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

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Abstract

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

1) Introduction

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

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

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

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

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

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

1) Material and Methods

Chemicals

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

BSFL Farming

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

Animals, diets and study design

Animals

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

Diets

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

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

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

*Study design (**Figure 1**)*

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

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

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

Kibbles containing insects rich in β-C or sweet potatoes

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

Liver and plasma β-C and retinoids

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

Quantification of β-C and retinoids by HPLC

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

Calculation of the conversion rate of β-C into VA

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

Calculation of bioconversion factors

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

Statistics

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

3) Results

Food consumption and growth performance.

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

Plasma retinol and β-C concentrations

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

Liver retinoids and β-C concentrations

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

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

Figure 4 shows the concentrations of the main retinyl esters found in the livers. The main retinyl ester was retinyl palmitate followed by retinyl oleate. The concentration of retinyl palmitate in the livers of gerbils fed the BSFL (0.25 ± 0.02 µmol/g), SP (0.34 ± 0.04 µmol/g), and C+ (0.34 ± 0.02 µmol/g) diets did not differ significantly ($p > 0.1$) (**Figure 4A**). Conversely it was significantly lower in gerbils fed the C- diet (0.08 ± 0.01 µmol/g; $p < 0.001$). Concerning retinyl oleate (**Figure 4B**), its liver concentration in gerbils fed the C-(0.03 ± 0.003 µmol/g) and BSFL (0.04 ± 0.004 µmol/g) diets were significantly lower ($p < 0.001$) than those of gerbils fed the SP (0.12 ± 0.01 µmol/g) and C+ (0.11 ± 0.01 µmol/g) diets, which were not significantly different. The concentrations of retinyl stearate (**Figure 4C**) in the livers of gerbils that had the BSFL (0.04 ± 0.003 µmol/g), SP (0.05 ± 0.005 µmol/g), and C+ diets (0.05 ± 0.004 µmol/g), were not significantly different ($p > 0.1$) but were significantly higher ($p < 0.001$) than that of the C- diet (0.01 ± 0.001 µmol/g). The liver concentrations of retinyl linoleate (**Figure 4D**) in the C+ (0.04 ± 0.003 µmol/g) and SP (0.04 ± 0.005 µmol/g) diets did not significantly differ ($p = 1$) but were significantly ($p < 0.001$) higher than those of the two other groups.

Finally, the retinyl linoleate concentration in the BSFL group (0.02 ± 0.001 µmol/g) was significantly higher than that in the C- group (0.01 ± 0.001 µmol/g).

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

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

Bioconversion factors

The bioconversion factor was 3.5 µg β-C equivalent to 1 µg of retinol for SP group and was higher for the BSFL group with a value of 7.

4) Discussion

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

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

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

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

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

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

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

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

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Abbreviations:

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

Supporting information:

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

Credit author statement:

Patrick Borel: had the idea of the research and has primary responsibility for final manuscript content; acquisition of funding, codesigned the protocol, project coordination, analyzed and interpreted data, drafting of the manuscript. **Lisa Morand-Laffargue:** codesigned the protocol, carotenoid analysis, analyzed and interpreted data, drew the figures, review the manuscript. **Benjamin Creton:** larvae rearing and larvae collection. **Damien Sabatier:** review & editing of

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

Conflicts of interest:

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

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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 (µmol/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 (µmol/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 (µmol/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

had bioaccumulated β-C, C+: standard VA diet. For each compound**,** bars with different letters are significantly different ($p < 0.05$; Student t-test or ANOVA followed by Tukey's HSD test). 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**
Body weight (g):				
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
**Weight gain (g):*				
After 60 days	14.3 ± 3.9	14.3 ± 5.8	12.5 ± 4.3	17.7 ± 6.0
Food consumption (g/day/gerbil):	5.5 ± 0.5[a]	5.7 ± 0.4[a]	5.1 ± 0.4[b]	5.8 ± 0.6[a]

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