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From feed to field: effect of dietary protein level and use of a blend of feed additives on gaseous emissions from growing-finishing pig slurry

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The environmental impact of livestock waste has driven the need for nutritional strategies to enhance digestive efficiency in pigs, aiming to reduce nutrient excretion and associated emissions of pollutants like ammonia (NH_3) and greenhouse gases (GHG). This study investigated the effects of dietary crude protein (CP) reduction by 1.5%, combined with supplementation of a dietary treatment that included feed additives and higher soluble fiber levels, on nutrient digestibility, slurry composition, and emissions across growing and finishing phases. Eighty male pigs were assigned to four different diets in a 13-week trial under a 2 × 2 factorial design: standard protein (SP) and low protein (LP) diets, with or without the dietary treatment. Key measurements included slurry composition, NH_3-N and CH_4 emissions, and crop yield when slurry was applied as fertilizer. The low-protein diet supplemented with additives (LPA) significantly reduced slurry pH ($P \leq 0.001$) and urinary NH_3-N excretion (interaction, $P = 0.03$), improving nutrient digestibility and lowering organic matter content in slurry ($P < 0.05$). NH_3 emissions from the room and slurry pit decreased by over 38%, while CH_4 emissions, although higher in LP diets, were mitigated with the LPA diet. Field application of slurry as fertilizer resulted in trends favoring sustainable wheat production, with increased yield and nitrogen use efficiency, alongside reduced CH_4 emissions ($P < 0.001$). These findings underscore the potential of combined dietary strategies to mitigate environmental impacts while enhancing agricultural sustainability.

KEYWORDS

ammonia emission, crude protein, feed additives, field application, slurry, swine

1 Introduction

Continued global demographic growth along with increasing purchasing power in developing countries, mainly in Asia and South America, is generating a growing consumption and demand of animal-based proteins, which is expected to double by 2050 (Rojas-Downing et al., 2017; Zappaterra et al., 2022). The current scenario requires an increase in food production by the agricultural and livestock sector, which must necessarily be accompanied by an improvement in the efficient utilization of nutrients and natural resources. Moreover, the livestock sector is under enormous social pressure for its contribution to environmental pollution and climate change, derived from the excretion of nutrients through manure including nitrogen (N) and phosphorous (P), but also for being an important source of polluting gases. Of the total greenhouse gases (GHG) of anthropogenic origin, it is estimated that livestock farming contributes to 14.5% of its emissions, being methane (CH_4) the gas with the highest proportion, with 6.7 gigatons CO_2-eq coming from the swine sector (Gerber et al., 2013). In addition, pig farms are an important source of ammonia (NH_3) emissions to the atmosphere, coming essentially from animal housing and slurry storage (Philippe et al., 2011). Its emissions have increased by 4.7% in Spain from 1990 to 2020, and of the 479 Kt produced, 96.8% is generated by agriculture and livestock farming (MITECO, 2023).

Dietary manipulation, but especially precision feeding has demonstrated to be a key strategy to improve nutrient efficiency of animals through adjusting the diet composition to animal needs (de la Fuente et al., 2018; Tullo et al., 2019), and thus reducing the excretion of nutrients that are supplied in excess. In this regard, lowering crude protein (CP) content, combined with a balanced crystalline amino acids addition, is a widely adopted measure to reduce N excretion and NH_3 emissions (Portejoie et al., 2004; Morazán et al., 2015; Seradj et al., 2018), with reductions of up to 8 to 10% per unit drop in dietary CP (Zhao et al., 2019; Trabue et al., 2020). In addition, dietary supplementation with additives including exogenous enzymes (e.g., phytases or carbohydrases), acidifying agents (e.g., benzoic acid), plant extracted essential oils, and their combination has received special recognition. The use of these products has demonstrated consistently an improved animal's performance as well as a more efficient nutrient utilization, mainly attributed to their positive effects on animal immune system, gut morphology, microbiota composition and enzyme activity (Torrallardona et al., 2007), thus enhancing the nutrient digestibility and absorption (Balasubramanian et al., 2020; Wang et al., 2020). Likewise, these dietary manipulations can modify the composition and physicochemical characteristics of slurry, directly impacting in the emission of pollutant gases (Wang et al., 2020). In this regard, including higher levels of soluble fiber has been appreciated as a measure towards reducing NH_3 emissions from pig slurry by stimulating hindgut fermentation, shifting volatile urinary N excretion to a more stable form in fecal N, and lowering slurry pH (Nguyen et al., 2019).

Furthermore, a proper destination of slurry as organic fertilizer is a concern of producers and a demand of society to reduce the use of mineral fertilizers (Debiase et al., 2016; Geng et al., 2019; Wei et al., 2020). The latter products impact economically but also environmentally on agricultural production systems (Osei et al., 2003; Rotz et al., 2011), negatively influencing the structure and composition of crop soils (Xia et al., 2017). Crops have nutrient requirements for their potential production, however the indiscriminate use of both organic and mineral fertilizers may lead environmental concerns, such as leaching of N and P into soil and water resources (Eghball et al., 1996; Álvaro-Fuentes et al., 2016; Demurtas et al., 2016; Aguilera et al., 2021). In addition, NH_3 and N_2O emissions from field application should not be underestimated. Therefore, the use of both types of fertilizers has been regulated by recent EU policies (European Environment Agency, 2018; Huygens et al., 2020), and any application of organic products as fertilizers should be tested for their efficacy in crop nutrition and toxicity for soil environment.

The objectives of the present study were to examine the impact of two levels of dietary CP, supplemented or not with a dietary treatment including a blend of feed additives as well as higher levels of soluble fiber in growing and finishing pigs, on slurry composition and gaseous emissions during both pig production cycle and soil application, along with wheat crop production evaluation when slurry was applied as fertilizer.

2 Materials and methods

2.1 Pig farm trial

The current study was carried out at the facilities of the Pig Research Centre (CEP; Torrelameu, Lleida, Spain). All procedures involving animal handling were performed in compliance with the regulations of the Spanish Policy for protection of animals employed in research and other scientific purposes (Real Decreto 53/2013, 2013), which meets the European Union guidelines (Directive 2010/63/EU, 2010) on the protection of animals used for experimentation. Protocols and experimental procedures were approved by the Ethics Committee for Animal Experiments of the University of Lleida, under the Project License Number 11498 (Generalitat de Catalunya).

2.1.1 Animals and experimental design

Eighty intact male Pietrain × (Landrace × Large White) pigs were used in a 13-week experiment, with a mean initial BW of 33.25 ± 0.43 kg (μ ± SE) and 12 weeks of age. Upon arrival at the experimental facilities, the pigs were weighed and randomly distributed into four separate rooms, each corresponding to one of the four diets under study and connected by isolation doors. Twenty pigs were allocated per room (five pigs per pen), with less than 1.5% variability in mean weight and no statistical differences between rooms at the start of the trial. Each room consisted of four pens distributed in two pens on each side of the room, whose slurry was first collected in a single-room slurry pit (145 cm × 150 cm × 40 cm) located underneath. At the end of each feeding phase, the slurry from each experimental diet was drained into a flexible tank of larger capacity. The individual pens (4.2 m^2 each) were 55% slat floor and were equipped with a single-space self-feeder in the concrete floor area and a square nipple drinker in the slatted floor area.

The first six weeks of the experiment were considered the growing (GRO) phase and the last seven weeks were defined as the finishing (FIN) phase. In both feeding phases, feed and drinking water were administered *ad libitum*, and the experiment followed a 2 × 2 factorial design, with the CP level and the inclusion of dietary treatment as the study factors.

The animals were kept under commercial-like production system, where temperature and relative humidity in the four rooms were monitored and recorded every 30 min. The average temperatures and humidity of the four rooms were of 23.7 ± 1.46 °C and 53.9 ± 5.18%, respectively during the GRO phase, and 20.90 ± 1.61 °C and 57.59 ± 6.54%, respectively in the FIN phase (Testo 174 H; Testo AG, Lenzkirch, Germany).

2.1.2 Diets

The ingredients and chemical composition of the experimental diets are shown in Table 1. The diets were pelleted and formulated to be isoenergetic for all four groups.

Two CP levels were established for each feeding phase. The standard protein (SP) diets followed FEDNA (2013) recommendations, with CP levels of 16% during the GRO phase and 15% during the FIN phase. In contrast, the low protein (LP) diets had CP levels reduced by 1.5 percentage points compared to SP diets, resulting in CP concentrations of 14.5% and 13.5% for the GRO and FIN phases, respectively. The reduction in CP aimed to investigate the potential impacts of protein sparing on animal performance and nutrient utilization.

In addition to CP concentration, half of the animals in each CP group received a dietary treatment consisting of a blend of feed additives and a higher inclusion level of soluble fiber. The additive blend included the following components: Vitazyme NSP (1000 mg kg^{-1}), a carbohydrase product containing xylanase. VevoVitall® (3000 mg kg^{-1}), composed mainly of benzoic acid. Biotronic® Top 3 (1000 mg kg^{-1}), a combination of organic acids. Digestarom® Finish (150 mg kg^{-1}), a blend of plant extracts designed to promote digestive efficiency.

The dietary treatment also included an elevated level of soluble fiber provided primarily through wheat bran and beet pulp for growing and finishing phases, respectively. Furthermore, all diets included phytase (Ronozyme® HiPhos, Switzerland) at 100 mg kg^{-1}, and the carbohydrases contained in the blend of additives were included using matrix values following the manufacturer recommendations for ME values.

As a result, the study implemented four diets: SPC: Standard protein without dietary treatment; SPA: Standard protein with dietary treatment; LPC: Low protein without dietary treatment; LPA: Low protein with dietary treatment.

2.1.3 Measurements and chemical analyses

Individual BW was obtained every two weeks throughout the experimental period to assess average daily gain (ADG), as well as the weight of feed supplied and refusals (at the pen level) to evaluate average daily feed intake (ADFI) and feed conversion ratio (FCR).

Slurry production was estimated every three weeks considering the volume of the pit, by measuring the depth of the slurry with a meter rule. Then, 1 kg of slurry samples were gathered by choosing three representative sampling points per pit, pooling them to obtain a single sample on which pH was recorded with a portable pH meter (Crison micropH 2000). To minimize N evaporation, the samples were immediately frozen at −20 °C. Dry matter (DM), ash, neutral detergent fiber (NDF) and N were determined using the techniques described by Morazán et al. (2015), and NH_3-N was analyzed by direct distillation with $Na_2B_4O_7$ according to the Kjeldahl method (ref. 976.05; AOAC (1990)). For P content, the samples were processed by high performance microwave digestion (Ethos Up; Milestone, Sorisole, Italy) and analyzed by inductively coupled plasma mass spectrometry (7700x, Agilent).

2.1.4 Gas collection, analyses and calculations

Two measurements of NH_3 and CH_4 emissions per feeding phase were performed every three weeks by simultaneous collection of air samples from the slurry pit level and total room gas production, both consisting of a continuous air inlet and outlet flow system. The description of the installation and air collection of both systems is described in detail in Seradj et al. (2018). Briefly, contaminated air was collected from the pit level through portable flow chambers (PFC), so that two PFC were used in each slurry pit, one for NH_3 emission and one for CH_4 uptake, each operating at different air flow rates. The inlet air was also analyzed to determine the gas concentrations reaching the inside of the PFC. Whereas, the total gaseous emission in the room were collected from the exhaust air outlet at the midpoint of each room, according to the procedure of AMCA (2011). A representative air sample from outside the farm facility was also collected and analyzed.

Ammonia was collected for 48 h using a vacuum air pump (KNF N035.3 AN.18 – IP20, USA) at a flow rate of 3 L min^{-1} measured by a flowmeter (LZQ-1 0-5 LPM). Following the model proposed by Goldman and Jacobs (1953) and updated by Antezana Julián (2014), the collected air was bubbled in an acid solution (100 mL of H_2SO_4; 0.5 M) contained in glass impingers trapping the gaseous NH_3 in aqueous ammonium (NH_4^+). After the measurement time elapsed, the acid solution was analyzed by Kjeldahl method (AOAC, 1990) and the NH_3 trapped (mg L^{-1}) was determined considering the N concentration, the volume of the solution and the molecular weight of NH_3, as well as the flow rate applied during each measurement. The NH_3 produced from the slurry was calculated as the difference between the NH_3 concentrations of the inlet and outlet air.

As for CH_4, all procedures were conducted following Seradj et al. (2018). Briefly, a flow rate of 10 mL min^{-1} (measured by Alltech electronic gas flowmeter, IL, USA) was generated by a peristaltic pump (Gilson, Minipulse 3, Le Bel Villiers, France) to collect the gas produced, which was stored in separate 20 L inert bags during 24 h. Air samples were taken from each bag using a 15 mL syringe and placed in 12 mL vials (model 039W, Labco, High Wycombe, UK). Samples were analyzed using a 7890A gas

TABLE 1 Ingredients and chemical composition of the experimental diets of two feeding phases, growing and finishing, fed standard (SP) or low (LP) levels of crude protein, combined with the presence (SPA and LPA) or absence (SPC and LPC) of dietary treatment.

	GROWING PHASE				FINISHING PHASE			
	SPC	SPA	LPC	LPA	SPC	SPA	LPC	LPA
Ingredients (g kg DM^{-1})								
Corn	200.0	200.0	200.0	200.0	128.0	128.0	128.0	128.0
Barley	889.3	877.6	901.9	855.2	1234.3	1201.5	1250.8	1125.6
Wheat	500.0	500.0	600.0	600.0	768.0	768.0	896.0	896.0
Wheat bran	–	40.00	–	75.40	–	–	–	83.58
Beet Pulp	–	–	–	–	–	64.00	–	73.03
Soy 47%	296.97	286.94	189.06	175.76	294.24	292.07	155.699	142.43
Fat	63.24	35.86	48.24	23.30	74.52	36.55	55.16	26.90
Carbonate	13.52	14.26	13.26	14.58	16.61	14.79	16.28	15.51
Monocalcium phosphate	4.98	4.72	5.84	5.42	4.14	4.14	5.24	4.94
Salt	8.48	8.20	8.48	8.02	10.85	10.47	10.8	10.06
L-Isoleucine	0.00	0.00	1.62	1.76	0.00	0.00	2.30	2.50
Lysine	10.16	10.34	13.46	13.70	12.97	12.95	17.20	17.43
DL-Methionine	2.80	2.84	3.74	3.82	3.04	3.12	4.27	4.48
L-Threonine	3.66	3.70	5.16	5.20	4.50	4.55	6.42	6.55
Tryptophan	0.40	0.40	0.90	0.88	0.43	0.46	1.07	1.10
Premix fattening pigs 0.3%	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Vitazyme NSP (200.000 FXUde WX/kg),0%	–	0.1	–	0.1	–	0.1	–	0.1
Vevovitall	–	0.3	–	0.3	–	0.3	–	0.3
Biotronic Top 3	–	0.1	–	0.1	–	0.1	–	0.1
Digestarom Finish	–	0.015	–	0.015	–	0.015	–	0.015
Chemical Characteristics (g 100 g DM^{-1})								
Humidity	9.62	9.62	9.58	9.59	9.43	9.45	9.40	9.43
Crude Protein	16.00	16.00	14.50	14.50	15.00	15.00	13.50	13.50
Crude Fiber	3.22	3.85	3.32	3.96	3.34	3.95	3.43	4.07
NDF	11.89	13.25	12.48	13.89	12.51	13.87	13.10	14.57
Fat	4.93	4.05	4.41	3.42	4.61	3.58	4.09	3.0
Ca	0.60	0.61	0.59	0.60	0.57	0.58	0.56	0.57
P	0.42	0.42	0.41	0.41	0.39	0.40	0.38	0.39
N	2.56	2.56	2.32	2.32	2.40	2.40	2.16	2.16
DM (g 100 g FM^{-1})	90.18	89.89	90.30	89.62	89.83	89.47	89.64	88.99
OM	95.69	95.24	96.09	95.90	95.95	95.81	95.91	95.70

chromatograph equipped with a flame ionization detector with a methanizer. An HP-Plot column (30 m long, 0.32 mm diameter) was used, together with a 15 m long pre-column. The injector and furnace temperatures were set at 50 ˚C and 250 ˚C, respectively, while 375 ˚C were set for the methanizer. Hydrogen was used as a carrier gas and N_2 as compensatory gas at 35 and 25 mL min^{-1}, respectively. The sample volume injected was 1 mL and the CH_4 production was calculated according to (Holland et al., 1999).

The CH_4 concentration values (ppm) obtained from gas chromatography were transformed to mass volume^{-1} concentration (mg (m^3)$^{-1}$) considering its molecular weight, and applying the ideal gas law as follows:

$$Cm = Cv \times M \times P \times R^{-1} \times T^{-1}$$

Where Cm is the mass volume^{-1} concentration (mg $(m^3)^{-1}$), Cv corresponds to the volume volume^{-1} concentration (ppm), M is the molecular weight of CH_4, P is the atmospheric pressure, R is the universal gas constant, and T is the temperature in degrees Kelvin. The absolute CH_4 emission was calculated as the difference between inlet and outlet concentration, considering the airflow used during the measurements.

2.2 Field trial

2.2.1 Crop field application

A commercial 7200 m^2 irrigated experimental field was set up at the beginning of the experiment in the proximity of the pig facility. An irrigated wheat crop was planted by late November 2021 following standard technological production practices used for this crop. The layout of the experiment was a factorial design containing the application of slurry from animals subjected to the four diets under evaluation explained in the previous chapter on a field-level crop, with three randomized replicates (see Figure 1).

2.2.2 Soil characteristics and slurry application

Prior to the start of the experiment, a soil analysis of the commercial plot was conducted to determine soil limiting factors by taking four composite samples from each plot at two depths (0–30 cm and 30–60 cm). The soil resulted to be a clay loamy soil, with a pH of 8.7, electric conductivity of 0.44 dS m^{-1} (no limiting for salinity), high levels of soil organic matter (25 mg kg^{-1}), high levels of soil fertility in P and K and normal in nitrate concentration (12 ppm NO_3^-N). The overall result of these analyses is that soil was not a limiting factor for the experiment.

Crop wheat nutrient requirements are about 100–150 kg N ha^{-1} for a regular yield in these conditions. Due to normal levels of NO_3^- N concentration in the soil and high organic matter levels, it was decided to apply 100 kg N ha^{-1} for an expected yield of 5–7 t ha^{-1}. All diets had approximately 5 to 6.5 kg N $(m^3)^{-1}$, then the slurry dosage for each group was calculated depending on this concentration. Slurry was applied as top-dressing fertilization of wheat in February 2022, with a separate tank for each group, which was loaded from the pig facilities.

2.2.3 Crop production and efficiency indicators

To assess the effect of the different diets on the soil and crop, a composite sample of three or four soil cores was taken from each plot three times (prior to sowing, before top dressing application of slurry and after harvest) and at two depths (0–30 cm, 30–60 cm). Soil water content was determined by gravimetric method. Soil NH_4^+-N and NO_3^-N contents were quantified by extracting 20 g of fresh soil with 100 mL of 1 M KCl by means of a continuous flow autoanalyzer (Seal Autoanalyzer 3, Seal Analytical, Norderstedt, Germany). To determine crop N content, two plant samples per plot were taken at harvest, and N content was determined by DUMAS combustion (Tru Spec CN; Leco Corporation, St. Joseph, MI, USA; ISO (2008)) for the grain and for the rest of the plant.

Each plot was harvested in July 2022 with 10% grain moisture in the field. Grain yield of each plot was measured by an automatic trolley equipment with a weighting scale. To determine resource use efficiencies, indicators of water use efficiency (WUE) for grain yield (Equation 1) and N use efficiency (NUE) for grain yield (Equation 2) were calculated:

$$\text{WUE (kg grain ha}^{-1}\text{mm}^{-1}) = \text{Grain yield} \times \text{WU}^{-1} \quad (1)$$

Where water use (WU) is calculated as the difference between soil water content at planting and at harvest, plus the cumulative rainfall and irrigation received between these two dates.

$$\text{NUE (kg kg}^{-1}) = \text{Grain yield} \times \text{N supply}^{-1} \quad (2)$$

2.2.4 Greenhouse gas collection

During the crop growth cycle, 15 samplings were done by a set of 24 non-steady-state chambers (Hutchinson and Mosier, 1981) to

FIGURE 1
Layout of the 7200 m^2 field experiment for agronomical and environmental evaluation of the application of the slurry on a wheat crop in Lleida, Spain. The slurry comes from the four different pig diets, which includes: T1: SPC; T2: SPA; T3: LPC; T4: LPA.

determine the concentration of three GHG: CH_4, N_2O and CO_2. During the winter months (November to March), gas measurements were conducted at 3-week intervals. Gas samples of 15 mL each were collected at intervals of 0, 20, and 40 min following chamber closure and were subsequently stored in 12 mL Exetainer® borosilicate vials (model 038 W, Labco, High Wycombe, UK). During the warmer seasons (April to July), soil gas fluxes were measured every two weeks. However, when the land underwent fertilization, the sampling frequency was intensified: samples were collected 24 h before fertilization and then at 2, 24, 48, and 72 h after fertilization.

Samples were analyzed by a gas chromatography system (7890A, Agilent, Santa Clara, CA, United States) equipped with a flame ionization detector coupled to a methanizer to determine CO_2, an electrical conductivity detector and a KRCIAES column (IA KRCIAES 6017: 250°C, 45 m long, 0.32 µm of section and 10 µm; Ingeniería Analítica., Barcelona, Spain) with a 15 m long pre-column of the same characteristics.

2.3 Statistical analysis

All data from the pig farm were analyzed using SAS System® software (SAS Institute Inc., Cary, NC, USA), employing an ANOVA mixed analysis model of repeated measures (PROC MIXED). The experimental design followed a randomized complete block design. The model consisted of fixed factors such as PH that represents the feeding phase (GRO and FIN) and considered as repeated measures, PL, representing the protein content of the diets (standard and low protein content), ADD represents the use of the dietary treatment with additives in the diets (with or without), and all possible interactions of the fixed factors as outlined below:

$$Y = \mu + PH_i + PL_j + ADD_k + (PH \times PL)_l + (PH \times ADD)_m + (PL \times ADD)_n + (PH \times PL \times ADD)_o + \sum_{ijklmno}$$

In cases (e.g., gas emissions) where the data did not conform to a normal distribution, a Log10 transformation was conducted prior to statistical analysis. Mean comparisons were performed using Tukey's test with a significance level of $P < 0.05$. Trends were considered with P values between 0.05 and 0.10.

Statistical analyses of field results were performed using JMP pro 15 (SAS Institute Inc, 2020) statistical software. Cumulative soil CH_4, CO_2 and N_2O emissions were quantified on a mass basis using the trapezoidal rule (Levy et al., 2017). The data were checked for normality, homoscedasticity and serial independence by Shapiro-Wilk, Bartlett, and Durbin-Watson test, respectively. Data were transformed when necessary to pass these tests. Outliers were checked using the Grubb's test with a statistical confidence level of 95%. Repeated measures analysis of variance (ANOVA) was performed with each protein level, additives use and their interactions as effects. When significant differences among diets were identified at 0.05 probability level of significance with Tukey HSD test, a mean separation was carried out.

3 Results and discussion

In this study, nutritional strategies were evaluated for their ability to modify pig digestive physiology to reduce enteric CH_4 emissions, as well as slurry features to minimize the emission of pollutant gases from its excretion and on-farm storage to crop application. Therefore, the combined effect of lowering dietary CP level and a dietary treatment including a blend of feed additives, which have previously shown scientific evidence to reduce NH_3 and CH_4 emissions, was investigated.

3.1 Animal trial

3.1.1 Pig production and slurry composition

Pig performance was within the expected results (see Supplementary Table S1). The reduction of dietary CP level, together with an appropriate supply of essential amino acids, allows to reduce costs and environmental impact (Wang et al., 2018) without altering growth performance (Wang et al., 2023), which was fully confirmed in our results. Regarding the use of the dietary treatment with feed additives, pigs fed the SPC diet in the FIN phase had higher ADG than the other groups, however, the analysis of the overall results showed little relevance of the interaction.

Regarding to the chemical composition of the slurry, the most relevant difference between the slurry produced in the two feeding phases was the DM content, which was much higher in the GRO phase in all diets, as can be seen in Table 2. These results can be explained by the increase in water consumption and subsequent urine production as the animals matured.

Dietary CP level had a significant effect on slurry pH in both feeding phases ($P < 0.001$), showing a lower pH when pigs were fed the LP diets, as has been widely described in the existing literature (Morazán et al., 2015; Trabue et al., 2021; Le Dinh et al., 2022). Lower CP content reduces the excess of excreted N, mainly in urine but also in feces (Wang et al., 2020), with NH_4^+ levels being the main alkalinizing factor. However, in the present study, no differences in total N concentration were found between SP and LP diets. A discrete reduction in the dietary CP (i.e., 1.5%) should not be excluded as the reason for the lack of significant differences in this parameter, as well as a collateral increase in fecal N excretion due to the higher fiber content in the LP diets. However, a higher NH_3 emission by pigs fed the SP diet, especially in the FIN phase, is also proposed and discussed below.

The incorporation of the aforementioned dietary treatment also had an effect on reducing the pH of the slurry, this was true in the FIN phase ($P = 0.001$) and in the LP group of the GRO phase, whereas young pigs fed the SPA diet showed the opposite response (significant interaction, $P < 0.001$). The decrease in pH may be explained by i) the presence of benzoic acid in the additive blend, whose hepatic conjugation with glycine leads to hippuric acid excretion in the urine, thus lowering the urine and slurry pH (Halas et al., 2010; Humphrey et al., 2022); and ii) the slightly

TABLE 2 Chemical composition (g (100 g DM)$^{-1}$) of slurry from pigs of two feeding phases, growing and finishing, fed standard (SP) or low (LP) levels of crude protein, combined with the presence (SPA and LPA) or absence (SPC and LPC) of the dietary treatment.

Chemical Composition[1]	SPC	SPA	LPC	LPA	SEM	P value		
						Protein	Additives	Interaction
GROWING PHASE								
pH	7.35^{b}	7.98^{a}	7.36^{b}	6.98^{c}	0.079	<0.001	0.142	<0.001
DM, g (100g FM)$^{-1}$	23.89^{b}	31.82^{a}	22.99^{b}	23.42^{b}	1.778	0.023	0.037	0.057
OM	96.19^{a}	93.71^{b}	95.72^{a}	95.57^{a}	0.296	0.037	0.0008	0.002
P	1.28	1.15	1.03	1.15	0.084	0.203	0.933	0.206
N	2.98	3.30	2.87	3.17	0.212	0.612	0.221	0.971
NH_3-N	0.73	0.89	0.82	0.70	0.07	0.58	0.83	0.14
FINISHING PHASE								
pH	7.73^{a}	7.39^{b}	7.16^{bc}	6.92^{c}	0.065	<0.001	0.001	0.535
DM, g (100g FM)$^{-1}$	6.98	14.61	7.90	13.71	1.938	0.994	0.004	0.637
OM	98.29	96.81	98.34	97.57	0.351	0.256	0.006	0.323
P	1.38	1.22	1.43	1.09	0.044	0.475	0.005	0.112
N	2.89	2.66	2.90	2.84	0.104	0.427	0.247	0.477
NH_3-N	0.65^{ab}	0.79^{a}	0.72^{ab}	0.52^{b}	0.06	0.16	0.64	0.03

$^{a-d}$ Mean values within a row with different superscript letters differ ($P < 0.05$).
[1]DM, Dry matter; OM, Organic matter; P, Phosphorus; N, Nitrogen; NH_3-N, Ammonia nitrogen.

higher fiber content of SPA and LPA diets in both feeding phases (see Table 1). Higher levels of fiber may reduce slurry pH either by improving the production of volatile fatty acids or by reducing urinary NH_3-N (and the NH_3-N alkalizing effect) in favor of fecal bacterial N (Kerr et al., 2006; Jarret et al., 2012), as was found especially in the slurry of LPA fed pigs in the FIN phase (significant interaction $P = 0.03$). As previously mentioned, the effect of the used dietary treatment was not as clear in the more immature piglet hindgut, and therefore this point needs to be further investigated.

In addition, feed acidifiers were also associated with an improvement in nutrient digestibility, as reported in several studies (Liu et al., 2018). This effect is explained by the improvement of intestinal morphology, the increase in the activity of some digestive enzymes such as pepsin, lipase or sucrose, and the modulation of the gut microbiota, making it more beneficial for the animal (Torrallardona et al., 2007; Halas et al., 2010; Xu et al., 2018), as well as an increased N retention (Humphrey et al., 2022). Our pH reductions in additive-supplemented diets agree well with those reported by Kerr et al. (2006), who used an *in vitro* dynamic system with continuous slurry addition.

Xylanases were included in the additive blend mainly to improve the digestibility of non-starch polysaccharides provided by the higher fiber level within the SPA and LPA diet and also to reduce the viscosity of the intestinal content. Moreover, xylanases might improve the availability of other nutrients such as amino acids retained in the fiber fraction (Woyengo et al., 2008). Consequently, the digestible energy content was also improved (Nortey et al., 2007), which explains the lower fat content required in SPA and LPA diets to obtain iso-energetic diets. In this sense, the reduction of OM content in the slurry of pigs fed the additive-supplemented diets in both feeding phases ($P \leq 0.05$) may be associated with improved nutrient digestibility (Boontiam et al., 2022). The increased availability of shorter oligosaccharides due to the action of xylanases on long arabinoxylan chains explains the compositional modulation in the intestinal microbiota (Boontiam et al., 2022); also associated with a great prebiotic potential. In this regard, it has been described that essential oils and other plant extracts can create a favorable intestinal environment for beneficial microbes, either through their antioxidant activity or by improving nutrient digestibility (Liu et al., 2018).

Regarding P concentration, the results indicate that despite the fact that the same amount of digestible P and phytase was provided in all experimental diets, pigs fed feed additive mixture had a lower excretion, especially in the FIN phase ($P = 0.005$), where the decrease of P was 12% in SP diets and up to 24% in LP diets. Several studies have demonstrated the positive effect of the addition of organic acids such as benzoic acid and essential oils (Sauer et al., 2009; Xu et al., 2018) on the apparent digestibility of P in growing pigs. The reduced pH by organic acids may have enhanced phytase activity on phytate P, while increasing mineral absorption through its association with acid anions (Nahm, 2004; Suiryanrayna and Ramana, 2015).

3.1.2 Gas emissions

Emissions of NH_3 and CH_4 were analyzed at 2 different measurement locations in the same room, at the slurry pit level just below the animals through the PFCs, and total room emissions. A summary of these gas emissions for the GRO and FIN phases is presented in Table 3.

TABLE 3 Gas emissions (g per animal per day) measured at the slurry pit level and total room gas production in the growing and finishing phases of pigs fed diets with two levels of crude protein, including or not a dietary treatment.

Gas emissions	SPC[1]	SPA[2]	LPC[3]	LPA[4]	SEM	P value		
						Protein	Additives	Interaction
GROWING PHASE								
NH_3 Emission								
Slurry pit	2.83	1.21	1.65	1.80	0.549	0.603	0.206	0.133
Total room	5.75^{a}	3.67^{ab}	3.61^{b}	2.25^{c}	0.695	0.025	0.029	0.609
CH_4 Emission								
Slurry pit	0.09	0.01	0.29	0.34	0.183	0.199	0.304	0.497
Total room	0.28^{b}	0.75^{b}	7.69^{a}	0.74^{b}	2.35	0.049	0.431	0.013
FINISHING PHASE								
NH_3 Emission								
Slurry pit	2.59^{a}	4.09^{a}	0.87^{b}	0.10^{b}	0.522	<0.001	0.501	0.049
Total room	7.12^{ab}	9.04^{a}	5.84^{b}	2.85^{c}	0.714	<0.001	0.485	<0.001
CH_4 Emission								
Slurry pit	1.23	0.92	1.63	2.31	0.959	0.605	0.388	0.059
Total room	9.68	3.29	31.35	3.44	10.87	0.757	0.182	0.823

$^{a-d}$ Mean values within a row with different superscript letters differ ($P < 0.05$).
[1]SPC, Standard protein; [2]SPA, Standard protein with dietary treatment; [3]LPC, Low protein; [4]LPA, Low protein with dietary treatment.

The recorded NH_3 emissions, especially those from total room production, were within the range reported by other authors (Philippe et al., 2007; Hansen et al., 2014; Le Dinh et al., 2022) for the GRO and FIN phases in pigs reared on slatted floors. In addition, these same NH_3 emissions increased numerically with pig maturation, as previously reported by Seradj et al. (2018). It was assumed that total room emissions correspond to those generated in the slurry pit plus those evaporated in the soiled portion of the floor and in the slat by direct contact of urine and feces prior draining into the slurry pit. This mixture leads to urea hydrolysis through the enzymatic action of ureases from fecal bacteria and the subsequent and rapid volatilization from NH_4^+ (Philippe et al., 2011; Fuertes et al., 2021). Accordingly, these latter NH_3 emissions should be numerically higher compared to those obtained in the slurry pit, where NH_3 formation and release occur in the long-term period and are exclusively subjected to the air-slurry interface, which limits the potential volatilization area. In the latter fraction, NH_3 emissions from the degradation of undigested proteins must also be considered, although they are proportionally much less important (Aarnink and Verstegen, 2007).

The dietary CP level had important effects on NH_3 emissions, which decreased in the rooms where LP diets were offered. This response was observed in the total room emissions of the GRO and FIN phases, with an overall reduction of 38% ($P = 0.025$) and 46% ($P < 0.001$), respectively, and also at the slurry pit level of the FIN phase, with a noticeable reduction of 85% ($P < 0.001$). The reduction of NH_3 emissions by lowering dietary CP levels has been widely reported in previous studies (Galassi et al., 2010; Hernández et al., 2011; Seradj et al., 2018; Le Dinh et al., 2022), associated with lower N excretion, mainly in the form of urine (Philippe et al., 2011; Wang et al., 2018). In this sense, the lack of significant differences in total N concentration between the two CP levels could be attributed to the higher NH_3 volatilization in the SP-fed pigs. In addition, the important effect on slurry pH discussed earlier may directly contribute to the lower conversion of manure NH_4^+ to NH_3 in the LP-fed pigs, thus reducing its emissions (Aarnink and Verstegen, 2007; Le Dinh et al., 2022). This effect was even more pronounced in pigs receiving the combination of the LP diet and the dietary treatment (i.e., LPA), which resulted in a reduced NH_3-N concentration especially in the FIN phase (significant interaction $P = 0.03$). It is well known that the reduction of NH_3-N in swine slurry reduces NH_3 volatilization, which in turn is a consequence of the reduced pH (van der Peet-Schwering et al., 1999).

Until recently, few studies have measured the emission of pollutant gases at the slurry pit level. In the present study, we observed that the overall reduction in NH_3 emissions by lowering the CP level was more intense at the slurry pit, up to almost 60%, than the total room emission, which was reduced by 43%. Likewise, in a recent study by Le Dinh et al. (2022), in which NH_3 emissions were measured at four heights, 1 and 10 cm above the slurry surface, and at floor and room level, they obtained more intense reductions in NH_3 emissions at locations closer to the volatilization surfaces when dietary CP was reduced by 2%.

In accordance with the above, the use of the dietary treatment under study also had significant effects on NH_3 emissions, although some differences were observed between both feeding phases. In the GRO phase, total room NH_3 emissions decreased significantly in the presence of the dietary treatment, by 36% in the SP and 38% in the LP group ($P < 0.029$). However, significant interactions were found in the FIN phase in both measurement locations ($P < 0.05$). While NH_3 emissions decreased significantly with the dietary treatment in the LP group, up to 89% at the slurry pit level and 51% in the total room emissions, no differences were found in the SP group, or even numerically increased. This slight increase could be consistent with the high values of NH_3-N in the slurry presented in Table 2. Furthermore, the greater reduction in total room NH_3 emissions shows that the inclusion of such dietary treatment may play a greater role when urine and feces are mixed prior to falling into the slurry pit, rather than the long-term emissions produced at the air-slurry interface of the slurry pit.

In line with the beneficial effects associated with the inclusion of feed additives on the dietary treatment discussed above, the meta-analysis by Ti et al. (2019), which evaluated several mitigation practices to reduce NH_3 emissions, concluded that the highest efficacy of feed additives, beyond reducing CP levels, can be explained by their effects on modifying the microbial population of the digestive tract, the nutrient composition, and the physicochemical properties of the slurry (e.g., pH), and thus gas emissions from both the digestive tract and the slurry.

Partitioning of emissions between the slurry pit level and total room emissions refers only to NH_3 evaporation. In the case of CH_4, the slurry pit emission is generated by anaerobic fermentation of slurry material, while the total CH_4 room emissions (excluding slurry pit emissions) correspond to the enteric CH_4 generated in the hindgut and eliminated by flatulence. Indeed, the CH_4 generated by the fermentation of fecal material during its transit toward the pit is considered negligible. Therefore, the results are presented and discussed in this context. Methane emissions showed considerable differences between the feeding phases, both at the slurry pit level and in total room emissions, which may be influenced by the different fiber source between phases. Emissions were higher in the FIN phase than in the GRO phase, in agreement with previous values from our group (Seradj et al., 2018). This is explained by a more developed and abundant microbial population in the hindgut of mature pigs (Sarri et al., 2021), resulting in a higher fermentative capacity and enteric production of CH_4 (Le Goff et al., 2002). However, it also coincides with higher dietary fiber content. Overall, CH_4 emissions showed a greater rate of variability between days of analysis compared to NH_3 emissions. This variability in the results led to a lack of statistical differences in most of the analyses performed for this gas.

Although in previous research (Seradj et al., 2018, 2020) we did not find differences in CH_4 emissions as a function of dietary CP variations, in the present study certain differences were obtained, especially in the GRO phase. Overall, CH_4 emissions increased by 6–8 times in the LP group of the GRO phase and by 2–3 times in the FIN phase, which was mainly due by a high rise in LPC diet, being significant in the total room emissions of the GRO phase ($P = 0.049$). However, this result was necessarily explained by its interaction with the presence of the dietary treatment (significant interaction $P = 0.013$). It should be noted that the fiber content in LP diets was 3% higher than in SP diets (Table 1), and therefore an increase in CH_4 emissions would have been expected. However, when the dietary treatment was included in the LP diets (i.e., LPA diet), an important reduction in CH_4 emissions was obtained in the total room emissions, reaching values close to those of the SP diet, even though the dietary treatment involved a 15% higher fiber content. These results suggest that the inclusion of additives may have helped to digest and absorb certain nutrients that would otherwise have been fermented in the hindgut, promoting both enteric and slurry pit CH_4 production (Balasubramanian et al., 2020). The specific effect of inclusion of additives seemed to be stronger in the enteric fermentation rather than the slurry pit emission, suggesting that it may target non-digestible but highly fermentable material that might be more available to the animal after enzymes action before reaching the hindgut.

On the other hand, the inclusion of feed additives in the dietary treatment could not involve a clear trend, as its effect varied with respect to control diets among phases and sampling locations. A previous study also using benzoic acid at 0.3% (Humphrey et al., 2022) showed no differences in CH_4 emissions when this acid was included in the diet. Moreover, investigations carried out by the Guide of emission reduction techniques in swine and poultry livestock (MAGRAMA, 2014), regarding the use of acidifiers in feed, indicate an 11% increase in CH_4 compared to control groups, despite being no significant, as in our case. Again, it has to be considered that the dietary treatment included a higher fiber content compared to control diets. This could mean that other components of the blend of additives could have a greater effect on the reduction of CH_4.

3.2 Field trial

3.2.1 Crop yield production

The first thing to be considered regarding the application of slurry on crop production and environmental assessment, is that this type of fertilization technology has low effect during the first year, and it is more relevant to study the cumulative effect over the years as well as the seasonal effect. Hence, the results obtained in this study can miss some of the long-term potential of using low CP diets on crop fertilization, limiting in this way the ability to assess the full scope of benefits. However, similar to what has been observed in other short-term studies (Franco-Otero et al., 2012; Jin et al., 2022), it remains valuable to identify trends in this particular matter that indicate the direction for further exploration in future studies.

Grain yield and grain protein content in the trial were similar to those obtained in the surrounding area (Zhang et al., 1998; Lloveras et al., 2004; Aranguren et al., 2021), with yields exceeding 5 t ha^{-1}, regardless of the diet, and an average protein content of 9.25%. Differences in crop yield and grain protein content for irrigated wheat among diets were not statistically significant, whether comparing protein levels, additives, or their interaction (Table 4). Therefore, the results should be interpreted as trends. In this sense, there was a positive trend towards increased wheat crop yield with the presence of feed additives, observed in both SP and LP diets. In terms of grain protein content, the slurry from pigs fed the dietary treatment led to a slightly higher protein content when combined with the LP diet, compared to when no dietary treatment was used. These results may suggest that LPA slurry had more accessible N, which could favor a

TABLE 4 Crop performance for wheat crop yield, grain protein content, water use efficiency (WUE) and N use efficiency (NUE) after slurry application.

Crop responses	SPC[1]	SPA[2]	LPC[3]	LPA[4]	SEM	P value		
						Protein	Additives	Interaction
Wheat crop yield, kg grain ha^{-1}	5632.1	5717.8	5348.4	5655.9	344.6	0.64	0.59	0.76
Grain protein content, %	9.64	8.92	9.02	9.42	0.38	0.88	0.69	0.18
WUE, kg grain ha^{-1} mm	13.4	13.6	12.7	13.5	0.67	0.58	0.53	0.72
NUE, kg grain ha^{-1} kg applied N	56.3	57.2	53.5	56.6	2.81	0.58	0.53	0.72

[1]SPC, Standard protein; [2]SPA, Standard protein with dietary treatment; [3]LPC, Low protein; [4]LPA, Low protein with dietary treatment.

faster incorporation of protein into the grain. Conversely, the opposite trend was observed at the SP level (Table 4).

The WUE in the wheat crop was below the area's average for irrigated conditions (Zhang et al., 1998; Katerji et al., 2008). The main hypotheses for this phenomenon revolve around the coincidence of grain filling stages with moderate temperatures and adequate soil moisture content during the tillering and flowering processes (Zahedi and Jenner, 2003). In this case, the results obtained should also be interpreted as trends. The primary observation was a better WUE when using the slurry from pigs supplemented with the dietary treatment, and a lower WUE values in the LPC slurry. Moreover, the NUE, considered as crop production vs. applied N was above 50 kg of grain per kg N ha^{-1}, which is considered a medium-high level of efficiency of the application of the slurry. Results were again not significant, despite it can be appreciated a trend towards a better NUE with the inclusion of the dietary treatment under study, in both SP and LP diets.

3.2.2 Greenhouse gas emissions

The GHG derived from slurry application to the field under study are shown for the four different diets in Table 5. In all of the

TABLE 5 Greenhouse gases accumulated emissions for CH_4, CO_2 and N_2O from the experimental field under the different diets of the study.

Gaseous Emissions	SPC[1]	SPA[2]	LPC[3]	LPA[4]	SEM	P value		
						Protein	Additives	Interaction
CH_4, kg ha^{-1}	0.26[a]	-0.03[bc]	-0.11[c]	-0.02[b]	0.02	<0.001	0.001	<0.001
CO_2, kg ha^{-1}	989.8	1064.6	1035.4	1003.9	331.1	0.98	0.94	0.87
N_2O, g ha^{-1}	269.7	205.5	230.1	360.6	89.4	0.52	0.71	0.28

[1]SPC, Standard protein;
[2]SPA, Standard protein with dietary treatment;
[3]LPC, Low protein;
[4]LPA, Low protein with dietary treatment.

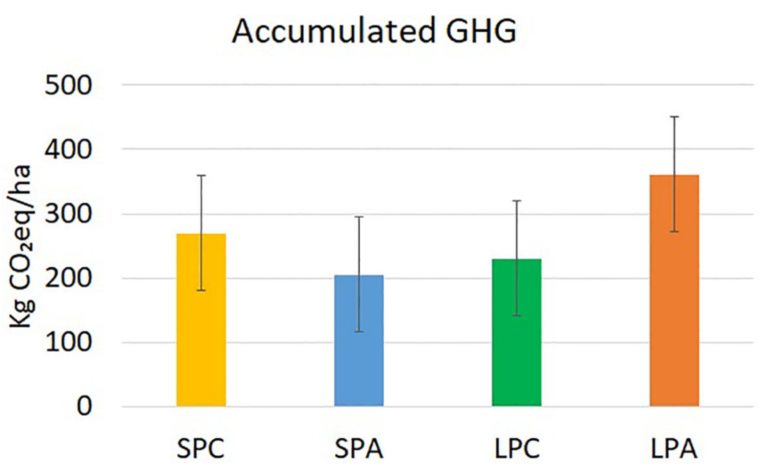

FIGURE 2
Accumulated emissions in kg CO_2 equivalents per ha.

three GHG analyzed (CH_4, CO_2 and N_2O) the level of the accumulated emission is consistent with the obtained in our Mediterranean conditions with irrigated wheat crop (Alhajj Ali et al., 2017; Oliveira et al., 2021).

The only GHG that showed statistical significance was CH_4, with values close to zero or even reaching negative balance in the emissions. This suggests that methanotrophic activity in the soil surpassed methanogenic activity due to the presence of aerobic conditions (Guo et al., 2022). This negative balance pattern is commonly observed in experiments conducted in the region under similar conditions (Franco-Luesma et al., 2019; Fernández-Ortega et al., 2024). In this way, some differences were found between groups. Diets including the dietary treatment significantly reduced CH_4 emissions when comparing with control ($P < 0.001$), as well as with the use of LP diets ($P < 0.001$), with a significant interaction between both parameters under study ($P < 0.001$). Considering the low emissions of CH_4, it is remarkable that possibly the LP diet combined with the dietary treatment could be contributing to lower CH_4 emissions added to a fixation of this as C in the soil.

No statistical differences were found for CO_2 between the four groups. That is consistent with the normal behavior of CO_2 emissions that are not usually affected by the application of fertilizers in a short period of one year, such as in this study.

Regarding N_2O, soils are the dominating source for atmospheric N_2O (Butterbach-Bahl et al., 2013). Deposited NH_3 can act as an indirect source of this gas, as it can be transformed into N_2O through nitrification-denitrification processes (IPCC, 2006). In our case, N_2O did not present significance for any treatment under study; nevertheless, its emissions showed a trend to be increased in LPA diets. Differences on the emission by diets may be associate to a more intense microbiological activity. Emission integration of CO_2, N_2O and CH_4 indicates the accumulated CO_2 emission equivalent and therefore its global warming potential. This GHG accumulation is presented in Figure 2, and despite there were no significant differences, numerically higher accumulated GHG emission in LPA diets can be appreciated ($P > 0.05$), mainly due to the aforementioned higher N_2O emissions. This may highlight the importance of considering the impact of N_2O, given its significant warming potential (EPA, 2023). On the other hand, the numerically lowest accumulated GHG emissions were found when the dietary treatment and the SP level were combined.

All human activity has an impact on GHG emission, but they are included in the natural cycles of the planet; therefore, any activity with a biological component produces emissions of CO_2, N_2O and CH_4. In this case, the best crop yields were observed with diets that included the dietary treatment, both for standard and low protein level. The numerically higher N_2O field emissions in the LPA slurry could be related to its lower emission of NH_3 during the animal trial, with a lower NH_3-N concentration in slurry (Table 2). Nevertheless, it may be possible that this slurry could still present a relevant organic N concentration, which may lead to higher N_2O emissions during field application (Dambreville et al., 2008). However, this effect needs to be further investigated in repeated slurry application as fertilizer in longer-term studies to better understand the interaction between N excretion forms and the subsequent GHG emissions when slurry is applied as fertilizer.

4 Conclusions

Results suggest that CP level in pigs can be reduced at some extent (1.5%) to reduce NH_3 emissions at the slurry pit level but also in the total room emissions, without compromising animal performance. Moreover, the use of feed additives combined with a higher soluble fiber content to mitigate NH_3 emissions led to significant reductions from slurry in both pit and room without affecting pig performance. Reduction was more effective in the FIN phase and especially in LP diets, where the inclusion of this dietary treatment led to a NH_3 reduction of more than 90% in the slurry pit and more than 50% at the room level. LP diets showed higher levels of CH_4 in both pit and room, although most of analysis weren´t conclusive for the high variability in the results. Regarding field application, LPA slurry had positive effects on the efficiency and yield of the crop despite not showing significance, suggesting some beneficial tendencies with the combination of LP and the dietary treatment with additives. Moreover, based on GHG emissions and global warming potential, LPA diets showed no significant negative effects of the application of the resulting slurry as top-dressing fertilization in the wheat crop.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

Ethics statement

The manuscript presents research on animals that do not require ethical approval for their study.

Author contributions

EF: Formal analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. LS: Conceptualization, Investigation, Methodology, Supervision, Writing – original draft, Writing – review & editing. RC: Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. EP-C: Funding acquisition, Project administration, Supervision, Visualization, Writing – original draft, Writing – review & editing. ÁC: Conceptualization, Data curation, Formal analysis, Supervision, Writing – original draft, Writing – review & editing. JB: Conceptualization, Funding acquisition, Resources, Supervision, Writing – original draft, Writing – review & editing. AS: Conceptualization, Methodology, Resources, Supervision, Writing – original draft, Writing – review & editing. CC-M: Formal analysis, Investigation, Methodology, Supervision, Writing – original draft, Writing – review & editing. JF-O: Formal analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. GD: Conceptualization, Investigation, Project administration, Supervision, Writing – original draft, Writing – review & editing.

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Conflict of interest

EP-C and AC were employed by DSM- Firmenich.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fanim.2025.1508660/full#supplementary-material

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