

Article

Ammonia, Nitrous Oxide, and Carbon Dioxide Emissions from Pig Production: An Exploratory Study on Single-Cell Protein Inclusion in Piglets' Diet

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Abstract

In animal production, gases such as ammonia and greenhouse gases (GHGs) originate from wastes in three main compartments: the animal house of an experimental facility in Northern Italy, storage sites and the slurry-spreading process on cultivated soils. Dietary composition can influence slurry characteristics and gaseous emissions from pig production systems. This exploratory study investigated relationships among slurry composition, volatile fatty acids (VFAs), and gaseous emissions in weaned pigs fed either a conventional diet or a reformulated diet. Faecal samples produced by piglets fed two different diets were collected at three time points: 14, 28 and 42 d of age. Piglets were housed in 32 pens (2 animals per pen, with 16 pens per treatment) on a slatted floor and assigned to either a control diet (C) or a reformulated diet (T) including single-cell protein (SCP). The piglets were administered a starter phase (0–14 days) and a grower phase (14–42 days). Slurry samples were analysed for physicochemical parameters, VFAs, NH_3 , N_2O , and CO_2 emissions. The reformulated diet was associated with higher total Kjeldahl nitrogen and volatile solids, together with changes in VFA profiles characterised by lower acetic and propionic acids and relatively higher lactic and n-butyric acids. NH_3 and CO_2 emissions tended to be lower in the reformulated treatment, whereas N_2O showed variable responses across sampling times. These findings indicate that dietary reformulation with SCP inclusion may influence manure characteristics and fermentation processes, with potential consequences for ammonia emission mitigation. Given the exploratory nature of the study and the non-iso-nutritional formulation of the diets, the results should be interpreted as treatment-level associations rather than evidence of direct causal effects. Further studies under controlled nutritional conditions are required to clarify the mechanisms linking dietary composition, manure properties, and environmental emissions in pig production.

Keywords: ammonia (NH_3); nitrous oxide (N_2O); carbon dioxide (CO_2); slurry composition; volatile fatty acids (VFA); single-cell protein (SCP)



Received: 30 April 2026

Revised: 26 June 2026

Accepted: 9 July 2026

Published: 14 July 2026

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1. Introduction

Nitrogen (N) is an essential nutrient sustaining global agricultural productivity; however, its inefficient utilisation across agro-food systems represents a major environmental challenge. A substantial proportion of nitrogen inputs is lost to the environment, contributing to ammonia (NH_3) volatilisation, nitrous oxide (N_2O) emissions, secondary particulate matter formation, nitrate leaching, eutrophication, soil acidification, and climate

change [1–4]. Improving nitrogen use efficiency (NUE) has therefore become a central objective for enhancing the sustainability of livestock production systems and reducing their environmental footprint [5].

Livestock production plays a key role in the global nitrogen cycle, particularly in intensive pig systems where dietary nitrogen represents the primary source of nitrogen excretion and subsequent gaseous emissions. In pigs, only 30–40% of ingested nitrogen is retained in body tissues, and the remaining fraction is excreted through urine and faeces [6,7]. The form in which nitrogen is excreted is highly relevant from an environmental perspective. Urinary nitrogen, mainly present as urea, is rapidly hydrolysed into ammonium (NH_4^+) and subsequently volatilised as ammonia, whereas faecal nitrogen is largely associated with organic compounds and microbial biomass, resulting in slower transformation processes and a different emission profile [8].

Consequently, dietary strategies that influence nitrogen intake and metabolic utilisation can directly affect manure composition and subsequent gaseous emissions.

Ammonia emissions from pig production are influenced by multiple interacting factors, including housing conditions (e.g., floor type, temperature, ventilation), animal characteristics (physiological stage, genotype, gender) [9], diet composition (crude protein, fibre, amino acid balance, feed additives), and manure management practices [10–12].

Among these, nutritional strategies are the primary step of mitigation tools in the pig production chain. Reducing dietary crude protein (CP), when balanced with essential amino acids (AA), is a well-established approach to decrease nitrogen excretion while maintaining animal performance [6,7]. However, changes in dietary protein levels may also alter manure's physicochemical characteristics, including pH, ammoniacal nitrogen concentration, and volatile fatty acid (VFA) profiles, which in turn influence microbial activity and gaseous emissions [13].

In this context, alternative protein sources derived from microbial fermentation are gaining increasing attention as sustainable feed ingredients. Crystalline amino acids are commonly produced through fermentation processes involving *Corynebacterium glutamicum* [14], generating a protein-rich co-product known as *Corynebacterium glutamicum* cell mass (CGCM). This microbial biomass has an amino acid profile comparable to conventional protein sources and is considered safe for animal feeding [15,16]. Its inclusion in pig diets may contribute to reducing reliance on land-intensive feed ingredients such as soybean meals, thereby lowering the environmental impact of feed production.

Despite the growing interest in microbial protein sources, current research has primarily focused on their effects on animal performance and gut health [17,18]. In contrast, their environmental implications remain insufficiently understood, particularly with respect to nitrogen transformations and gaseous emissions occurring after excretion. Given that a large proportion of dietary nitrogen is excreted rather than retained, post-excretion processes in manure represent a critical stage in determining the overall environmental footprint of pig production. These processes regulate the formation and release of gaseous nitrogen compounds such as ammonia (NH_3) and nitrous oxide (N_2O), whose emission dynamics depend on both manure composition and microbial activity.

Because gaseous emissions originate from the interaction between manure composition and microbial processes, understanding how dietary modifications affect these downstream stages is essential for improving the environmental sustainability of pig production systems.

Therefore, the present study provides an exploratory investigation of the effects of a dietary reformulation including *Corynebacterium glutamicum* cell mass (SCP) on faecal characteristics, volatile fatty acid profiles, and post-excretion gas emissions in weaned pigs, with particular attention to NH_3 , N_2O and carbon dioxide (CO_2) emissions.

2. Materials and Methods

2.1. Experimental Design

The study was performed by collecting slurry produced by piglets of 28 days of age, with an initial body weight of approximately 8 kg, fed different diets, reared in the same building. Piglets were housed in an experimental facility in Northern Italy, divided into 32 pens (2 animals per pen) on a slatted floor and assigned to either a control diet (C) or a reformulated diet (T), with 16 pens per treatment.

The experimental period lasted 42 days and included a starter phase (0–14 days) and a grower phase (14–42 days). Slurry sampling was performed at three time points during the experimental period (days 14, 28 and 42). The starter diet for the two groups, C and T, had the following chemical characteristics (g 100 g DM^{−1}): 7% humidity, 17.1–17.5% CP, 6.2–6.5 of fats, and crude fibre (CF) 4.2–4.9%. The grower diets had 7% humidity, 17.8% CP, 5.6–6.2% fat, and 1.8–3.8% CF. SCP was included in the piglets' treatment at 6% in the starter and at 8% in the grower diet.

For each dietary treatment and sampling time, faecal material was collected from the corresponding pens to obtain approximately 4 L of slurry per treatment. Samples were pooled and thoroughly homogenised to generate one representative composite sample for each treatment at each sampling time.

From each composite sample, four 1 L aliquots were prepared: two subsamples were used for physicochemical analyses, and two subsamples were used for gaseous emission measurements.

The dietary treatments differed in ingredient composition and nutritional characteristics; therefore, the present study evaluates treatment-associated differences in slurry composition, volatile fatty acid profiles and gaseous emissions rather than the response to a single dietary ingredient.

2.2. Physicochemical Analyses

In physicochemical analyses, total solids (TS), volatile solids (VS), pH, total Kjeldahl nitrogen (TKN), total phosphorus (P), potassium (K), and volatile fatty acids (VFA)—including lactic, isobutyric, n-butyric, isovaleric, valeric, acetic, and propionic acids—were measured.

TS, VS, and total carbon were calculated gravimetrically after drying at 40 °C for 24 h, followed by 105 °C for another 24 h; dried samples were then ground and sieved to 1 mm. The pH of fresh samples was measured potentiometrically (Hach® HQ Series portable metres, Hach® Company, Loveland, CO, USA). Total Nitrogen was determined on fresh samples using the Kjeldahl method. The measurements of P and K were conducted using ICP spectrometry (Varian Inc., Palo Alto, CA, USA), following the procedure of acid digestion. VFA concentrations were determined using isocratic HPLC with calibration performed using certified standards and blanks. The total VFA concentration was calculated as the sum of individual acids.

2.3. Emission Measurements

Gas emissions of NH₃, N₂O and CO₂ from animal faeces were quantified using a static chamber method.

Each sample was placed in Pyrex chambers (V/A ratio 0.24 m) and connected through 0.048 m diameter Teflon tubing to a multi-gas photoacoustic analyser (INNOVA 1512, Lumasense Technologies, Fort Collins, CO, USA), with an uncertainty of 7%, a detection limit of 0.1 ppb, and a measurement precision of 0.25 ppb. Gas concentrations were continuously monitored over 40 min for each sample to capture temporal flux dynamics.

To maintain a closed-loop configuration, exhaust gas from the analyser was recirculated into the chamber. Specific fluxes (F), expressed as emission rates in $\text{mg m}^{-2} \text{h}^{-1}$, were calculated for each monitored gas using the formula:

$$F = \frac{\Delta C}{\Delta t} \times \frac{V}{A}$$

where ΔC represents the change in gas concentration over the measurement interval (mg m^{-3}), Δt is the time interval (h), V is the chamber volume (m^3), and A is the surface area of the sample (m^2). Only regression analyses with $R^2 \geq 0.60$ were considered.

2.4. Statistical Analysis

Statistical analyses were performed using SAS 9.4 software (SAS Institute Inc., Cary, NC, USA).

Physicochemical characteristics, volatile fatty acid (VFA) concentrations, and gaseous emission fluxes were analysed using a General Linear Model (GLM), including dietary treatment (Control vs. Treatment), sampling time (days 14, 28, and 42), and their interaction as fixed effects.

Gas emission fluxes were calculated from replicated chamber measurements and are presented as mean $\pm$ standard error (SE).

Results are presented as means $\pm$ standard deviation (SD). Statistical significance was declared at $p < 0.05$.

3. Results

3.1. Faecal Physicochemical Characteristics

The physicochemical characteristics of faeces collected from pigs fed the experimental diets (C and T) are reported in Table 1 as mean values and respective standard deviations.

Table 1. Physicochemical characteristics of faeces from pigs fed experimental diets (control, C; treated, T), expressed as mean $\pm$ standard deviation (SD). Different superscript letters within the same row indicate significant differences ($p < 0.05$).

Group	TKN g/100 g	Ashes g/100 g	VS %	P mg/kg	K mg/kg	pH
C	3.19 ^A ± 0.22	9.65 ± 0.35	21.89 ^A ± 2.65	8207.33 ± 720.02	10,925.67 ± 400.18	6.59 ± 0.18
T	3.48 ^B ± 0.17	9.14 ± 0.13	23.02 ^B ± 2.74	8414.67 ± 298.09	10,956.33 ± 231.40	6.56 ± 0.08

No interaction effect was detected in these analyses, as for the other related to VFA and gaseous emission.

Total Kjeldahl Nitrogen (TKN) was affected by dietary treatment; higher TKN concentrations were observed in the treated group (3.48 ± 0.17 g/100 g) compared to the control (3.19 ± 0.22 g/100 g) ($p = 0.05$).

Volatile solids (VSs) were also significantly higher in T ($23.02 \pm 2.74\%$) than in C ($21.89 \pm 2.65\%$) ($p = 0.04$).

Ashes content showed a numerical decrease in T compared to C, although this difference was not significant. Regarding mineral composition, phosphorus (P) and potassium (K) concentrations were slightly higher in T (8414.67 ± 298.09 mg/kg and $10,956.33 \pm 231.40$ mg/kg, respectively) than in C (8207.33 ± 720.02 mg/kg and $10,925.67 \pm 400.18$ mg/kg).

Faecal pH showed a slight decrease in the treated group (6.56 ± 0.08) compared with the control (6.59 ± 0.18).

3.2. Volatile Fatty Acids Profile

The mean concentrations of volatile fatty acids (VFAs) in the two experimental groups (C and T), calculated over the entire experimental period (42 days), are shown in Figure 1.

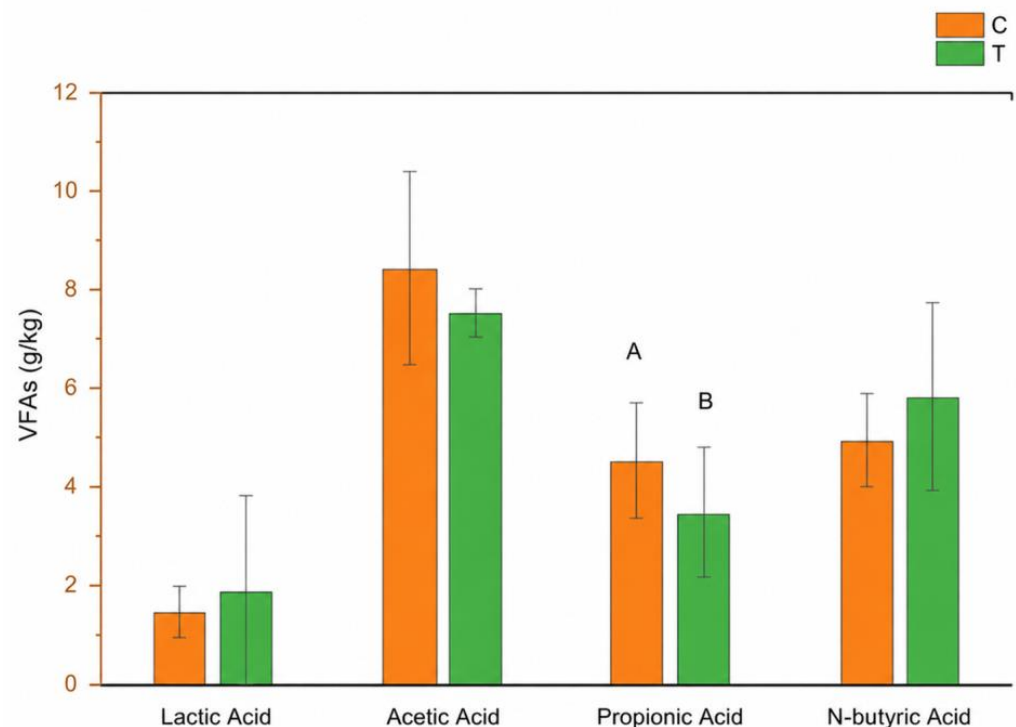


Figure 1. Mean concentrations of volatile fatty acids (VFAs) $\pm$ standard deviation (SD) in faeces from pigs fed the control diet (C) and the treated diet (T), measured over the entire experimental period (42 days). Values represent mean concentrations of individual VFAs (acetic, propionic, n-butyric, and lactic acid) in each experimental group. (Iso-butyric, iso-valeric, and valeric acids were always below detection levels (<0.25 g/kg)) (Different letters within the same row indicate significant differences ($p < 0.05$)).

Overall, acetic acid was the predominant VFA in both groups, with higher mean values in the control (C) than in the treated group (T). Propionic acid showed comparable values between groups, with a slight reduction in T. By contrast, n-butyric acid was higher in T than in C, while lactic acid showed higher mean values in T than in the control. The T group exhibited a partially altered VFA profile compared with the control, characterised by a reduction in acetic acid and a relative increase in lactic and n-butyric acids.

Propionic acid was significantly affected by dietary treatment ($p = 0.045$), with lower values observed in the treated group compared to the control.

3.3. Gaseous Emissions

In Figure 2, NH_3 emission fluxes measured at the three sampling time points are shown for the control (C) and treated (T) groups.

At timepoint 1, NH_3 flux was higher in the control group (C) compared with the treated group (T). At timepoint 2, emissions increased both in C and T, resulting in higher values in C. At timepoint 3, NH_3 fluxes markedly increased in C, while T showed a smaller increase, maintaining lower overall values compared with C.

Overall, mean NH_3 emissions were $0.46 \pm 0.23 \text{ mg m}^{-2} \text{ h}^{-1}$ for C and $0.33 \pm 0.21 \text{ mg m}^{-2} \text{ h}^{-1}$ for T.

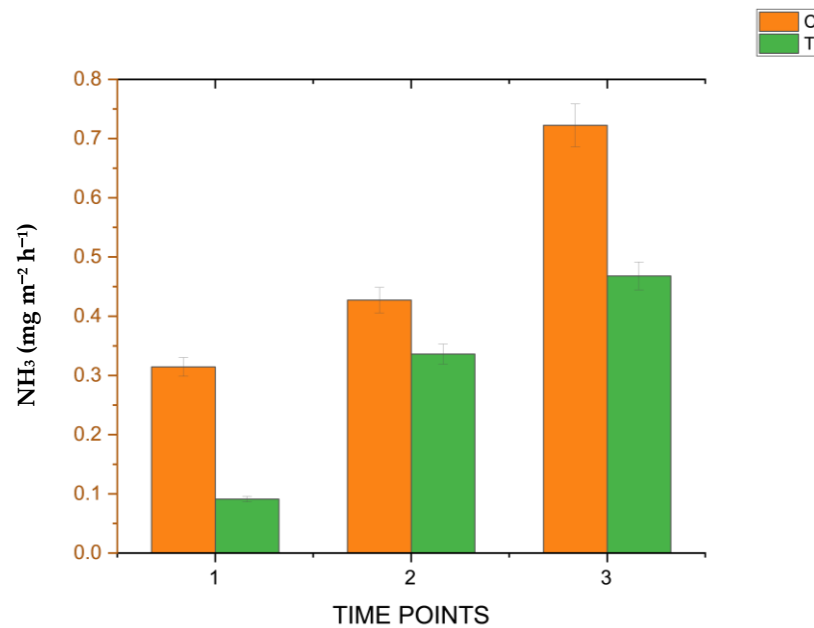


Figure 2. Ammonia (NH₃) emission fluxes measured at three sampling time points in pigs fed the control diet (C) and the treated diet (T). Values are expressed as mean $\pm$ standard error (SE).

In Figure 3, N₂O emission fluxes measured at the three sampling time points are reported for the control (C) and treated (T) groups.

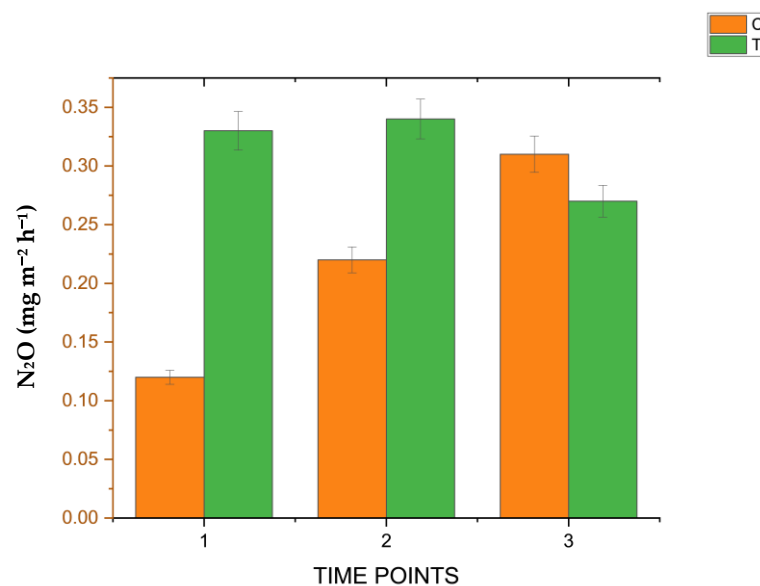


Figure 3. Nitrous oxide (N₂O) emission fluxes measured at three sampling time points in pigs fed the control diet (C) and the treated diet (T). Values are expressed as mean $\pm$ standard error (SE).

At timepoint 1, N₂O fluxes were higher in the treated group (T) compared with the control (C). At timepoint 2, emissions increased in both groups, remaining higher in T than in C. At timepoint 3, N₂O fluxes further increased in the control group, whereas the treated group showed a slight reduction, maintaining values comparable between treatments.

Overall, mean N₂O emissions were 0.26 ± 0.12 mg m⁻² h⁻¹ for C and 0.27 ± 0.10 mg m⁻² h⁻¹ for T.

Carbon dioxide (CO₂) emission fluxes measured at the three sampling time points are presented in Figure 4.

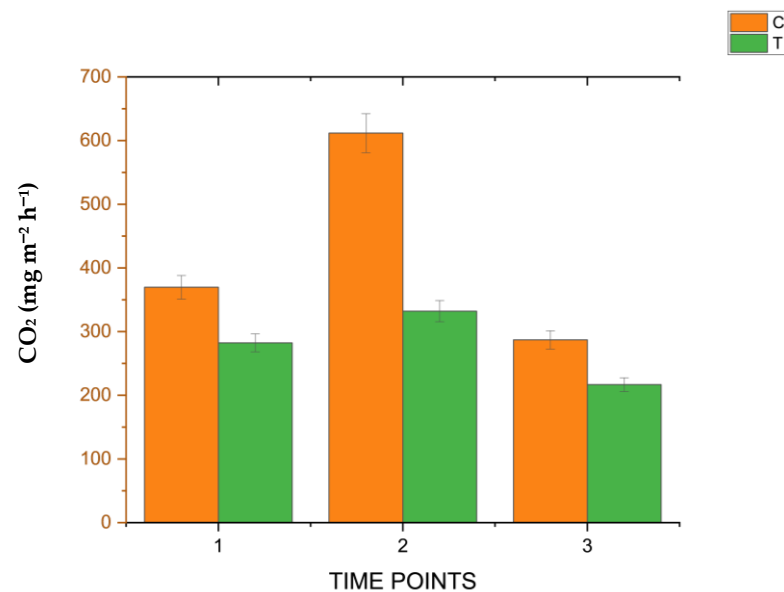


Figure 4. Carbon dioxide (CO₂) emission fluxes measured at three sampling time points in pigs fed the control diet (C) and the treated diet (T). Values are expressed as mean emission fluxes (mg m⁻² h⁻¹) ± standard error (SE).

At the first sampling timepoint, CO₂ emissions were higher in the control group (C) compared with the treated group (T). This difference became more pronounced at the second sampling timepoint, when the control group exhibited the highest CO₂ fluxes recorded during the experimental period. At the final sampling timepoint, CO₂ emissions decreased in both treatments, while the treated group maintained lower values than the control.

Overall, CO₂ emissions were consistently lower in the treated group throughout the experimental period. Mean CO₂ emissions were 422.80 ± 178.70 mg m⁻² h⁻¹ in the control group and 277.11 ± 57.80 mg m⁻² h⁻¹ in the treated group.

4. Discussion

The present study explored the effects of dietary reformulation including *Corynebacterium glutamicum cell mass* (SCP at 75% inclusion) on faecal characteristics, fermentation profiles, and post-excretion nitrogen emissions in weaned pigs. Because the experimental diets differed not only in SCP inclusion but also in the composition of conventional ingredients, the observed responses should be interpreted as the result of the overall dietary strategy rather than the effect of a single ingredient. Specifically, the higher amount of crude fibre and crude protein in the SCP diets represented a potential higher contribution to ammonia emissivity, since it is well reported in the literature that dietary fibres may interfere with apparent ileal digestibility of crude protein in a many ways, fibres can encapsulate proteins by the cell wall matrix, and the soluble fraction of fibre is able affect digesta physicochemical characteristics, and, as a consequence, to reduce protein hydrolysis and amino acid absorption [19].

This aspect is reflected in the increase in total Kjeldahl nitrogen (TKN) observed in the treated group (T), together with higher faecal volatile solids (VS), indicating that dietary reformulation modified the composition of excreted organic matter and nitrogen. Similar relationships between diet composition and manure characteristics have been previously reported, highlighting how changes in protein and fibre supply can alter nutrient partitioning and manure composition [20].

The higher faecal TKN observed in the treated group may reflect altered nutrient utilisation and digestive efficiency. Previous studies have shown that dietary modifications, including alternative protein sources and fermentation-derived ingredients, can influence nitrogen digestibility and utilisation in piglets [21]. Increased faecal nitrogen has also been associated with reduced protein digestibility and greater flow of dietary nitrogen to the distal gut [22,23]. However, digestibility was not directly measured, so these mechanisms remain inferential.

In addition to undigested dietary nitrogen, a contribution of microbial nitrogen cannot be excluded. Increased availability of fermentable substrates may stimulate microbial growth and incorporation of nitrogen into microbial biomass, thereby contributing to faecal nitrogen output [24,25]. Nitrogen recycling via urea transfer to the gut may further support microbial protein synthesis in the large intestine [26,27].

Importantly, these responses are unlikely to be attributable solely to SCP. Its inclusion was accompanied by changes in protein and cereal sources, including reduced whey and soybean products and increased fibre-rich components. Consequently, the dietary reformulation altered both fibre supply and overall diet structure, key drivers of nutrient utilisation and manure composition.

The observed modulation of VFA profiles supports an effect of dietary reformulation on hindgut fermentation. Acetic acid remained dominant, whereas propionic acid decreased and lactic and n-butyric acids increased in the treated group. Diet composition is a major determinant of fermentation patterns, as substrate availability and microbial competition rapidly adapt to changes in ingredient profile [28–30]. Temporal variability further suggests dynamic adaptation rather than a static response.

Differences in microbial biomass ingredients may also have contributed to the observed responses. The physical structure of microbial protein sources can influence nutrient accessibility and microbial utilisation in the gut [31], potentially contributing to treatment differences.

From an environmental perspective, gaseous emissions like ammonia fluxes were generally lower in the treated group, although variability across time points was observed. This suggests that dietary reformulation according to SCP inclusion influenced ammonia volatilisation through combined effects on nitrogen availability, manure pH, and organic matter composition [20], rather than a single driver.

In contrast, N₂O emissions showed no consistent treatment effect, with comparable average fluxes and marked temporal variation. This indicates that the dietary changes were insufficient to consistently alter nitrification–denitrification processes under the present conditions. However, previous studies have suggested that microbial-derived protein sources, including those from *Corynebacterium glutamicum*, may influence intestinal microbial ecology and gut health outcomes [18,32,33].

Carbon dioxide emissions were consistently lower, even if not significantly, in the treated group, suggesting a potential reduced microbial respiratory activity and/or lower degradability of organic substrates. Although CO₂ is not a regulated pollutant like NH₃ or N₂O, it is a relevant indicator of microbial metabolism and carbon oxidation in manure systems.

This reduction is consistent with higher volatile solids and altered VFA profiles in the treated group, indicating changes in substrate quality and fermentability. Fibre characteristics and diet structure likely influenced microbial access to carbon substrates, thereby affecting carbon mineralisation dynamics [34,35]. However, CO₂ emissions are also influenced by other factors such as temperature, moisture, and oxygen availability, and mechanistic attribution remains limited.

Overall, CO₂ trends aligned with NH₃, VFA, and VS patterns, supporting the interpretation that dietary reformulation induced a coordinated shift in manure biochemical dynamics rather than isolated effects on individual nitrogen pathways.

The observed relationships among gaseous emissions and fermentation parameters seem to suggest that different microbial pathways may have contributed to NH₃ and N₂O dynamics. While NH₃ emissions generally followed trends similar to those observed for CO₂, N₂O appeared to be more closely associated with changes in organic matter content and fermentation products. This pattern is consistent with the hypothesis that NH₃ volatilisation and CO₂ production are linked to overall microbial decomposition processes, whereas N₂O formation may depend more strongly on substrate availability and specific nitrogen transformation pathways.

In addition, the tendency for lower pH values to occur alongside variables associated with N₂O production may indicate that slurry acidity influenced microbial activity and nitrogen conversion processes. Although causal relationships cannot be established from the present dataset, these observations support the existence of interconnected relationships among slurry composition, fermentation characteristics and gaseous emissions [36].

Overall, the results indicate that dietary reformulation influenced manure composition, fermentation profiles, and post-excretion nitrogen dynamics. Rather than isolated effects, the data highlight interconnected relationships between diet composition, microbial activity, and gaseous emissions.

Several limitations should be considered. This was an exploratory study using composite faecal samples, and diets differed in multiple formulation aspects beyond SCP inclusion. Therefore, the results reflect treatment-level dietary effects rather than single-ingredient responses. Future studies should apply iso-nutritional designs and include direct measurements of digestibility, microbial ecology, and nitrogen and carbon transformation pathways to better resolve underlying mechanisms.

5. Conclusions

This preliminary study explored the effects of dietary reformulation including *Corynebacterium glutamicum* cell mass (SCP) on faecal composition, volatile fatty acid profiles, fermentation profiles, and post-excretion nitrogen emissions in weaned pigs.

The treated diet resulted in higher faecal total Kjeldahl nitrogen (TKN) and volatile solids concentrations and was associated with lower overall NH₃ emissions, whereas no consistent effects were observed for N₂O emissions. In addition, CO₂ emissions were consistently lower in the treated group, suggesting a potential effect of dietary reformulation on microbial respiratory activity and organic matter degradation processes. In parallel, differences in volatile fatty acid profiles suggest that dietary reformulation influenced microbial fermentation processes occurring within the faecal material.

Because the evaluated diets differed in multiple formulation components, the observed responses should be interpreted as effects of the overall dietary strategy rather than of SCP alone. Nevertheless, the results indicate that dietary composition may influence manure characteristics and subsequent gaseous nitrogen and carbon losses after excretion, highlighting the importance of considering downstream environmental processes when evaluating alternative feed ingredients.

Given the limited number of samples analysed in this study, further studies based on iso-nutritional experimental designs and direct measurements of digestibility, microbial ecology, and nitrogen and carbon transformation pathways are required to clarify the mechanisms linking dietary reformulation, manure composition, and emissions in pig production systems.

Author Contributions: Conceptualisation, E.B. and A.C.; methodology, A.C. and E.B.; software, E.B.; validation, A.C. and E.B.; formal analysis, E.B., A.C. and M.E.M.; investigation, A.C., E.B.; data curation, E.B. and A.C.; writing—original draft preparation, E.B. and A.C.; writing—review and editing, E.B., A.C. and M.E.M.; visualisation, A.C.; supervision, M.E.M.; project administration, M.E.M.; funding acquisition, M.E.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by Università degli Studi di Milano “Piano di Sostegno alla Ricerca (Linea 2)—Progetto DIVAS—CORE” and “Piano di Sostegno alla Ricerca 2023—Linea 2 Azione A.”

Data Availability Statement: Data is contained within the article.

Conflicts of Interest: The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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