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(Article begins on next page)

1 **Reducing ammonia and GHG emissions from rabbit production through a feed additive from**  
2 **green urban residues**

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8

9 **ABSTRACT**

10

11 The present paper proposes a new strategy, based on the use of a new healthy animal diet additive  
12 obtained from urban biowaste, to reduce the environmental impact from livestock production. The  
13 diet supplement is a water soluble biopolymer (SB) obtained from urban gardening wastes. The paper  
14 reports the results of a case study in which 35-day old rabbits were fed a conventional diet as control  
15 and with a test diet made up of the conventional diet and 0.05-1% SB. Urine and faeces were  
16 collected, stored for 18 days at room temperature to simulate farm storage, and the resulting gaseous  
17 emissions were analysed. The manure from the animals fed the 0.25% SB diet produced significantly  
18 lower emissions ( $p < 0.05$ ): e.g., 30% less ammonia ( $\text{NH}_3$ ), 25% less methane ( $\text{CH}_4$ ), 9% less nitrous  
19 oxide ( $\text{N}_2\text{O}$ ) and 8% less carbon dioxide ( $\text{CO}_2$ ) than the control group. The SB feeding strategy can  
20 be applied on farms of any size and does not lead to any extra costs for the farm, except for the  
21 negligible cost of 0.003-0.007 €  $\text{kg}^{-1}$  for the SB supplement that has to be added to the normal animal  
22 diet. Full implementation of the SB strategy at a European Union level could lead to global yearly  
23 reductions of 1.1 Mt  $\text{NH}_3$ , 0.06 Mt  $\text{N}_2\text{O}$ , 2.2 Mt  $\text{CH}_4$ , 0.92 Gt  $\text{CO}_2$ -eq. emissions. The relevance of  
24 these results at a European Union level is discussed and the perspectives of previous work are  
25 accounted for to demonstrate that SBs can be used as chemical specialities in the chemical industry  
26 and in agriculture. The implementation of SB production and application at an industrial and  
27 commercial level could lead to important environmental and socio-economic benefits for several  
28 sectors of a bio-based economy.

29

30 *Keywords:* Municipal bio-waste, livestock, manure, gaseous emissions, biopolymers

31 *Abbreviations:* SB, soluble biopolymers from the same source; SBs, soluble biopolymers from  
32 different sources; MBW, municipal bio-wastes; D, anaerobic digestate from the bio-organic  
33 (humid) fraction of solid urban waste; CV, composted urban gardening residues (V); CVD,  
34 composted mix of D and V; CVF, composted mix of V and sewage sludge (F); CVDF, composted  
35 mix of V, D, F.

36

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39

## 40 1. Introduction

41 The breeding of animals is responsible for about 80% of the negative environmental impact of  
42 the agriculture sector on the quality of water and air (Allen et al., 2018). The Food and Agriculture  
43 Organisation has reported that the total gaseous emissions from global livestock production amount  
44 to 7.1 Gt yr<sup>-1</sup> of CO<sub>2</sub>-eq (FAO, 2020). This corresponds to 14.5% of all anthropogenic greenhouse  
45 gases (GHG) emissions. Manure storage, processing, and its application to soil, together with  
46 animal feed production and enteric fermentation are the main causes of gaseous emissions. These  
47 include unpleasant odours, as well as ammonia (NH<sub>3</sub>), methane (CH<sub>4</sub>) and nitrous oxide (N<sub>2</sub>O)  
48 emissions.

49 There are basically two strategies to control or mitigate air emissions from livestock operations:  
50 1) the animal feeding and health (AFH) strategy, 2) the manure management (MM) strategy  
51 (National Research Council, 2003). The experimental work described in the present paper focuses  
52 on a novel diet that includes a new diet additive, which allows animals to produce manure with  
53 reduced gaseous emissions. Thus, *subSection 1.1* of the introduction below reports what has been  
54 done so far in the AFH strategy. Section 3 shows that the experimental results offer sustainable  
55 perspectives for substituting and/or dismissing the MM strategy and shows that the implementation  
56 of the here reported novel diet may contribute indirectly to solving the economic and environmental  
57 impacts of the MM strategy. Thus, in order to fully evidence the innovation potential and purpose of  
58 the present study, the state of art of the MM strategy and its drawbacks are also reviewed in  
59 *subSection 1.2* in the introduction below.

60

### 61 1.1. The AFH strategy

62 The animal feeding and health strategy is aimed at increasing the production per animal in order  
63 to decrease the number of animals that have to be bred to satisfy the market demand for animal  
64 products, and therefore at decreasing the amount of animal dejections. This is pursued by  
65 formulating diets to match the animal requirements of optimum growth and productivity. However,  
66 the effects of this strategy on gas emissions from animal manure have rarely been studied. In one  
67 case, it was reported that synthetic amino acid diet supplements, such as lysine, tryptophan,  
68 threonine and methionine, lowered ammonia and total nitrogen in freshly excreted manure by 28%  
69 ([National Research Council, 2003](#)).

70 The present paper reports a new animal diet supplement produced from green urban residues and  
71 its effects on gaseous emissions from rabbit manure. This product is the result of R&D work that  
72 has been carried since 2004 by the University of Torino (Italy) with the aim of solving  
73 environmental problems and producing revenues from biowastes (Montoneri, 2017). A recent paper  
74 (Tabasso et al., 2020) has reported an update of this work, in which new water soluble biopolymers  
75 (SBs) have been obtained from the hydrolysis of fermented municipal biowaste (MBW). The SBs  
76 are constituted by mixes of macromolecules with 5-over 750 kDa molecular weight. The  
77 macromolecules are made of aliphatic C chains, aromatic rings, functional groups with variable acid

78 strength and complexing power, which bond several mineral elements. The whole assembly is  
79 reminiscent of the chemical features of the proximates contained in pristine biowastes. These  
80 features endow SBs with several properties and allow them to be used in different agriculture and  
81 chemical industry sectors.

82 For the specific animal sector addressed in the present work, Montoneri et al. (2013) reported the  
83 results of an *in-vitro* study carried out on the caecal fermentation of pigs fed a protein diet  
84 containing five SBs obtained from different fermented MBWs. These SBs were produced by the  
85 hydrolysis of the digestate (D) obtained from the anaerobic fermentation of food wastes, by the  
86 hydrolysis of composted green residues (CV) and of composted mixes of green residues with D  
87 (CVD), with D and sewage sludge (CVDF), and with sewage sludge (CVF). The study tested seven  
88 feed slurry concentrations, ranging from 0.0 (control) to 1.3 V/V% for each SB. The results showed  
89 that the highest effects were exhibited by the diets supplemented with 0.2% CV SB and CVD SB.  
90 In short, the CV SB and CVD SB diet produced a 33-44% lower gas volume than the control diet,  
91 and both diets produced less ammonia, that is, 17 and 8% less, respectively. The profile of the  
92 ammonia production vs. SB concentration showed some particular features. The ammonia  
93 production decreased as the SB concentration increased; it reached the lowest value at 0.2% SB and  
94 then increased significantly for higher SB concentrations. The compost-sourced SB diet at a 0.2%  
95 SB concentration generally decreased the ammonia production, compared to the control diet. On the  
96 other hand, the D SB diet increased the ammonia production since the lowest content, that is, 0.1%  
97 SB, up to the highest content, 1.3% SB.

98 After the above *in-vitro* studies, other authors carried out *in-vivo* animal health studies with CV  
99 SB. In two trials (Biagini et al., 2016), 131 and 120 35-day old rabbits were fed a conventional diet  
100 supplemented with 0.0-1.0 % CV SB for 2 months. The live and slaughtering performance, diet  
101 digestibility and health status of the animals were monitored. Generally, the CV SB diet supplement  
102 did not affect the growth performances and the carcass and meat traits. The rabbits revealed no  
103 signs of toxicity or pathologies caused by SB. The heavy metals in the feeds and meat were well  
104 below the legal threshold (European Commission, 2005) and not significantly different in the  
105 control and test groups.

106 Morlacchini et al. (2017) carried out an *in-vivo* animal health study in which 106 pigs were fed a  
107 protein diet supplemented with 0.1-0.2% CV SB. The study lasted 42 days. The average weight of  
108 the animals was 7.7 kg at the start and 26 kg at the end. No significant differences in the health  
109 performance of the animals were evidenced between the CV SB supplement diet and the control (no  
110 added CV SB) diet.

111 The results of the above rabbits and pig studies offered scope for the hereinafter reported case  
112 study in which the effects of CV SB on gas emissions from manure produced by rabbits were tested.

## 113 114 1.2. The MM strategy

115  
116 Animal manure production in the European Union amounts to 1400 Mt yr<sup>-1</sup> (European  
117 Commission, 2014). Livestock housing, manure storage, urine and dung deposition in grazed  
118 pastures, and manure spreading on agricultural land are responsible for emission of 3.6 Mt yr<sup>-1</sup> of

119 NH<sub>3</sub> (Eurostat, 2017), and GHG CO<sub>2</sub>-eq emissions of 185 N<sub>2</sub>O Mt yr<sup>-1</sup> and 245 CH<sub>4</sub> Mt yr<sup>-1</sup>  
120 (Eurostat, 2018a) calculated from emission weights of 0.70 Mt N<sub>2</sub>O and 8.75 Mt CH<sub>4</sub>, respectively.  
121 The application of manure as soil fertilisation is a common practice (European Union 27, 2020).  
122 The application of manure at dose rates in excess, compared to the plant uptake capacity (Khan et  
123 al., 2018), causes the leaching of nutrients (e.g., nitrogen and phosphorus) through the soil into  
124 ground water. Excessive manure applications to soil also cause GHG and NH<sub>3</sub> emissions. Ammonia  
125 from animal manure affects both the air and water. NH<sub>3</sub> 5–35 ppm concentrations in air, against 25  
126 ppm threshold level (Ji et al., 2006), and 10-18 g pig<sup>-1</sup>d<sup>-1</sup> (Szogi et al., 2015) emissions on pig  
127 farms, have been reported. A high NH<sub>3</sub> level can harm both animals and human health. The  
128 leaching of ammonium nitrogen through soil and water causes acidification and eutrophication  
129 (Leip et al., 2015).

130 In this context, manure management strategies include a number of different approaches. One is  
131 to move off manure from a farm where intensive livestock production is practiced to another farm  
132 with nutrient-deficit cropland. In principle, this would allow nitrogen (N) and phosphorus (P)  
133 manure leaching to be reduced in soil and water. Unfortunately, in most cases, the direct application  
134 of untreated manure to soil is not likely to meet the current legislation requirements. Moreover, the  
135 cost of manure transportation increases as the distance from its production site increases. This leads  
136 to the need to treat manure prior to land application and/or transportation. The objective is to obtain  
137 a nutrient-rich product that is more uniform, easier to transport and which meets the sanitation  
138 requirements according to the legislation in force.

139 A range of alternative technologies may be adopted for a safe eco-friendly sustainable manure  
140 management. These include physical, biochemical, chemical and thermochemical treatments. A  
141 fairly recent review (Szogi et al., 2015) has reported the advantages and drawbacks of each  
142 treatment. All these treatments require capital (CAPEX) and operating (OPEX) costs, which may  
143 not be sustainable for average sized farms. There are about 10.5 million farms in the European  
144 Union. Two-thirds of these cover areas of less than 5 hectares. The total amount of agricultural land  
145 in the European Union is 173 million hectares, that is, 39% of total land area of the European Union  
146 (Eurostat, 2018b). Over 55% of the European Union's agricultural holdings have livestock  
147 (Eurostat, 2019).

148 Traditionally, agriculture and animal wastes were handled at a farm level for recycling as  
149 fertilizers. Livestock manure, in both liquid and solid form, is generally stored outdoors and left  
150 uncovered, thereby allowing NH<sub>3</sub> and the GHG produced by fermentation processes to be released  
151 into the atmosphere. Anaerobic fermentation is a practice that is also used on farms. In this case,  
152 manure is stored in a closed vessel where it is converted, in an oxygen-free environment, into  
153 biogas, a gas mixture mainly composed of CO<sub>2</sub> and CH<sub>4</sub>, that can be used as a renewable energy  
154 source. Commercial bioreactors of different sizes for biogas production are available. Apart from  
155 biogas, the process yields digestate that contains the residual recalcitrant lignocellulosic matter and  
156 ammonia formed from the biodegradation of the pristine proteins (Riggio et al., 2017). In order to  
157 comply with the European Union's Nitrate Directive 91/676/EEC (Musacchio et al., 2020), the  
158 disposal of the digestate in soil is restricted in order to reduce NH<sub>3</sub> emissions into the air, nitrate  
159 formation in soil and leaching into ground water.

160 Excess inorganic N may be removed from the digestate through a number of chemical and  
161 biochemical technologies. However, these imply additional costs (Francavilla et al., 2016a). For  
162 example, the cost of the biochemical Anammox process is 1.6 \$ kg<sup>-1</sup> N. The cost of other physico-  
163 chemical processes ranges from 1 to 13 \$ kg<sup>-1</sup> N. These processes require high CAPEX costs that  
164 can only be sustained only in large centralised installations, and not at farm level. The problem is  
165 rather challenging, considering the figures given above in *subSection 1.1*. Globally, 1.4 10<sup>9</sup> t yr<sup>-1</sup> of  
166 manure is produced on European Union farms. Only 8% of this (Riggio et al., 2017) is processed. A  
167 strategy to reduce CAPEX and OPEX costs for the treatment of manure is to create consortia  
168 constituted by a sufficiently large number of neighbouring farms that can construct and manage  
169 centralised installations with sustainable economies of scale. This approach would improve the cost  
170 effectiveness of manure treatment in the local area. Moreover, the centralised installations would  
171 facilitate research and development (R&D) work directed towards optimising the manure  
172 processing technology. However, in these circumstances, the most desirable approach would be to  
173 develop a simple treatment strategy that could be applied locally in on-farm installations of any  
174 size. This would not lead to unsustainable CAPEX and OPEX costs derived from the collection and  
175 transportation of manure or its anaerobic digestate to centralised plants where secondary treatments  
176 are performed.

177 The above review of the state of art of the MM strategy has been a further incentive for the  
178 authors of the present study to develop the hereinafter reported change of the diet formulation and  
179 to evaluate its potential to solve the environmental and economic drawbacks of the MM strategy.

180

## 181 **2. Materials and methods**

182

183 The animal feeding trials were conducted according to Italian legislation (Gazzetta Ufficiale, 2001)  
184 and complied with the University of Torino protocol.

185

### 186 *2.1. Manure production and collection*

187

188 Sixty weaned crossbred rabbits (Grimaud x Hycole) were individually reared in the DISAFA  
189 rabbit farm in Carmagnola (TO), Italy (44°51'002N, 7°43'002E, at an altitude of 240 m a.s.l.). The  
190 rabbits were reared under semi-controlled environmental conditions (temperature 22±5 °C, light  
191 duration >8 h), in California type cages (30 x 30 x 40 cm; 0.12 m<sup>2</sup> head<sup>-1</sup>) according to the standard  
192 procedures. The rabbits were randomly assigned to five groups (12 heads each), while respecting a  
193 1:1 sex ratio. At the beginning of the trial, the animals were 35 days old and had an average body  
194 weight of about 1 kg. During the experimental period, the rabbits were fed ad libitum, for 55 days,  
195 with isoenergetic (18.8 MJ kg<sup>-1</sup> of dry matter) and isoproteic (179 g kg<sup>-1</sup> crude protein on dry  
196 matter) diets to which CV SB was added. CV SB was isolated from the alkaline hydrolysate of  
197 composted urban gardening and park trimming residues and characterised, as reported by  
198 Montoneri et al. (2013). The SB supplementations levels were: 0 (control group; 0-SB), 0.5 (0.5-  
199 SB), 2.5 (2.5-SB), 5 (5-SB), 10 g kg<sup>-1</sup> (10-SB). The SB levels cover the same range of values as  
200 those tested in the previous work of the authors (Biagini et al., 2016). The performances of the  
201 rabbits during the trial have been presented and discussed in a previous paper (Biagini et al., 2016)

202 and have shown that the addition of SBs to rabbit diets does not affect the live or slaughtering  
203 performances of the animals. In fact, the experimental groups, compared to the control ones, have  
204 shown an average daily feed intake, an average daily weight gain and an average daily feed  
205 conversion rate of 101 vs 98 g, 36 vs 36 g and 3.4 vs 3.4 g g<sup>-1</sup>, respectively, and the same dressing  
206 percentage (59%).

207

208

## 209 *2.2. Preparation of the samples for analyses*

210

211 The urine and faeces produced by the rabbits were collected daily for six days (from 49 to 54  
212 days of age) according to the European reference method for the *in-vivo* determination of diet  
213 digestibility in rabbits (Perez et al., 1995). In order to collect the excreta, four animals from each of  
214 the five different groups were housed in individual metabolic cages that allowed the separate  
215 collection of faeces and urine. A small amount of each sample was placed into a two-layer plastic  
216 bag to prevent moisture loss and immediately frozen at -20 °C. Each sample was then thawed,  
217 mixed thoroughly, pooled and then ground in a homogeniser fabricated by Tecator, Herndon, VA,  
218 the USA. Representative sub-samples were then taken and weighed in an aluminium foil pan, dried  
219 in a draft oven at 80 °C to a constant weight, and then stored for later chemical analysis. The  
220 excreta were maintained at 4 °C for the gaseous emission trials.

221

## 222 *2.3. pH measurement and chemical analysis of the manure*

223

224 The pH was measured with a Crison portable pH-meter (Crison Instruments, S.A., Alella, ES)  
225 fitted with a spear-type, automatic, temperature-compensation electrode. Proximate composition  
226 analyses of the faeces were performed on duplicate samples, according to the AOAC (2006)  
227 methods for the preparation of analytical samples (reference method no. 950.02) and for the  
228 analysis of the dry matter (DM; reference method no. 934.01), organic matter (OM; reference  
229 method no. 942.05), total nitrogen (TN; reference method no. 984.13), total ammonia nitrogen  
230 (TAN; reference method no. 941.04), neutral detergent fibre (NDF; reference method no. 2002.04),  
231 acid detergent fibre (ADF) and acid detergent lignin (ADL; reference method no. 973.18) contents.

232

## 233 *2.4. Measurement of the gaseous emissions from storage manure*

234

235 The faeces and urine produced by each rabbit housed in metabolic cages were collected  
236 separately to avoid the release of ammonia as a result of urease activity and then mixed in a 1 to 2  
237 ratio, according to the physiological production (Gamberini, 2001). Sub-samples of 0.50 kg were  
238 then placed in 1.5 L vessels (0.15 m height, 0.113 m diameter, 0.65 L headspace volume after  
239 placing the manure) and stored for 18 days in a climatic room which was kept at 20±5 °C. Such a  
240 temperature was chosen to approximate the average annual air temperature of most rabbit  
241 production areas in Italy.

242

243 The NH<sub>3</sub>, CO<sub>2</sub>, CH<sub>4</sub>, and N<sub>2</sub>O emissions from each stored manure sample (four samples per  
treatment, totalling twenty over the five treatments) were measured by means of a dynamic chamber

244 method, using a gas trace analyser (1412 Photoacoustic Multi-gas Monitor, Innova Air Tech  
 245 Instruments), according to the procedures proposed by Dinuccio et al. (2019). Specifically, gaseous  
 246 emissions were measured and recorded three times per week during the storage trials for a total of  
 247 eight times during the 18-day experimental period. All the investigated manure samples were also  
 248 analysed at the beginning of the storage period for DM, OM, TN, TAN, fibre content (NDF, ADF,  
 249 ADL) and pH using the previously described procedures. The NH<sub>3</sub>, CO<sub>2</sub>, CH<sub>4</sub>, and N<sub>2</sub>O readings  
 250 (mg m<sup>-3</sup>) of the photoacoustic analyser were converted into emission fluxes (F, mg m<sup>-2</sup> h<sup>-1</sup>) from  
 251 each vessel as follows:

$$252 \quad F = Q (C_{\text{out}} - C_{\text{in}}) / A$$

253 where: Q is the airflow rate (m<sup>3</sup> h<sup>-1</sup>) supplied to the vessels, C<sub>in</sub> (mg m<sup>-3</sup>) is the air inlet gas  
 254 concentration, C<sub>out</sub> (mg m<sup>-3</sup>) is the vessel air outlet gas concentration, and A (m<sup>2</sup>) is the emitting  
 255 surface area of the vessel.

256 The cumulative emissions (g m<sup>-2</sup>) of each gas recorded during the experimental period were  
 257 calculated, according to Pampuro et al. (2016), and expressed in CO<sub>2</sub>-eq (IPCC, 2013), which was  
 258 obtained by multiplying the single gaseous emissions by conversion factors of 1, 28, 265 and 2.65  
 259 for CO<sub>2</sub>, CH<sub>4</sub>, N<sub>2</sub>O and NH<sub>3</sub>, respectively.

260

## 261 *2.5. Statistical analyses*

262

263 Single emission data (g m<sup>-2</sup> h<sup>-1</sup>) of all the investigated gaseous emissions at each reading time  
 264 were analysed after testing their normal distribution (Shapiro-Wilk test) using the GLM ANOVA  
 265 procedure (IBM SPSS Statistics 26.0, SPSS Inc., Chicago, IL) with the following model:

$$266 \quad y = \mu + \alpha_i + \varepsilon_{ij}$$

267 where:  $\mu$  = general mean;  $\alpha_i$  = session effect;  $\varepsilon_{ij}$  = random error effect.

268 As the five groups of manure had a different initial averages of TN, the N<sub>2</sub>O and NH<sub>3</sub> emission data  
 269 were tested, using the GLM ANCOVA procedure (IBM SPSS Statistics 26.0, SPSS Inc., Chicago,  
 270 IL), according to the following model:

$$271 \quad y = \mu + \alpha_i + \beta(x_{ij} - x) + \varepsilon_{ij}$$

272 where  $\mu$  is the general mean;  $\alpha_i$  is the SB integration effect;  $\beta(x_{ij}-x)$  is the effect linearly associated  
 273 with the TN content;  $\varepsilon_{ij}$  is the random error effect.

274 Differences in the mean values were tested by means of Tuckey's test, using a first class error value  
 275 set at  $\alpha = 0.05$  to accept the differences as significant.

276

## 277 **3. Results**

278 Table 1 reports the proximate content in the rabbit manure collected before storage. The SB  
 279 content of the diet affected the manure composition. The DM content of the animal dejections  
 280 resulted to be within the range reported for solid manure from livestock and poultry production  
 281 (Bicudo, 2009). The manure obtained from the 5-SB and 10-SB groups contained significantly  
 282 ( $P < 0.05$ ) more DM than the control and the other two 0.5-SN and 2.5-SB groups. Other significant



283 differences pertaining to the SB diets, compared to the control diet, are evident for the TN, TAN,  
 284 NDF, ADF, ADL and pH indicators.

285 Fig. 1 reports the mean emission rates for all of the investigated gases at each measurement  
 286 during manure storage. Each group shows a production peak for the emissions of N<sub>2</sub>O on day 7,  
 287 when the 0-SB groups releases the maximum flux recorded over the experimental period (0.306 mg  
 288 m<sup>-2</sup> h<sup>-1</sup>). The flow shows a decreasing trend, with the lowest value recorded on the last day of the  
 289 test when the 5-SB group releases the lowest amount, that is, of 0.066 mg m<sup>-2</sup> h<sup>-1</sup>. The manure  
 290 obtained from the groups of rabbits fed with the 5-SB and 10-SB diets shows the lowest N<sub>2</sub>O  
 291 emissions of all the 5 experimental groups. Generally, through the trials, the diets containing SB  
 292 exhibit lower N<sub>2</sub>O emission than the control group. The mean N<sub>2</sub>O fluxes of the diets containing SB  
 293 range from 0.25 on day 7 for the 0.5-SB and 2.5-SB groups to 0.08 mg m<sup>-2</sup> h<sup>-1</sup> on day 18 for the 5-  
 294 SB group. Similarly, the ammonia emissions show a peak value on day 7, when the maximum, that  
 295 is, of 49.71 mg m<sup>-2</sup> h<sup>-1</sup> occurs for the 5-SB group, and the lowest value, that is, of 11.04 mg m<sup>-2</sup> h<sup>-1</sup>  
 296 is shown for the 2.5-SB group. As far as ammonia is concerned, the groups showing the highest  
 297 emissions are the same as those that produced the lowest N<sub>2</sub>O emissions (groups 5-SB and 10-SB).

298 The CH<sub>4</sub> and CO<sub>2</sub> emission patterns are different from the N<sub>2</sub>O and NH<sub>3</sub> emissions, as the  
 299 former already reach peak values on day 2. The highest CH<sub>4</sub> emission (7.08 mg m<sup>-2</sup> h<sup>-1</sup>) occurs on  
 300 day 2 for the 0.5-SB group. The lowest emission (1.43 mg m<sup>-2</sup> h<sup>-1</sup>) occurs on day 18 for the 10-SB  
 301 group. At the end of the trial, the CH<sub>4</sub> flux averaged over all groups results 1.73 mg m<sup>-2</sup> h<sup>-1</sup>. The  
 302 highest CH<sub>4</sub> emission, that is, 5.45 mg m<sup>-2</sup> h<sup>-1</sup>, averaged over all the groups, is on day 7, and the  
 303 lowest, 1.70 mg m<sup>-2</sup> h<sup>-1</sup>, occurs on day 16. The mean CO<sub>2</sub> fluxes range from 384.91 on day 7 to  
 304 111.28 mg m<sup>-2</sup> h<sup>-1</sup> on day 18. The 5-SB group exhibits the highest emission values between day 2  
 305 and 14, and then decreases more rapidly than the others groups, reaching the lowest emission value  
 306 recorded for all the groups.

307 Table 2 reports the cumulative emission values for all investigated gases over the entire 0-18  
 308 days of the trials. The manure produced by the rabbits fed the 5-SB diet shows the lowest N<sub>2</sub>O  
 309 cumulative emission value. The diets rank in the following statistical order of increasing N<sub>2</sub>O  
 310 emissions: 5-SB < 10-SB < 2.5-SB = 0.5-SB = 0-SB. On the other hand, the manure produced by  
 311 the rabbits fed the 2.5-SB diet shows the lowest cumulative NH<sub>3</sub> emission value. The diets rank in  
 312 the following order of increasing NH<sub>3</sub> emissions: 2.5-SB < 0.5-SB ≤ 0-SB ≤ 10-SB ≤ 5-SB. The 5-  
 313 SB diet, which produces the lowest amount of N<sub>2</sub>O emissions, also produces the highest amount of  
 314 NH<sub>3</sub> emissions. However, the 2.5-SB, which produces the highest N<sub>2</sub>O emissions, produces the  
 315 lowest NH<sub>3</sub> emissions.

316 Different trends are evident for the two other GHG gases. The cumulative CH<sub>4</sub> emission tends to  
 317 decrease as the SB level increases. It shows a significantly lower value for the 10-SB groups. The 5-  
 318 SB group exhibits the highest emission value for CO<sub>2</sub>.

319

#### 320 4. Discussion

321 The experimental data show that the CV SB content in the diet affects the manure composition,  
 322 the emission pattern and the level of the different gases. The effects of SBs have been reported in  
 323 many different sectors, where these biopolymers have been tested (Montoneri, 2017). These reports  
 324 not only cover the animal sector, as specifically addressed in the present work, but also a variety of

325 other agricultural fields and uses in the chemical industry. As a result of their surfactant properties,  
326 SBs have shown to be efficient in the remediation of soil and water contaminated with industrial  
327 organic and trace pollutants (Tabasso et al., 2020), in the formulation and fabrication of chemical  
328 speciality products, such as detergents (Savarino et al., 2010), and in auxiliaries for textile dyeing  
329 (Savarino et al., 2009). Because of their polymeric nature, SBs have been used for the fabrication of  
330 mulch films (Nisticò et al., 2017). Because of the presence of plant nutrient mineral elements, SBs  
331 are also efficient soil fertilisers (Sortino et al., 2014) and plant growth biostimulants (Massa et al.,  
332 2016). The production cost of SBs has been estimated as 0.2 € kg<sup>-1</sup>, against a potential selling value  
333 that ranges from 1 to 800 € kg<sup>-1</sup>, depending on the market sector where the product is allocated  
334 (Montoneri, 2017). On the basis of their properties, performance, production cost and potential  
335 market value, SBs can undoubtedly be considered sustainable products. The capacity of SBs to  
336 reduce the ammonia content in the anaerobic fermentation digestates of municipal kitchen wastes  
337 (Francavilla et al., 2016) and cow manure (Riggio et al., 2017) is particularly relevant, in relation to  
338 the MM strategy reviewed in *subSection 1.2*.

339 The issue in all the previous work carried out with SBs has always been that of finding the  
340 scientific explanation for the specific investigated use. This issue has remained open, mainly  
341 because of the compositional complexity of SBs. These biological source products are mixes of  
342 molecules, which differ from each other as far as their molecular weight and chemical composition  
343 are concerned. The heterogeneity of the products is the reason for the many properties and uses of  
344 SBs. However, this heterogeneity makes it difficult to identify the molecules that perform as  
345 effective active principles in the specific investigated applications.

346 Plausible explanations for the effects of SBs have been given in some cases. For example, in the  
347 case of environmental remediation, the performance of SBs has been correlated with the  
348 hydrophilic-lipophilic balance, the surface activity and the solution conformational behaviour of the  
349 molecular pool (Tabasso et al., 2020). In the case of plant cultivation (Sortino et al., 2014), the  
350 effect of SBs on promoting plant growth and productivity has been related to the presence of Fe and  
351 Si ions (Massa et al., 2016). These ions, bonded to and/or complexed by the SB acid and basic  
352 functionalities, are soluble in water at soil pH, can be taken up more rapidly by the plant and  
353 participate in the foliar photosynthesis process. Under different conditions, the soluble SB Fe ions  
354 at circumneutral pH have been assumed to catalyse the mineralisation of organic C in industrial  
355 waste waters as the result of the simple exposure to solar light (Gomis et al., 2014). The aromatic  
356 rings in SBs derive from the aromatic moieties that are present in the parent native lignin contained  
357 in the pristine biowaste from which they are obtained. These moieties contribute mechanical  
358 stiffness. They have been assumed responsible for the lack of film forming capability of SBs, the  
359 poor plastic properties of SBs and the higher mechanical strength (capacity to keep the original  
360 shape under mechanical stress) of composite mulch films fabricated from SBs and synthetic  
361 polymer blends, compared to films obtained from neat synthetic polymers (Nisticò et al., 2017). The  
362 compositional difference between D SB and CV SB (Montoneri et al., 2013), with the former  
363 containing more crude proteins, less acid detergent fibres and ashes, relatively more aliphatic C than  
364 aromatic C and relatively more lipophilic than hydrophilic moieties, makes D SB a better surfactant  
365 (Tabasso et al., 2020) and CV SB a better animal diet supplement that is able to lower the ammonia  
366 content in the anaerobic digestate (Montoneri et al., 2013). The reasons for the different effects of

367 the D SB and the CV SB are certainly related to the different microbial interactions with the two  
 368 SBs.

369 In the specific case of the Table 1 data, the CV SB interaction with the microbial population is  
 370 the likely cause of the differences in the manure composition. These interactions can vary to a great  
 371 extent, and depend on several factors (Huck et al., 1991). For example, the TAN concentration in  
 372 manure (Table 1) could be affected by the microbial production of urease, which transforms ureic-N  
 373 to ammonia-N. The highest TAN is here observed for the 0.5-SB and 2.5-SB diets. These two diets  
 374 also exhibit the highest TN, NDF, ADF and pH. The available data do not allow the reasons for the  
 375 effects of the SB diet content on the manure composition to be clearly defined. From the practical  
 376 operational point of view addressed in the present work, the analysis of the gas emissions is much  
 377 more useful than the analysis of the manure composition.

378 The data in Table 2 show that the effect of the diet SB content on the gas emissions is not linear.  
 379 Moreover, the lowest ammonia emission from the 2.5-SB diet ( $144 \text{ g m}^{-2}$ ) is accompanied by the  
 380 lowest  $\text{CO}_2$  eq ( $3498 \text{ g m}^{-2}$ ) emissions. Similar findings are reported (see *subSection 1.1* above) for  
 381 the *in-vitro* caecal fermentation of SB supplemented pig diets (Montoneri et al., 2013). Apart from  
 382 the reasons for these trends, the consistency of the data obtained in the present manure study and in  
 383 previous work on the *in-vitro* caecal fermentation of pigs, and in the anaerobic fermentation of  
 384 urban food wastes (Francavilla et al., 2016) and cow manure (Riggio et al., 2017) performed in  
 385 closed bioreactors validates the reliability of the SB effect on lowering the emissions of noxious  
 386 gases.

387 The authors are well aware of the scientific limitations of the data reported in the present work.  
 388 Nevertheless, in spite of the constraints deriving from the complexity of the chemical composition  
 389 of SBs and the consequent lack of knowledge, the reported data are still highly relevant for farmers  
 390 and land owners, from the practical point of view.

391

#### 392 4.1. The benefits and costs of the SB strategy for the livestock production sector

393

394 In the present work, the authors have considered rabbits as a case study since they constitute a  
 395 relevant population in the animal production sector, both because of their number and farm type  
 396 distribution. The number of rabbit stocks worldwide is around 300 million (FAO-STAT, 2018). In  
 397 Europe, 180 million rabbits are reared for meat consumption (Trocino et al., 2019). They are  
 398 distributed over commercial farms (66%) and backyard farms (34%). In spite of their increasing  
 399 importance for the world's production of meat, rabbits have been less studied than other meat-  
 400 producing animals, although several researches on feed additives in their diets have been published.  
 401 Fish oil (Tres et al., 2014), organic acidifiers of drinking water (Zhu et al., 2014), phytogenic feed  
 402 additives (Hasem et al., 2017), enzymes, organic acids,  $\beta$ -pro (Sherif, 2018) have been investigated  
 403 as diet supplements because of their effects on the growth and meat quality of the animals.  
 404 However, no study has reported on the effect of diet additives on the gas emissions from animal  
 405 manure. In the present study the CV SB was chosen as diet supplement, since most of the previous  
 406 work by the authors was carried out with it and/or demonstrated that CV SB was more efficient than  
 407 the SBs obtained from the other MBW composts or from the MBW anaerobic digestate (Montoneri  
 408 et al., 2013).

409 The available literature reports gas emission data in different units and from different  
 410 experimental set ups, which make it difficult to conduct comparative evaluations. In order to  
 411 circumvent this difficulty, Table 3 reports the gaseous emissions from manure as percentage  
 412 changes of the gaseous emissions affected by the CV SB supplemented diets, relatively to emissions  
 413 from the manure from rabbits fed the control diet (no added SB). The data show the significant  
 414 effect of the 2.5-SB diet on causing a 30% decrease in ammonia emissions from the manure of  
 415 rabbits fed this diet, along with decreases of the other GHG emissions, that is, 9% for N<sub>2</sub>O, 25% for  
 416 CH<sub>4</sub> and 8% for CO<sub>2</sub>. On the other hand, the *in-vitro* caecal fermentation of pig diets supplemented  
 417 with 2 g kg<sup>-1</sup> CV SB (Montoneri et al., 2013) produced 17% less ammonia and 36% less cumulative  
 418 biogas volume, compared than the control diet containing no SB. The consistency of the SB effects  
 419 under different experimental conditions, involving different animals and different control diets, is  
 420 remarkable. This result seems to suggest that the SB effects could be replicated for other animals in  
 421 other environments. The effects of the 2.5-SB diet are even more remarkable, when compared with  
 422 those reported by Dinuccio et al. (2019). These authors tested the practice of incorporating manure  
 423 into soil, which is recommended by the Italian Government regulation as a strategy to abate  
 424 ammonia and GHG emissions, instead of spreading manure on the land surface. They reported that,  
 425 compared to the deposition of manure on the soil surface, the incorporation of rabbits' manure into  
 426 the soil yielded an ammonia abatement of 42.0%, but the N<sub>2</sub>O, CH<sub>4</sub> and CO<sub>2</sub> emissions increased  
 427 by 37.0%, 57.3% and 34.8%, respectively. These data show that, although manure soil  
 428 incorporation may in principle be a practice sustainable in farms of any size, it worsens the impact  
 429 of GHG emissions from manure. However, the SB diet approach may allow the impact of both  
 430 ammonia and other GHG emissions to be reduced.

431 The results prospect a scenario in which any farm, regardless of its size, can significantly reduce  
 432 the environmental problem caused by livestock production on site, just by adding a small amount of  
 433 SB to the animal diets. In such a situation, the farm would not need to face CAPEX and OPEX cost  
 434 of complex chemical facilities, or transportation costs to large centralised manure processing  
 435 facilities and the related tipping fees, as described in above *subSection 1.2*. The only operational  
 436 cost of the prospected strategy stems from the SB supplement that has to be added to the normal  
 437 animal diet. SB is not a commercial product. No data about the cost of its production and market  
 438 selling value under real operational conditions are available. The production cost of SB has been  
 439 estimated, on the basis of previous R&D work and pilot studies on the optimisation of the SB  
 440 production process (Montoneri,2017), as 0.2 € kg<sup>-1</sup>. An estimate of its potential selling value can  
 441 only be made on the basis of the selling value of commercial products with dietary effects on  
 442 animal breeding with similar effects to SB. There is no known commercial feed supplement  
 443 counterpart that has the same properties as SB. Synthetic amino acid supplements, such as lysine,  
 444 tryptophan, threonine and methionine, have been reported to lower ammonia and total nitrogen in  
 445 freshly excreted manure by 28 % ([National Research Council, 2003](#)). The market price of these  
 446 products ranges from 1.2- 2.7 € kg<sup>-1</sup> ([Chinniah, 2014](#)). On the basis of these figures, the potential  
 447 added specific cost of the SB diet, relatively to the control diet, results negligible (ca. 0.003-0.007 €  
 448 kg<sup>-1</sup>).

449 The experimental GHG emission data are related to the storage of rabbit manure. For this  
 450 specific case, the percentage emission reduction of the 2.5 SB diet in Table 3 allows the potential

451 reduction of GHG and ammonia from rabbit manure storage to be estimated at a world level upon  
 452 full implementation of the SB strategy. Extrapolating the values given in Table 3 to the world's  
 453 rabbit population (FAOSTAT, 2018) and their gaseous emissions during 35 days' manure storage  
 454 (Dinuuccio et al., 2019), it is possible to estimate reductions of 9.0 N<sub>2</sub>O, 4328 NH<sub>3</sub>, 836.4 CH<sub>4</sub> and  
 455 48,354 CO<sub>2</sub> kt yr<sup>-1</sup>. These reductions are equivalent to 98,563 kt yr<sup>-1</sup> CO<sub>2</sub> eq. Taking in  
 456 consideration the results of the present work and those obtained in previous work on the effects of  
 457 SB in the *in vitro* pig caecal fermentation (Montoneri et al., 2013) and the anaerobic fermentation of  
 458 cow manure (Riggio et al., 2017), it is possible to expect that the SB effects may be general for all  
 459 livestock. Under this hopeful hypothesis, and considering the level of the European Union global  
 460 livestock emissions of ammonia and GHG given in *Section 1* and *1.2*, as well as hypothesizing that  
 461 the order of magnitude of the emission reduction in Table 3 could be replicated across most of the  
 462 European Union livestock population, it is possible to imagine the full potential environmental  
 463 benefit of the SB strategy at a European Union level.

464 The authors are aware that, under the experimental constraints of the present study, a reliable  
 465 estimate of the full benefits of the implementation of the SB strategy cannot be obtained at a  
 466 European Union level. The question is how much rabbit farming affect the total livestock  
 467 production, not only in terms of the quantity of the animals bred, but also and especially in terms of  
 468 value and emissions. According to FAOSTAT (2018) data, if the number of rabbits bred in the  
 469 world (300 million heads) is taken as 100, cattle's head are 484, pig's 318, goat's 340 and sheep's  
 470 393. Considering head numbers and body weights of the different animals, rabbits contribute by far  
 471 the least to gaseous emissions. Nevertheless, the data on rabbits, which are reported in the present  
 472 work, offer scope to carry on further studies and to assess for which other animal species the SBs  
 473 effects could be replicated.

474

#### 475 *4.2. The SBs added values for waste management plants and the biobased industry.*

476

477 The full relevance of the present and previous (Montoneri et al., 2017) work on the valorisation  
 478 of SBs may be appreciated by considering their many properties, potential uses and the benefits for  
 479 the growing biobased economy (Tabasso et al., 2020). SBs are products that are sourced from urban  
 480 biowastes and applied in various sectors of agriculture and the chemical industry. In this context,  
 481 they represent a virtuous link that connects urban, rural and industrial environments. Producing SBs  
 482 from MBW and using them on farms as soil fertilisers, plant growth biostimulants, animal diet  
 483 supplements, additives for closed fermentation bioreactors to produce biogas and digestate with a  
 484 low ammonia content, or using them for the manufacturing of biobased chemical specialities,  
 485 returns renewable C in cities to agricultural land. This promotes the production of biomass for  
 486 human consumption that generates more MBW, from which further SBs are produced. This  
 487 scenario realises a virtuous C cycle, which involves developing a circular biobased economy with  
 488 environmental, economic and social benefits. Compared to commercial products for the same uses,  
 489 the potential market value of SBs has been estimated as 1.5 to 800 € kg<sup>-1</sup>, against a production cost  
 490 of 0.2 € kg<sup>-1</sup> (Montoneri et al., 2017). The market value depends on the type of SB obtained from  
 491 the type of sourced MBW and on the market sector where the SB may be allocated. For example, in  
 492 the agriculture sector, because of their performance as soil fertilisers (Sortino et al., 2014) and plant

493 biostimulants (Massa et al., 2016), D SB and CVD SB are potentially worth 1-3 € kg<sup>-1</sup>, D and  
 494 CVDF SB, because of their use as plant disease suppressants (Jindrichova et al., 2018), can be sold  
 495 as much as 800 € kg<sup>-1</sup>, while D SB, because of its use as a specialised surfactant (Montoneri et al.,  
 496 2020) can reach a market value of 150 € kg<sup>-1</sup>. Currently, MBW treatment plants process urban  
 497 wastes through fermentation and produce biogas, digestate and/or compost. The selling value of  
 498 these products, relative to their production cost, is quite low. The excess cost is covered by the  
 499 tipping fees municipalities pay to the MBW processing plants. By integrating their fermentation  
 500 facilities with chemical facility producing SBs from the anaerobic digestate and composts of the  
 501 plant, the MBW plant revenue could be enhanced 100 to 1000 times. This would be enough to  
 502 allow citizens' taxes to lowered. An estimate of the environmental and socio-economic impacts at a  
 503 European Union level for the full implementation of SB at an industrial and commercial level has  
 504 been published (Montoneri et al., 2017).

505

## 506 **5. Conclusions**

507

508 Rabbits fed diets containing 2.5 g kg<sup>-1</sup> of CV SB produce manure that emits 30% less ammonia  
 509 and significantly lower GHG (- 9% N<sub>2</sub>O, - 25% CH<sub>4</sub> and - 8.0% CO<sub>2</sub>) emissions, than rabbits fed a  
 510 control diet containing no CV SB. These results and those of previous work (Montoneri, 2017)  
 511 suggest two alternative sustainable strategies for use on farms: 1) the SB assisted anaerobic  
 512 fermentation of manure in closed reactors; 2) the production of low gaseous emission manure by  
 513 animals fed SB supplemented diets. These alternatives are low cost strategies. They both require the  
 514 negligible costs for SB consumption. The former alternative also involves CAPEX cost of the  
 515 bioreactor. Commercial anaerobic reactors of different sizes and processing capacities are available  
 516 at reasonable prices. SB is not commercially available yet. However, thanks to the European  
 517 Union's funding of the currently running Lifecab (2020) project, a prototype reactor, with a 5 kt yr<sup>-1</sup>  
 518 SB production capacity, has been built. This will allow enough SB to be produced to test and  
 519 demonstrate the replicability of the two strategies in different urban and rural environments. This is  
 520 a step forward along the route to transferring the SB-based technology to a real operational and  
 521 commercial level.

522 The environmental and economic benefits derived from the use of SB in managing livestock  
 523 manure are not restricted to the animal sector. Previous work (Montoneri, 2017) proved that SBs  
 524 can also be applied in agriculture as soil fertilisers and plant growth biostimulants, and in the  
 525 chemical industry for the manufacturing of chemical specialities to use in place of commercial  
 526 products derived from fossil sources. The published data demonstrate that the production and  
 527 consumption of SBs is environmentally and economically sustainable. In such R&D context, the  
 528 results obtained in the present work add further evidence in favour of the implementation of SBs to  
 529 industrial and commercial level. They further support the feasibility of a virtuous renewable C cycle  
 530 based on the production and use of SBs, which encompasses the urban, rural and industrial  
 531 domains. The proposed scenario is well in line with the objectives of the new developing biobased  
 532 economy for agriculture (Diakosavvas, 2019), the livestock sector (Šperanda et al., 2019), the  
 533 chemical industry (European Commission, 2019) and, generally, for all sectors in different  
 534 countries worldwide (FAO, 2018).

535

536 **Declaration of competing interest**

537 None.

538

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543

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702 **Table 1**  
 703 Analytical values (w/w%)<sup>a, b</sup> for manure produced by rabbits fed with the control diet (0-SB) and  
 704 with the diets containing 0.5 (0.5-SB), 2.5 (2.5-SB), 5 (5-SB) and 10 (10-SB) g L<sup>-1</sup> of CV SB.

	0-SB	0.5-SB	2.5-SB	5-SB	10-SB
DM	19.34±0.51 b	19.25±0.51 b	19.00±0.51 b	21.78±0.58 a	21.77±0.51 a
OM	88.78±1.32 a	83.94±1.32 a	83.84±1.32 a	86.85±1.52 a	83.60±1.32 a
TN	3.26±0.17 b	4.33±0.17 a	4.28±0.17 a	3.38±0.19 b	3.46±0.17 b
TAN	0.51±0.95 b	1.00±0.95 a	0.98±0.95 a	0.77±0.11 ab	0.84±0.95 ab
NDF	66.72±1.60 ab	69.16±1.60 ab	71.19±1.60 a	63.87±1.85 bc	59.27±1.60 c
ADF	45.33±1.01 b	52.13±1.01 a	51.08±1.01 a	43.34±1.23 bc	40.18±1.01 c
ADL	12.70±0.47 b	15.64±0.47 a	13.42±0.47 b	12.56±0.54 b	11.68±0.47 b
pH	7.25±0.12 b	8.37±0.12 a	8.25±0.12 a	7.52±0.14 b	7.57±0.12 b

705 <sup>a</sup> Mean ± standard error values of measurements on 4 samples, as% w/w values of dry matter (DM)  
 706 in the collected manure, and as% w/w values referred to DM for organic matter (OM), total nitrogen  
 707 (TN), total ammonia nitrogen (TAN), neutral detergent fibre (NDF), acid detergent fibre (ADF),  
 708 acid detergent lignin (ADL).

709 <sup>b</sup> Values within rows, followed by different letters with (a, b and c), are significantly different (P <  
 710 0.05).

711

712 **Table 2**

713 Cumulative gaseous emissions ( $\text{g m}^{-2}$ )<sup>a,b</sup> from manure produced by rabbits fed with the control diet  
 714 (0-SB) and with the diets containing 0.5 (0.5-SB), 2.5 (2.5-SB), 5 (5-SB) and 10 (10-S)  $\text{g L}^{-1}$  of CV  
 715 SB over the 18 days' duration of the trials.

	0-SB	0.5-SB	2.5-SB	5-SB	10-SB
N <sub>2</sub> O	1.60±0.06 a	1.47±0.06 a	1.43±0.06 a	1.04±0.06 c	1.22±0.05 b
NH <sub>3</sub>	206.20±5.97 bc	193.57±5.97 c	143.69±5.97 d	246.74±5.97 a	227.66±5.97 ab
CH <sub>4</sub>	35.18±0.58 a	33.33±0.58 a	26.47±0.58 b	27.75±0.58 b	22.80±0.58 c
CO <sub>2</sub>	1852±148 b	1779±148 b	1993±148 ab	2533 ±148 a	2034±148 ab
CO <sub>2</sub> eq	3804±234 ab	3618±234 ab	3498±234 b	4226±234.15 a	3596±234 ab

716 <sup>a</sup> Mean ± standard error values calculated from measurements on 4 samples.

717 <sup>b</sup> Values within rows, followed by different letters (a, b, c and d) are significantly different ( $P < 0.05$ ).

718

719 **Table 3**

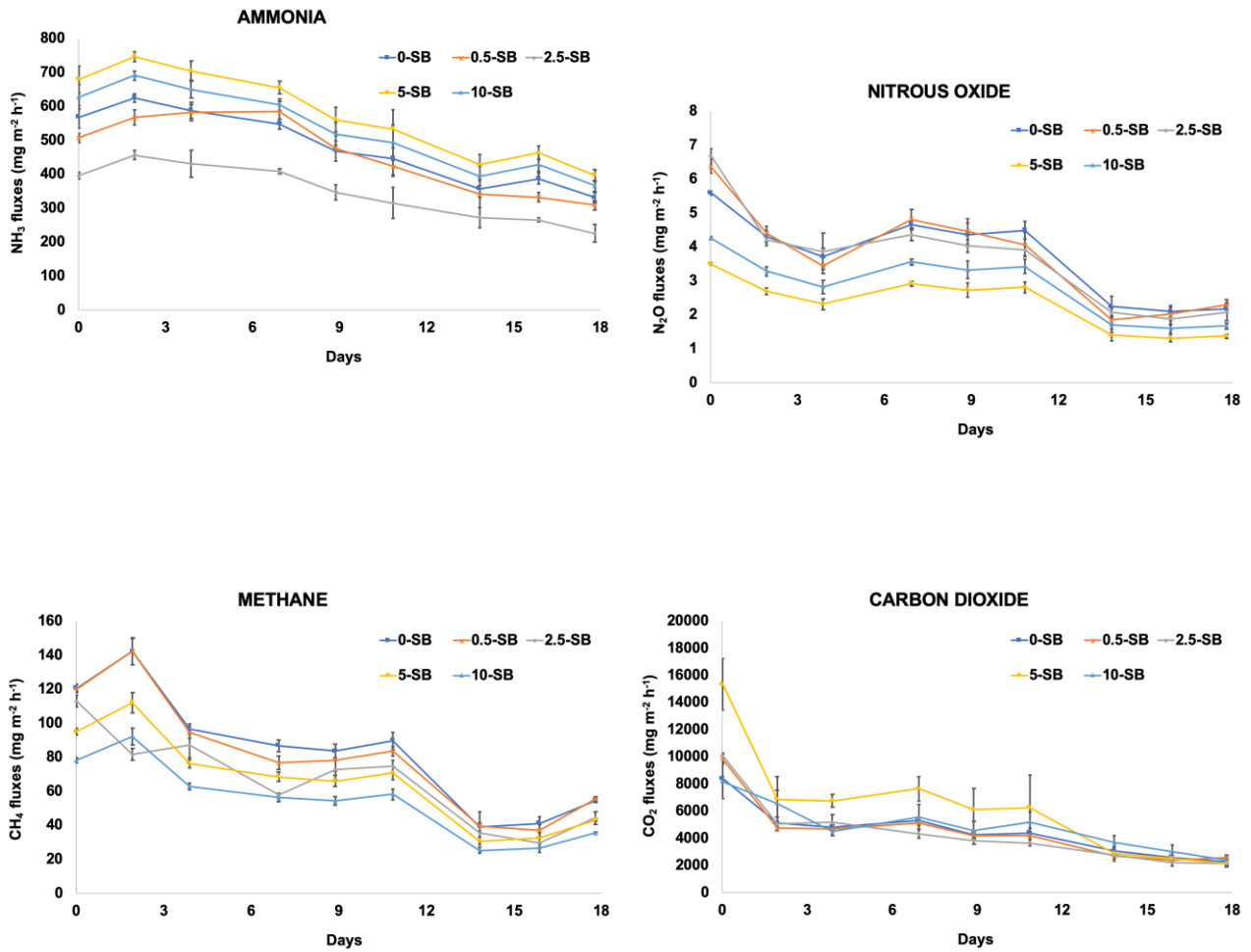
720 Percentage changes<sup>a</sup> in the cumulative gaseous emissions from manure produced by rabbits fed  
 721 diets containing SB (0.5 to 10-SB), relative to the manure emissions from the rabbits in the control  
 722 group (0-SB). The control and test groups are the same as in Table 2.

	0.5-SB	2.5-SB	5-SB	10-SB
N <sub>2</sub> O	-6.92	-10.8	-35.0	-23.8
NH <sub>3</sub>	-6.13	-30.3	19.7	10.5
CH <sub>4</sub>	-5.26	-24.8	-21.1	-35.2
CO <sub>2</sub>	-3.96	-8.01	36.7	9.80
CO <sub>2</sub> eq	-1.96	-13.0	14.5	-2.56

723 <sup>a</sup>Percent change (PCC) calculated from the Table 2 data according to the equation:

724 
$$\text{PCC} = 100 (i\text{-SB value} - 0\text{-SB value}) / 0\text{-SB value, where } i = 0.5 \text{ to } 10.$$

725



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729

**Fig. 1.** Gaseous emissions ( $\text{g m}^{-2} \text{h}^{-1}$ ) recorded during the trials from manure produced by rabbits fed the control diet (0-SB) and the diets containing 0.5 (0.5-SB), 2.5 (2.5-SB), 5 (5-SB) and 10 (10-S)  $\text{g L}^{-1}$  of CV SB.