Clinoptilolite (E567), a natural zeolite, inclusion in heavy-pig diets: Effect on the productive performance and gaseous emissions during fattening and manure storage

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Abstract

Intensive pig rearing systems produce several air pollutant emissions, mainly associated with housing and slurry storage. Dietary strategies based on the use of feed additives can effectively mitigate such impacts. This work has been aimed at evaluating the effectiveness of dietary zeolites in mitigating ammonia (NH_3), carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) emissions from piggery and slurry storage on finishing pig farms. An experimental trial, in which three groups of approximately 500 pigs each were reared, has been carried out on a commercial pig farm. The three groups were fed the same diet, with the addition of 0 g/kg (Z0, control), 10 g/kg (Z1), and 20 g/kg (Z2) of micronized clinoptilolite (E567), respectively. The emissions from housing facilities and the live and slaughtering animal performances, were assessed. In addition, manure samples were collected during the rearing period to evaluate, at a laboratory scale, the NH_3, CO_2, CH_4, and N_2O emission potential during the subsequent slurry storage phase prior to land application. The results have shown that the addition of dietary zeolite can be considered a valid strategy to reduce gaseous emissions from pig houses without affecting animal performances or the system's overall productivity. Treatment Z2 gave the best results and resulted in a 25% and 36% reduction of NH_3 and CO_2 equivalent emission fluxes, respectively, compared to those recorded for the control. The laboratory-scale experiment revealed no significant effect of dietary clinoptilolite inclusion on NH_3 or the greenhouse gas emission potential during slurry storage.

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Introduction

The management of pig wastes, such as slurry and manure, produces ammonia (NH_3) emissions (Finzi *et al*., 2019) and greenhouse gases (carbon dioxide, CO_2; methane, CH_4; nitrous oxide, N_2O) (IPCC, 2014; Steinfeld *et al*., 2006). Ammonia is one of the environmental pollutants of most significant concern, as it is responsible for the formation of airborne particulate matter, the acidification of soils, and water eutrophication (Davidson *et al*., 2005; Philippe *et al*., 2011; Philippe and Nicks, 2015). Furthermore, a high concentration of NH_3 inside pig production buildings negatively affects animal and human health (Michiels *et al*., 2015). Greenhouse gas (GHG) emissions are also of great concern, not only because of their effect on global warming but also because the rise in the average temperatures, as a result of high GHG concentrations, could lead to an increase in NH_3 emissions from pig houses, thereby neutralising some of the efforts made to mitigate them (Schauberger *et al*., 2018). The proposed solutions for the abatement of polluting gas emissions from pig farms include a combination of nutrition strategies, techniques for treating the air against pollutants within buildings (*e.g*., an air scrubbing technology, bio-filters), and improved manure management practices.

Zeolites are crystalline aluminosilicate minerals characterised by high porosity and the ability to exchange cations (Mumpton and Fishman, 1977; Reháková *et al*., 2004). Among the many different types of zeolites, clinoptilolite has been widely used in animal husbandry as a feed additive (Mumpton and Fishman, 1977) because of its strong exchange capacity with ammonium and its deodorising effect. Interest in this technique is due to the better efficiency in feed utilisation and the lower incidence of intestinal disease (Cevolani, 2010) observed. In addition, the adsorption capacity of zeolites leads to a reduction in the intestinal concentration of ammonia, which is then slowly released during digestion, thereby promoting a better utilisation of feed nitrogen, lower nitrogen excretion, and, in turn, lower NH_3 emissions (Mercurio *et al*., 2016; Mumpton and Fishman, 1977). The capacity of zeolites of binding ammonia and, therefore, of reducing NH_3 emissions has been confirmed by many authors (Fokas *et al*., 2004; Milić *et al*., 2006; Poulsen and Oksbjerg, 1995). However, the information about the effects of zeolite integration on animal performances is contrasting, since some authors have observed practically no

effects or even slightly worse performances (Fokas *et al*., 2004; Poulsen and Oksbjerg, 1995), while others have reported substantial improvements (Leung *et al*., 2007; Yannakopoulos *et al*., 2000).

Furthermore, there is still a lack of information on the influence of such a dietary strategy on GHG emissions from pig houses and manure management. The influence of zeolites on feed utilization efficiency could reduce CH_4 emissions from pigs, which depend on the fermentative capacity of pig's hindgut and the digestion transit time (Philippe and Nicks, 2015). Moreover, the adsorption effect of zeolites (Arefi Pour *et al*., 2015; Hao *et al*., 2018; Kennedy *et al*., 2019) could exert some effect on GHG emissions during slurry storage.

This work has aimed to evaluate the efficacy of adding zeolite (clinoptilolite type) to pig growing and fattening diets as a technique to mitigate gaseous emissions from pig houses and slurry storage.

Materials and methods

All the procedures involving animals were conducted according to the Italian Law that regulates animal welfare in scientific experiments (Legislative Decree D.Lgs 146/2001).

Clinoptilolite characteristics

Clinoptilolite is a natural form of zeolite (empirical formula: $(Ca, K_2, Na_2, Mg)_4 Al_8 Si_{40} O_{96} \cdot 24H_2O$). The zeolitic material used in the experiment (ZeoS: feed additive E567, produced by Zeocem, Bystré, Prešov region, the Slovak Republic) had a particle size of 50 µm, an average specific weight of 2320 kg/m^3 and contained clinoptilolite (875 g/kg), plagioclase (95 g/kg) and illite (40 g/kg). The chemical composition was 684 g/kg silicon dioxide (SiO_2), 124 g/kg aluminium oxide (Al_2O_3), 39 g/kg calcium oxide (CaO), 28 g/kg potassium oxide (K_2O), 12 g/kg ferric oxide (Fe_2O_3), 8 g/kg magnesium oxide (MgO), 7 g/kg sodium oxide (Na_2O), 2 g/kg titanium dioxide (TiO_2) and 96 g/kg water (H_2O). The maximum total cation exchange capacity of the material was 1.5 mol/kg, and the ammonium cation ($NH4^+$) substitution capacity was 8500 mg/kg.

Animals: housing and diets

The experimental trial was conducted on a commercial fattening pig farm on the Po plain in North West Italy (Genola, Cuneo, Italy; 44°34′53″N, 7°39′08.4″E, at 340 m a.s.l.) which produces 'Prosciutto di Parma' cured pork ham for the protected designation of origin (PDO) supply chain.

During a fattening period that lasted about 5 months (from 31st May to 27th October), an initial group of 1550 pigs (commercial hybrid L 1050, by PIC Italy, Perugia, both females and castrated males in an average 1:1 ratio) was reared inside a north-south oriented building (Figure 1) with a total area of 1928.0 m^2 (120.5 m length × 16.0 m width), a height of 3.5 m at the eaves, and 6.5 m at the roof ridge. The building was made up of three consecutive rooms, separate from each other. Each room, used for one different treatment, was provided with mechanical ventilation and had 28 pens (2.80×6.50 m), while the floor was totally slatted. The ventilation system consisted of two series of 2 fans (EOLOSTAR ES-120, GigolA®, Brescia, Italy) installed on the two opposite sides of each room. Fresh air entered each room through openable windows located along the eaves. The ventilation system was equipped with automatic controls to provide an appropriate level of air exchange through the rooms and limit rises in temperature in the facility during the summer. The opening of the windows was adjusted automatically to maintain a negative pressure of approx. 20 Pa between the inside of each room and the outside. The pits in the three rooms were also independent and were equipped with a vacuum system to remove the slurry.

The animals arrived at the fattening farm after the weaning phase. They were randomly assigned to each room and treatment at their arrival, assuring homogeneity for the weight (38 kg of average body weight, BW) and sex ratio (1:1 between castrated male and female). The animal density inside the pens (18.4 pig/pen, at least 1 m^2 per pig at the end of the fattening period taking into account pig mortality) complies with the specific European Law requirements (European Council Directive 2008/120/EC) for the protection of pigs. The three animal groups were fed a wet diet based on whey and two commercial feedstuffs (M-90 and M-120, Martini SpA, Longiano, FC, Italy) containing corn, triticale, wheat bran, dehulled soybean, peas, calcium carbonate, and sodium chloride, according to a two-phase diet programme (the first phase lasted 76 days, from 50 till 120 kg of average BW, and the second one lasted 73 days till slaughtering, at

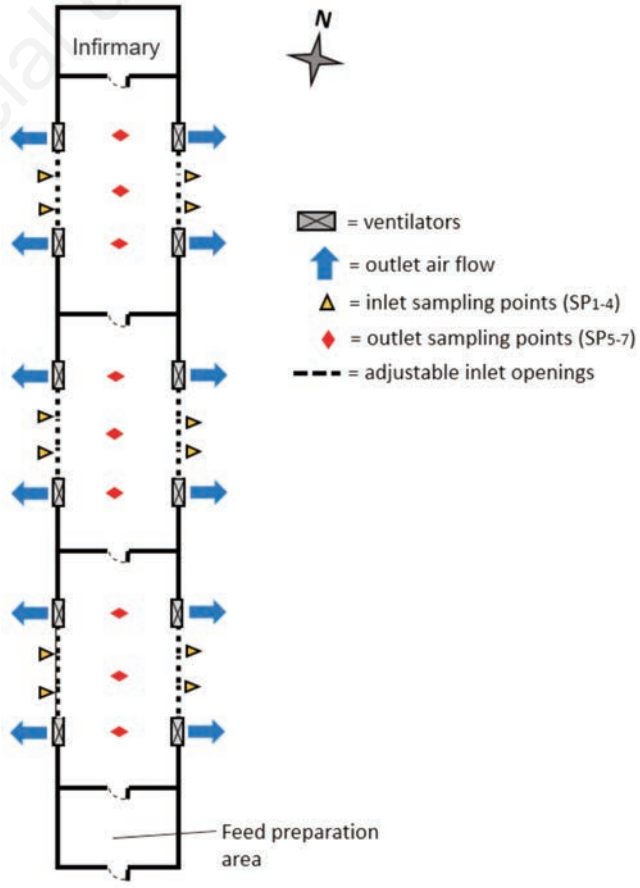

Figure 1. Scheme of the three consecutive rooms, with the positioning of the inlet and outlet air sampling points, ventilators, and adjustable windows for air inflow (dimensions are not drawn to scale).

about 170 kg of average BW). The whey addition ranged between 2:1 to 3:1 on the weight basis of feedstuff given according to the animal weight. After a 30-day adaptation period, the animals were weighed again, and the experimental period began; the feedstuff was integrated with the addition of 0 g/kg (control diet, Z0), 10 g/kg (Z1), and 20 g/kg (Z2), of ZeoS, on a wet basis (WB) before whey addition, with a total cost (purchase plus delivery to the farm) of 0.305 €/kg. The feed characteristics given by the feedstuff company are reported in Table 1. The feed was sampled monthly after whey addition to verify the diet composition, determined according to the following AOAC (2006) methods: preparation of an analytical sample (950.02 method), dry matter (DM) content (934.01); total ash (942.05 method); crude protein (CP) content (984.13 method); ether extract (EE) content (2003.05 method); neutral detergent fibre (aNDF) content (2002.04 method); acid detergent fibre (ADF) content (973.18 method). The net energy (NE) of feed was calculated based on the caloric content of the nutritional components detected with the chemical analysis.

Live and slaughtering performances

The initial and final BW (kg) of the pigs and the feed intake were recorded by trained operators during the experimental period. The pigs were weighed individually, using platform scales (Model EC2000, Tru-Test Limited, Auckland, New Zealand) to determine the initial and final BW. In addition, the feed intake was recorded per pen, the distributed feed was weighed, and its total consumption was verified. The average daily gain (ADG, kg/d) and feed conversion rate (FCR, kg WB/kg BW) were calculated based on these data. At the end of the fattening period, the pigs were slaughtered in an authorised slaughterhouse. The carcass weight, the dressing percentage, the rib muscle thickness, the backfat depth, and the EUROP carcass grade were determined at slaughtering, using online weight scales and a Fat-O-Meater II^{TM} instrument (Frontmatec, Kolding, Denmark), according to the European Commission Implementing Decision 2014/38/EU.

Since slaughtering was performed without detachment of some anatomical parts (*e.g.*, flare fat, kidneys, and diaphragm), the carcass weight was corrected according to the European legislation (attachment V part B Council Regulation (EC) 1234/2007) to obtain the standard carcass weight.

Evaluation of the emissions derived from the housing facilities

In order to evaluate the gaseous emissions from the pig house rooms, weekly measurements of the environmental concentrations of CO_2, CH_4, N_2O, and NH_3 were carried out using an infrared photoacoustic multi-gas analyser (INNOVA 1412, AirTech Instruments, Ballerup, Denmark). Seven gas measuring points were identified in each room in order to obtain a representative dataset of the gaseous emission rates (Figure 1): four (Sp_{1-4}) for the inlet gas concentrations and three (Sp_{5-7}) for the outlet ones. The Sp_{1-4} sampling points were located outside, close to the air inlets, and were arranged symmetrically (two on each side of the room). The Sp_{5-7} sampling points were inside the room and were spaced equally along the longitudinal symmetry line at the same height as the rotation axis of the ventilation fans.

Before starting each measurement, the flow rate of the fans was measured using a vane-type anemometer (Model 416, Testo Ltd, Alton, Hampshire, UK) connected to a Testo 400 data logger. The multi-gas analyser simultaneously measured the concentration

Table 1. Composition and mineral-vitamin-enzymatic supplementation per kg as fed of feedstuff.

	First phase (until 120 kg BW)	Second phase (until slaughter)
Crude protein (g)	133	110
Ether extract (g)	42	43
Crude cellulose (g)	40	29
Ash (g)	42	34
Lysine (g)	8	5.9
Methionine (g)	2.1	1.8
Ca (g)	0.54	0.47
P (g)	0.39	0.33
Na (g)	0.20	0.20
Vitamin A (U.I.)	6500	5200
Vitamin D3 (U.I.)	1500	1200
Vitamin E (mg)	55	44
Biotin (mg)	0.10	0.08
Vitamin K3 (mg)	4.0	3.2
Niacin (mg)	30	24
Folic acid (mg)	0.80	0.64
Vitamin B1 (mg)	2.5	2.0
Vitamin B2 (mg)	5.0	4.0
Vitamin B6 (mg)	3.8	3.0
Vitamin B12 (mg)	0.030	0.024
6-phyitase (FYT)	1000	1000

BW, body weight.

of the target gases (CO_2, CH_4, N_2O, and NH_3) plus relative humidity (RH) in air samples. The measurement time for each of the measuring points lasted 10 min. The instrument needed 2 min to analyse one air sample; thus, five values were recorded for each of the measuring points on each measurement occasion. The air temperature inside each room was also detected during each measurement, using temperature data loggers (Model U12-014, HOBO, Onset Computer Corporation, Bourne, MA, USA).

The detected NH_3 and GHG concentrations (mg/m^3) were related to the air ventilation rate and expressed per pig. As a result, the net emission flux of each gas (F, mg/h/head) was calculated as follows:

$$F = (Cout - Cin) * Q/n \quad (1)$$

where $Cout$ is the outlet gas concentration (mg/m^3), Cin is the air inlet gas concentration (mg/m^3), Q is the airflow rate (m^3/h) and n is the number of animals housed in the room at the time of each measurement.

The total NH_3, CO_2, CH_4, and N_2O (Ec, kg/head) emitted during the fattening period were estimated as follows:

$$Ec=\sum_{n=1}^{n} (Fm\ t)/10^6 \quad (2)$$

where Fm is the average net emission flux value (mg/h/head) of two consecutive measurements; n is the number of measurements carried out during the trial; t is the time-lapse duration between two measurements (h). The CO_2 equivalent (CO_2eq) emissions were calculated by multiplying the CO_2, CH_4, and N_2O emissions by their 100-year global warming powers (1, 28, and 265, respectively), as suggested by IPCC (2014).

Evaluation of the emission potential during slurry storage

In order to evaluate whether the dietary addition of ZeoS could influence NH_3 and GHG emissions during storage, a laboratory experiment was carried out on slurry samples collected during pig rearing. Slurry sub-samples were taken monthly; this involved inserting a specific slurry sampler into an inspection well when the pit was being emptied. The inspection well was placed on the pipeline connecting the under-floor slurry pit of each room to the storage tank outside the building. The collected slurry sub-samples were stored at +4°C in sealed plastic barrels and were used to produce three composite slurry samples (one per treatment) for the storage trial.

Before starting the trial, the composite slurry samples were analysed to determine the dry matter content (DM; g/kg on a wet basis, WB), volatile solid content (VS; g/kg on DM), the total nitrogen content (TN; g/kg on WB, the 984.13 method in AOAC, 2006), the ammonia nitrogen content (NH_3-N; g/kg on WB, the 941.04 method in AOAC, 2006) and pH. The DM of the slurries was determined by drying weighed slurry samples in an oven (Model ABS 220-4, Kern & Sohn gmbH, Balingen, Germany) at 105°C for 24 h. The volatile solids content was determined by igniting the weighed slurry samples in a muffle furnace (Model TCN115, Argo Lab, Carpi, MO, Italy) at 450°C for 4 hours. The pH was determined using a pH-meter (Model HI 9026, Hanna Instruments Italia srl, Ronchi di Villafranca Padovana, PD, Italy).

During the laboratory test, three 4 L homogeneous slurry aliquots of each treatment were stored, for thirty days, in nine experimental 5 L capacity glass jars. The storage was performed at room temperature (17.01±2.2°C). Gaseous emissions were measured using a ventilated chamber system and using the infrared photoacoustic multi-gas analyser (INNOVA 1412, AirTech Instruments, Ballerup, Denmark), as described by Dinuccio *et al*. (2008, 2011, 2019). Accordingly, the gas concentrations at the outlet of each jar were recorded for 10 min, to have five measurements for each slurry sample.

The emission fluxes (F, mg/h/m^2) of NH_3 and GHG from each jar were calculated according to the following formula:

$$F = i * Q/S \quad (3)$$

Where i is the gas concentration detected by the photoacoustic analyser in mg/m^3; Q is the air exchange rate inside the jars (0.06 m^3/h); S is the free slurry surface area (m^2).

The average daily emission rates (Er, mg/m^2/d^1) were then calculated as follows:

$$Er=\sum_{n=1}^{n}(Fv*t)/d \quad (4)$$

where Fv is the average emission flux value (mg/h/m^2) between two consecutive measurements; n is the number of measurements carried out during the trial; t is the number of hours that elapsed between two measurements; d is the overall duration of the storage period (days).

The CO_2eq emissions were estimated as described in the section above.

Statistical analysis

The collected data were analysed by statistical means, using the GLM (IBM SPSS, 2017) procedure. The data relating to the initial BW (kg) of the animals, to the environmental condition of the fattening rooms (temperature and relative humidity), and to the gas emissions, from both housing (kg/pig) and storage (g/m^2/d), were assessed, after testing their normal distribution and their heteroscedasticity (Shapiro-Wilk test and Levene test), using the GLM ANOVA procedure (IBM SPSS, 2017), according to the following model:

$$y = \mu + \alpha_i + \varepsilon_{ij} \quad (5)$$

where μ is the general mean value; α_i is the ZeoS integration effect; ε_{ij} is the random error effect.

Moreover, given that the three groups of animals had a different average BW at the beginning of the trial (*i.e.*, after the 30-day adaptation period) and the end of the experimental period, the data related to the live and slaughtering performances were tested using the GLM ANCOVA procedure (IBM SPSS, 2017), according to the following model:

$$y = \mu + \alpha_i + \beta(x_{ij}-x) + \varepsilon_{ij} \quad (6)$$

where μ is the general mean value; α_i is the ZeoS integration effect; $\beta(x_{ij}-x)$ is the effect linearly associated with the initial BW (for live performances) and with the final BW (for slaughtering performances); ε_{ij} is the random error effect.

Differences in the mean values were tested using the Duncan test, using a first-class error α=0.05 to accept the differences as significant.

Results

Effects on animal performances

During the experimental period, the composition of the diets used for the different animal groups (Table 2) showed no differences between the three groups for both the two feeding phases. The live and slaughtering performances of the three tested animal groups are shown in Table 3. At the beginning of the trial (*i.e.*, after the 30-day adaptation period), the Z0 group showed a slightly higher initial BW than the other groups (P<0.05). Although the three experimental groups did not result balanced in terms of weight, in order to avoid increasing the stress conditions among the piglets, which could have affected the live performance of some subjects (feed consumption, weight gain, *etc.*), it was decided not to move the animals at this stage. This decision allowed any uncontrollable variables to be eliminated. The GLM ANCOVA analysis revealed that the final BW, the weight gain, and ADG had higher estimated means in the Z0 and Z2 groups than in Z1 (P<0.05), whereas the Z2 group showed the most favourable FCR (P<0.05). As far as the slaughtering performance is concerned, the Z0 group showed higher dressing percentages and carcass weights than the Z1 and Z2 groups (P<0.01). The treatment did not affect the rib muscle thickness, but the back fat depth was greater in the Z0 and Z2 groups than in the Z1 group (P<0.01). Consequently, the carcass grade was also affected, and the Z0 and Z2 groups had more carcasses classified as E and U than the Z1 group, and therefore a higher lean meat yield.

Effects of housing on the emissions

Temperature and relative humidity trends during fattening are presented in Figure 2. The average air temperatures measured inside the rearing facility during the trial were 26.41±SD 3.18, 26.55±SD 3.00, and 26.25±SD 2.65°C for Z0, Z1, and Z2, respectively, with no significant difference (P>0.05) between the three rooms. Likewise, average relative humidity was equal to 74.8±SD 6.36, 76.4±SD 6.78, and 74.9±SD 5.76% in Z0, Z1, and Z2, respectively, with no significant difference (P>0.05) between the three rooms. Similarly, no statistically different average airflow rates (P>0.05) were recorded during the gas emission measurements, which ranged from 157 to 173 m^3/head/h, between the control room (Z0) and the treatment pig-rearing rooms (Z1, Z2). Therefore, it was possible to make a meaningful comparison between the emission rates in the three rooms.

As can be seen in Table 4, the addition of ZeoS to the diets led to significantly (P<0.05) lower cumulated NH_3 emissions in the Z1

Table 2. Diet composition in the two feeding phases for the three groups of pigs.

	First phase (until 120 kg BW)					Second phase (until slaughter)				
	Z0	Z1	Z2	SEM	P	Z0	Z1	Z2	SEM	P
DM (g/kg)	192.15	193.83	199.26	3.143	0.636	172.17	171.41	175.83	1.122	0.279
Ash (g/kg DM)	63.76	64.76	66.05	0.349	0.053	72.80	74.44	76.48	0.911	0.304
CP (g/kg DM)	152.66	153.50	152.15	1.096	0.881	133.13	132.18	131.37	1.226	0.844
EE (g/kg DM)	43.28	39.20	41.31	0.951	0.246	43.23	47.49	44.23	0.652	0.061
aNDF (g/kg DM)	136.25	136.43	137.66	1.510	0.917	112.89	118.56	119.09	2.163	0.463
ADF (g/kg DM)	49.12	51.83	51.86	0.688	0.209	44.17	47.01	48.07	2.017	0.725
NE (MJ/kg DM)	8.84	8.78	8.78	0.020	0.382	8.90	8.90	8.81	0.031	0.372

BW, body weight; Z0, control diet with 0 g/kg of ZeoS; Z1, a diet with the addition of 10 g/kg of ZeoS; Z2, a diet with the addition of 20 g/kg of ZeoS; SEM, standard error of the mean (calculated on 6 replicates); DM, dry matter; CP, crude protein; EE, ether extract; aNDF, neutral detergent fibre; ADF, acid detergent fibre; NE, net energy.

Table 3. Live performances [adjusted for an initial body weight (BW)=51.69] and slaughtering performances (adjusted for a final BW=175.65) for the three groups of pigs.

	Z0	Z1	Z2	SEM	P
Live performances					
Initial BW (kg)	54.43^{a}	50.38^{b}	50.46^{b}	0.567	0.013
Final BW (kg)	177.16^{a}	173.53^{b}	176.25^{a}	0.455	0.024
Weight gain (kg)	125.46^{a}	121.83^{b}	124.55^{a}	0.455	0.024
ADG (kg/d)	0.80^{a}	0.78^{b}	0.80^{a}	0.003	0.024
FCR (kg WB/kg BW)	3.33^{a}	3.35^{a}	3.25^{b}	0.013	0.011
Slaughtering performances					
Carcass yield (kg/100 kg BW)	84.81^{A}	83.94^{B}	83.36^{C}	0.067	<0.001
Hot standard carcass weight (kg)	144.30^{A}	142.85^{B}	141.85^{C}	0.112	<0.001
Cold standard carcass weight (kg)	141.42^{A}	139.99^{B}	139.02^{C}	0.110	<0.001
Back fat depth (mm)	36.08^{B}	37.46^{A}	35.86^{B}	0.229	0.009
Rib muscle thickness (mm)	68.81	69.29	70.41	0.290	0.066
Carcass classification (1E-5P)	2.62^{B}	2.79^{A}	2.60^{B}	0.024	0.002

Z0, control diet with 0 g/kg of ZeoS; Z1, a diet with the addition of 10 g/kg of ZeoS; Z2, a diet with the addition of 20 g/kg of ZeoS; SEM, standard error of the mean (calculated on 28 and 252 replicates for live performances and slaughtering performances respectively); BW, body weight; ADG, average daily gain; FCR, feed conversion rate; WB, wet basis. $^{a\text{-}b,A\text{-}C}$Treatment means with the same letter are not significantly different (P>0.05).

and Z2 groups than in the control group (Z0). The greenhouse gas emissions were also significantly reduced particularly the CO_2 and CH_4 emission levels, which were lowered by 18% and 12% (in the Z1 group) and by 51% and 31% (in the Z2 group), respectively. The cumulated N_2O emissions were found to only be affected slightly, with a significant ($P<0.05$) 5.13% reduction in the Z2 group. The total GHG emission reductions in the Z1 and Z2 groups, in terms of CO_2 equivalents, were equal to 13% and 36%, respectively.

Effects on the slurry composition and emission potential during storage

The composition of the control slurry (Z0) and the slurries from the treated groups (Z1, Z2) are shown in Table 5. Comparisons of the mean values of the measured slurry parameters exhibited significant ($P<0.05$) variations with respect to DM, VS, and pH. The DM content varied from 50.70 g/kg in Z2 to 50.30 g/kg in Z0 and 42.70 g/kg in Z1. At the same time, the VS/DM ratio was equal to 0.65 in Z0, 0.64 in Z2, and 0.64 in Z1. The pH, on average, was equal to 7.47 and was higher for Z0 than for Z1 and Z2. Nevertheless, there was no significant difference ($P>0.05$) between treatments, in terms of TN and NH_3-N content, with overall means of 4.40 and 2.30 g/kg, respectively, for all the slurries. Similarly, the GHG and NH_3 emissions that occurred during slurry storage did not vary significantly ($P>0.05$) for the three treatments (Table 6).

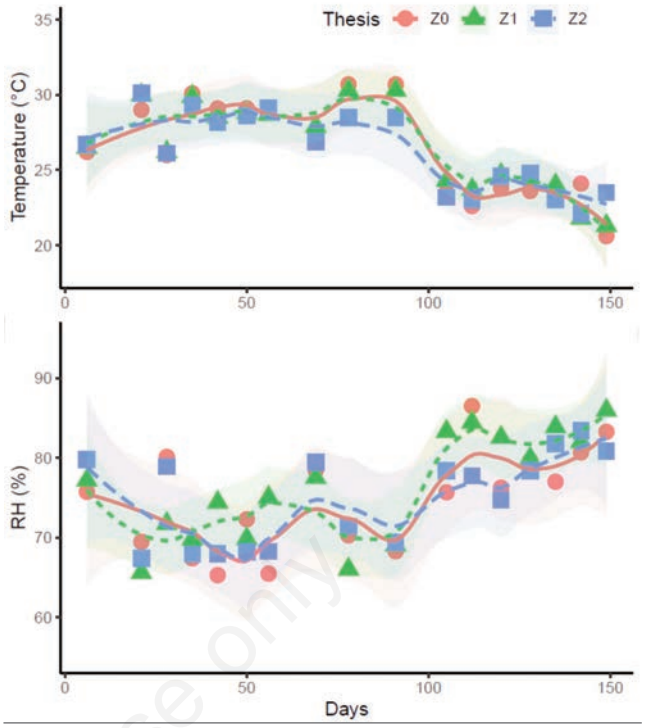

Figure 2. Temperature (T) and relative humidity (RH) trends during fattening (each point represents the T and RH value averaged over three sampling points, with 10 measures per point, in each chamber; n=30); graphs are obtained using the 'geom_smooth' function of R package ggplot2 and adopting a 'loess' smoothing method (Wickham, 2016; R core team, 2019).

Table 4. Total gaseous emissions from pig houses for the three groups of pigs.

	Z0	Z1	Z2	SEM	P
NH_3 (kg/pig)	1.79[a]	1.62[b]	1.34[c]	0.019	<0.001
CO_2 (kg/pig)	1358.00[a]	1194.90[b]	934.80[c]	30.670	<0.001
CH_4 (kg/pig)	25.39[a]	20.79[b]	12.43[c]	0.848	<0.001
N_2O (kg/pig)	0.39[a]	0.38[a]	0.37[b]	0.004	0.006
CO_2eq (kg/pig)	2172.76[a]	1878.41[b]	1379.86[c]	53.801	<0.001

Z0, control diet with 0 g/kg of ZeoS; Z1, a diet with the addition of 10 g/kg of ZeoS; Z2, a diet with the addition of 20 g/kg of ZeoS; SEM, standard error of the mean (calculated on 3 replicates); NH_3, ammonia; CO_2, carbon dioxide; CH_4, methane; N_2O, nitrous oxide; CO_2eq, carbon dioxide equivalent. [a-c]Treatment means with the same letter are not significantly different ($P>0.05$).

Table 5. Main slurry characteristics for the three groups of pigs.

	Z0	Z1	Z2	SEM	P
DM (g/kg)	50.27[b]	42.74[c]	50.70[a]	0.642	<0.001
VS (g/kg WB)	32.87[a]	27.14[c]	32.59[b]	0.548	0.010
TN (g/kg WB)	4.55	4.19	4.52	0.009	0.063
NH_3-N (g/kg WB)	2.32	2.24	2.30	0.006	0.597
pH	7.55[a]	7.47[b]	7.39[c]	0.007	0.021

Z0, control diet with 0 g/kg of ZeoS; Z1, a diet with the addition of 10 g/kg of ZeoS; Z2, a diet with the addition of 20 g/kg of ZeoS; SEM, standard error of the mean (calculated on 3 replicates); DM, dry matter; VS, volatile solids; TN, total nitrogen; WB, wet basis; NH_3-N, ammonia nitrogen. [a-c]Treatment means with the same letter are not significantly different ($P>0.05$).

Table 6. Gaseous emissions during slurry storage for the three groups of pigs.

	Z0	Z1	Z2	SEM	P
NH_3 (g/m^2/day)	0.71	0.93	0.78	0.081	0.231
CO_2 (g/m^2/day)	110.10	113.29	116.68	2.630	0.287
CH_4 (g/m^2/day)	7.86	12.72	11.30	1.830	0.233
N_2O (g/m^2/day)	0.03	0.02	0.02	0.002	0.900
CO_2eq (g/m^2/day)	335.53	461.64	440.48	53.291	0.276

Z0, control diet with 0 g/kg of ZeoS; Z1, a diet with the addition of 10 g/kg of ZeoS; Z2, a diet with the addition of 20 g/kg of ZeoS; SEM, standard error of the mean (calculated on three replicates); NH_3, ammonia; CO_2, carbon dioxide; CH_4, methane; N_2O, nitrous oxide; CO_2eq, carbon dioxide equivalent.

Discussion

Despite the addition of ZeoS, that according to the experimental plan is 10-20 g/kg of feedstuff before the whey addition (and therefore corresponding only to 2-4 g/kg of DM increment to the final diet), the ash content of the different diets did not change significantly between groups and phases (Table 2). Moreover, the regulation of the PDO (which pigs are intended) recommended a diet ash content in the second phase feed between 4 and 8% on DM. The commercial feedstuff of the first and second phases had an ash content of 4.2 and 3.5% as fed, respectively, but the whey (varying composition according to the lot supplied) had a higher ash concentration (about +2%) in the last period than in the first one, and this affected the total ash content of the diet determined by analysis. The addition of clinoptilolite to the diet slightly affected the live performance of the pigs, albeit only slightly (Table 2). The Z2 group showed the same live performances as the Z0 one, except for the FCR. Although zeolite, and clinoptilolite, in particular, is usually added to animal feeds at a level of 20-25 g/kg (Fokas *et al*., 2004), the 20 g/kg of zeolite supplementation used in our trial may have been too low to trigger an improvement in animal performances. Fokas *et al*. (2004) conducted a study on the effects of the addition of 20 g/kg zeolite to the diet of pigs and did not find any significant effect on the live performances of the animals.

On the other hand, a study in which a higher concentration of zeolites (50 g/kg) had been used (Mumpton and Fishman, 1977) showed some improvements in terms of weight gain. Similarly, Yannakopoulos *et al*. (2000) observed improvements in weight gain and FCR after adding 60 g/kg of clinoptilolite-rich tuff to finishing pig diets. Moreover, it should be noted that in our study, the ZeoS inclusion only pertained to the growing and finishing phases. This could have affected the obtained results. In fact, Alexopoulos *et al*. (2007) found that the long-term dietary use of clinoptilolite, at inclusion of 20 g/kg, appeared to enhance the performance of growing and fattening pigs without adversely affecting their health status. However, they already recorded a higher weight gain during the weaning stage (70 days), which also affected the performance of the whole growing period. Prvulovic *et al*. (2007) found that, during the first 90 days of an experiment with a diet inclusion of 5 g of clinoptilolite per kilogram of feed-in growing pigs, the treated group showed a higher body weight gain than with the control one, and the growth parameters were significantly lower in the finishing phase (–4.8%), results that would seem to confirm our results. The observed variations in slaughtering performance (Table 3) did not affect the quantity or quality of the obtained productions to any great extent. To the best of our knowledge, this is the first study to have reported the effect of zeolite addition to the diet on the slaughtering performance of heavy pigs. Further studies should include this aspect, particularly regarding the carcass grade, a key parameter for the production of PDO ham in Italy.

The cumulated NH_3 and GHG emissions from the housing facilities resulted in being influenced greatly by the addition of ZeoS to the diets (Table 4).

As expected, the ammonia emissions were reduced ($P<0.05$) in the Z1 and Z2 groups, by 9% and 25%, respectively, compared to the control group (Z0). This result is similar to the one obtained by Milić *et al*. (2006), who observed a 33% NH_3 emission reduction in piglets after implementing an integration of 20 g/kg of zeolite in their diet. Similarly, the CO_2 and CH_4 emission levels (Table 4) resulted significantly ($P<0.05$) higher for Z0 than for Z1 and Z2 groups. Although little information is currently available in the literature on dietary clinoptilolite supplementation as a GHG emission mitigation technique on pig farms, the adsorption properties of clinoptilolite, with respect to CO_2 and CH_4, has been well documented (Arefi Pour *et al*., 2015; Hao *et al*., 2018; Kennedy *et al*., 2019), thus making it a potential tool for gas purification. The adsorption effect of clinoptilolite on CH_4 could have been exerted both during the digestion phase, by reducing the enteric CH_4 production, and on the CH_4 produced by anaerobic microbial degradation of the slurry organic matter in the slurry pit (Philippe and Nicks, 2015). Moreover, the capacity of clinoptilolite to adsorb CH_4 is related to its surface area and pore volume (Arefi Pour *et al*., 2015) and to the specific ions that clinoptilolite is cation-exchanged with (Kennedy *et al*., 2019); it, therefore, depends on the particular type of clinoptilolite that is used.

The net NH_3 emission fluxes recorded during the rearing period (Table 4) for the Z0 group were 0.53 mg/h/head^{-1} on average, equivalent to an annual amount of 3.81 kg/head/year. The latter figure falls within the range of those given for typical heavy-pig rearing systems in Italy, ranging from 1.7 (Guarino *et al*., 2003) to 6.29 (Costa, 2017) kg/pig/year. However, the average annual N_2O (0.832 kg/pig) and CH_4 (54.2 kg/pig) EFs estimated in this study were 2.9 and 3.2 times higher than those reported by Costa and Guarino (2009) for fattening pigs with more than 110 kg of live weight. The higher N_2O and CH_4 emissions found in our study could be attributed to several factors, including differences in diet composition and housing conditions (Philippe and Nicks, 2015). Moreover, the measurements in our study were performed under summer-autumn conditions, with an average internal temperature ranging from 20.6 to 30.7°C. At the same time, the EFs reported by Costa and Guarino (2009) were based on measurements performed in three different periods of the year, including winter conditions (room temperature ranging from 15.0 to 21.0°C). The presence of a forced ventilation system (instead of a natural one) could also have determined higher gaseous emissions, as pointed out in previous studies (Gallmann *et al*., 2003; Philippe *et al*., 2007; Blanes-Vidal *et al*., 2008), although NH_3 emissions measured in our study do not seem to reflect this effect.

The GHG and NH_3 emissions that occurred during slurry storage did not vary significantly for the three treatments (Table 6). This absence of variation, especially in terms of NH_3 emissions, could be attributed to the low concentration of ZeoS in the slurry biomass. Considering that the ZeoS in the diets did not accumulate in the animal bodies and the total mass of slurry produced during the rearing cycle (about 1.5 m^3/head), which was estimated using the reference guideline values reported in the Piedmont region regulations (DPGR 10/R, 2007), the concentration of ZeoS in the stored slurry was calculated to be approximately 0.17% (on WB). This concentration is lower than the one adopted by Lefcourt and Meisinger (2001), who observed an NH_3 emission reduction in the dairy slurry of about 50% due to adding 6.25% zeolites. Moreover, the capacity of ZeoS to mitigate NH_3 and GHG emissions could have been depleted during housing, thereby having no further effect in the subsequent phases. On the other hand, there seems to have been an increasing NH_3 and CH_4 emission trend (even though no significant variation was detected) as the zeolite concentration was increased. Therefore, it could be hypothesised that the adsorption of NH_3 and CH_4 during housing can increase storage NH_3 and CH_4 emissions due to a delayed release of the pollutant.

Conclusions

The dietary addition of ZeoS, at both 10 and 20 g/kg, was able

to reduce NH_3 and GHG emissions from pig houses. Of the two ZeoS concentrations tested, the 20 g/kg one resulted in a higher mitigation effect, reducing NH_3 and GHG emissions by about 25% and 36%, respectively. The increase in the feeding cost per head, as a result of a 20 g/kg supplementation in the diet, can be calculated as approximately € 0.02 per day, about 1.5% of the current selling price of heavy-pigs in Italy. This cost could be acceptable at a farm level, but this depends on the general production costs and on the sale price of the pigs, which vary over time according to market dynamics. Nevertheless, the manure storage trial showed an increasing trend in CH_4 emissions as ZeoS concentration in the diet increased, thus suggesting that the adsorption of CH_4 during housing could increase storage CH_4 emissions due to a delayed release of the pollutant. Therefore, ZeoS could be a valid tool to mitigate CH_4 emissions during housing, but only if coupled with other mitigation strategies (such as covering the storage tank) to prevent the loss of saved CH_4 in the subsequent phases of the manure management cycle.

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