealworm (*Tenebrio molitor* larva) as a novel food
490 pursuant to Regulation (EU) 2015/2283. EFSA Journal 19: e06778.
491 https://doi.org/10.2903/j.efsa.2021.6778.

492 EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP), Bampidis, V.,
493 Azimonti, G., de Lourdes Bastos, M., Christensen, H., Dusemund, B., Kouba, M., Kos
494 Durjava, M., López-Alonso, M., López Puente, S., Marcon, F., Mayo, B., Pechová, A.,
495 Petkova, M., Ramos, F., Sanz, Y., Villa, R.E., Woutersen, R., Bories, G., Costa, L.G., Gropp,
496 J., Lundebye, A.-K., Renshaw, D., Holczknecht, O., Vettori, M.V. and Aquilina, G., 2019.
497 Safety and efficacy of lutein and lutein/zeaxanthin extracts from *Tagetes erecta* for poultry for
498 fattening and laying (except turkeys). EFSA journal. European Food Safety Authority 17:
499 e05698. https://doi.org/10.2903/j.efsa.2019.5698.

500 European Commission, 2022. Directorate-General for Agriculture and Rural Development, EU
501 agricultural outlook for markets, income and environment 2021-2031 –, Publications Office of
502 the European Union. Available at : https://data.europa.eu/doi/10.2762/753688.

503 FAO, 2021. Looking at edible insects from a food safety perspective: Challenges and opportunities for
504 the sector. Food and Agriculture Organization of the United States (FAO), Rome, Italy, 108
505 pp https://doi.org/10.4060/cb4094en.

506 Feng, L., Nie, K., Jiang, H. and Fan, W., 2019. Effects of lutein supplementation in age-related
507 macular degeneration. PLOS ONE 14: e0227048.
508 https://doi.org/10.1371/journal.pone.0227048.

509 Finke, M.D., 2013. Complete Nutrient Content of Four Species of Feeder Insects: Nutrient Content of
510 Feeder Insects. Zoo Biology 32: 27–36. http://doi.wiley.com/10.1002/zoo.21012.

511 Garrett, D.A., Failla, M.L. and Sarama, R.J., 2000. Estimation of carotenoid bioavailability from fresh
512 stir-fried vegetables using an in vitro digestion/Caco-2 cell culture model. The Journal of
513 Nutritional Biochemistry 11: 574–580. https://doi.org/10.1016/S0955-2863(00)00122-4.

514 Goncalves, A., Gleize, B., Roi, S., Nowicki, M., Dhaussy, A., Huertas, A., Amiot, M.-J. and Reboul,
515 E., 2013. Fatty acids affect micellar properties and modulate vitamin D uptake and basolateral
516 efflux in Caco-2 cells. The Journal of Nutritional Biochemistry 24: 1751–1757.
517 https://doi.org/10.1016/j.jnutbio.2013.03.004.

518 Goncalves, A., Gontero, B., Nowicki, M., Margier, M., Masset, G., Amiot, M.-J. and Reboul, E.,
519 2015. Micellar lipid composition affects micelle interaction with class B scavenger receptor
520 extracellular loops. Journal of Lipid Research 56: 1123–1133.-
521 https://doi.org/10.1194/jlr.M057612.

522 Goupy, P., Genot, C., Hammaz, F., Halimi, C., Caris-Veyrat, C. and Borel, P., 2020. Mechanisms
523 Governing the Transfer of Pure and Plant Matrix Carotenoids Toward Emulsified
524 Triglycerides. Molecular Nutrition & Food Research 64:
525 1900911.https://doi.org/10.1002/mnfr.201900911.

526 Heath, J., Cipollini, D. and Stireman, J., 2013. The role of carotenoids and their derivatives in
527 mediating interactions between insects and their environment. Arthropod-Plant Interactions 7
528 https://doi.org/10.1007/s11829-012-9239-7. https://doi.org/10.1007/s11829-012-9239-7.

529 Hien, T.T.T., Loc, T.V., Tu, T.L.C., Phu, T.M., Duc, P.M., Nhan, H.T. and Liem, P.T., 2022. Dietary
530 Effects of Carotenoid on Growth Performance and Pigmentation in Bighead Catfish (*Clarias*
531 *macrocephalus* Günther, 1864). Fishes Multidisciplinary Digital Publishing Institute, 7: 37.
532 https://doi.org/10.3390/fishes7010037.

533 Honda, M., Kageyama, H., Hibino, T., Ichihashi, K., Takada, W. and Goto, M., 2020a. Isomerization
534 of Commercially Important Carotenoids (Lycopene, β-Carotene, and Astaxanthin) by Natural
535 Catalysts: Isothiocyanates and Polysulfides. Journal of Agricultural and Food Chemistry
536 American Chemical Society, 68: 3228–3237. https://doi.org/10.1021/acs.jafc.0c00316.

537 Honda, M., Kageyama, H., Hibino, T., Sowa, T. and Kawashima, Y., 2020b. Efficient and
538 environmentally friendly method for carotenoid extraction from *Paracoccus carotinifaciens*
539 utilizing naturally occurring Z-isomerization-accelerating catalysts. Process Biochemistry 89:
540 146–154. https://doi.org/10.1016/j.procbio.2019.10.005.

541 Humphries, J.M. and Khachik, F., 2003. Distribution of lutein, zeaxanthin, and related geometrical
542 isomers in fruit, vegetables, wheat, and pasta products. Journal of Agricultural and Food
543 Chemistry 51: 1322–1327. https://doi.org/10.1021/jf026073e.

544 Ites, S., Smetana, S., Toepfl, S. and Heinz, V., 2020. Modularity of insect production and processing
545 as a path to efficient and sustainable food waste treatment. Journal of Cleaner Production 248:
546 119248. https://doi.org/10.1016/j.jclepro.2019.119248.

547 Johnson, E.J., 2014. Role of lutein and zeaxanthin in visual and cognitive function throughout the
548 lifespan. Nutrition Reviews 72: 605–612. https://doi.org/10.1111/nure.12133.

549 Langi, P., Kiokias, S., Varzakas, T. and Proestos, C., 2018. Carotenoids: From Plants to Food and
550 Feed Industries: Methods and Protocols. Methods in molecular biology (Clifton, N.J.). , pp.
551 57–71. https://doi.org/10.1007/978-1-4939-8742-9_3.

552 Leni, G., Maistrello, L., Pinotti, G., Sforza, S. and Caligiani, A., 2022. Production of carotenoid-rich
553 *Hermetia illucens* larvae using specific agri-food by-products. Journal of Insects as Food and
554 Feed 1–12. https://doi.org/10.3920/JIFF2022.0016.

555 Liu, C.-S., Glahn, R.P. and Liu, R.H., 2004. Assessment of carotenoid bioavailability of whole foods
556 using a Caco-2 cell culture model coupled with an in vitro digestion. Journal of Agricultural
557 and Food Chemistry 52: 4330–4337. https://doi.org/10.1021/jf040028k.

558 Lokaewmanee, K., Yamauchi, K., Komori, T. and Saito, K., 2011. Enhancement of Yolk Color in
559 Raw and Boiled Egg Yolk with Lutein from Marigold Flower Meal and Marigold Flower
560 Extract. The Journal of Poultry Science 48: 25–32. https://doi.org/10.2141/jpsa.010059.

561 Mamatha, B.S. and Baskaran, V., 2011. Effect of micellar lipids, dietary fiber and β-carotene on lutein
562 bioavailability in aged rats with lutein deficiency. Nutrition (Burbank, Los Angeles County,
563 Calif.) 27: 960–966. https://doi.org/10.1016/j.nut.2010.10.011.

564 Maoka, T., Kawase, N., Hironaka, M. and Nishida, R., 2021. Carotenoids of hemipteran insects, from
565 the perspective of chemo-systematic and chemical ecological studies. Biochemical
566 Systematics and Ecology 95: 104241. https://doi.org/10.1016/j.bse.2021.104241.

567 Maoka, T., Kawase, N., Ueda, T. and Nishida, R., 2020. Carotenoids of dragonflies, from the
568 perspective of comparative biochemical and chemical ecological studies. Biochemical
569 Systematics and Ecology 89: 104001. https://doi.org/10.1016/j.bse.2020.104001.

570 Nabi, F., Arain, M.A., Rajput, N., Alagawany, M., Soomro, J., Umer, M., Soomro, F., Wang, Z., Ye,
571 R. and Liu, J., 2020. Health benefits of carotenoids and potential application in poultry
572 industry: A review. Journal of Animal Physiology and Animal Nutrition 104: 1809–1818.
573 https://doi-org.lama.univ-amu.fr/10.1111/jpn.13375.

574 Nguyen, T.T.X., Tomberlin, J.K. and Vanlaerhoven, S., 2015. Ability of Black Soldier Fly (Diptera:
575 Stratiomyidae) Larvae to Recycle Food Waste. Environmental Entomology 44: 406–410.
576 https://doi.org/10.1093/ee/nvv002.

577 O'Neill, M.E., Carroll, Y., Corridan, B., Olmedilla, B., Granado, F., Blanco, I., Van den Berg, H.,
578 Hininger, I., Rousell, A.M., Chopra, M., Southon, S. and Thurnham, D.I., 2001. A European
579 carotenoid database to assess carotenoid intakes and its use in a five-country comparative
580 study. The British Journal of Nutrition 85: 499–507. https://doi.org/10.1079/BJN2000284.

581 Patterson, P.H., Acar, N., Ferguson, A.D., Trimble, L.D., Sciubba, H.B. and Koutsos, E.A., 2021. The
582 impact of dietary Black Soldier Fly larvae oil and meal on laying hen performance and egg
583 quality. Poultry Science 100: 101272. https://doi.org/10.1016/j.psj.2021.101272.

584 Perry, A., Rasmussen, H. and Johnson, E.J., 2009. Xanthophyll (lutein, zeaxanthin) content in fruits,
585 vegetables and corn and egg products. Journal of Food Composition and Analysis 22: 9–15.
586 https://doi.org/10.1016/j.jfca.2008.07.006.

587 Reboul, E. and Borel, P., 2011. Proteins involved in uptake, intracellular transport and basolateral
588 secretion of fat-soluble vitamins and carotenoids by mammalian enterocytes. Progress in Lipid
589 Research 50: 388–402. https://doi.org/10.1016/j.plipres.2011.07.001.

590 Reis, E.C., Galuch, M.B., Silveira, R. da, Santos, H.M.C. dos, Sary, C., Magon, T.F.S., Figueiredo,
591 I.L., Ribeiro, R.P., Visentainer, J.V. and Santos, O.O., 2018. Fatty Acid Composition and
592 Carotenoids in Raw and Grilled Tilapia GIFT Fillets Supplemented with Lycopene, β-
593 Carotene and Lutein. Journal of the Brazilian Chemical Society Sociedade Brasileira de
594 Química, 29: 1742–1750. https://doi.org/10.21577/0103-5053.20180049.

595 Sánchez-Muros, M.-J., Barroso, F.G. and Manzano-Agugliaro, F., 2014. Insect meal as renewable
596 source of food for animal feeding: a review. Journal of Cleaner Production 65: 16–27.
597 https://doi.org/10.1016/j.jclepro.2013.11.068.

598 Secci, G., Bovera, F., Nizza, S., Baronti, N., Gasco, L., Conte, G., Serra, A., Bonelli, A. and Parisi, G.,
599 2018. Quality of eggs from Lohmann Brown Classic laying hens fed black soldier fly meal as
600 substitute for soya bean. Animal: An International Journal of Animal Bioscience 12: 2191–
601 2197. https://doi.org/10.1017/S1751731117003603.

602 Shumo, M., Osuga, I.M., Khamis, F.M., Tanga, C.M., Fiaboe, K.K.M., Subramanian, S., Ekesi, S., van
603 Huis, A. and Borgemeister, C., 2019. The nutritive value of black soldier fly larvae reared on
604 common organic waste streams in Kenya. Scientific Reports 9: 10110.
605 https://doi.org/10.1038/s41598-019-46603-z.

606 Siddiqui, S.A., Ristow, B., Rahayu, T., Putra, N.S., Widya Yuwono, N., Nisa', K., Mategeko, B.,
607 Smetana, S., Saki, M., Nawaz, A. and Nagdalian, A., 2022. Black soldier fly larvae (BSFL)
608 and their affinity for organic waste processing. Waste Management 140: 1–13.
609 https://doi.org/10.1016/j.wasman.2021.12.044

610 Song, X., Li, X., Chen, Z.-Z., Wang, L., Huan, D. and Leng, X., 2017. Effect of dietary xanthophyll
611 on the growth and body color of discus fish (*Symphysodon aequifasciata*). Journal of Fisheries
612 of China 41: 566–572. https://doi.org/10.11964/jfc.20160710484.

613 Taufek, N.M., Lim, J.Z.Y. and Abu Bakar, N.H., 2021. Comparative evaluation of *Hermetia illucens*
614 larvae reared on different substrates for red tilapia diet: effect on growth and body
615 composition. Journal of Insects as Food and Feed Wageningen Academic Publishers, 7: 79–
616 88. https://doi.org/10.3920/JIFF2019.0058.

617 The Age-Related Eye Disease Study 2 (AREDS2) Research Group*, 2013. Lutein + Zeaxanthin and
618 Omega-3 Fatty Acids for Age-Related Macular Degeneration: The Age-Related Eye Disease
619 Study 2 (AREDS2) Randomized Clinical Trial. JAMA 309: 2005–2015.
620 https://doi.org/10.1001/jama.2013.4997.

621 The European Commission, 2021. Commission Implementing Regulation (EU) 2021/420 of 9 March
622 2021 correcting Implementing Regulation (EU) 2020/1097 concerning the authorisation of
623 lutein-rich and lutein/zeaxanthin extracts from Tagetes erecta as feed additives for poultry
624 (except turkeys) for fattening and laying and for minor poultry species for fattening and
625 laying. Official Journal of the European Union Available at: https://eur-lex.europa.eu/legal-
626 content/EN/TXT/PDF/?uri=CELEX:32021R0420&from=EN.

627 Tu, N.P.C., Ha, N.N., Linh, N.T.T. and Tri, N.N., 2022. Effect of astaxanthin and spirulina levels in
628 black soldier fly larvae meal-based diets on growth performance and skin pigmentation in
629 discus fish, Symphysodon sp. Aquaculture 553: 738048.
630 https://doi.org/10.1016/j.aquaculture.2022.738048.

631 Van Huis, A., Van Itterbeeck, J., Klunder, H., Mertens, E., Halloran, A., Muir, G. and Vantomme, P.,
632 2013. Edible insects: future prospects for food and feed security. FAO Forestry Paper no. 171.
633 Food and Agriculture Organization of the United Nations (FAO), Rome, Italy, 187 pp.
634 Available at: http://www.fao.org/docrep/018/i3253e/i3253e.pdf.

635 Yang, C., Zhang, H., Liu, R., Zhu, H., Zhang, L. and Tsao, R., 2017. Bioaccessibility, Cellular Uptake,
636 and Transport of Astaxanthin Isomers and their Antioxidative Effects in Human Intestinal
637 Epithelial Caco-2 Cells. Journal of Agricultural and Food Chemistry American Chemical
638 Society, 65: 10223–10232. https://doi.org/10.1021/acs.jafc.7b04254.

639 Yuasa, M., Kitamura, A., Maoka, T., Sakudoh, T., Shimada, T. and Tsuchida, K., 2014. Astaxanthin
640 and lutein compete for accumulation into the middle silk gland via *Yellow cocoon* gene (*C*)-
641 dependent control and produce a red cocoon of *Bombyx mori*. The Japanese Society of
642 Sericultural Science Available at: https://doi.org/10.11416/jibs.83.1_001.