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Reduction of chosen gaseous pollutants from rabbit excrement in *ex situ* conditions, using natural manure additives

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Abstract: Animal production is a major source of environmental pollutants, so it is becoming crucial to search for new methods to reduce their release while maintaining animal welfare. The aim of the study was to apply natural additives to rabbit manure in *ex situ* conditions to reduce the volume of released gaseous pollutants like ammonia (NH_3), methane (CH_4) and hydrogen sulphide (H_2S). The study was carried out in two stages, each with a control group and five experimental groups with additives, natural sorbents (zeolite, bentonite biochar, perlite, mixtures in various proportions of zeolite, biochar and bentonite as well as perlite and biochar) or dried plants containing saponins (*Tribulus terrestris* and *Lysimachia nummularia*). Pollutants from each group were measured continuously for one month. In stage 1, both in the case of NH_3 and CH_4, statistically significant differences were observed between the tested groups. The use of sorbent mix and *Tribulus terrestris* was shown to reduce the release of both NH_3 (by 80% and 83%, respectively) and CH_4 (by 17% and 25%, respectively). The greatest reduction of NH_3 in stage 2 was achieved when perlite with the addition of biochar was used (56%), and CH_4 was achieved when *Bacillus azotofixans* sp. nov. bacteria were used (38%).

Keywords: ammonia, gaseous pollutants, hydrogen sulphide, methane, natural sorbents, rabbit manure

INTRODUCTION

Awareness of problems associated with pollution of the natural environment is continually growing (Naidu *et al.*, 2021; Ahmed *et al.*, 2022; Tonhauzer, Zetochová and Szemesová, 2023). The most attention is paid to agricultural pollution in dominant sectors of animal production, such as pigs, poultry, and dairy cows, without considering small animals such as rabbits. Interest in production of rabbit meat is continually growing, and Poland is a valued producer, resulting in increased manure production and a greater burden on the environment (Kowalska, Gugołek and Strychalski, 2016; Składanowska-Baryza, 2017; Dinuccio *et al.*, 2019; Tonhauzer, Zetochová and Szemesová, 2023). According to the Announcement of the Marshal of the Sejm of the Republic of Poland (Obwieszczenie, 2023), manure is an organic fertiliser which requires appropriate management. The measures implemented contribute to a more positive perception of animal production. There are numerous programmes based on legal guidelines for limiting pollution from sectors of the economy, including animal production (Directive, 2016; Obwieszczenie, 2023). One important aspect of this problem is emissions of gaseous pollutants, such as ammonia (NH_3), methane (CH_4), and hydrogen sulphide (H_2S). Released pollutants can have serious health effects for people, animals, and entire ecosystems (Nowakowicz-Dębek *et al.*, 2014; Marszałek, Kowalski and Makara, 2018; Lins and Lins, 2020; Nowakowicz-Dębek *et al.*, 2020; Ossowski *et al.*, 2022). Douglas *et al.* (1994) exposed young rabbits to gaseous pollutants and found that the initial exposure to an allergen determines the animals' sensitivity later on. The researchers observed hyperreactivity of the respiratory tract and an increase in class E antibodies in the experimental animals (Douglas, Price and Page, 1994).

Ammonia and hydrogen sulphide, in addition to producing unpleasant odours, can negatively affect air quality and the state of the aquatic environment (Richard *et al.*, 2023). Air quality on farms is influenced by rabbit feeding and housing (Calvet *et al.*, 2008; Nowakowicz-Dębek *et al.*, 2020). Rabbits excrete about 60% of the nitrogen they take in as urine and faeces, so the resulting manure is rich in these compounds (Nowakowicz-Dębek *et al.*, 2020). Methane is a gas which is permanently present in agricultural pollution. Methane released from animal waste has the potential to increase the greenhouse effect and accelerate negative climate changes (Kweku *et al.*, 2018). Rabbit breeding differs from other animal production in terms of maintenance conditions and management of excrements, which directly influences the amount of gaseous pollutants released (Calvet *et al.*, 2011). Apart from chemical pollutants on rabbit farms, high concentrations of pollution in the form of biological aerosol are suspended in the air on dust particles. Maintaining optimal conditions on the farm is a crucial element of the environmental control system, especially given that pathogens can be emitted even 45 m from vents (Song *et al.*, 2023). The presence of organic dust is linked to the aetiology of numerous respiratory diseases in rabbits, as well as in people. The concentration of fungal aerosols may be correlated with the dust concentration, due to the characteristics of the feed and bedding material used. At the same time, vector transmission of respiratory diseases, including zoonotic diseases, can take place via inhalation (Song *et al.*, 2023). Particulate air pollution (PM2.5) may also be correlated with chemical pollutants such as NH_3 and H_2S. Pu *et al.* (2022), in a study of air pollution in a pig-fattening house, reported strong relationships between NH_3 and H_2S and between NH_3 and CO_2, indicating feed and manure as potential sources of the pollutants. The scientific literature contains little information on the negative environmental impact of commercial breeding of rabbits. There are also few studies on nitrogen losses resulting from poorly balanced rabbit diets, which influence the amount of pollutants released to the atmosphere. Therefore, there is a need to develop and implement effective methods to reduce gaseous pollutants, in order to minimise the negative impact of this aspect of animal production on the natural environment. Natural additives provide an opportunity to reduce the release of gaseous pollutants at the level of the farm (Stavi and Lal, 2013; Sejian *et al.*, 2015). These additives may be natural sorbents or dried plants containing saponins (such as *Lysimachia nummularia* or *Tribulus terrestris*). Natural sorbents are mainly aluminosilicates (including bentonite, zeolite), activated carbon and their mixtures, known for their sorption properties (Pliś *et al.*, 2015; Kwaśny *et al.*, 2020). Thanks to their unique structure, their properties can be used to reduce gas emissions in agriculture or animal production (Wlazło *et al.*, 2016). The Code for counteracting odour nuisance (Kodeks, 2016) indicates the use of saponin as one of the methods to reduce emissions. *Lysimachia nummularia* and *Tribulus terrestris* contain saponins, which have a killing effect against uricolitic bacteria (Liu *et al.*, 2023). The aim of the study was to determine the level of reduction of gaseous pollutants from rabbit droppings in *ex situ* conditions using natural sorbents or dried plants as manure additives.

MATERIALS AND METHODS

The materials used in the study were rabbit manure, natural sorbents, and ground dried plants containing saponins. The *ex situ* study was carried out in two stages due to the limitations of the analyser, which can take six gas samples at one time. The additives were allocated to the two stages. In each stage there was a control group as a reference for the measurements.

In stage 1, samples of rabbit manure were divided into six groups: a control group and five experimental groups in which natural additives were used (Tab. 1).

The characteristics of the rabbit manure used in the experiment are presented in Table 2. The analyses were performed in an accredited laboratory of District Chemical and Agricultural Station (DCAS) in Lublin.

The rabbit manure was transported to the laboratory and a bulk sample was prepared from one batch and mixed. The material prepared in this way was weighed on a Radwag scale

Table 1. Experimental groups in stages 1 and 2

Stage 1		Stage 2	
group	**description**	**group**	**description**
C	control group without sorbent additives	C1	control group without sorbent additives
D1	with 1% addition of zeolite	B1	with 1% addition of perlite
D2	with 1% addition of biochar	B2	with 1% addition of a mixture of sorbents: perlite and biochar
D3	with 1% addition of bentonite	B3	with 1% addition of a mixture of sorbents: perlite and creeping jenny (*Lysimachia nummularia*)
D4	with 1% addition of a mixture of sorbents: zeolite, biochar and bentonite in proportions of 1:1:1	B4	with 1% addition of *Lysimachia nummularia* – LN
D5	with 1% addition of *Tribulus terrestris* – TT	B5	with 1% addition of *Bacillus azotofixans* bacteria

Source: own elaboration.

Table 2. Characteristics of rabbit manure used in the experiment of fresh sample weight

Parameter	Measurement unit	Concentration of ingredients
pH	–	6.7
Conductivity	$mS \cdot m^{-1}$	7.8
Dry matter	%	37.6
NH_4-N	$Mg \cdot kg^{-1}$	2.67
N	%	1.29
P	%	0.36
P_2O_5	%	0.81
K	%	1.76
K_2O	%	2.12
Ca	%	0.65
Mg	%	0.20

Source: own study.

(Radwag, Poland) – 100 g from each batch of material. The tested additives in the form of natural sorbents, powdered dried *Tribulus* and sage moths, and *Bacillus azotofixans* bacteria were prepared in advance in order to combine and mix each sample immediately after making manure samples. The basic composition of manure is shown in Table 2. The prepared samples were placed in incubation containers connected with a polytetrafluoroethylene tube to a Fresenius GA220 gas analyser (GmbH, Germany). The multi-gas analyser used in the study operates in electrochemical reaction technology conducted in the non-dispersive infrared (NDIR) system in measurement cuvettes heated to 80°C. The experiment was conducted for a month at an average temperature of 25°C and a relative humidity of 50.9%. The multi-gas analyser was calibrated for quantitative measurements of NH_3, CH_4 and H_2S and equipped with a reference system (Ossowski *et al.*, 2022). For each group, 180 measurement cycles were recorded over 24 hours and the results were averaged to obtain daily averages. The reduction in released gaseous pollutants for each group in each stage was calculated according to the Equation (1) (Szymula *et al.*, 2021):

$$Re = 100\% - \left(\frac{CG \cdot 100\%}{CC}\right) \tag{1}$$

where: *Re* = reduction in NH_3, CH_4 or H_2S (%), *CG* = average concentration of NH_3, CH_4 or H_2S in group, *CC* = average concentration of NH_3, CH_4 or H_2S in control group.

Statistical analysis of the results was conducted separately for the two stages, using Statistica ver. 13.3. For each of the pollutants tested (NH_3, CH_4 and H_2S), the mean (*M*) and standard deviation (*SD*) were determined for each group. ANOVA was used to determine differences between groups within stages, after testing the distribution for normality. If the criteria were met, ANOVA was performed; otherwise Kruskal–Wallis ANOVA was used. For both tests the level of significance was $p < 0.05$. Statistically significant values were designated with letters (a, b, …).

RESULTS AND DISCUSSION

At the start of the first stage of the experiment, a high NH_3 concentration was observed in group C (control), followed by a marked decrease in all groups (Fig. 1). The average concentration of this pollutant in stage 1 ranged from 1.26 to 253.21. Both extreme values were in group C (control).

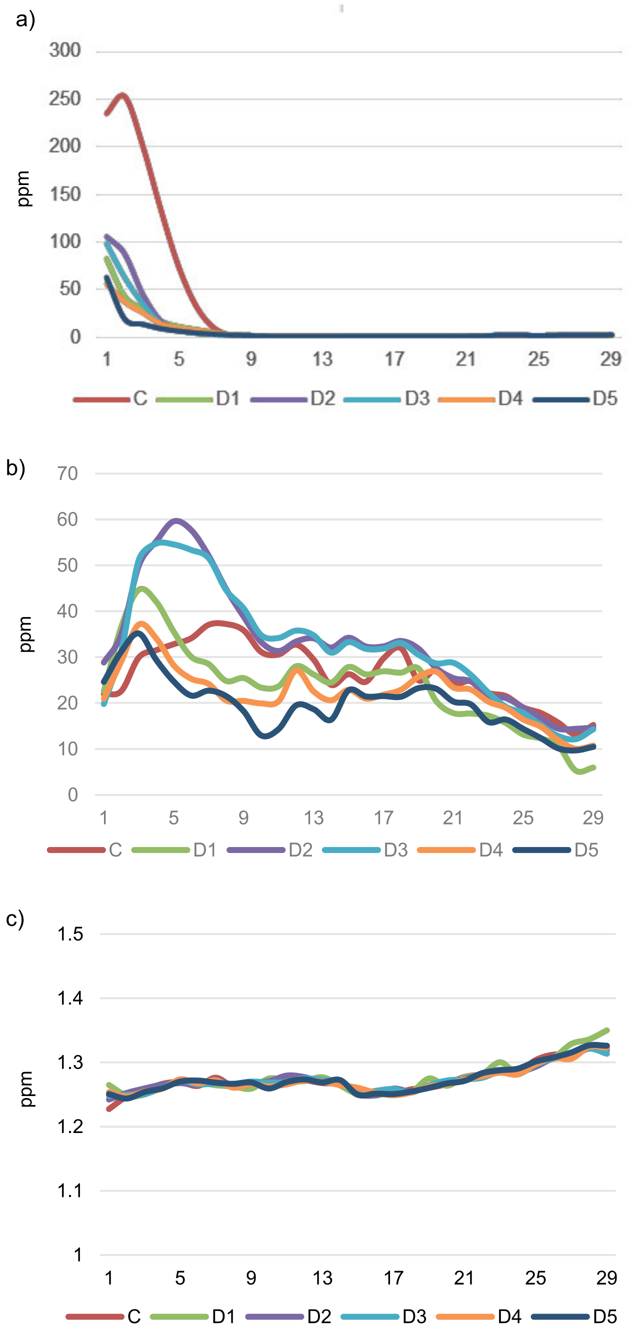

Fig. 1. Concentration of investigated gaseous pollutants in each group in stage 1 (ppm): a) ammonia, b) methane, c) hydrogen sulphide; C, D1–D5 as in Tab. 1; 1–29 = days of experiment; source: own study

The CH_4 concentration in stage 1 ranged from 5.25 (D1 – zeolite) to 59.58 (D2 – biochar) – Figure 2. The concentration of H_2S in stage 1 was similar in all groups, averaging 1.28 ppm. There were slight fluctuations in the concentration of the gas during the experiment, but they were not statistically significant

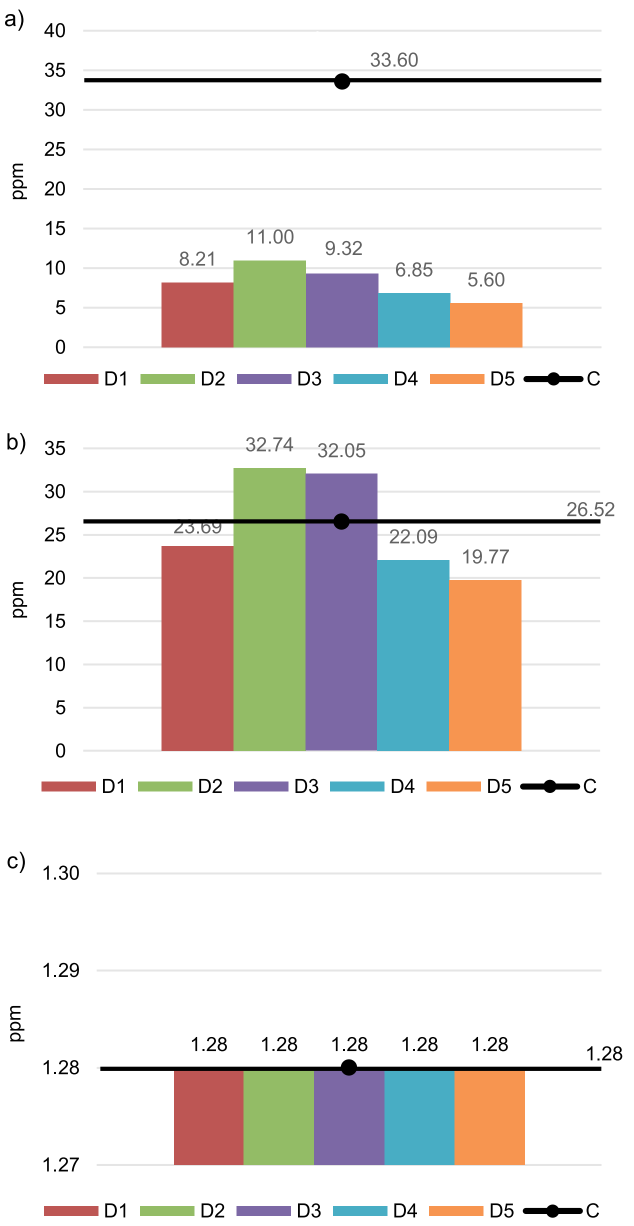

Fig. 2. Concentration reduction of investigated gaseous pollutants (ppm) in experimental groups in relation to the control group in stage 1: a) ammonia, b) methane, c) hydrogen sulphide; C, D1–D5 as in Tab. 1; source: own study

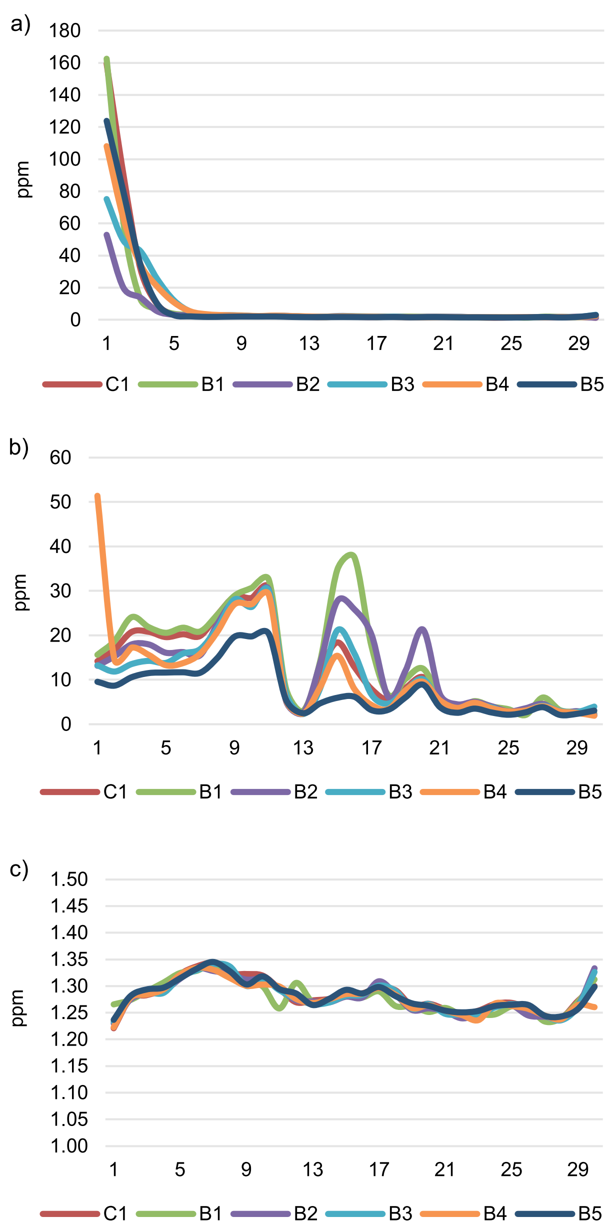

Fig. 3. Concentration (ppm) of investigated gaseous pollutants in each group in stage 2: a) ammonia, b) methane, c) hydrogen sulphide; C, D1–D5 as in Tab. 1; 1–29 = as in Fig. 1; source: own study

(Fig. 3). The level of the reduction in individual gaseous pollutants in all groups in stage 1 of the experiment is presented in Figure 2.

The average concentration of NH_3 in the experimental groups ranged from 5.6 (D5 – TT) to 33.6 (C – control). Reduction in NH_3 in relation to the control group C (33.6 ppm) was noted in every of the experimental group. In the case of CH_4, not every study group noted a reduction. In the groups D2 (biochar) and D3 (bentonite) was recorded an increase of the CH_4 level. In the rest groups there was reduction, the most (25%) in the D5 (TT). The concentration of H_2S was not shown to be reduced by the additives. In the first few days of stage 2 of the experiment, a high NH_3 concentration was observed in all groups, followed by a significant decrease. After a week the values stabilised until the end of the experiment (Fig. 3, Tab. 3).

The CH_4 concentration in stage 2 of the experiment ranged from 1.89 (B4 – LN) to 51.38 (B4 – LN). The concentration of this pollutant changed dynamically in group B5, ultimately attaining its lowest level – 1.89. In the case of H_2S, as in the first part of the experiment, the levels were similar in experimental groups and ranged from 1.22 to 1.35 ppm. In stage 2 there was a reduction in NH_3 released from rabbit manure in all groups. The greatest reduction was noted in groups B2 (biochar), in which the NH_3 level was reduced by 57% (Fig. 4).

Similarly, levels of reduction of CH_4 were obtained in stage 2 of the study, where in the two of the groups wasn't noticed the reduction. The remaining three groups achieved pollution levels lower than those in the control group. The best level of reduction (38%) was achieved by the group B5 (*Bacillus azotofixans* bacteria). The concentration of H_2S released from rabbit manure

Table 3. Statistical analysis of the results in stages 1 and 2

Group	Statistics	NH_3	CH_4	H_2S
Stage 1				
C	*M* (ppm)	33.6a	26.52	1.28
	SD (ppm)	73.72	6.62	0.02
D1	*M* (ppm)	8.21	23.69a	1.28
	SD (ppm)	17.21	9.43	0.03
D2	*M* (ppm)	11	32.74bc	1.28
	SD (ppm)	25.43	12.79	0.02
D3	*M* (ppm)	9.32	32.05bc	1.28
	SD (ppm)	21.48	12.72	0.02
D4	*M* (ppm)	6.85bc	22.09ad	1.28
	SD (ppm)	12.51	6.06	0.02
D5	*M* (ppm)	5.6bc	19.77ad	1.28
	SD (ppm)	11.82	6.12	0.02
p-value		0.02	0.00	0.99
Stage 2				
C1	*M* (ppm)	11.35	12.1	1.28
	SD (ppm)	32.62	9.09	0.03
B1	*M* (ppm)	9.84	14.75	1.28
	SD (ppm)	30.78	11.06	0.03
B2	*M* (ppm)	4.83	13.08	1.28
	SD (ppm)	9.88	9.17	0.03
B3	*M* (ppm)	8.65	10.83	1.28
	SD (ppm)	17.22	8.18	0.03
B4	*M* (ppm)	9.71	11.47	1.28
	SD (ppm)	22.52	10.99	0.03
B5	*M* (ppm)	9.85	7.48	1.28
	SD (ppm)	26.35	5.57	0.03
p-value		0.94	0.07	0.98

Explanations: *M* = means; *SD* = standard deviation; a, b, … = values with different letters are significantly different at $p < 0.05$.

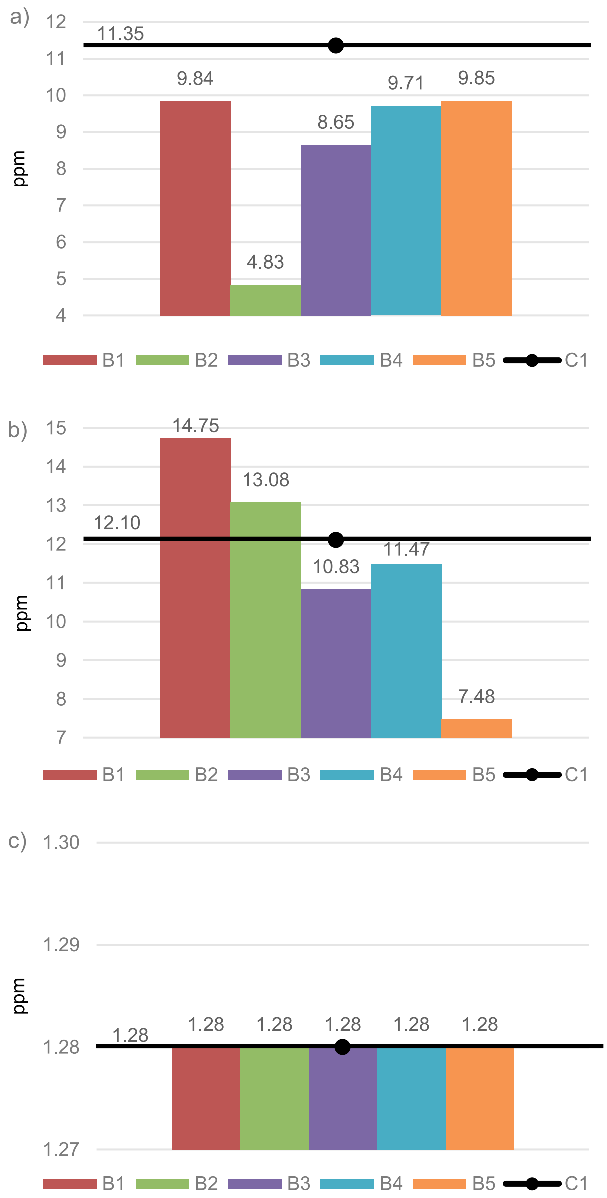

Fig. 4. Concentration reduction of investigated gaseous pollutants (ppm) in experimental groups in relation to the control group in stage 2: a) ammonia, b) methane, c) hydrogen sulphide; C, D1–D5 as in Tab. 1; source: own study

was similar in both stages of the experiment. The reduction of this pollutant was negligible in all experimental groups.

In stage 1 of the study, NH_3 concentration was higher in the control group (C) and was statistically significant at $p < 0.05$. In turn, the amount of CH_4 released was statistically significant between the experimental groups (Tab. 3). In recent years many studies have dealt with the problem of air pollution in animal production, associated with emissions of odorous substances as well as with biogenic emissions from manure heaps into the environment (Calvet *et al.*, 2011; Nowakowicz-Dębek *et al.*, 2014; Dinuccio *et al.*, 2019; Szymula *et al.*, 2021). Safe manure utilisation technologies are sought with the aim of reducing nutrient losses (Petersen *et al.*, 2007; Petersen and Sørensen, 2008; Dinuccio *et al.*, 2019). According to Dinuccio *et al.* (2019), there is little information on emissions of NH_3 and greenhouse gases (GHGs) from rabbit manure. The increased demand for rabbit meat necessitates pro-environmental action, as according to Dinuccio *et al.* (2019), the release of nitrogen compounds increases with the growth of these animals. In a study of manure in dynamic chambers, the authors obtained the highest levels of NH_3 (315 $mg \cdot m^{-2} \cdot h^{-1}$) and N_2O (0.7 $mg \cdot m^{-2} \cdot h^{-1}$) on day 7, followed by a sharp decrease. The authors noted an increase in NH_3 concentration in the first period, similarly to their own research. This is due to the peculiarities of manure decomposition, where the energy released from decomposition causes an increase in temperature and an increase in gas release. An increase in oxygen availability in individual phases may affect the dynamics of this process. The obtained results were consistent with the research of Olszewski, Dach and Jędruś (2005) and Dach and Zbytek (2008). For CH_4, the highest values were obtained at the start of the experiment, and by day 4 the level had dropped significantly to 30 $mg \cdot m^{-2} \cdot h^{-1}$. According to the authors, the release of pollutants from manure depends not only on the amount of N in the faeces, but also on air exchange. The levels of pollutants obtained in the present study were significantly lower.

Wang *et al.* (2022) used microbes, enzymes, and natural sorbents (calcium or biochar) in composting of manure. These authors found that these additives accelerate the composting process through changes in physicochemical parameters and the production of passive mechanisms on the surface, which affects the content of nitrates (V). In addition, it creates a good environment for the development of microbes, including *Bacillus*, *Peptostreptococcus*, and *Clostridium*, especially in the thermophilic phase. According to Gómez-Brandón, Lores and Domínguez (2013), composting of rabbit manure is an environmentally friendly form of manure processing, but takes a long time – more than 200 days.

Szymula *et al.* (2021) used natural sorbents, in particular biochar and a mixture of bentonite and zeolite, to reduce NH_3 from cattle waste, obtaining significant reductions in this pollutant (42.56 and 24.06%, respectively). Similar results were reported by Kaikiti, Stylianou and Agapiou (2021), who used biochar to absorb volatile organic compounds and certain inorganic gases. Following the use of the sorbent, the authors noted a reduction in emissions of NH_3 and volatile organic compounds (VOCs) at levels of 90 and 60%. Shah *et al.* (2018) demonstrated that zeolite added to bedding also has a beneficial effect by reducing levels of NH_3 released from animal faeces. The reduction in this gas following the application of biochar was similar to the values obtained in the present study.

In choosing natural additives to reduce the release of pollutants, it should be borne in mind that they may also modify processes taking place in bedding, in digestion, or in fermentation. In an experiment carried out by Emmerling, Krein and Junk (2020), the NH_3 reduction strategy successfully reduced the NH_3 level, but at the same time increased the level of N_2O. Moreover, the reduction in CH_4 was associated with a measurable increase in the level of CO_2. Similarly, in the present study, a reduction in the release of one pollutant did not always correspond to a reduction in the concentrations of the other gases. Vinci and Rapa (2019) used various substrates, including perlite, vermiculite, peat, and sand, in hydroponic systems to assess the effects of environmental changes. In the life cycle assessment), the perlite substrate showed certain inconsistencies.

However, the authors emphasise that it has a low carbon footprint compared to the other substrates tested. Rangling *et al.* (2022) showed that compost from rabbit manure can be used to grow seedlings due to its low moisture level, low content of heavy metals, high content of lignocellulose, and good fertilising effects. Cabanillas, Stobbia and Ledesma (2013) report that compost from rabbit manure can be an effective alternative to urea in the production of basil (*Ocimum basilicum* L.), especially as a partial replacement for peat. The use of rabbit manure as fertiliser requires storage and composting (Rangling *et al.*, 2022). It may be an effective solution to the problem of emissions of pollutants from composted manure and rabbit excrements, and it will reduce emissions of GHGs (Meng *et al.*, 2022).

According to Lonardo di *et al.* (2021), the use of rabbit manure alone as fertiliser has certain physicochemical limitations. Preparing manure by mixing it with natural sorbents will give it better fertilising properties and minimise emissions of gases. Research by Vinci and Rapa (2019) and Meng *et al.* (2022) indicates that the natural sorbents perlite and vermiculite are the most commonly used additives in growing media, especially for regulating ventilation and retaining moisture in excrement. The need to conduct further research on the enrichment of rabbit manure with natural sorbents and to establish their optimal proportions is demonstrated by Zhang, Duan and Li (2012), Çelebi (2019), and Li *et al.* (2022), who prepared growing media by mixing peat and perlite. Unfortunately, the literature contains very little information on the possibility of replacing peat with rabbit manure, although this use of animal excrement seems to be very promising and should be verified in terms of agronomic and economic effects as well as environmental protection. Only Rangling *et al.* (2022), who used 25% perlite, 25% vermiculite and rabbit manure in fertiliser mixtures, show that it is a promising fertiliser with suitable physicochemical properties. The authors indicate that growing media based on rabbit manure have similar or better properties (e.g. porosity, bulk density, organic matter and nutrient effects) than standard peat substrate (Li *et al.*, 2022).

Appropriate management of rabbit manure and its use for agricultural purposes prove to be able to reduce GHG emissions from excrements themselves, and furthermore, preparation of fertilisers using rabbit manure enriched with natural sorbents can limit the amount of GHGs emitted during peat extraction. Therefore, manure piles should be monitored with the aim of minimising the level of pollutants released to the environment. The use of a suitably chosen additive to the manure pile will then make it possible to reduce emissions of pollutants into the environment.

CONCLUSIONS

The ammonia (NH_3) level in all experimental groups was reduced compared to the control group, with similar values of the other pollutants tested – NH_3, H_2S. The best effect in the first stage of the experiment was recorded in manure containing *Tribulus terrestris* (group D5). The methane (CH_4) reduction was over 25% less compared to the control group, and the NH_3 reduction was over 83% less. A significant level of NH_3 reduction was also achieved in the remaining research groups and ranged from over 67% in the group with the addition of activated carbon to over 83% in the group with the addition of *Tribulus terrestris*. In the second stage of the research, a high level of NH_3 reduction was achieved in group (B2), where perlite and activated carbon were used, and the reduction level was over 57%. In the group with the addition of the *Bacillus azotofixans* sp. bacteria (D5) enzyme, a decrease in the levels of NH_3 and CH_4 was demonstrated, by over 13% and over 38%, respectively. This leads to the conclusion that it is the best tested manure additive. However, these tests require confirmation in real farm conditions. At the same time, expanding further research to include the directions of agricultural use of rabbit manure may turn out to be the best available strategy to counteract the environmental burden caused by this sector of animal production.

CONFLICT OF INTERESTS

All authors declare no conflicts of interest.

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