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► To cite this version:

Lisa Morand-Laffargue, Stéphane Delbecq, Benjamin Creton, Damien Sabatier, Marie Papin, et al.. Bioaccumulated provitamin A in black soldier fly larvae is bioavailable and capable of improving vitamin A status of gerbils. Food Research International, 2023, 171, pp.113064. 10.1016/j.foodres.2023.113064 . hal-04155345

HAL Id: hal-04155345

<https://hal.inrae.fr/hal-04155345>

Submitted on 11 Sep 2023

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1 **BIOACCUMULATED PROVITAMIN A IN BLACK SOLDIER FLY**
2 **LARVAE IS BIOAVAILABLE AND CAPABLE OF IMPROVING**
3 **VITAMIN A STATUS OF GERBILS**

4

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14

15 **Keywords:** β -carotene; insect; vitamin A deficiency; *Hermetia illucens*.

16

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24

25 **Abstract**

26

27 The aim was to study whether provitamin A (proVA), which can bioaccumulate in black
28 soldier fly larvae (BSFL), is bioavailable and can restore VA status in mammals. A model for
29 studying the metabolism of this vitamin, the gerbil, was either fed a standard diet (C+ group), a
30 diet without VA (C-), a diet in which VA was provided by β -carotene (β -C) from sweet
31 potatoes (SP), or a diet in which VA was provided by β -C from BSFL that had been fed sweet
32 potatoes (BSFL). The animals were killed at the end of the supplementation period and β -C,
33 retinol and retinyl esters were measured in plasma and liver. As expected β -C was not detected
34 in plasma and liver of the C+ and C- groups. β -C concentrations were lower ($p < 0.05$) in
35 plasma and liver of the BSFL group as compared to the SP group. Liver retinol and retinyl ester
36 concentrations were lower in the C- group than in all the other groups ($p < 0.05$). These
37 concentrations were not significantly different in the C+ and SP groups while they were lower
38 in the BSFL group ($p < 0.05$ for retinyl oleate and retinyl linoleate). In total, the liver stock of
39 retinol equivalent was almost twice lower in the BSFL group than in the SP group. Thus, β -C
40 present in the BSFL matrix is bioavailable and capable of improving VA status, but this matrix
41 decreases its effectiveness by a factor of around two compared to the sweet potato matrix.

42

43 **1) Introduction**

44

45 Vitamin A (VA) deficiency remains a public health problem in many countries (Sahile
46 et al., 2020; Wolde & Tessema, 2023; Xu et al., 2021), although the richest sources of this
47 vitamin are well known. We also know very well the needs of different groups of the
48 population and we have a lot of data on the factors that influence its bioavailability, in
49 particular that of proVA carotenoids (Bohn et al., 2017; Desmarchelier & Borel, 2017). Finally,
50 we have at our disposal several strategies to combat this deficiency (Bruins & Kraemer, 2013)
51 such as regular supplementation with high doses of VA, or nutritional recommendations aimed
52 at increasing the consumption of local foods richest in this vitamin, such as orange sweet potato
53 (Bechoff & Dhuique-Mayer, 2017; Mulwa, Heck, Maru, Mwema, & Campos, 2022).

54 The different strategies used to tackle deficiency, although complementary and chosen
55 according to the socio-economic and/or cultural context of the country where the deficiency is
56 rife, are clearly not sufficient. Indeed, the deficiency is still present in many countries (Mason,
57 Greiner, Shrimpton, Sanders, & Yukich, 2015). It is also very likely that this deficiency will
58 increase in the decades to come due to population growth, which will be particularly strong in
59 many countries where this deficiency is already rife, and the scarcity of food resources of VA,
60 due to the climate change and its consequences on agricultural yields (Semba, Askari, Gibson,
61 Bloem, & Kraemer, 2022).

62 In this context, it is relevant to seek new sources of VA that are sustainable and that can
63 be consumed by populations in which this deficiency still prevails. We recently showed that the
64 black soldier fly, and probably other edible insects, could be a significant source of proVA
65 carotenoids if its larvae feed on a substrate rich in these micronutrients (Borel, Hammaz, et al.,
66 2021). This new source of VA would have the double advantage of providing energy and
67 quality protein to malnourished populations (Baiano, 2020; Dicke, 2018; Jantzen da Silva

68 Lucas, Menegon de Oliveira, da Rocha, & Prentice, 2020), and make it possible to recover
69 plant waste, and recycle energy and VA which is irretrievably lost in this waste (Ojha, Bussler,
70 & Schluter, 2020).

71 Nevertheless, although we demonstrated that these larvae can become a significant
72 source of proVA, and that this provitamin has a bioaccessibility equivalent to that of the usual
73 plant sources of proVA (Borel, Hammaz, et al., 2021), it remained to be demonstrated that this
74 provitamin is truly bioavailable *in vivo*, i.e. that it is indeed found in the bloodstream after the
75 assimilation of the insect matrix enriched in proVA. Indeed, it cannot be excluded that the
76 insect matrix contains one or more factors that could inhibit the intestinal uptake of this
77 micronutrient. It also remained to be demonstrated that the proVA provided in the insect matrix
78 can be effectively converted into VA in the body. Again, it must be verified that the insect
79 matrix does not provide an inhibitor of the main enzyme for converting proVA into VA in
80 mammals, i.e. BCO1 (Amengual et al., 2013; Lobo, Amengual, Palczewski, Babino, & von
81 Lintig, 2012).

82 The purpose of this study was therefore both to compare the bioavailability of proVA
83 when it was provided by an insect matrix or by a plant matrix naturally rich in proVA, and to
84 compare the ability of these two matrices to restore the status in VA from a laboratory animal
85 which is often used as a model for studying the metabolism of this vitamin, the gerbil (Lee,
86 Lederman, Hofmann, & Erdman, 1998; Pollack, Campbell, Potter, & Erdman, 1994).

87 **1) Material and Methods**

88

89 *Chemicals*

90 Solvents used for HPLC (ethanol, *n*-hexane, dichloromethane, methyl-tert-butyl-ether,
91 methanol and acetonitrile) were purchased from Carlo Erba reagents (Peypin, France). Retinol,
92 retinyl palmitate, β -carotene (β -C) (HPLC purity > 95%), β -apo-8'-carotenal and α -tocopherol
93 acetate were from Sigma-Aldrich (Saint-Quentin-Fallavier, France).

94

95 *BSFL Farming*

96 BSFL were reared on chopped sweet potatoes. The rearing protocol was performed by
97 BioMiMetiC in Avignon, France. The rearing procedure was conducted as previously described
98 (Borel, Hammaz, et al., 2021). To obtain 1 kg of larvae reared on sweet potatoes,
99 approximately 200 mg of eggs were sowed on the substrate. Considering this desired quantity
100 of BSFL and the relative humidity of the food substrates, the amount of substrate required was
101 estimated to be approximately 20 kg of sweet potatoes, which were coarsely cut. Briefly, the
102 rearing procedure was as follows: firstly, after collecting the eggs, the substrate was prepared
103 with hatching devices placed on top of the substrate, already cut, and placed in a rearing room
104 for 14 days at $29 \pm 1^\circ\text{C}$ and $65 \pm 5\%$ relative humidity. At the end of this period, larvae were
105 isolated from their food substrates by sieving (2 x 2 mm) and washed using tap water. Finally,
106 the larvae were filtered again through a sieve (2 x 2 mm) and dried using absorbent paper
107 before being frozen at -80°C .

108

109 *Animals, diets and study design*

110 *Animals*

111 Male Mongolian SPF gerbils (n = 29), 7 weeks old, were obtained from Janvier (S^t
112 Berthevin, France). Animals were housed four by four or three by three in plexiglass cages
113 (enriched with wood or cardboard toys) at $22 \pm 1^{\circ}\text{C}$, subjected to a 12 h light/dark cycle and
114 free access to food and water. Animals and food were weighed three times a week. Animals
115 were handled in compliance with European Union rules and according to the guidelines of the
116 National Institute of Health and the Committee for Animal Care at the University of
117 Montpellier (France). The project authorization by the Ethics committee has been approved in
118 April 2022 (referral number: 1930-35390).

119

120 *Diets*

121 Four different diets were used in this study. The first one was the standard diet of these
122 animals, i.e. standard diet A04 (Specific rodent diet) (SAFE: Scientific Animal Food and
123 Engineering; Augy, France), a positive control group called C+. The second diet, also
124 manufactured by SAFE, was the same standard diet but it did not contain VA and proVA. It
125 was called the negative control group (C-). The third diet was the VA deficient diet to which
126 sweet potatoes were added as a source of proVA. It was called the Sweet Potato diet (SP). The
127 fourth diet was the VA deficient diet to which BSFL rich in proVA were added. It was called
128 the BSFL diet (BSFL).

129 The incorporation of sweet potatoes and insect larvae into the SP and BSFL diets
130 respectively was carried out as follows: boiled sweet potatoes and larvae were freeze-dried
131 during 72 h by a high vacuum line (Christ bioblock scientific, Rungis, France), before being
132 crushed with a mortar. The incorporation of freeze-dried sweet potato in extruded diet was
133 done by society SAFE while the incorporation of insects was done by the galenic service of
134 Faculty of Pharmacy (Montpellier). The apparatus for simulation of compression was the

135 Medelpharm model, Stylone Evolution Lyon. β -C concentrations in the VA deficient diet
136 supplemented with SP or BSFL were 19.4 ± 2.5 and 19.5 ± 4 mg/kg, respectively and were thus
137 non-significantly different. It is important to specify that we chose these concentrations of β -C,
138 and therefore the proportions of sweet potato and BSFL incorporated into the kibbles, so as to
139 provide quantities of proVA that allow its good detection in the tissues of the gerbils (Poulaert
140 et al., 2014).

141

142 *Study design (Figure 1)*

143 During the acclimatization period (4 d) the gerbils were fed with the standard diet.
144 Then, 23 of the 29 gerbils were subjected to the C- diet for 5 weeks. The 6 remaining gerbils
145 consumed the C+ diet during the same 5-week period. The 23 gerbils depleted of VA were
146 randomly divided into 3 groups for the experimental diet period (3 weeks). One group (n=7)
147 continued to consume the VA depleted diet (C- group). One group (n=8) consumed the C+ diet
148 supplemented with 6.6% freeze-dried orange-fleshed sweet potatoes (SP group). One group
149 (n=8) consumed the VA depleted diet supplemented with 10% freeze-dried black soldier fly
150 larvae (BSFL group). Finally, the 6 gerbils that had consumed the standard diet during the first
151 5-week period continued to consume this standard diet during the experimental diet period (C+
152 group). At the end of the experimental period, the gerbils were deprived of food overnight.
153 Prior to sample collection, the animals were placed under deep general anaesthesia by
154 intraperitoneal injection of Ketamine (100 mg/kg) and Xylazine (10 mg/kg) supplied by the
155 animal manager according the drug regulations (cf. referral document cited above). Fasting
156 blood samples were collected by cardiac puncture before euthanasia which was performed by
157 taking an intracardiac blood sample. Blood samples were centrifuged at 2200 g for 10 min to
158 collect plasma which was stored at -80°C until analysis. Liver was collected and weighted after

159 being washed with ice-cold saline solution (0.9% NaCl, w/v) and immediately frozen in liquid
160 nitrogen and stored at -80°C until analysis.

161
162 *Extraction procedure for β -C and retinoids to allow their quantification by HPLC*

163 *Kibbles containing insects rich in β -C or sweet potatoes*

164 β -C extraction from kibbles, carried out according to Poulaert et al. (Poulaert et al.,
165 2014) has been optimized for insect kibbles. Briefly, 0.5 g of grounded kibbles were mixed
166 with 4 ml of water and 300 μ l of ethanol containing β -apo-8'-carotenal as internal standard
167 (stock solution at around 50 μ M). Then a volume of hexane was added respecting the ratio
168 hexane/ethanol (700/400; v/v). The mixture was stirred vigorously then the hexane phase was
169 recovered. A second addition of hexane was made and the procedure repeated. The two hexane
170 phases were pooled and the solvent was evaporated under nitrogen. The dry residue was
171 dissolved in 500 μ l of dichloromethane and 500 μ L of methyl-tert-butyl-ether
172 (MTBE)/methanol mixture (4:1, v/v) in an amber vial before injection in HPLC.

173
174 *Liver and plasma β -C and retinoids*

175 One hundred mg of gerbil livers were suspended in 1 mL of PBS, ground 5 min at 30
176 rotations/min using two 1 mm-diameter and one 3 mm-diameter stainless steel balls in 2 mL
177 Eppendorf tubes with a MM301 ball mill (Retsch, Eragny sur Oise, France). For their
178 extraction, 500 μ L of hepatic samples and 500 μ L of a solution containing approximatively 5
179 ng/ μ L of α -tocopherol acetate as internal standard were used. Concerning the plasma samples,
180 400 μ L were used. The volume was adjusted to 500 μ L with distilled water. Then, 500 μ L of
181 the internal standard solution were added. The rest of the extraction procedure is identical to
182 that described previously (Borel et al., 2022).

183

184 *Quantification of β -C and retinoids by HPLC*

185 Dry extracts of liver and plasma samples were solubilized in 200 μ L of
186 acetonitrile/dichloromethane/methanol (70/20/10, v/v/v) and injected into the HPLC system.
187 Injection volumes were established after a first injection and adjusted to obtain signals in the
188 calibration range. Thus, 50 μ L of resolubilized kibbles samples, 25 μ L of resolubilized hepatic
189 samples and 180 μ L of resolubilized blood samples were injected into the HPLC system. The
190 HPLC system and method used (column, mobile phase, flow rate, detection method and
191 spectral analysis software) were the same as previously described (Borel et al., 2022; Borel,
192 Troadec, et al., 2021). Retinol, β -C and retinyl palmitate were identified by retention times and
193 absorption spectra coincident with authentic standards. Retinyl linoleate, retinyl oleate and
194 retinyl stearate were identified by spectral analysis and quantified regarding their molar
195 extinction coefficient ratio compared to retinyl palmitate. Chromatograms obtained for a
196 sample of gerbil livers (group SP) are available in the **supplemental figure**.

197

198 *Calculation of the conversion rate of β -C into VA*

199 This conversion rate was estimated by calculating the percentage of VA that is found in
200 the form of retinoids (retinol plus retinyl esters) in the liver compared to the total quantity of
201 VA found in the liver, i.e. the sum of retinoids plus that of β -C. This calculation was made by
202 converting each retinoid and β -C into retinol equivalents, i.e. 1 mole of retinyl ester = 1 mole of
203 retinol and 1 mole of β -C = 2 moles of retinol.

204

205 *Calculation of bioconversion factors*

206 The bioconversion factors were based on the ratio of total quantity of β -C ingested on
207 total liver retinoid in treatment groups, i.e. SP and BSFL groups, corrected by the negative
208 control group, i.e. the C- group.

209

210 *Statistics*

211 Results in the text are expressed as means \pm SEM, but note that we have shown the
212 distribution of the data as box plots in the figures to provide additional informations about the
213 symmetry, variance, and potential outliers of the data. Differences between diets for each
214 retinoid or β -C were tested using either one-way ANOVA or Student's t-test. The homogeneity
215 of variances ($p > 0.05$) were checked by Levene's test. For ANOVA, in case of heterogeneity
216 of variances, data were log-transformed and for student's test, Welch's correction was applied.
217 Q-Q plots of standardized residuals were used to assess the normality of the data. For ANOVA,
218 when a significant effect was detected, post-hoc Tukey-Kramer tests were used to compare
219 means from the different groups. Values of $p < 0.05$ were considered significant. All statistical
220 analyses were performed using R version 4.1.1 for Windows (R Core Team, 2021).

221 **3) Results**

222 *Food consumption and growth performance.*

223 The daily consumption of gerbils varied from 5.09 ± 0.44 to 5.79 ± 0.59 g kibbles/gerbil
224 (**Table 1**). Only the gerbils belonging to the SP group had a significant lower consumption but
225 these animals also presented lower body weight and their low consumption were equally noted
226 in depletion phase where gerbils were fed with the same standard diet. The gerbils gained
227 weight during the entire study. The final body weights were homogenous and ranged from
228 79.86 ± 6.34 g to 84.66 ± 5.87 g. Liver weights were also non-significantly different and varied
229 from 1.9 ± 0.3 to 3.1 ± 0.2 g (data not shown). The experimental protocol did not induce severe
230 changes in food consumption and growth performance.

231

232 *Plasma retinol and β -C concentrations*

233 The concentration of retinol in the plasma of gerbils fed with the C- (0.77 ± 0.04
234 $\mu\text{mol/L}$), BSFL(0.89 ± 0.07 $\mu\text{mol/L}$), SP (0.83 ± 0.05 $\mu\text{mol/L}$), or C+ (0.85 ± 0.05 $\mu\text{mol/L}$) diet
235 did not differ significantly ($p = 0.59$) (**Figure 2A**). Conversely, the concentrations of β -C in the
236 plasma of gerbils fed with BSFL (0.002 ± 0.0005 $\mu\text{mol/L}$) and SP (0.02 ± 0.003 $\mu\text{mol/L}$) diet
237 differed significantly ($p < 0.01$) and there was no detectable β -C in the plasma of the C- and C+
238 groups (**Figure 2B**).

239

240 *Liver retinoids and β -C concentrations*

241 **Figure 3** shows the main chemical species of VA found in the gerbil liver, i.e. retinyl
242 esters, retinol and β -C. It also shows the total VA concentration, i.e. the sum of the VA species
243 expressed in equivalent moles of retinol. The concentration of retinyl esters in gerbil livers did
244 not differ ($p = 1.0$) between SP (0.57 ± 0.06 $\mu\text{mol/g}$) and C+ (0.55 ± 0.03 $\mu\text{mol/g}$) diets
245 (**Figure 3A**). However, the retinyl ester concentration in the livers of C- fed gerbils ($0.14 \pm$

246 0.01 $\mu\text{mol/g}$) differed significantly ($p < 0.001$) from that of the other diets, including BSFL-fed
247 gerbils ($0.34 \pm 0.03 \mu\text{mol/g}$). For retinol (**Figure 3B**), livers from gerbils fed BSFL ($0.02 \pm$
248 $0.001 \mu\text{mol/g}$), SP ($0.02 \pm 0.002 \mu\text{mol/g}$) and C+ ($0.02 \pm 0.001 \mu\text{mol/g}$) diets had
249 concentrations that did not differ ($p > 0.4$) but did differ ($p < 0.01$) from those of gerbils fed C-
250 diet ($0.009 \pm 0.001 \mu\text{mol/g}$). β -C was found in the livers of only gerbils consuming sweet
251 potatoes ($0.03 \pm 0.004 \mu\text{mol/g}$) and BSFL ($0.005 \pm 0.0006 \mu\text{mol/g}$) (**Figure 3C**), furthermore
252 their concentrations differed significantly ($p < 0.001$). Finally, the total liver retinoid
253 concentrations of gerbil fed with the SP ($0.66 \pm 0.07 \mu\text{mol/g}$) and C+ ($0.57 \pm 0.03 \mu\text{mol/g}$)
254 diets did not differ ($p = 0.87$) but differed from the BSFL ($0.37 \pm 0.03 \mu\text{mol/g}$) and C- ($0.15 \pm$
255 $0.01 \mu\text{mol/g}$) groups (**Figure 3D**).

256 **Figure 4** shows the concentrations of the main retinyl esters found in the livers. The
257 main retinyl ester was retinyl palmitate followed by retinyl oleate. The concentration of retinyl
258 palmitate in the livers of gerbils fed the BSFL ($0.25 \pm 0.02 \mu\text{mol/g}$), SP ($0.34 \pm 0.04 \mu\text{mol/g}$),
259 and C+ ($0.34 \pm 0.02 \mu\text{mol/g}$) diets did not differ significantly ($p > 0.1$) (**Figure 4A**).
260 Conversely it was significantly lower in gerbils fed the C- diet ($0.08 \pm 0.01 \mu\text{mol/g}$; $p < 0.001$).
261 Concerning retinyl oleate (**Figure 4B**), its liver concentration in gerbils fed the C- (0.03 ± 0.003
262 $\mu\text{mol/g}$) and BSFL ($0.04 \pm 0.004 \mu\text{mol/g}$) diets were significantly lower ($p < 0.001$) than those
263 of gerbils fed the SP ($0.12 \pm 0.01 \mu\text{mol/g}$) and C+ ($0.11 \pm 0.01 \mu\text{mol/g}$) diets, which were not
264 significantly different. The concentrations of retinyl stearate (**Figure 4C**) in the livers of gerbils
265 that had the BSFL ($0.04 \pm 0.003 \mu\text{mol/g}$), SP ($0.05 \pm 0.005 \mu\text{mol/g}$), and C+ diets (0.05 ± 0.004
266 $\mu\text{mol/g}$), were not significantly different ($p > 0.1$) but were significantly higher ($p < 0.001$) than
267 that of the C- diet ($0.01 \pm 0.001 \mu\text{mol/g}$). The liver concentrations of retinyl linoleate (**Figure**
268 **4D**) in the C+ ($0.04 \pm 0.003 \mu\text{mol/g}$) and SP ($0.04 \pm 0.005 \mu\text{mol/g}$) diets did not significantly
269 differ ($p = 1$) but were significantly ($p < 0.001$) higher than those of the two other groups.

270 Finally, the retinyl linoleate concentration in the BSFL group ($0.02 \pm 0.001 \mu\text{mol/g}$) was
271 significantly higher than that in the C- group ($0.01 \pm 0.001 \mu\text{mol/g}$).

272

273 *Comparison of β -C conversion rate when it was provided by SP or BSFL*

274 The conversion rates were $88.0 \pm 0.9 \%$ and $97.5 \pm 0.3 \%$ for SP and BSFL diets,
275 respectively (data not shown). These means were significantly different ($p < 0.001$).

276

277 *Bioconversion factors*

278 The bioconversion factor was $3.5 \mu\text{g } \beta\text{-C}$ equivalent to $1 \mu\text{g}$ of retinol for SP group and
279 was higher for the BSFL group with a value of 7.

280

281 **4) Discussion**

282

283 The nutritional intervention was designed to investigate the ability of proVA-enriched
284 BSFLs to restore VA status. We included two control groups: one with a standard VA diet
285 (C+), which provide the amount of VA needed for this animal species, and one with a VA
286 deficient diet (C-) to assess the ability of the two diets that provided VA only as β -C, i.e. the SP
287 and the BSFL diets, to restore the VA status. Note that we adjusted the quantities of sweet
288 potatoes and larvae that were incorporated into the kibbles in order to compare the
289 bioavailability of this proVA according to the matrix in which it was provided, i.e. sweet
290 potatoes or larvae, for the same quantity of β -C provided in the diet. Nevertheless, we found
291 that the SP gerbils consumed significantly less food, and therefore β -C, than the BSFL ones
292 (**Table 1**), which must be considered when interpreting the results.

293 The measurement of retinol, retinyl-ester and β -C concentrations in the blood and the
294 liver, which is by far the main storage organ for VA (Borel & Desmarchelier, 2017), gives both
295 an indication of the quantity of VA, or proVA, which was absorbed from the diets, but it also
296 gives a precise indication of the VA status of the animals, the concentration of VA in the liver
297 being considered as the best marker of the VA status (Borel & Desmarchelier, 2017). Finally, it
298 gives an idea of the conversion rate of β -C in VA by calculating the β -C/(retinol + retinyl-esters
299 + β -C) ratio when the only dietary source of VA is β -C (Borel, Troadec, et al., 2021). The
300 discussion will therefore consist in interpreting the data obtained in the plasma then in the liver
301 and then in making a synthesis of these observations.

302 The fact that plasma retinol concentrations were not significantly different between the
303 C+, SP and BSFL groups was not very surprising. Indeed, there was VA or proVA in the diet
304 of all these groups and it is well established that serum retinol is very well regulated and only
305 begins to decline when hepatic VA stores are nearly depleted (Borel, Troadec, et al., 2021).

306 Thus the fact that plasma retinol concentrations were not significantly lower in the C- group, as
307 compared to the other groups, simply shows that this group was not deficient enough to affect
308 hepatic retinol secretion and hence retinolemia (Borel et al., 2022; Borel, Troadec, et al., 2021).

309 With regard to plasma β -C, the first observation is that there was none detectable in the
310 plasma of the C+ and C- groups. This is exactly what was expected since these two diets did
311 not contain this provitamin which is not synthesized by mammals. Concerning the two other
312 groups, the fact that plasma β -C concentration was significantly lower in the BSFL group than
313 in the SP group can be explained by two mechanisms. The first that comes to mind is a lower
314 absorption efficiency of the β -C present in the BSFL matrix than in the sweet potato matrix.
315 Nevertheless, we have shown in a previous study that the bioaccessibility, i.e. the rate of
316 incorporation of β -C in the mixed micelles, was equivalent between these two matrices (Borel,
317 Hammaz, et al., 2021). If there is a difference in absorption efficiency then it may be due to a
318 difference in uptake efficiency by the intestinal cells, but we have no hypothesis that could
319 support this mechanism. The second mechanism that could explain the lower plasma β -C
320 concentration in the BSFL group, as compared to the SP one, may be a difference in the
321 efficiency of intestinal conversion of β -C to VA, with gerbils fed the BSFL diet having a higher
322 β -C conversion efficiency than those fed the SP diet. This hypothesis is supported by the result
323 of the calculation of the β -C conversion efficiency which showed that gerbils fed the BSFL diet
324 had a conversion factor twice as high as those fed the SP diet. We suggest that this was because
325 the amount of β -C absorbed was markedly lower in the gerbils of the BSFL group than in those
326 of the SP group. Indeed, a lower β -C absorption efficiency will lead to a lower VA status in the
327 long term and it has been established that the decrease in VA status increases in the expression
328 of *BCO1* via ISX (Lobo et al., 2013). The increase in the activity of BCO1 at the intestinal
329 level will result in a lower secretion of β -C in the blood and therefore reduce its plasma
330 concentration.

331 Concerning liver VA, the first key observation is that the C- gerbils had significantly
332 lower concentrations of retinyl esters and retinol than the other groups, and they had no β -C in
333 the liver. This is very reassuring because it shows that the experiment worked very well
334 because we expected to have a lower VA status in this group and not to detect β -C. Concerning
335 the SP gerbils, their liver retinyl esters and retinol concentrations were not significantly
336 different from those of the C+ gerbils, which means that the amount of β -C present in the SP,
337 and which was the only source of VA in this diet, was perfectly able to restore the VA status in
338 this group of gerbils who had previously been VA deficient. The results of the BSFL group are
339 more complex to interpret. They first show that the concentrations of retinol and of the various
340 retinyl esters in the liver of these gerbils were always lower than those observed in the SP and
341 C+ groups, sometimes this was significant, sometimes not. These results also show that the
342 concentration of β -C in the liver was significantly lower in the BSFL group than in the SP
343 group. It can therefore be concluded that the VA status was lower in the BSFL group than in
344 the SP group. More precisely, the total amount of VA present in the liver, which was estimated
345 in retinol equivalent concentration, was significantly lower, by a factor of approximately 2, in
346 the liver of the BSFL group as compared to the SP group. We thus concluded that the capacity
347 of the BSFL matrix enriched in β -C to improve the VA status was half that of the sweet potato
348 matrix. The most likely hypothesis to explain this difference, while the gerbils of the BSFL
349 group have consumed more β -C than the gerbils of the SP group (**Table 1**), is that β -C present
350 in the insect matrix was less bioavailable than that of the SP matrix. This seems very surprising
351 at first since the insect matrix provided more lipids than the sweet potato matrix and it has been
352 shown that lipids improve the bioavailability of β -C (Jayarajan, Reddy, & Mohanram, 1980;
353 Mokady & Benamotz, 1991). Nevertheless, it seems that a minimal quantity of lipids is
354 necessary to have a good bioavailability of β -C but that higher quantities of lipids do not
355 improve the bioavailability any more (Ribaya-Mercado et al., 2007; Roodenburg, Leenen, Hof,

356 Weststrate, & Tijburg, 2000). Thus, knowing that the basal diet of gerbils already contained
357 lipids, the additional lipids provided by the insect matrix therefore probably did not have a
358 positive effect on the bioavailability of β -C. On the other hand, it is clear that a component of
359 the insect matrix partially impaired the bioavailability of β -C. The first component that comes
360 to mind is chitin since this polysaccharide, which is part of the composition of the exoskeleton
361 of insects, behaves like vegetable dietary fiber in the digestive tract, and it has been observed
362 that dietary fiber inhibits the bioavailability of β -C (Erdman & Fahey, 1986; Riedl, Linseisen,
363 Hoffmann, & Wolfram, 1999; Zanutto, Jordao Junior, Meirelles, Favaro, & Vannucchi, 2002).
364 Nevertheless, no study is available on the effect of chitin on β -C bioavailability. Thus, further
365 experiments must be performed to verify this hypothesis.

366 In conclusion, this study shows for the first time that the β -C which is present in BSFL
367 is bioavailable and readily converted into VA in the organism. It can therefore be concluded
368 that BSFL enriched in proVA could be a significant source of VA for farm animals and
369 indirectly for humans. Nevertheless, the fact that the bioavailability of β -C from BSFL was at
370 least twice lower than that of β -C present in orange sweet potatoes, if it were to be confirmed in
371 other studies carried out on other animal models and possibly on humans, should be considered
372 to calculate the quantities of VA provided by this new sustainable source of proVA.

373 **Acknowledgments:**

374 The authors thank Adrien Aubert (Technical support - Laboratoire de Pharmacie Galénique et
375 Génie Pharmaceutique-ICGM UMR5253 - D1 Chimie et Matériaux Moléculaires) for
376 incorporation of insect larvae in kibbles.

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379 document and will be applied to all subsequent versions up to the Author Accepted Manuscript
380 arising from this submission.



381

382

383 **Abbreviations:**

384 BSFL (black soldier fly larvae), VA (vitamin A), β -C (β -carotene).

385

386 **Supporting information:**

387 The costs of this project were covered equally by the own budget of P. Borel's research team,
388 which came mainly from INRAE endowments, by Cirad C. Dhuique-Mayer's research and by
389 the BioMiMetiC company.

390

391 **Credit author statement:**

392 **Patrick Borel:** had the idea of the research and has primary responsibility for final manuscript
393 content; acquisition of funding, codesigned the protocol, project coordination, analyzed and
394 interpreted data, drafting of the manuscript. **Lisa Morand-Laffargue:** codesigned the protocol,
395 carotenoid analysis, analyzed and interpreted data, drew the figures, review the manuscript.

396 **Benjamin Creton:** larvae rearing and larvae collection. **Damien Sabatier:** review & editing of

397 the manuscript. **Marie Papin:** review & editing of the manuscript. **Claudie Dhuique-Mayer:**
398 Management and coordination responsibility for the animal experimental protocol (planning
399 and execution), performed the nutritional study, participation to euthanasia and collected the
400 blood and liver, interpretation of the data, review the manuscript. **Stephane Delbecq:** Follow-
401 up of animal experimentation, euthanasia of the gerbils according actual ethic rules and
402 harvested the blood and liver.

403

404 **Conflicts of interest:**

405 BC and DS work in the BioMiMetiC company. This company conducts research and
406 development activities aimed at enhancing the value of insect-based bioconversion of a wide
407 variety of organic materials generated in the area at all levels of the food value chain.

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506

507 **Figure legends**

508

509 **Figure 1. Diagram of the nutritional intervention on gerbils.** C- : VA deficient diet, SP:
510 sweet potato diet providing VA as β -C, BSFL: diet containing BSFL that were fed with sweet
511 potatoes and that had bioaccumulated β -C, C+ : standard VA diet.

512

513 **Figure 2. Plasma retinol (A) and plasma β -C (B) concentrations ($\mu\text{mol/L}$) of gerbils fed**
514 **diets containing different sources and concentrations of VA.** C-: VA deficient diet, SP:
515 sweet potato diet providing VA as β -C, BSFL: diet containing BSFL that were fed with sweet
516 potatoes and that had bioaccumulated β -C, C+: standard VA diet. For each compound, bars
517 with different letters are significantly different ($p < 0.05$; Student t-test or ANOVA followed by
518 Tukey's HSD test). N=7 (C- group), n=8 (BSFL and SP group) and n=6 (C+ group).

519

520 **Figure 3. Liver VA concentrations ($\mu\text{mol/g liver}$) of gerbils fed diets containing different**
521 **sources and concentrations of VA.** **A:** sum of retinyl esters, **B:** retinol, **C:** β -C, **D:** total
522 retinoids, i.e. sum of retinol equivalents. C-: VA deficient diet, SP: sweet potato diet providing
523 VA as β -C, BSFL: diet containing BSFL that were fed with sweet potatoes and that had
524 bioaccumulated β -C, C+: standard VA diet. For each compound, bars with different letters are
525 significantly different ($p < 0.05$; Student t-test or ANOVA followed by Tukey's HSD test).
526 N=7 (C- group), n=8 (BSFL and SP group) and n=6 (C+ group).

527

528 **Figure 4. Concentrations ($\mu\text{mol/g liver}$) of the main retinyl esters in livers of gerbils fed**
529 **diets containing different sources and concentrations of VA.** **A:** retinyl palmitate, **B:** retinyl
530 oleate, **C:** retinyl stearate, **D:** retinyl linoleate. C-: VA deficient diet, SP: sweet potato diet
531 providing VA as β -C, BSFL: diet containing BSFL that were fed with sweet potatoes and that

532 had bioaccumulated β -C, C+: standard VA diet. For each compound, bars with different letters
533 are significantly different ($p < 0.05$; Student t-test or ANOVA followed by Tukey's HSD test).
534 N=7 (C group), n=8 (BSFL and SP group) and n=6 (C+ group).

535

536 **Table 1:** Growth performance and food consumption of the gerbils.

537

	Experimental diets			
	C-	C+	SP	BSFL
<i>Body weight (g):</i>				
Initial	65.7 ± 6.2	69.3 ± 3.4	67.3 ± 4.3	67.0 ± 1.4
After 60 days	80.0 ± 6.0	83.5 ± 5.4	79.9 ± 6.3	85.0 ± 5.9
<i>*Weight gain (g):</i>				
After 60 days	14.3 ± 3.9	14.3 ± 5.8	12.5 ± 4.3	17.7 ± 6.0
<i>Food consumption</i>				
(g/day/gerbil):	5.5 ± 0.5 ^a	5.7 ± 0.4 ^a	5.1 ± 0.4 ^b	5.8 ± 0.6 ^a

538 C-: VA deficient diet, C+: standard VA diet, SP: sweet potato diet providing VA as β -C, BSFL: diet containing BSFL that
539 were fed with sweet potatoes and that had bioaccumulated β -C. Values are means \pm SEM. N=7 for the C- group, n=8 for
540 the BSFL and SP groups, and n=6 for the C+ group. The different groups were compared with each other for each
541 variable with an ANOVA followed by Tukey's HSD test. Means that have different superscript letters are significantly
542 different ($p < 0.05$).