Reducing ammonia and GHG emissions from rabbit rearing through a feed additive produced from green urban residues

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

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ABSTRACT

The present paper proposes a new strategy, based on the use of a new healthy animal diet additive obtained from urban biowaste, to reduce the environmental impact from livestock production. The diet supplement is a water soluble biopolymer (SB) obtained from urban gardening wastes. The paper reports the results of a case study in which 35-day old rabbits were fed a conventional diet as control and with a test diet made up of the conventional diet and 0.05-1% SB. Urine and faeces were collected, stored for 18 days at room temperature to simulate farm storage, and the resulting gaseous emissions were analysed. The manure from the animals fed the 0.25% SB diet produced significantly lower emissions ($p<0.05$): e.g., 30% less ammonia (NH_3), 25% less methane (CH_4), 9% less nitrous oxide (N_2O) and 8% less carbon dioxide (CO_2) than the control group. The SB feeding strategy can be applied on farms of any size and does not lead to any extra costs for the farm, except for the negligible cost of 0.003-0.007 € kg^{-1} for the SB supplement that has to be added to the normal animal diet. Full implementation of the SB strategy at a European Union level could lead to global yearly reductions of 1.1 Mt NH_3, 0.06 Mt N_2O, 2.2 Mt CH_4, 0.92 Gt CO_2-eq. emissions. The relevance of these results at a European Union level is discussed and the perspectives of previous work are accounted for to demonstrate that SBs can be used as chemical specialities in the chemical industry and in agriculture. The implementation of SB production and application at an industrial and commercial level could lead to important environmental and socio-economic benefits for several sectors of a bio-based economy.

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

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

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1. Introduction

The breeding of animals is responsible for about 80% of the negative environmental impact of the agriculture sector on the quality of water and air (Allen et al., 2018). The Food and Agriculture Organisation has reported that the total gaseous emissions from global livestock production amount to 7.1 Gt yr^{-1} of CO_2-eq (FAO, 2020). This corresponds to 14.5% of all anthropogenic greenhouse gases (GHG) emissions. Manure storage, processing, and its application to soil, together with animal feed production and enteric fermentation are the main causes of gaseous emissions. These include unpleasant odours, as well as ammonia (NH_3), methane (CH_4) and nitrous oxide (N_2O) emissions.

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

1.1. The AFH strategy

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

The present paper reports a new animal diet supplement produced from green urban residues and its effects on gaseous emissions from rabbit manure. This product is the result of R&D work that has been carried since 2004 by the University of Torino (Italy) with the aim of solving environmental problems and producing revenues from biowastes (Montoneri, 2017). A recent paper (Tabasso et al., 2020) has reported an update of this work, in which new water soluble biopolymers (SBs) have been obtained from the hydrolysis of fermented municipal biowaste (MBW). The SBs are constituted by mixes of macromolecules with 5-over 750 kDa molecular weight. The macromolecules are made of aliphatic C chains, aromatic rings, functional groups with variable acid strength and complexing power, which bond several mineral elements. The whole assembly is reminiscent of the chemical features of the proximates contained in pristine biowastes. These features endow SBs with several properties and allow them to be used in different agriculture and chemical industry sectors.

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

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

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

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

1.2. The MM strategy

Animal manure production in the European Union amounts to 1400 Mt yr^{-1} (European Commission, 2014). Livestock housing, manure storage, urine and dung deposition in grazed pastures, and manure spreading on agricultural land are responsible for emission of 3.6 Mt yr^{-1} of

NH_3 (Eurostat, 2017), and GHG CO_2-eq emissions of 185 N_2O Mt yr^{-1} and 245 CH_4 Mt yr^{-1} (Eurostat, 2018a) calculated from emission weights of 0.70 Mt N_2O and 8.75 Mt CH_4, respectively. The application of manure as soil fertilisation is a common practice (European Union 27, 2020). The application of manure at dose rates in excess, compared to the plant uptake capacity (Khan et al., 2018), causes the leaching of nutrients (e.g., nitrogen and phosphorus) through the soil into ground water. Excessive manure applications to soil also cause GHG and NH_3 emissions. Ammonia from animal manure affects both the air and water. NH_3 5–35 ppm concentrations in air, against 25 ppm threshold level (Ji et al., 2006), and 10-18 g $pig^{-1}d^{-1}$ (Szogi et al., 2015) emissions on pig farms, have been reported. A high NH_3 level can harm both animals and human health. The leaching of ammonium nitrogen through soil and water causes acidification and eutrophication (Leip et al., 2015).

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

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

Traditionally, agriculture and animal wastes were handled at a farm level for recycling as fertilizers. Livestock manure, in both liquid and solid form, is generally stored outdoors and left uncovered, thereby allowing NH_3 and the GHG produced by fermentation processes to be released into the atmosphere. Anaerobic fermentation is a practice that is also used on farms. In this case, manure is stored in a closed vessel where it is converted, in an oxygen-free environment, into biogas, a gas mixture mainly composed of CO_2 and CH_4, that can be used as a renewable energy source. Commercial bioreactors of different sizes for biogas production are available. Apart from biogas, the process yields digestate that contains the residual recalcitrant lignocellulosic matter and ammonia formed from the biodegradation of the pristine proteins (Riggio et al., 2017). In order to comply with the European Union's Nitrate Directive 91/676/EEC (Musacchio et al., 2020), the disposal of the digestate in soil is restricted in order to reduce NH_3 emissions into the air, nitrate formation in soil and leaching into ground water.

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

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

2. Materials and methods

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

2.1. Manure production and collection

Sixty weaned crossbred rabbits (Grimaud x Hycole) were individually reared in the DISAFA rabbit farm in Carmagnola (TO), Italy (44°51¢002N, 7°43¢002E, at an altitude of 240 m a.s.l.). The rabbits were reared under semi-controlled environmental conditions (temperature 22±5 °C, light duration >8 h), in California type cages (30 x 30 x 40 cm; 0.12 m^2 $head^{-1}$) according to the standard procedures. The rabbits were randomly assigned to five groups (12 heads each), while respecting a 1:1 sex ratio. At the beginning of the trial, the animals were 35 days old and had an average body weight of about 1 kg. During the experimental period, the rabbits were fed ad libitum, for 55 days, with isoenergetic (18.8 MJ kg^{-1} of dry matter) and isoproteic (179 g kg^{-1} crude protein on dry matter) diets to which CV SB was added. CV SB was isolated from the alkaline hydrolysate of composted urban gardening and park trimming residues and characterised, as reported by Montoneri et al. (2013). The SB supplementations levels were: 0 (control group; 0-SB), 0.5 (0.5-SB), 2.5 (2.5-SB), 5 (5-SB), 10 g kg^{-1} (10-SB). The SB levels cover the same range of values as those tested in the previous work of the authors (Biagini et al., 2016). The performances of the rabbits during the trial have been presented and discussed in a previous paper (Biagini et al., 2016) and have shown that the addition of SBs to rabbit diets does not affect the live or slaughtering performances of the animals. In fact, the experimental groups, compared to the control ones, have shown an average daily feed intake, an average daily weight gain and an average daily feed conversion rate of 101 *vs* 98 g, 36 *vs* 36 g and 3.4 *vs* 3.4 g g^{-1}, respectively, and the same dressing percentage (59%).

2.2. Preparation of the samples for analyses

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

2.3. pH measurement and chemical analysis of the manure

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

2.4. Measurement of the gaseous emissions from storage manure

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

The NH_3, CO_2, CH_4, and N_2O emissions from each stored manure sample (four samples per treatment, totalling twenty over the five treatments) were measured by means of a dynamic chamber method, using a gas trace analyser (1412 Photoacoustic Multi-gas Monitor, Innova Air Tech Instruments), according to the procedures proposed by Dinuccio et al. (2019). Specifically, gaseous emissions were measured and recorded three times per week during the storage trials for a total of eight times during the 18-day experimental period. All the investigated manure samples were also analysed at the beginning of the storage period for DM, OM, TN, TAN, fibre content (NDF, ADF, ADL) and pH using the previously described procedures. The NH_3, CO_2, CH_4, and N_2O readings (mg m^{-3}) of the photoacoustic analyser were converted into emission fluxes (F, mg m^{-2} h^{-1}) from each vessel as follows:

$$F = Q\,(C_{out} - C_{in}) / A$$

where: Q is the airflow rate (m^3 h^{-1}) supplied to the vessels, C_{in} (mg m^{-3}) is the air inlet gas concentration, C_{out} (mg m^{-3}) is the vessel air outlet gas concentration, and A (m^2) is the emitting surface area of the vessel.

The cumulative emissions (g m^{-2}) of each gas recorded during the experimental period were calculated, according to Pampuro et al. (2016), and expressed in CO_2-eq (IPCC, 2013), which was obtained by multiplying the single gaseous emissions by conversion factors of 1, 28, 265 and 2.65 for CO_2, CH_4, N_2O and NH_3, respectively.

2.5. Statistical analyses

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

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

where: μ = general mean; α_i = session effect; ε_{ij} = random error effect.

As the five groups of manure had a different initial averages of TN, the N_2O and NH_3 emission data were tested, using the GLM ANCOVA procedure (IBM SPSS Statistics 26.0, SPSS Inc., Chicago, IL), according to the following model:

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

where μ is the general mean; α_i is the SB integration effect; $\beta(x_{ij}-x)$ is the effect linearly associated with the TN content; ε_{ij} is the random error effect.

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

3. Results

Table 1 reports the proximate content in the rabbit manure collected before storage. The SB content of the diet affected the manure composition. The DM content of the animal dejections resulted to be within the range reported for solid manure from livestock and poultry production (Bicudo, 2009). The manure obtained from the 5-SB and 10-SB groups contained significantly ($P<0.05$) more DM than the control and the other two 0.5-SN and 2.5-SB groups. Other significant differences pertaining to the SB diets, compared to the control diet, are evident for the TN, TAN, NDF, ADF, ADL and pH indicators.

Fig. 1 reports the mean emission rates for all of the investigated gases at each measurement during manure storage. Each group shows a production peak for the emissions of N_2O on day 7, when the 0-SB groups releases the maximum flux recorded over the experimental period (0.306 mg m^{-2} h^{-1}). The flow shows a decreasing trend, with the lowest value recorded on the last day of the test when the 5-SB group releases the lowest amount, that is, of 0.066 mg m^{-2} h^{-1}. The manure obtained from the groups of rabbits fed with the 5-SB and 10-SB diets shows the lowest N_2O emissions of all the 5 experimental groups. Generally, through the trials, the diets containing SB exhibit lower N_2O emission than the control group. The mean N_2O fluxes of the diets containing SB range from 0.25 on day 7 for the 0.5-SB and 2.5-SB groups to 0.08 mg m^{-2} h^{-1} on day 18 for the 5-SB group. Similarly, the ammonia emissions show a peak value on day 7, when the maximum, that is, of 49.71 mg m^{-2} h^{-1} occurs for the 5-SB group, and the lowest value, that is, of 11.04 mg m^{-2} h^{-1} is shown for the 2.5-SB group. As far as ammonia is concerned, the groups showing the highest emissions are the same as those that produced the lowest N_2O emissions (groups 5-SB and 10-SB).

The CH_4 and CO_2 emission patterns are different from the N_2O and NH_3 emissions, as the former already reach peak values on day 2. The highest CH_4 emission (7.08 mg m^{-2} h^{-1}) occurs on day 2 for the 0.5-SB group. The lowest emission (1.43 mg m^{-2} h^{-1}) occurs on day 18 for the 10-SB group. At the end of the trial, the CH_4 flux averaged over all groups results 1.73 mg m^{-2} h^{-1}. The highest CH_4 emission, that is, 5.45 mg m^{-2} h^{-1}, averaged over all the groups, is on day 7, and the lowest, 1.70 mg m^{-2} h^{-1}, occurs on day 16. The mean CO_2 fluxes range from 384.91 on day 7 to 111.28 mg m^{-2} h^{-1} on day 18. The 5-SB group exhibits the highest emission values between day 2 and 14, and then decreases more rapidly than the others groups, reaching the lowest emission value recorded for all the groups.

Table 2 reports the cumulative emission values for all investigated gases over the entire 0-18 days of the trials. The manure produced by the rabbits fed the 5-SB diet shows the lowest N_2O cumulative emission value. The diets rank in the following statistical order of increasing N_2O emissions: 5-SB < 10-SB < 2.5-SB = 0.5-SB = 0-SB. On the other hand, the manure produced by the rabbits fed the 2.5-SB diet shows the lowest cumulative NH_3 emission value. The diets rank in the following order of increasing NH_3 emissions: 2.5-SB < 0.5-SB ≤ 0-SB ≤ 10-SB ≤ 5-SB. The 5-SB diet, which produces the lowest amount of N_2O emissions, also produces the highest amount of NH_3 emissions. However, the 2.5-SB, which produces the highest N_2O emissions, produces the lowest NH_3 emissions.

Different trends are evident for the two other GHG gases. The cumulative CH_4 emission tends to decrease as the SB level increases. It shows a significantly lower value for the 10-SB groups. The 5-SB group exhibits the highest emission value for CO_2.

4. Discussion

The experimental data show that the CV SB content in the diet affects the manure composition, the emission pattern and the level of the different gases. The effects of SBs have been reported in many different sectors, where these biopolymers have been tested (Montoneri, 2017). These reports not only cover the animal sector, as specifically addressed in the present work, but also a variety of other agricultural fields and uses in the chemical industry. As a result of their surfactant properties, SBs have shown to be efficient in the remediation of soil and water contaminated with industrial organic and trace pollutants (Tabasso et al., 2020), in the formulation and fabrication of chemical speciality products, such as detergents (Savarino et al., 2010), and in auxiliaries for textile dyeing (Savarino et al., 2009). Because of their polymeric nature, SBs have been used for the fabrication of mulch films (Nisticò et al., 2017). Because of the presence of plant nutrient mineral elements, SBs are also efficient soil fertilisers (Sortino et al., 2014) and plant growth biostimulants (Massa et al., 2016). The production cost of SBs has been estimated as 0.2 € kg^{-1}, against a potential selling value that ranges from 1 to 800 € kg^{-1}, depending on the market sector where the product is allocated (Montoneri, 2017). On the basis of their properties, performance, production cost and potential market value, SBs can undoubtedly be considered sustainable products. The capacity of SBs to reduce the ammonia content in the anaerobic fermentation digestates of municipal kitchen wastes (Francavilla et al., 2016) and cow manure (Riggio et al., 2017) is particularly relevant, in relation to the MM strategy reviewed in *subSection 1.2*.

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

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

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

The data in Table 2 show that the effect of the diet SB content on the gas emissions is not linear. Moreover, the lowest ammonia emission from the 2.5-SB diet (144 g m^{-2}) is accompanied by the lowest CO_2 eq (3498 g m^{-2}) emissions. Similar findings are reported (see *subSection 1.1* above) for the *in-vitro* caecal fermentation of SB supplemented pig diets (Montoneri et al., 2013). Apart from the reasons for these trends, the consistency of the data obtained in the present manure study and in previous work on the *in-vitro* caecal fermentation of pigs, and in the anaerobic fermentation of urban food wastes (Francavilla et al., 2016) and cow manure (Riggio et al., 2017) performed in closed bioreactors validates the reliability of the SB effect on lowering the emissions of noxious gases.

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

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

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

The available literature reports gas emission data in different units and from different experimental set ups, which make it difficult to conduct comparative evaluations. In order to circumvent this difficulty, Table 3 reports the gaseous emissions from manure as percentage changes of the gaseous emissions affected by the CV SB supplemented diets, relatively to emissions from the manure from rabbits fed the control diet (no added SB). The data show the significant effect of the 2.5-SB diet on causing a 30% decrease in ammonia emissions from the manure of rabbits fed this diet, along with decreases of the other GHG emissions, that is, 9% for N_2O, 25% for CH_4 and 8% for CO_2. On the other hand, the *in-vitro* caecal fermentation of pig diets supplemented with 2 g kg^{-1} CV SB (Montoneri et al., 2013) produced 17% less ammonia and 36% less cumulative biogas volume, compared than the control diet containing no SB. The consistency of the SB effects under different experimental conditions, involving different animals and different control diets, is remarkable. This result seems to suggest that the SB effects could be replicated for other animals in other environments. The effects of the 2.5-SB diet are even more remarkable, when compared with those reported by Dinuccio et al. (2019). These authors tested the practice of incorporating manure into soil, which is recommended by the Italian Government regulation as a strategy to abate ammonia and GHG emissions, instead of spreading manure on the land surface. They reported that, compared to the deposition of manure on the soil surface, the incorporation of rabbits' manure into the soil yielded an ammonia abatement of 42.0%, but the N_2O, CH_4 and CO_2 emissions increased by 37.0%, 57.3% and 34.8%, respectively. These data show that, although manure soil incorporation may in principle be a practice sustainable in farms of any size, it worsens the impact of GHG emissions from manure. However, the SB diet approach may allow the impact of both ammonia and other GHG emissions to be reduced.

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

The experimental GHG emission data are related to the storage of rabbit manure. For this specific case, the percentage emission reduction of the 2.5 SB diet in Table 3 allows the potential reduction of GHG and ammonia from rabbit manure storage to be estimated at a world level upon full implementation of the SB strategy. Extrapolating the values given in Table 3 to the world's rabbit population (FAOSTAT, 2018) and their gaseous emissions during 35 days' manure storage (Dinuccio et al., 2019), it is possible to estimate reductions of 9.0 N_2O, 4328 NH_3, 836.4 CH_4 and 48,354 CO_2 kt yr^{-1}. These reductions are equivalent to 98,563 kt yr^{-1} CO_2 eq. Taking in consideration the results of the present work and those obtained in previous work on the effects of SB in the *in vitro* pig caecal fermentation (Montoneri et al., 2013) and the anaerobic fermentation of cow manure (Riggio et al, 2017), it is possible to expect that the SB effects may be general for all livestock. Under this hopeful hypothesis, and considering the level of the European Union global livestock emissions of ammonia and GHG given in *Section 1* and *1.2,* as well as hypothesizing that the order of magnitude of the emission reduction in Table 3 could be replicated across most of the European Union livestock population, it is possible to imagine the full potential environmental benefit of the SB strategy at a European Union level.

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

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

The full relevance of the present and previous (Montoneri et al., 2017) work on the valorisation of SBs may be appreciated by considering their many properties, potential uses and the benefits for the growing biobased economy (Tabasso et al., 2020). SBs are products that are sourced from urban biowastes and applied in various sectors of agriculture and the chemical industry. In this context, they represent a virtuous link that connects urban, rural and industrial environments. Producing SBs from MBW and using them on farms as soil fertilisers, plant growth biostimulants, animal diet supplements, additives for closed fermentation bioreactors to produce biogas and digestate with a low ammonia content, or using them for the manufacturing of biobased chemical specialities, returns renewable C in cities to agricultural land. This promotes the production of biomass for human consumption that generates more MBW, from which further SBs are produced. This scenario realises a virtuous C cycle, which involves developing a circular biobased economy with environmental, economic and social benefits. Compared to commercial products for the same uses, the potential market value of SBs has been estimated as 1.5 to 800 € kg^{-1}, against a production cost of 0.2 € kg^{-1} (Montoneri et al., 2017). The market value depends on the type of SB obtained from the type of sourced MBW and on the market sector where the SB may be allocated. For example, in the agriculture sector, because of their performance as soil fertilisers (Sortino et al., 2014) and plant biostimulants (Massa et al., 2016), D SB and CVD SB are potentially worth 1-3 € kg^{-1}, D and CVDF SB, because of their use as plant disease suppressants (Jindrichova et al., 2018), can be sold as much as 800 € kg^{-1}, while D SB, because of its use as a specialised surfactant (Montoneri et al., 2020) can reach a market value of 150 € kg^{-1}. Currently, MBW treatment plants process urban wastes through fermentation and produce biogas, digestate and/or compost. The selling value of these products, relative to their production cost, is quite low. The excess cost is covered by the tipping fees municipalities pay to the MBW processing plants. By integrating their fermentation facilities with chemical facility producing SBs from the anaerobic digestate and composts of the plant, the MBW plant revenue could be enhanced 100 to 1000 times. This would be enough to allow citizens' taxes to lowered. An estimate of the environmental and socio-economic impacts at a European Union level for the full implementation of SB at an industrial and commercial level has been published (Montoneri et al., 2017).

5. Conclusions

Rabbits fed diets containing 2.5 g kg^{-1} of CV SB produce manure that emits 30% less ammonia and significantly lower GHG (- 9% N_2O, - 25% CH_4 and – 8.0% CO_2) emissions, than rabbits fed a control diet containing no CV SB. These results and those of previous work (Montoneri, 2017) suggest two alternative sustainable strategies for use on farms: 1) the SB assisted anaerobic fermetation of manure in closed reactors; 2) the production of low gaseous emission manure by animals fed SB supplemented diets. These alternatives are low cost strategies. They both require the negligible costs for SB consumption. The former alternative also involves CAPEX cost of the bioreactor. Commercial anaerobic reactors of different sizes and processing capacities are available at reasonable prices. SB is not commercially available yet. However, thanks to the European Union's funding of the currently running Lifecab (2020) project, a prototype reactor, with a 5 kt yr^{-1} SB production capacity, has been built. This will allow enough SB to be produced to test and demonstrate the replicability of the two strategies in different urban and rural environments. This is a step forward along the route to transferring the SB-based technology to a real operational and commercial level.

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

Declaration of competing interest

None.

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Table 1

Analytical values (w/w%)[a, b] for manure produced by rabbits fed with the control diet (0-SB) and with the diets containing 0.5 (0.5-SB), 2.5 (2.5-SB), 5 (5-SB) and 10 (10-SB) g L^{-1} 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

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

[b] Values within rows, followed by different letters with (a, b and c), are significantly different ($P < 0.05$).

Table 2

Cumulative gaseous emissions (g m^{-2})[a,b] from manure produced by rabbits fed with the control diet (0-SB) and with the diets containing 0.5 (0.5-SB), 2.5 (2.5-SB), 5 (5-SB) and 10 (10-S) g L^{-1} of CV SB over the 18 days' duration of the trials.

	0-SB	0.5-SB	2.5-SB	5-SB	10-SB
N_2O	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_3	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_4	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_2	1852±148 b	1779±148 b	1993±148 ab	2533 ±148 a	2034±148 ab
CO_2 eq	3804±234 ab	3618±234 ab	3498±234 b	4226±234.15 a	3596±234 ab

[a] Mean ± standard error values calculated from measurements on 4 samples.

[b] Values within rows, followed by different letters (a, b, c and d) are significantly different ($P < 0.05$).

Table 3

Percentage changes[a] in the cumulative gaseous emissions from manure produced by rabbits fed diets containing SB (0.5 to 10-SB), relative to the manure emissions from the rabbits in the control 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_2O	-6.92	-10.8	-35.0	-23.8
NH_3	-6.13	-30.3	19.7	10.5
CH_4	-5.26	-24.8	-21.1	-35.2
CO_2	-3.96	-8.01	36.7	9.80
CO_2 eq	-1.96	-13.0	14.5	-2.56

[a] Percent change (PCC) calculated from the Table 2 data according to the equation: PCC = 100 (i-SB value – 0-SB value)/0-SB value, where i = 0.5 to 10.

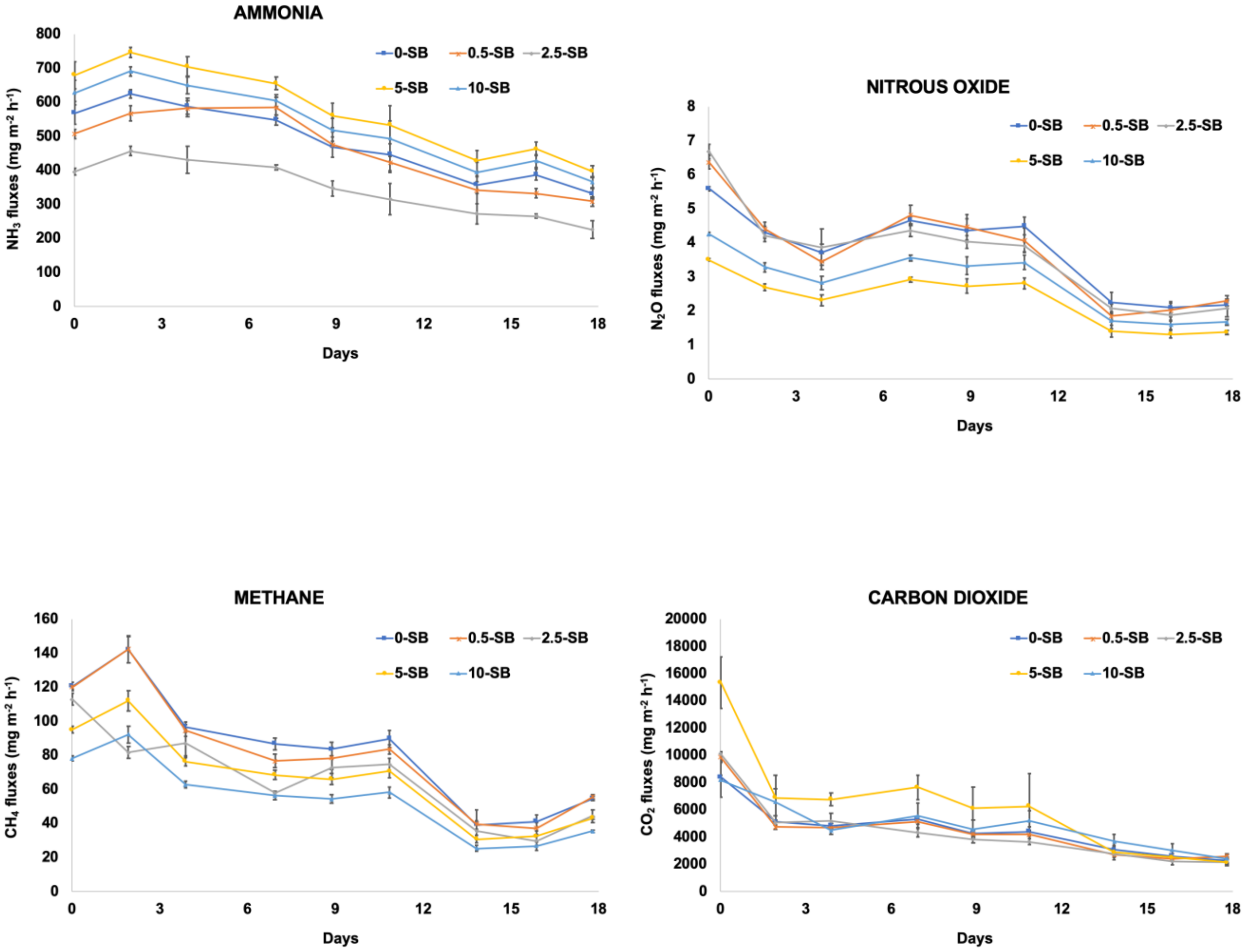

Fig. 1. Gaseous emissions (g m $^{-2}$ h^{-1}) recorded during the trials from manure produced by rabbits fed the control diet (0-SB) and the diets containing 0.5 (0.5-SB), 2.5 (2.5-SB), 5 (5-SB) and 10 (10-S) g L^{-1} of CV SB.