

1 **Productive performance of *Tenebrio molitor* larvae in response to the protein level in the**
2 **substrate**

3
4 Running title: Protein requirements for the growth of *Tenebrio molitor* larvae

5
6 G. Fondevila and M. Fondevila*

7
8 Departamento. Producción Animal y Ciencia de los Alimentos, Instituto Agroalimentario de
9 Aragón (IA2), Universidad de Zaragoza-CITA, M Servet 177, 50013 Zaragoza, Spain

10 * mfonde@unizar.es
11
12

13 **Abstract**

14 Insect production for animal feeding is receiving increasing interest worldwide. The dietary
15 protein level is a key factor influencing overall production and feeding costs. Thus, a growth
16 performance trial was conducted with *Tenebrio molitor* larvae for 28 days using substrate feeds
17 based on wheat grain and barley straw with increasing levels of soybean meal to reach 100
18 (10P), 120 (12P), 140 (14P) and 160 (16P) g crude protein (CP) per kg dry matter (DM). Four
19 trays per treatment, with 40 larvae and 24 g substrate, were set up. Average mortality was 0.142
20 and did not differ among treatments. Feed intake decreased linearly (L, $P < 0.001$) and
21 quadratically (Q, $P < 0.01$) and larval growth (mg/d per larvae) tended to decrease (Q, $P =$
22 0.065) as the dietary CP increased from 100 to 160 g/kg DM. Protein intake, however, increased
23 (L, $P < 0.001$) with the CP content of the diet. Feed to gain ratio decreased (L, $P < 0.01$) as the
24 dietary CP level increased but total production per tray of DM, CP or ether extract, was not
25 affected. In conclusion, an increase in the dietary CP content over 100 g/kg DM by including
26 soybean meal in the substrate might not be needed to optimize larval growth. A CP content in
27 the diet of 100 g/kg might improve the economic results because of lower feeding costs.

28 **Keywords:** Insect feeding, protein level, larval growth, larval composition.
29

30 There are no conflicts of interests.
31

32 **Introduction**

33 The crude protein (CP) content is often the most relevant nutrient of the feed affecting
34 productive performance with major implications in feeding costs and production viability.
35 Consequently, if insect production is aimed to be established as a nutrient source for domestic
36 animals, the CP requirements of larvae should be considered to assess the economic production
37 profitability. Nevertheless, the optimum dietary CP level for larval growth of *Tenebrio molitor*
38 is controversial. Mancini *et al.* (2019) evaluated different substrates with CP levels of 66, 112
39 and 180 g/kg. In this approach, the lower protein level tended to be limiting for optimal larval
40 growth, whereas the higher level was probably over the insect requirements and could make
41 diet cost inviable, as CP is often the most expensive component of diet.

42 In many cases, the optimum CP level is established from a wide range and with diets
43 varying in ingredient or chemical composition (Broekhoven *et al.*, 2015; Ooninx *et al.* (2015)
44 Alves *et al.*, 2016; Rumbos *et al.*, 2020). However, these experimental conditions do not allow
45 to make accurate recommendations in most cases. Larvae show a selective feeding behaviour
46 by giving preference to certain ingredients to maintain a constant CP intake (Morales-Ramos *et*
47 *al.*, 2011) but the potential selection for CP intake should be distinguished from a preference
48 for an ingredient itself. As a result of the huge differences among dietary ingredients, some
49 authors (Ooninx *et al.*, 2015; Mancini *et al.*, 2019) suggested that larvae do not respond to the
50 dietary CP content. In contrast, Davis and Sosulski (1973) and Ramos-Elorduy *et al.* (2002)
51 recommended CP levels of 100 and 200 g/kg, respectively, for optimum larval growth. In any
52 case, a comparison among different protein levels in diets with a homogeneous ingredient and
53 nutrient composition is required to establish the optimal CP content. In this work, CP levels
54 ranging from 100 to 160 g/kg DM, chosen as an acceptable range considering both productive
55 performance and dietary costs, were tested for production of *T. molitor* larvae. Growth
56 performance and chemical composition of the larvae were evaluated. Soybean meal was chosen
57 as a common, high quality protein source for avoiding confusion between ingredients and
58 protein levels.

59

60 **Materials and methods**

61 *Experimental diets*

62 Combinations of wheat grain, barley straw and soybean meal were formulated in four
63 diets with the same level of starch but with equally spaced CP levels: 100 (10P), 120 (12P), 140
64 (14P) and 160 (16P) g CP/kg DM (Table 1). Nutrient composition was estimated from the
65 chemical analysis of the ingredients, that were ground to 2 mm particle size before being mixed
66 and used as insect substrate. In addition, 1.8 to 2.2 g of fresh carrot (909 g moisture/kg) was
67 weekly added to each tray as a source of water for larvae.

68

69 *Experimental procedures*

70 The trial was carried out in a room with average minimum and maximum daily
71 temperatures between 25 and 28 °C and relative humidity values between 37 and 58% and kept
72 in darkness except for sampling procedures. The duration of the experiment was limited to 28
73 days to minimise the possible effect of insect metamorphosis that could bias treatment
74 comparison (Ghali and Alkoaik, 2009). Four open plastic trays (15 × 9 × 6 cm) supplemented
75 with 24 g of substrate were prepared for each experimental treatment. Forty larvae (approximate
76 initial size 3-5 mm), selected by sieving the larvae colony through a 1 mm screen, were included
77 on each tray to reach a density of 30 larvae per dm². Initial larval weight (n = 40) was 2.80 ±
78 0.102 g per tray (average initial weight of 70.0 mg per larvae) and no differences among
79 treatments were detected. Weight of larvae and the remaining substrate, as well as larval
80 mortality, were weekly monitored to estimate mass gain (total increase in larval weight) and
81 substrate intake per tray, as well as the daily weight growth per larvae. Dry carrot remaining

82 from the previous offer was weekly removed. Feed to gain ratio (F:G) was calculated on fresh
83 and DM basis, without considering the weight of carrot provided. In cases when metamorphosis
84 occurred on a specific tray, the number and weight of pupae appearing (the average proportion
85 of pupae in total insects was 0.008 and 0.063 on days 21 and 28, respectively) were recorded
86 and removed from the tray. Pupae were not considered in the counts, as *T. molitor* does not
87 consume feed during this physiological stage, but pupal weight was also considered in the
88 calculation of F:G ratio. At the end of the experiment, larvae were collected from the trays,
89 weighed, frozen at -80°C, lyophilised and stored until chemical analysis. Total DM, CP and
90 ether extract (EE) production per tray was calculated, and protein efficiency was estimated as
91 the amount of CP retained per unit of ingested CP. Feed residue was also sampled for
92 determination of its DM, CP, EE and neutral detergent fibre (aNDF) content.

93 *Chemical and statistical analyses*

94 Substrate feeds were ground to 1 mm size and analysed for DM, organic matter (OM),
95 CP and EE following the AOAC procedures ref. 934.01, 942.05, ref. 976.05 and 2003.05
96 (AOAC, 2005), respectively. Besides, the concentration of aNDF was analysed as described by
97 Mertens (2002) with an Ankom 200 Fibre Analyser (Ankom Technology, New York), using α -
98 amylase and sodium sulphite, and the results were expressed exclusive of residual ashes. Total
99 starch content was determined enzymatically from samples ground to 0.5 mm using a
100 commercial kit (Total Starch Assay Kit K-TSTA 07/11; Megazyme, Bray, Ireland). Larvae
101 were also analysed for DM, OM, CP and EE, as reported for substrate feeds. Substrate and
102 larval CP content was expressed as total N x 6.25.

103 Results were analysed as a completely randomised design with 4 treatments (10P, 12P,
104 14P and 16P) by ANOVA with the Statistix 10 package (Analytical Software, 2010). Each
105 treatment was replicated 4 times considering the tray as the experimental unit for all
106 measurements. Initial larval weight was considered as a covariable for correcting its effect on
107 experimental results. When significant differences were detected, average means were
108 separated using the Tukey test. Mean differences at $P < 0.05$ were considered significant and
109 $0.10 \geq P \geq 0.05$ were considered as a trend for significance. Polynomial (lineal, L and quadratic,
110 Q) contrasts were also planned to estimate the trend in the response to the dose of dietary
111 protein.

112 **Results**

113 Productive performance along the 28-d experimental period is shown in Table 2.
114 Accumulated mortality averaged 0.142 and did not differ among dietary CP levels, in part
115 because of the variability observed in this parameter (coefficient of variation of 0.25). Feed
116 intake both in fresh and DM basis decreased both linearly ($P < 0.001$) and quadratically ($P < 0.01$)
117 as the CP level of the substrate increased from 100 to 160 g/kg. In contrast, protein intake
118 increased (L, $P < 0.001$) with the dietary protein level. Final larval weight tended to decrease (Q,
119 $P = 0.066$) as the dietary CP level increased, with the highest and lowest values observed with
120 10P and 12P, respectively ($P < 0.05$). No differences among treatments were observed on larval
121 mass gain per tray ($P > 0.10$). When average individual growth was estimated, however, larval
122 growth tended to decrease (Q, $P = 0.065$) with the dietary CP level, with the highest and lowest
123 values observed with 10P and 12P, respectively ($P < 0.05$). Feed to gain decreased (L, $P < 0.01$)
124 with the level of dietary protein, either when expressed in fresh or DM basis.

125 The total production of DM, CP and EE and protein efficiency of *T. molitor* larvae is
126 shown in Table 2. No differences were detected for DM, CP or EE production in response to
127 the level of dietary CP. Protein efficiency (amount of larval CP produced per unit of dietary CP
128 used) decreased (L, $P = 0.016$; L, $P = 0.034$) as the dietary CP content increased from 100 to 160
129 g/kg DM.

132 Data on the chemical composition of the residual substrate and the larvae after 28 d of
133 experiment are presented in Table 3. These data were not analysed statistically but numerically,
134 the contents in the residual substrate increased in CP and decreased in NDF as the dietary CP
135 increased, following a similar trend compared to the original feeds. However, the EE content
136 of the residual substrate increased numerically with the dietary CP content, contrasting with the
137 similar EE content of the original feeds. In general, the increase in the CP content of the diet
138 did not result in apparent differences in larval composition in terms of DM and CP but a
139 decrease in EE was numerically observed.

141 Discussion

142 In order to avoid any bias caused by qualitative differences among ingredients on the
143 comparison of dietary protein levels, the experimental substrates were designed to include
144 similar contents of ground wheat grain but variable proportions of soybean meal and barley
145 straw. In this respect, soybean meal was selected as the reference protein source because of the
146 similar amino acid composition compared to *T. molitor* larvae (Bovera *et al.*, 2015). Wheat bran
147 is commonly used as basal ingredient for production of *T. molitor* larvae but its CP content
148 (around 150 g/kg) makes difficult to formulate diets with protein levels at the lower range
149 studied in the current research. Consequently, barley straw was included as a source of fibre
150 that should not affect the balance of other nutrients such as CP, starch or fat.

151 Mortality was lower than results from Morales-Ramos *et al.* (2011), who observed
152 mortality rates from 0.44 to 0.56, but remained at a similar level to that reported by van
153 Broekhoven *et al.* (2015), who agreed in the lack of differences caused by dietary CP level.
154 Also, Ooninx *et al.* (2015) evaluated the effects of 6 different diets varying in protein and fat
155 contents and reported mortality rates ranging from 0.07 to 0.20 in most cases but raising to 0.85
156 for one of the treatments. Probably, the wide variation observed in this parameter within and
157 among research might explain the absence of significant differences among dietary CP levels.

158 Average final larval weight was 147 mg, which is in the upper range reviewed by
159 Ribeiro *et al.* (2018). No apparent response to dietary CP levels ranging from 100 and 160 g/kg
160 DM was detected in larval growth when considered per tray (larval mass). In contrast, when
161 corrected per living larvae, larval growth dropped quadratically as the dietary CP content
162 increased. In fact, 10P resulted in the highest larval growth as a consequence of a higher feed
163 intake both in fresh and DM basis. However, this response is opposite to that previously
164 observed when the CP levels tested ranged from 120 to 290 g/kg (Ooninx *et al.*, 2015; van
165 Broekhoven *et al.*, 2015; Alves *et al.*, 2016). Also, Paulicks *et al.* (2021) evaluated the effect
166 of dietary CP contents from 90 to 280 g/kg DM in form of wheat gluten and reported maximum
167 growth rates at a CP level of 120 g/kg DM. The results reported herein suggest that protein
168 requirements of mealworms are probably below the currently assumed levels. In any case, total
169 DM or CP production per tray did not differ among treatments, suggesting that larvae are able
170 to compensate a low protein level in diet with a higher substrate intake. Similarly, Barragan-
171 Fonseca *et al.* (2021) observed that levels as low as 8-10% CP should be enough for maximising
172 larval survival rates and larval yield in Black soldier fly.

173 *Tenebrio molitor*, commonly known as mealworm, naturally grows on feed substrate
174 based on cereal grains, ingredients that are low in protein and amino acids. Probably, a possible
175 explanation to the lack of response in larval growth to an increase in dietary CP could be that
176 10P, which included wheat grain as the only source of amino acids, already satisfied the protein
177 requirements of *T. molitor* larvae. These results are in agreement with those reported by
178 Paulicks *et al.* (2021) using wheat gluten as protein source. This information suggest that any
179 amino acid deficiency of wheat protein could be discarded and consequently, supplementation
180 with soybean meal as a source of amino acids should not be quantitatively necessary. In any

181 case, further studies should be conducted to determine amino acid requirements of different
182 larval stages of insects for optimising their production.

183 In insect production, F:G ratio is considerably lower than that observed in conventional
184 animal production systems such as cattle, pigs or even poultry, as it has been compiled in several
185 reviews (Halloran *et al.*, 2016; de Souza-Vilella *et al.*, 2019). However, the reported results are
186 often variable, in part because of differences among experiments in feeding and environmental
187 conditions, population density and observed mortality rates (Halloran *et al.*, 2016). In the
188 current research, average F:G ratio was 2.038 and decreased linearly as the dietary CP
189 increased. The linear impairment in F:G ratio observed with the lower dietary CP contents
190 might be related to the increase in DM intake to compensate the reduction in CP. In this respect,
191 the estimation of F:G ratios from the results reported by Morales-Ramos *et al.* (2011) did not
192 show differences among CP levels ranging from 126 to 148 g/kg. In contrast, Oonincx *et al.*
193 (2015) reported average F:G values of 5.3-6.1 for diets with 130 to 140 g CP/kg that decreased
194 to 3.8-4.8 with 170 to 220 g CP/kg, in agreement with the current results. However, the
195 variability in ingredient composition among the diets used by Oonincx *et al.* (2015) precludes
196 the formulation of any hypothesis regarding the effect of the protein content. In any case, it has
197 to be considered that larvae are expected to be commercialised as dry, and larval DM content
198 usually ranges between 380 to 480 g/kg (Ghali and Alkaik, 2009; Alves *et al.*, 2016; Rumbos
199 *et al.*, 2020). In our experiment, the larval DM content was, on average, 443 g/kg which falls
200 within the range reported. Therefore, expression of F:G ratio on a DM basis should give a
201 clearer idea of the economic impact of this production (Oonincx and de Boer, 2012; Oonincx
202 *et al.*, 2015; Halloran *et al.*, 2016). In this sense, our results indicate that a range of 3.8 to 4.6
203 kg dry feed should be used per kg DM of produced *T. molitor* larvae according to the considered
204 dietary CP content.

205 Protein retention efficiency in *T. molitor* larvae decreased as dietary CP increased from
206 100 to 160 g/kg. This information suggests that insects can compensate a low dietary protein
207 content with a more efficient use of it. In this respect, a higher N excretion as uric acid may be
208 expected with high levels of dietary CP (Oonincx *et al.*, 2015), provided that protein intake
209 might be adequate to satisfy larval requirements with the lowest level assumed here. Similarly,
210 Mancini *et al.* (2019) reported a lower protein efficiency when dietary protein level was
211 intermediate, although in their case the variability in ingredient dietary composition made
212 difficult to draw a firm conclusion in this sense.

213 Larval CP content was estimated as $N \times 6.25$ (Jones *et al.*, 1941), despite it has been
214 reported that the N proportion in insect protein (considering only aminoacidic N) is
215 considerably lower. In fact, the proportion of non-protein N in insect protein is 12 to 23%. As
216 a result, the coefficient for the estimation of the CP content should be close to 4.76 for whole
217 larvae and 5.60 for extracted protein (Janssen *et al.*, 2017). This consideration does not affect
218 the comparison of results from protein production from *T. molitor* among experimental
219 treatments but a potential alteration of the estimation of protein efficiency cannot be
220 overlooked. Also, true protein coefficients for vegetal sources (including soybean meal) should
221 be around 5.4-5.5 (Mariotti *et al.*, 2008) and consequently, differences among coefficients
222 should be also considered for a sound comparison. In any case, for an easier comparison with
223 results from other references, the use of the conventional 6.25 coefficient has been maintained
224 here to estimate for insect CP content (Finke, 2007; Oonincx and Finke, 2021).

225 The observed chemical composition of *T. molitor* larvae (on average, 424 g CP and 333
226 g EE per kg DM) was within the range of previous references (Makkar *et al.*, 2014; Sanchez-
227 Muros *et al.*, 2016; Ribeiro *et al.*, 2018), although the protein content was at the lower edge of
228 those reviews. Other published works (Ramos-Elorduy *et al.*, 2002; Ravzanaadii *et al.*, 2012;
229 van Broekhoven *et al.*, 2015) reported CP values between 464 and 477 g/kg DM and Mancini
230 *et al.* (2019) cited a range of CP content of 373 to 513 g/kg, with extreme values corresponding

231 to maximal and minimal dietary protein values (70 and 190 g CP/kg). However, a potential
232 effect of dietary protein content on the chemical composition of *T. molitor* larvae is unclear.
233 According to van Broekhoven *et al.* (2015), no effects in larval protein content are manifested
234 even when fed diets differing 2-3-fold in dietary protein proportions. Similarly, although the
235 current composition results were not compared statistically, the numerical differences in protein
236 content of larvae were minimal despite the dietary protein level (Table 3). This homogeneity in
237 the CP content of larvae among diets was not reflected in that of EE, which was higher with
238 10P and 12P than with 14P and 16P. This variation might be related with differences in the
239 dietary energy to protein ratio. However, this treatment effect was not manifested on the total
240 EE produced per tray although it seems that larvae tend to retain more fat when the level of
241 protein is low. In any case, the plasticity of body composition of larvae respect to the dietary
242 CP level of protein by increasing protein retention at the expense of fat as suggested by Mancini
243 *et al.* (2019) was not so apparent here. In this respect, Oonincx and Finke (2021) reported that
244 holometabolous and hemimetabolous species showed a constant amino acid pattern unaffected
245 by diet composition. This information suggests that, despite larval EE content might depend on
246 diet characteristics, their CP proportion might be relatively constant and difficult to modify by
247 the CP content of the diet.

248 In the current research, the diet with 100 g CP/kg resulted in the lowest larval protein
249 intake, which increased linearly as the dietary CP content increased to 160 g CP/kg. Probably,
250 the higher DM intake observed with the lowest CP content in the diet was not enough to
251 compensate the increase in the protein level of the other treatments in terms of protein
252 consumption. In any case, a low dietary inclusion of soybean meal should result in a reduction
253 in feeding costs. In this respect, considering commercial prices (average price for 2021 in
254 market of Barcelona, Spain) and assuming ingredient costs respect to 47% CP soybean meal of
255 61% for wheat grain and 15% for barley straw, the price of the dietary mixtures 12P, 14P and
256 16P would result in 110, 118 and 127% that of 10P, respectively. According to F:G results, this
257 would be reflected in feeding costs of 1.076, 1.145 and 1.214 per kg of *T. molitor* larvae
258 produced with successive increases in two percentual units of CP respect to a diet without
259 soybean meal as protein source (100 g CP/kg). Consequently, the choice of the dietary protein
260 level would depend on the current prices of raw materials, without a major concern on larval
261 production.

262

263 **Conclusions**

264 Growth performance of *T. molitor* larvae tended to decrease as dietary CP increased
265 from 100 to 160 g/kg. Larvae compensated a low CP concentration by increasing total substrate
266 intake. Feed to gain ratio, however, decreased with the dietary CP level. Apparently, larvae
267 composition is barely affected by the CP content of the diet. The economic implications of
268 increasing the CP content of the diet suggest that the inclusion of soybean meal in the diet might
269 not be necessary to optimize larval productivity and that 100 g CP/kg DM should result in lower
270 feeding costs per unit of larval weight produced, according to the actual estimation.

271

272 **Acknowledgements**

273 Work supported by the company INSECTOPIA 2050, with the Department of Industry
274 and Innovation of the Government of Aragón and the European Social Fund.

275

276 **References**

277 Alves, A.V., Sanjinez-Argandoña, E.J., Linzmeier, A.M., Lima Cardoso, C.A. and Rodrigues
278 Macedo, M.L., 2016. Food value of mealworm grown on *Acrocomia aculeata* pulp flour.
279 PLoS ONE 11, e0151275.
280 Analytical Software, 2010. Statistix 10 for Windows. Analytical Software, Tallahassee, FL.

281 AOAC, 2005. Official Methods of Analysis, 18th ed. Horwitz, W. and Latimer, G.W. (eds.),
282 Association of Official Analytical Chemists, Gaithersburg, MD, USA.

283 Barragan-Fonseca, K.B., Gort, G., Dicke, M. and van Loon, J.J.A., 2021. Nutritional plasticity
284 of the black soldier fly (*Hermetia illucens*) in response to artificial diets varying in protein
285 and carbohydrate concentrations. *Journal of Insects as Food and Feed* 7: 51-61.

286 Bovera, F., Piccolo, G., Gasco, L., Marono, S., Loponte, R., Vassalotti, G., Mastellone, V.,
287 Lombardi, P., Attia, Y.A. and Nizza, A., 2015. Yellow mealworms larvae (*Tenebrio molitor*
288 L.) as protein source for broilers: Effects on growth performance and blood profiles. *British*
289 *Poultry Science* 56: 569–575.

290 Davis, G.R.F. and Sosulski, W., 1973. Protein nutrition of *T. molitor* L. XVI. Effects of dietary
291 protein concentration on larvae of race F. *Archives Internationales de Physiologie et de*
292 *Biochimie* 81: 661-665.

293 de Souza-Vilella, J., Andrew, N.R. and Ruhnke, I., 2019. Insect protein in animal nutrition.
294 *Animal Production Science*. 59: 2029-2036.

295 Finke, M.D., 2007. Estimate of chitin in raw whole insects. *Zoo Biology* 26: 105-115.

296 Ghali, A.E. and Alkokaik, F.N., 2009. The yellow mealworm as a novel source of protein.
297 *American Journal of Agricultural and Biological Sciences* 4: 319-331.

298 Halloran, A., Roos, N., Eilenberg, J., Cerutti, A. and Bruun, S., 2016. Life cycle assessment of
299 edible insects for food protein: a review. *Agronomy for Sustainable Development* 36, 57.

300 Janssen, R.H., Vincken, J.P., van den Broeck, L.A.M., Fogliano, V. and Lakemond, C.M.M.,
301 2017. Nitrogen-to-protein conversion factors for three edible insects: *Tenebrio molitor*,
302 *Alphitobius diaperinus*, and *Hermetia illucens*. *Journal of Agricultural and Food Chemistry*
303 65: 2275-2278.

304 Jones, D.B., 1941. Factors for converting percentages of nitrogen in foods and feeds into
305 percentages of protein. US Department of Agriculture-circ. 183. Washington, DC.

306 Makkar, H.P.S., Tran, G., Heuzé, V. and Ankers, P., 2014. State-of-the-art on use of insects as
307 animal feed. *Animal Feed Science and Technology* 197: 1-33.

308 Mancini, S., Fratini, F., Turchi, B., Mattioli, S., Dal Bosco, A., Tuccinardi, T., Nozic, S. and
309 Paci, G., 2019. Former foodstuff products in *Tenebrio molitor* rearing: effects on growth,
310 chemical composition, microbiological load, and antioxidant status. *Animals* 9, 489.

311 Mariotti, F., Tomé, D. and Patureau Mirand, P., 2008. Converting nitrogen into protein—
312 Beyond 6.25 and Jones' factors. *Critical Reviews in Food Science and Nutrition* 48: 177-
313 184.

314 Mertens, D.R., 2002. Gravimetric determination of amylase-treated neutral detergent fiber in
315 feeds with refluxing in beakers or crucibles: collaborative study. *Journal of AOAC*
316 *International* 85: 1217-1240.

317 Morales-Ramos, J.A., Rojas, M.G., Shapiro-Ilan, D.I. and Tedders, W.L., 2011. Self-selection
318 of two diet components by *Tenebrio molitor* (Coleoptera: Tenebrionidae) larvae and its
319 impact on fitness. *Environmental Entomology* 40: 1285-1294.

320 Oonincx, D.G.A.B. and de Boer, I.J.M., 2012. Environmental impact of the production of
321 mealworms as a protein source for humans - a life cycle assessment. *PLoS ONE* 7, e51145.

322 Oonincx, D.G.A.B. and Finke, M.D., 2012. Nutritional value of insects and ways to manipulate
323 their composition. *Journal of Insects as Food and Feed* 7: 639-659.

324 Oonincx, D.G.A.B., van Broekhoven, S., van Huis, A. and van Loon, J.J.A., 2015. Feed
325 conversion, survival and development, and composition of four insect species on diets
326 composed of food by-products. *PLoS ONE* 10, e0144601.

327 Paulicks, B.R., Vorndran, A., Kuenz, S. and Windisch, W., 2021. Effect of dietary crude protein
328 on growth performance of mealworms (*Tenebrio molitor*). 72nd Annual Meeting EAAP,
329 Davos (Switzerland), 30 August to 3 September 2021, p. 145.

- 330 Ramos-Elorduy, J., Avila González, E., Rocha Hernández, A. and Pino, J.M., 2002. Use of
331 *Tenebrio molitor* (Coleoptera: Tenebrionidae) to recycle organic wastes and as feed for
332 broiler chickens. *Journal of Economic Entomology* 95: 214-220.
- 333 Ravzanaadii, N., Kim, S.H., Choi, W.H., Hong, S.J. and Kim, N.J., 2012. Nutritional value of
334 mealworm, *Tenebrio molitor* as food source. *International Journal of Industrial Entomology*
335 25: 93-98.
- 336 Ribeiro, N., Abelho, M. and Costa, R., 2018. A review of the scientific literature for optimal
337 conditions for mass rearing *Tenebrio molitor* (Coleoptera: Tenebrionidae). *Journal of*
338 *Entomological Science* 53: 434-454.
- 339 Rumbos, C.I., Karapanagiotidis, I.T., Mente, E., Psafakis, P., Christos, G. and Athanassiou,
340 C.G., 2020. Evaluation of various commodities for the development of the yellow
341 mealworm, *Tenebrio molitor*. *Scientific Reports* 10: 11224.
- 342 Sánchez-Muros, M.J., Barroso, F.G. and Manzano-Agugliaro, F., 2014. Insect meal as
343 renewable source of food for animal feeding: a review. *Journal of Cleaner Production* 65:
344 16-27.
- 345 van Broekhoven, S., Oonincx, D.G.A.B., van Huis, A. and van Loon, J.J.A., 2015. Growth
346 performance and feed conversion efficiency of three edible mealworm species (Coleoptera:
347 Tenebrionidae) on diets composed by organic by-products. *Journal of Insect Physiology* 73:
348 1-10.
349

350 **Table 1:** Ingredient (g/kg) and estimated chemical composition (g/kg dry matter, DM) of
 351 experimental diets.
 352

	10P	12P	14P	16P
Ingredients:				
Wheat grain	830	830	830	830
Barley straw	170	120	75	30
Soybean meal	0	50	95	140
Chemical composition:				
Organic matter	966	969	971	974
Crude protein	99	123	145	166
Ether extract	16	16	16	17
Starch	561	562	562	564
Neutral detergent fibre	203	172	143	115

353
 354

355 **Table 2:** Mortality (as proportion of initial larvae), growth performance, feed to gain ratio (F:G), total nutrient production and protein efficiency
 356 (mg CP retained/mg CP ingested) of *T. molitor* larvae fed diets with increasing CP levels for 28 days.

	10P	12P	14P	16P	SEM	<i>P</i> -value ¹	Regression (<i>P</i> -value)	
							Linear	Quadratic
Mortality	0.164	0.124	0.159	0.122	0.0180	0.270	0.324	0.935
Final weight (mg)	153a	141b	147ab	146ab	2.138	0.023	0.183	0.066
Mass gained (mg/d per tray)	84.4	78.5	77.5	85.3	5.33	0.730	0.947	0.288
Larval growth (mg/d per larvae)	3.07a	2.64b	2.87ab	2.81ab	0.029	0.023	0.182	0.065
Intake (mg/d, fresh basis)	192.6a	157.6b	146.2b	158.9b	5.45	<0.001	<0.001	<0.01
Intake (mg/d, DM basis)	171.4a	134.0b	129.5b	140.5b	4.38	<0.001	<0.001	<0.01
Protein intake (mg/d)	19.86b	21.66ab	22.44a	23.68a	0.484	0.002	<0.001	0.624
F:G (g/g, fresh basis)	2.326a	2.013ab	1.916ab	1.896b	0.0875	0.033	<0.01	0.171
F:G (g/g, DM basis)	4.620a	4.178ab	3.751b	3.779b	0.1741	0.031	<0.01	0.262
Total production (mg)								
Dry matter	1058	941	985	1059	66.6	0.577	0.884	0.236
Crude protein	457	402	408	448	28.4	0.536	0.888	0.175
Ether extract	375	354	294	333	22.0	0.237	0.103	0.268
Protein efficiency	0.817a	0.663b	0.646b	0.674ab	0.0327	0.027	0.016	0.034

357 Within lines, values not sharing a common letter are significantly different ($P < 0.05$)

358 SEM: standard error of means; DM: dry matter; CP: crude protein; EE: ether extract

359 ¹*P*-values from the analysis of variance

360

361 **Table 3:** Dry matter content (mg/g) and chemical composition (mg/g DM) of the residual
 362 substrate and *T. molitor* larvae after 28 days receiving diets with increasing levels of CP.

	10P	12P	14P	16P
Feed residue				
Dry matter	895	896	896	892
Crude protein	92.7	113.4	136.2	164.4
Ether extract	8.8	10.6	12.2	12.1
Neutral detergent fibre	298	240	208	160
Larval composition				
Dry matter	448	428	453	444
Crude protein	432	427	415	423
Ether extract	355	360	305	311

363 Within lines, values not sharing a common letter are significantly different ($P < 0.05$)

364 SEM: standard error of means; DM: dry matter; CP: crude protein; EE: ether extract.

365 ¹ P -values from the analysis of variance

366

367