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Using Spirulina (*Limnospira platensis*) as an alternative feedstuff for poultry: Effects on ammonia and greenhouse gas emissions from excreta during storage

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ABSTRACT

To identify sustainable alternative solutions to soybean meal, novel feedstuffs have been extensively investigated. Among these, Spirulina stands out due to its interesting composition and widespread use in animal nutrition. It is also well-known that animal excreta contribute significantly to global greenhouse gases (GHG), particularly ammonia (NH_3) emissions. The latter are particularly affected by storage conditions and excreta intrinsic characteristics namely pH, which can lead to increased Nitrogen losses. This study investigates the impact of including an alternative protein source, specifically Spirulina at a 15 % inclusion rate on chicken feeding, on GHG and ammonia NH_3 emissions during excreta storage. Two slow-growing broiler strains (naked neck and fully feathered) were raised under either thermoneutral (23 °C) or heat stress (30 °C) conditions. Each feeding trial lasted 12 weeks, during which excreta were collected, frozen, and later stored at ambient temperature for 60 days. A total of eight excreta types, each with three replicates, were analyzed. Results showed that excreta from birds raised under heat stress (30 °C) exhibited greater NH_3 and CO_2 emissions compared to those under thermoneutral conditions. Spirulina inclusion significantly increased NH_3 emissions by 11 % compared to normal diet and nitrous oxide (N_2O) by 17 %, regardless of temperature. Methane emissions remained low (< 0.075 μg/kg) across all treatments and were not significantly affected by diet nor temperature. Spirulina offers potential as a sustainable protein source; however, the inclusion level evaluated in the present study (15 %) was associated with increased nitrogen losses during manure storage, suggesting that high inclusion rates may increase the environmental footprint of broiler production.

Introduction

World population is expected to reach 9.2 billion by 2075 (Alexandratos and Bruninsma, 2012). To feed such a population, in particular in developing countries and with increased urbanization, it is necessary to increase agricultural outputs, particularly of animal-origin products such as meat, dairy or eggs (Crosson et al., 2011; Chaves et al., 2021). Among the different animal production sectors, poultry has the potential to meet such demands, due to its relatively low production costs, short production cycle and high efficiency (Alkhtib et al., 2023). Furthermore, poultry meat is easy to produce, has a high nutritional value, particularly regarding its high protein and essential amino acid contents, being free from religious or cultural constraints (Pestana et al., 2020). However, the rapid expansion of the poultry sector over the past 60 years has raised significant environmental sustainability issues.

Broiler chicken production depends heavily on corn and soybean meal as primary sources of energy, lipids, proteins, and amino acids. However, soybean cultivation and international trade have been major drivers of land-use, (Song et al., 2021) linked to greenhouse gas emissions, soil erosion, deforestation, biodiversity loss, high water consumption and ecosystem degradation, raising environmental and sustainability concerns (Foley et al., 2005; Pimm et al., 2014; Gibbs

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et al., 2015).

In order to address the challenges associated to soybean meal dependency in poultry production, several alternative feedstuffs have been proposed. Among these, Spirulina *(Limnospira platensis)*, has emerged as a promising candidate due to its high crude protein content, balanced amino acid profile and abundance of essential vitamins and minerals (Pestana et al., 2020; Mullenix et al., 2022). Microalgae, such as Spirulina, are particularly interesting, not only for their adaptability to extreme environments, but also the fact that they can grow using non-arable land and furthermore may use brackish or even wastewater, minimizing competition with conventional agriculture (Madacussengua et al., 2025). Additionally, microalgae contribute to environmental sustainability by enhancing carbon sequestration through their high carbon dioxide fixation rates (Costa et al., 2024). Microalgae, particularly Spirulina, can be used at low (typically below 1 %) or high (5-20 %) inclusion levels. While Spirulina has been extensively studied as a feed additive at low dietary inclusion levels rendering interesting results regarding enhanced feed conversion ratios, increased antioxidant enzyme activity, and improved total tract digestibility therefore reducing N excreted at low dosages (Evans et al., 2015; Park et al., 2018; Moustafa et al., 2021). Nevertheless, at high inclusion rates, the recalcitrant cell wall—characterized by a high content of indigestible carbohydrates—hinders nutrient digestion, thereby reducing digestibility and increasing digesta viscosity (Pestana et al., 2020; Zampiga et al., 2023). Such changes in digestive efficiency may be linked to increased ammonia emissions during excreta storage due to the incomplete breakdown of dietary nitrogen in the gastrointestinal tract. Furthermore, ammonia emissions may also be influenced by additional factors, such as environmental temperatures and poultry genetics, which can modulate the extent of these effects.

On the other hand, poultry excreta is rich in nitrogen (N) as well as in phosphorus (P) and potassium (K), with the former present as residues from protein absorption (70 %) and uric acid (30 %) (Nahm, 2003). Upon excretion, the N transformation process is highly influenced by housing and storage conditions including temperature, humidity, ventilation and pH of the material (Webb et al., 2005; Dróżdż et al., 2020; Bist et al., 2023; Gržinić et al., 2023). As a consequence, poultry excreta is a relevant source of ammonia (NH_3) and greenhouse gases (GHGs), including nitrous oxide (N_2O), methane (CH_4), carbon dioxide (CO_2), throughout its lifecycle comprising storage and soil application (Dróżdż et al., 2020). Globally, poultry production accounts for 8 % of livestock-related greenhouse gas (GHG) emissions, totaling 606 Mt of CO_2-equivalent annually (Gerber et al., 2008). Nitrous oxide emissions which account for 92.8 % of the overall emissions from non-ruminant livestock, within the poultry sector originate predominantly from manure storage and processing (Du Toit et al., 2014). In Europe, agricultural activities account for approximately 80–90 % of NH_3 emissions, with animal production being one of the major contributors (Wyer et al., 2022).

To mitigate such environmental impacts, it is crucial to explore alternative protein sources, such as microalgae in general and Spirulina in particular. Several studies have examined the effects of dietary microalgae inclusion on poultry performance, as well as egg and meat quality (Boskovic Cabrol et al., 2022; Madacussengua et al., 2024; Fernandes et al., 2024; Spínola et al., 2025). However, and to the best of our knowledge, no studies have quantified the GHG emissions associated with the excreta of animals fed diets containing microalgae. These gaps hinder the ability to produce accurate and concrete estimates on the environmental sustainability of this practice. Addressing such knowledge gaps is thus essential for determining whether Spirulina-based feeds can effectively reduce the environmental footprint of animal production systems.

The present study evaluates the effects of chicken strain (FF = fully feathered; NN = naked neck) and Spirulina dietary inclusion on the chemical composition, ammonia and greenhouse gas emissions of excreta from chickens raised under thermoneutral and heat stress conditions. By analyzing the interaction between chicken raising temperature, strain and emissions, this study provides insights into optimizing poultry production systems for higher levels of sustainability. Understanding how these factors influence environmental impacts, particularly manure emissions, is crucial for developing climate-resilient and environmentally sustainable broiler production practices.

Materials and methods

Excreta production and collection

Given the role of temperature as an environmental factor in terms of growth performances in this study, slow-growth broilers were reared at two distinct temperature ranges: 23°C (TNZ: adequate temperature conditions) and 30°C (HS: heat stress conditions). These conditions were selected to simulate environmental challenges and assess how broilers respond to thermal stress. In accordance, two broiler strains were compared: fully feathered and naked neck. The naked neck strain, characterized by a dominant autosomal gene reducing feather coverage in the cervical region by 20–40 %, has been shown to enhance thermal dissipation efficiency and improve resilience to heat stress (Cahaner et al., 2008). Poultry is particularly vulnerable to high temperatures due to their lack of sweat glands, making heat stress a critical factor that adversely affects growth rates, feed intake, body weight, egg production, and immune function (Khan et al., 2023). However, the reduced plumage of the naked neck strain enhances heat tolerance, which can result in faster growth rates and higher productive performance under heat stress. These traits make the naked neck phenotype a cost-effective and viable solution, particularly in regions with warm climates (Fernandes et al., 2023a). The animal housing and experimental diet protocols for this trial have been thoroughly detailed by Fernandes et al. (2024). Authors evaluated productive performance and meat traits. As such, the *in vivo* experimental design is briefly addressed here: two distinct strains of slow-growth broilers were used in this study: SA31A x XL44N (Naked Neck) and SA31A x C44 (Fully Feathered). A total of 80 one-day old male broilers (40 per strain), divided in two experiments of 40 animals each, were housed individually in cages at the animal experimentation facilities at the School of Agronomy of the University of Lisbon (Portugal). Animals were randomly allocated into individual cages with *ad libitum* access to water and feed. The study lasted 84 days and was conducted under two separate 12-week trials, one at 23°C (TNZ) and the other at 30°C (HS). Each trial included four experimental treatments, with 10 animals per treatment, defined by strain and diet as follows: CFF: Control diet + Fully Feathered; CNN: Control diet + Naked Neck; SPFF: 15 % Spirulina Inclusion + Fully Feathered; SPNN: 15 % Spirulina Inclusion + Naked Neck. Therefore, leading to eight different experimental group as reported in Table 1. Excreta samples were collected daily using trays lined with aluminum foil placed beneath each cage. During sample collection, excreta from all birds within the same treatment were pooled together without distinguishing between individual animals or separating feathers. Samples were placed in labeled bags and immediately stored at −20°C. This sampling procedure was conducted daily for one week. Subsequently, weekly pooled samples were thawed and subjected to further analyses. Birds had ad libitum access to feed and water. Experimental diets were formulated to be isoenergetic and isonitrogenous within each feeding phase (starter, grower, and finisher) (Table S1 and Table S2). For full details on the experimental design, kindly refer to Fernandes et al. (2024) and Fernandes et al. (2025).

Excreta analysis

The excreta were thawed at 4°C for 72 hours and subsequently dried in a ventilated oven at 60°C. This procedure has been described by Chaves et al. (2024). Dried samples were ground to a particle size of 1 mm using a mesh mill (SK100 comfort miller, Retsch, Haan, Germany).

Table 1
Description of experimental treatments and corresponding abbreviations used throughout the manuscript. Treatments CTNZ, SPTNZ, CHS, and SPHS represent diet × environmental condition groups obtained after excluding the genetic component from the statistical analyses.

Treatment	Description
CFF_TNZ	Control diet + Fully Feathered + Thermoneutral Zone
CNN_TNZ	Control diet + Naked Neck + Thermoneutral Zone
SPFF_TNZ	15 % Spirulina Inclusion + Fully Feathered + Thermoneutral Zone
SPNN_TNZ	15% Spirulina Inclusion + Naked Neck + Thermoneutral Zone
CFF_HS	Control diet + Fully Feathered
CNN_ HS	Control diet + Naked Neck
SPFF_ HS	15% Spirulina Inclusion + Fully Feathered
SPNN_ HS	15% Spirulina Inclusion + Naked Neck
CTNZ	Control + Thermoneutral Zone
SPTNZ	Spirulina + Thermoneutral Zone
CHS	Control + Heat Stress
SPHS	Spirulina + Heat Stress

Excreta were analyzed for dry matter (DM), ash and crude protein (CP) following the procedure described by EGRAN (Gidenne et al., 2001). The dry matter content was determined by placing the sample in an oven at 103°C overnight, followed by cooling in a desiccator until constant weight at room temperature. Ash content was determined through incineration in a muffle furnace at 550°C for 3 hours. Total nitrogen was quantified using the Kjeldahl method (Horneck and Miller, 1998) using the complete procedure (digestion, distillation and titration), while crude protein content was estimated using a conversion factor of 6.25. The pH was measured in a suspension of the sample in deionized water (1:10, m/v), after 1 h of occasional agitation using an Orion 3 Star pH meter (Thermo Fisher Scientific, USA). For further details, kindly refer to Chaves et al. (2024).

Gaseous measurements

It should be noted that ammonia volatilization may occur throughout the entire manure management chain, prior to manure collection. Consequently, emissions measured during storage represent losses from the residual nitrogen pool remaining after volatilization occurring in the housing environment. Since excreta from all treatments were subjected to the same rearing and collection procedures, potential pre-storage ammonia losses were assumed to affect all treatments equally.

Gaseous measurements during excreta storage were conducted between May and July 2023 in a ventilated building (Lisbon, Portugal, N:38.708300, W: −9.185308). Samples were never exposed to sunlight. Room temperature varied between 21 and 27°C (Fig. S1) to simulate storage conditions. Before experiment set-up, excreta were thawed at 4°C, homogenized, and then placed in glass jars (diameter of 25.5 cm and height of 28.5 cm, 4.1 L capacity) in order to occupy 1/3 of the jar's volume and leave an open headspace between the surface of the excreta and the glass lid.

Each excreta type, resulting from the previously described experiments (Fernandes et al., 2024 and Fernandes et al., 2025) was studied in triplicate, leading to a total of 24 glass jars under the same conditions (Fig. S2). An additional empty jar was included as control to account for background gas concentrations. To determine the net emissions from each excreta surface, gas concentrations measured in the treatment chambers were corrected by subtracting the background concentrations recorded in the control jar. All jars were closed using a modified lid, were similar to those described by Pereira et al. (2022), two tubes and their respective valves were attached representing system's inlet and outlet. If necessary, silicone was added to the lid-jar interface to ensure isolation conditions. Once closed, jars were not moved nor opened until the end of the experiment. Air Temperature was measured daily and a vacuum air pump (ACO-388. Hailea, Chaozhou, China) was used to draw air through the headspace of the jars at a constant air flow rate of 1.8 L min^{-1} controlled through flow controllers equipped with needle valves. The flow rate was checked before each measurement using a digital flow controller (Dwyer Instruments, RMA-21-SS, Michigan City, IN, United States of America). Teflon tubes were used in all the connections.

Each jar (Fig. 1) was connected to an acid trap filled with 0.05 M orthophosphoric acid (350 g of acid at day 0 and then as emissions decreased over time, the quantity was reduced to 250 g), Orthophosphoric acid was replaced at every measurement. The acid trap was used to capture any emissions of ammonia from the airflow through the outlet. The placement of the air outlet at the center of the jar lid, combined with the use of an air pump to force airflow through the outlet, ensured that the air sampling accurately represented the atmospheric conditions within the jar. The NH_3 absorbed by the acid was quantified through the measurement of the ammonium concentration in the solution employing automated segmented-flow spectrophotometric techniques as described by Temminghoff and Houba (2004). The acid trap was also connected to an Erlenmeyer flask containing water (200 mL) to remove any acid present in the air. A filter was installed to safeguard the pump from any liquid that might inadvertently entered the system and reached the pump.

Greenhouse gases concentrations were measured by temporarily stopping the pump and connecting the photoacoustic gas monitor via two valves located at the beginning of the air inlet and outlet tubes positioned in the lid. Spot measurement were conducted and validated as described by Chrysanthopoulos et al. (2025), with each measurement lasting 60 s followed by a 60 s break before proceeding with the next jar. Initially, GHG fluxes were measured daily, including weekends (until d11) however, once the system was stabilized (from day 21 onwards), measurements were conducted every Monday, Wednesday, and Friday. The concentrations of N_2O, CO_2 and CH_4 were taken using a gas trace analyzer (Photoacoustic Gas Monitor - INNOVA 1512, Innova AirTech Instruments, Ballerup, Denmark). The photoacoustic monitor was equipped with an optical filter for water vapor (filter type SB0527, Lumasense Technologies, Ballerup, Denmark) and optical filters for N_2O (filter type UA0985, Lumasense Technologies, Ballerup, Denmark), CO_2 (filter type UA0982, Lumasense Technologies, Ballerup, Denmark), and CH_4 (filter type UA0969, Lumasense Technologies, Ballerup, Denmark). The system was built following the setup of previous studies (Fangueiro et al., 2008; Prado et al., 2020; Pereira et al., 2022) where no problem with cross sensitivities was reported. The CO_2, CH_4 and N_2O concentrations in the airflows were measured with the same pattern of the NH_3 emissions. The cumulated GHG emissions were also expressed as CO_2-equivalents using the conversion factors of 273 and 27.2 for N_2O and CH_4, respectively (IPCC, 2021).

Calculations and statistical analysis

Once measurements were obtained, actual gaseous emissions were obtained using the Eq. (1).

$$GE = (C_{outlet} \; - \; - \; C_{inlet}) \; \times \frac{273 \; x \; m(gas) \; x \; flux \; x \; 60}{22.4 \; x \; (T + 273) \; x \; A \; x \; 1000} \qquad (1)$$

Where: C_{outlet} and C_{inlet} corresponds to the gas concentration (ppm) read through the INOVA device, m(gas) stands for the molecular mass of the gas measured in g/mol, flux is equal to the flow rate measured each day through the use of a flowmeter (dm^3/min), T is the temperature expressed in°C and A as the surface of emissions (m^2).

Subsequently, cumulative emissions (CV) were estimated by averaging the flux between two sampling occasions and multiplying by the time interval between the measurements, as detailed in Eq. (2).

$$CV(n) = CV(n\text{-}1) + GE(n) + (n_{current} - n_{previous}) \; x \; (GE(n\text{-}1) + GE(n)) \; /2 \qquad (2)$$

Where $CV(n-1)$ corresponds to the cumulative value of the previous

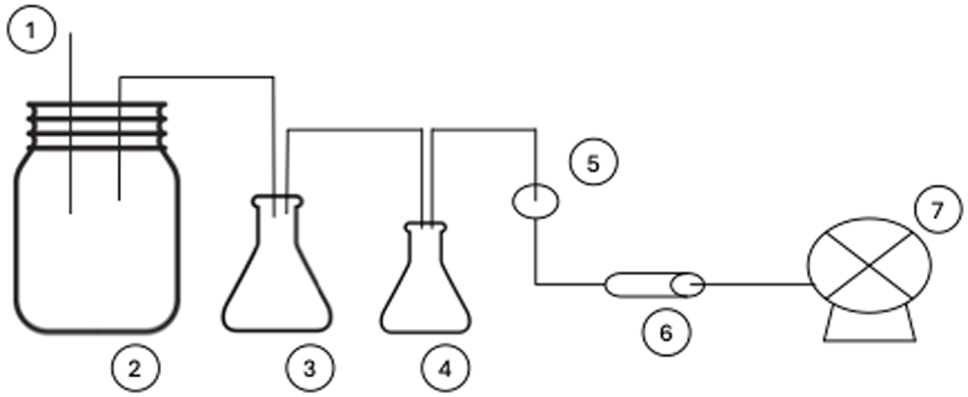

Fig. 1. Schematic representation of the system used to conduct the ammonia emissions analysis. The system is designed to enable air exchange within a jar (2). It includes an air inlet and outlet to facilitate this process (1), an acid trap (3) positioned downstream to capture ammonium emissions, an Erlenmeyer flask (4) filled with water to ensure proper airflow, a filter (5) and a flow regulator (6). A pump is connected to the setup, continuously drawing air through the system (7).

day, GE(n) to the gas emission of the current day, $n_{current} - n_{previous}$ as the difference in days between current and previous cumulative values while GE(n − 1) +GE(n)/2 corresponds to the average of the gas emissions for the previous day and the current day.

The characteristics of the excreta were averaged, and standard errors (SE) calculated based on the number of replicates (n = 3). Statistical analyses were conducted using R studio software (Version 2024.09.1 + 394). Normality of variance were assessed using the Shapiro test. Dataset resulted to be not normally distributed therefore, the Wilcoxon rank-sum test was used to assess the influence of genetic type, diet, and temperature conditions on each treatment (Tables S2, Table S3 and Table S4). The test results showed that p-values for genetic type were the highest (data not shown), indicating a lack of statistical significance. Consequently, genetic type (FF and NN) was excluded from further analysis, as it was not a significant factor in gas emissions. As a result, cumulative emissions were categorized as CTNZ: Control + Thermoneutral Zone, SPTNZ: Spirulina + Thermoneutral Zone, CHS: Control + Heat Stress and SPHS: Spirulina + Heat Stress (Table 1).

Following a significant effect detected by the Kruskal-Wallis test, a generalized linear model (GLM) using the quasipoisson family to account for overdispersion was performed to evaluate the effects of diet, temperature, and their interaction on emissions. Type III analysis of variance (ANOVA) was performed to determine the significance of main effects and interactions. Tukey's Honestly Significant Difference (HSD) test was used as a post-hoc analysis to compare treatment means, with significance declared when $P < 0.05$.

Results

Characteristics of the excreta

The initial composition of each type of excreta used is shown in Table 2. Although all materials had an average dry matter content of approximately 94 %, the SPNN_TNZ material exhibited the lowest dry matter content, while the highest value was observed in the CNN_HS material (94.161 % vs. 94.972 %, respectively). At the beginning of the experiment, N content showed highest values for the materials SPFF_TNZ and SPNN_TNZ (50.904 ± 0.392 and 52.820 ± 0.166 g/kg, respectively), whereas significantly lower values were recorded in the CNN_TNZ and SPNN_HS materials (42.088 ± 0.426 and 43.238 ± 0.127 g/kg, respectively). However, the ammonium fraction of nitrogen was minimal, showing little variability, ranging from 1.190 ± 0.049 g/kg (CNN_TNZ) to 1.457 ± 0.018 g/kg (SPFF_HS). Nonetheless, total carbon concentrations were markedly elevated across all treatment treatments, with values as follows: CFF_TNZ = 459.246 ± 0.558 g/kg; CNN_TNZ = 458.660 ± 0.100 g/kg; SPFF_TNZ = 429.872 ± 0.353 g/kg; SPNN_TNZ = 428.267 ± 0.204 g/kg; CFF_HS = 455.933 ± 0.296 g/kg; CNN_HS = 454.991 ± 0.227 g/kg; SPFF_HS = 427.303 ± 0.237 g/kg; and SPNN_HS = 422.414 ± 0.064 g/kg. The pH was higher in the materials derived from Spirulina enriched diet.

Gaseous emissions

Ammonia and Greenhouse gas emissions are reported in Table 3. In the same table, Global Warming Potential is also shown.

Nitrogen emissions. The ammonia emission flux over the 62 days of storage is depicted in Fig. 2. All treatments followed a similar pattern

Table 2
Characteristics of the excreta at the beginning of storage. Only pH is reported both at the beginning and at the end of the experiment. Mean and SEM (n = 3) are provided.

	CFF_TNZ	CNN_TNZ	SPFF_TNZ	SPNN_TNZ	CFF_HS	CNN_HS	SPFF_HS	SPNN_HS
pH_i	6.23 ± 0.05	6.82 ± 0.06	8.38 ± 0.09	7.93 ± 0.09	6.76 ± 0.03	6.55 ± 0.03	7.56 ± 0.07	8.04 ± 0.09
pH_f	7.96 ± 0.02	7.78 ± 0.08	10.11 ± 0.07	10.02 ± 0.08	8.63 ± 0.07	8.84 ± 0.44	10.33 ± 0.08	10.34 ± 0.01
Total N g/kg	45.682 ± 0.254	42.088 ± 0.426	50.904 ± 0.392	52.820 ± 0.166	45.729 ± 1.334	45.250 ± 0.240	47.885 ± 0.173	43.238 ± 0.127
NH_4^+ g/kg	1.382 ± 0.014	1.190 ± 0.049	1.409 ± 0.013	1.213 ± 0.028	1.248 ± 0.038	1.317 ± 0.031	1.457 ± 0.018	1.203 ± 0.026
Total C g/kg	459.246 ± 0.558	458.660 ± 0.100	429.872 ± 0.353	428.267 ± 0.204	455.933 ± 0.296	454.991 ± 0.227	427.303 ± 0.237	422.414 ± 0.064
C:N	10.054 ± 0.067	10.900 ± 0.108	8.446 ± 0.071	8.108 ± 0.027	9.987 ± 0.285	10.056 ± 0.057	8.924 ± 0.031	9.770 ± 0.027
DM %	94.515 ± 0.073	94.600 ± 0.016	94.626 ± 0.040	94.161 ± 0.012	94.921 ± 0.016	94.972 ± 0.009	94.429 ± 0.009	94.787 ± 0.004

pH_i: pH of the material at the beginning of the experiment; pH_f: pH of the material at the end of the experiment; Total C: total carbon at the beginning of the experiment; CFF_TNZ: Control diet fully feathered strain in thermoneutral conditions; CNN_TNZ: Control diet naked neck strain in thermoneutral conditions; SPFF_TNZ: Spirulina inclusion diet fully feathered strain in thermoneutral conditions; SPNN_TNZ: Spirulina inclusion diet naked neck strain in thermoneutral conditions; CFF_HS: Control diet fully feathered strain under heat stress; CNN_HS: Control diet naked neck strain under heat stress; SPFF_HS: Spirulina inclusion diet fully feathered strain under heat stress; SPNN_HS: Spirulina inclusion diet naked neck strain under heat stress.

Table 3
Cumulative emissions of carbon dioxide, nitrous oxide, methane and ammonia during a 63d storage period. Mean and standard deviation are given ($n = 3$). Values presented with different lowercase letters within rows are significantly different ($p < 0.05$), as per a Tukey test.

NH_3	CTNZ	SPTNZ	CHS	SPHS
Cumulative emissions (g/kg)	3.174 ± 1.585[d]	6.719 ± 0.842[c]	9.859 ± 1.641[a]	7.781 ± 0.848[b]
% N released as NH_3	8.623 ± 4.007[d]	15.732 ± 1.916[c]	26.381 ± 4.691[a]	20.781 ± 2.385[b]
N_2O				
Cumulative emissions (mg/kg)	8.187 ± 1.310[d]	9.197 ± 1.158[c]	12.140 ± 1.539[b]	14.518 ± 3.055[a]
% N released as N_2O	0.0185 ± 0.0023[a]	0.01756 ± 0.0022[a]	0.0266 ± 0.0032[a]	0.0322 ± 0.0086[a]
CO_2				
Cumulative emissions (mg/kg)	20.765 ± 8.133[c]	20.568 ± 5.126[c]	147.206 ± 126.957[b]	457.628 ± 511.311[a]
% C released as CO_2	0.0045 ± 0.0018[a]	0.0048 ± 0.0012[a]	0.0323 ± 0.0278[a]	0.1083 ± 0.1211[a]
CH_4				
Cumulative emissions (μg/kg)	0.037 ± 0.006[a]	0.046 ± 0.009[a]	0.059 ± 0.018[a]	0.073 ± 0.021[a]
GWP				
mgCO_2eq/kg	3574.25 ± 588.01[d]	3984.91 ± 530.93[b]	5732.99 ± 1108.93[c]	7887.55 ± 3034.55[a]
%N_2O	0.3586 ± 0.0018[a]	0.3593 ± 0.0013[a]	0.3353 ± 0.02336[a]	0.3067 ± 0.0591[a]
%CH_4	0.0014 ± ± 0.0002[a]	0.0015 ± 0.0002[a]	0.0014 ± 0.0004[a]	0.0014 ± 0.0006[a]

CTNZ: Control diet in thermoneutral conditions; SPTNZ: Spirulina inclusion diet in thermoneutral conditions; CHS: Control diet under heat stress; SPHS: Spirulina inclusion diet under heat stress.

with an initial period of higher activity followed by a decreasing trend once the system stabilizes (around day 14). Ammonia emission rates ranged from approximately 0 to 3.5 g N/kg/day across all treatments throughout the duration of the experiment. NH_3 emissions peaked in all treatments at approximately days 11, 18, and 58. The CNN_TNZ treatment shows the minimum emission values throughout the storage period, while notably higher patterns were observed for CNN_ HS, SPFF_HS, and SPNN_HS treatments.

Cumulative ammonia emissions (Table 3) were highest in the CHS treatment (9.859 g/kg; a), followed by SPHS (7.781 g/kg; b) and SPTNZ (6.719 g/kg; c). The lowest emissions were observed in the CTNZ treatment (3.174 g/kg; d). Statistically significant differences in ammonia emissions were observed among the four experimental treatments. Nitrogen losses via NH_3 emissions accounted for 8–26 % of the total initial nitrogen, with the lowest proportion in CTNZ and the highest in CHS. SPHS and SPTNZ contributed to 20.8 % and 15.7 % of the initial nitrogen losses, respectively.

Fig. 3 illustrates the daily N_2O fluxes, revealing an overall trend characterized by an initial stabilization phase, a sustained plateau, and a subsequent increase in emissions between days 51 and 58. In the SPNN_HS treatment, noteworthy emission peaks were observed on days 3 (3.78 mg N_2O/kg), 53 (0.84 mg N_2O kg/day), and 56 (0.72 mg N_2O/kg/day). In contrast, the CFF_HS treatment showed two distinct emission peaks on days 3 (1.24 mg N_2O/kg/day) and 17 (1.16 mg N_2O/kg/day). Among all treatments, the highest N_2O emissions were recorded in the SPNN_HS treatment, followed by SPNN_TNZ and SPFF_HS.

Cumulative emissions (Table 3) revealed significant differences among treatment, with SPHS and CHS displaying higher values compared to their respective control treatments (SPHS = 14.518 mg/kg (a) and CHS = 12.140 mg/kg (b) vs. SPTNZ = 9.197 mg/kg (c) and CTNZ = 8.187 mg/kg (d)). No statistically significant differences ($p > 0.05$) were observed between treatments for the percentage of nitrogen released as N_2O, with values remaining consistently low for all groups (CTNZ=0.0185 %, SPTNZ=0.01756 %, CHS=0.0266 % and SPHS=0.0322 %).

Carbon emissions. Regarding CO_2 emissions fluxes (Fig. 4), the SPNN_HS and CFF_HS treatments showed significant peaks within the first five days of storage, distorting the overall graph. To better resolve the temporal patterns of the other treatments in Fig. 4B, a graph close-up from day 5 to day 60 was also displayed. In the absence of SPNN_HS and CFF_HS, a general decline in CO_2 fluxes was observed after the first 14 days of the trial. Nonetheless, intermittent peaks were still evident, particularly in the SPFF_HS treatment on days 32 (0.93 mg CO_2/kg/day), 37 (0.68 mg CO_2/kg/day), and 42 (0.77 mg CO_2/kg/day). Additionally, elevated fluxes were detected for the CNN_HS and SPNN_TNZ treatments on days 21 (SPNN_TNZ=1.12 mg CO_2/kg/day) and 25 (CNN_HS=0.99 mg CO_2/kg/day), respectively.

No significant differences were observed in CO_2 cumulative emissions (Table 3) between the CTNZ and SPTNZ (CTNZ=20.765 mg/kg (c) and SPTNZ=20.568 mg/kg (c)) while differences were recorded in the CHS and SPHS alongside with higher emissions CHS=147.206 mg/kg (b) and SPHS=457.628 mg/kg (a) respectively, they were also statistically different. The percentage of carbon released as CO_2 did not differ significantly between treatments that were all below 0.1 %.

As expected, CH_4 emissions (Table 3) were markedly low across all treatments (CTNZ=0.037, SPTNZ=0.046, CHS=0.059 and SPHS=0.073 μg/kg), with no significant differences recorded.

Total GHG emissions. Total GHG emissions, expressed in mg CO_2-equivalents per kilogram (mg CO_2eq/kg), were calculated based on the cumulative emissions of CH_4 and N_2O. NH_3 was excluded from the calculation due to its negligible contribution to overall GHG emissions, as reported by Berg et al. (2006). The highest total GHG emissions were observed in the SPHS and CHS treatments, with values of 7887.55 mg CO_2eq/kg (a) and 5732.99 mg CO_2eq/kg (c), respectively. Statistically significant differences were found between all treatments, with SPHS and CHS values markedly exceeding those of CTNZ (3574.25 mg CO_2eq/kg; d) and SPTNZ (3984.91 mg CO_2eq/kg; b). Notably, the contributions of CH_4 and N_2O to the total GHG emissions remained minimal across treatments, accounting for less than 0.0015 % and 0.36 %, respectively (Table 3).

Discussion

Heat stress is an increasingly critical concern in broiler production, not only in tropical and subtropical regions but also in temperate climates. Indeed, heat stress negatively affects poultry by disrupting physiological and metabolic functions, impairing immune responses, and ultimately reducing feed intake, growth performance, and reproductive efficiency (Khan et al., 2023). To mitigate these effects, nutritional and genetic strategies have been studied. Dietary supplementation with Spirulina *(Limnospira plantensis)* has demonstrated promising results, including enhanced antioxidant enzyme activity, improved nutrient digestibility, and reduced ammonia emissions in broiler excreta (Park et al., 2018). Heat-tolerant broiler strains such as those carrying the naked neck (Na) gene exhibit improved thermoregulation due to reduced feather coverage in the neck region (20 %−40 %), leading to increased resilience under heat stress (Cahaner et al., 2008; Fernandes et al., 2023a).

While considerable research has focused on the impacts of heat stress on broiler productivity, studies investigating the emissions of noxious gases — such as NH_3 and GHGs — from broiler excreta produced at different thermal conditions remain limited. Most available studies have assessed emissions from poultry litter (Moore et al., 2011; Anderson et al., 2021; Liu et al., 2022), which includes bedding material, making it difficult to isolate the contribution of excreta alone. For instance, Calvet et al. (2011a) evaluated litter emissions across seasons but did not consider dietary factors that might have influenced the results.

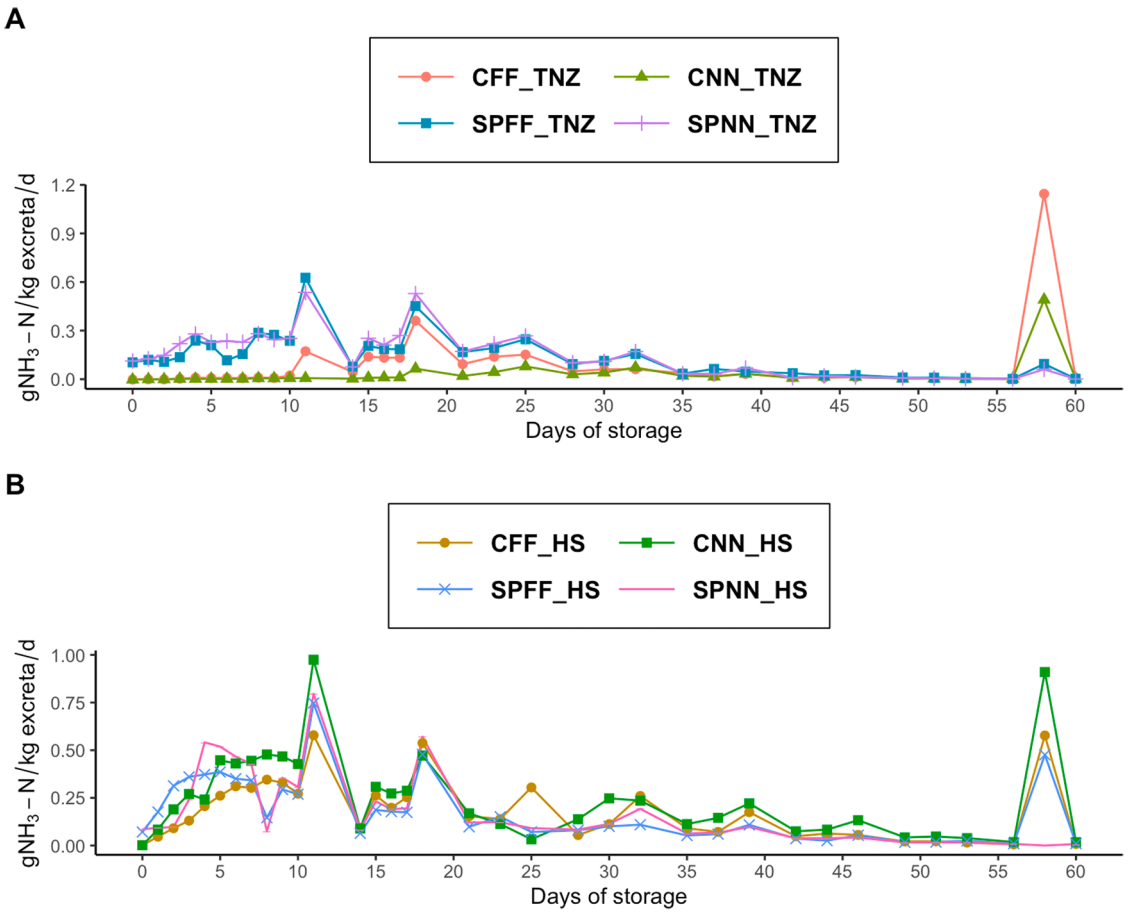

Fig. 2. Daily ammonia emission rates observed during excreta storage. Values presented are arithmetic means of three replicates. (A) CFF_TNZ: Control diet + Fully Feathered + Thermoneutral Zone; CNN_TNZ: Control diet + Naked Neck + Thermoneutral Zone; SPFF_TNZ: 15% Spirulina Inclusion + Fully Feathered + Thermoneutral Zone and SPNN_TNZ: 15% Spirulina Inclusion + Naked Neck + Thermoneutral Zone. (B) CFF_HS: Control diet + Fully Feathered; CNN_ HS: Control diet + Naked Neck; SPFF_ HS 15%: Spirulina Inclusion + Fully Feathered and SPNN_ HS: 15% Spirulina Inclusion + Naked Neck.

Similarly, while Park et al. (2018) investigated the effects of Spirulina supplementation on excreta characteristics, their study was limited to the final three days of the rearing period and did not include long-term collection period and storage duration.

In the present study, this knowledge gap was addressed by quantifying NH_3 and greenhouse gas (GHG) emissions from broiler excreta during a 60-day storage period. We assessed the effects of Spirulina supplementation, broiler genotype, and rearing temperature to provide a more comprehensive understanding of emission dynamics under both thermoneutral and heat stress conditions. To achieve this objective, excreta storage conditions were standardized and carefully controlled to ensure reliable comparisons among dietary treatments and to minimize environmental variability that could confound treatment effects. However, in commercial poultry production systems, excreta is mixed with various types litter are often stored in open piles or outdoor facilities, sometimes covered with plastic sheets or roofs, and are therefore exposed, to varying degrees, to environmental factors such as solar radiation, rainfall, wind, and temperatures (Ershadi et al., 2020). Sunlight exposure and elevated temperatures may further accelerate organic matter degradation and ammonia volatilization, whereas rainfall influence physicochemical processes occurring during excreta storage by altering microbial activity, increasing moisture content, and potentially causing nitrogen losses of up to approximately 30 % of total nitrogen, as well as increasing the risk of polluted runoff (Ogejo and Junior, 2009). Conversely, drying effects induced by wind and solar radiation may reduce microbial activity over time.

The results of this study indicate that the highest pH values of excreta were recorded in the SPFF_TNZ group, with an initial pH of 8.38 and a final pH of 10.11. Final pH levels in a similar range, were also observed in the SPNN_TNZ, SPFF_HS, and SPNN_HS groups. Increased pH levels (above 7) are known to promote ammonia (NH_3) volatilization from manure, as reported by Chen et al. (2021). In this trial, all treatments receiving Spirulina supplementation had higher pH levels. This could be due to the dietary inclusion of Spirulina. This observation aligns with the findings of Fernandes et al. (2024), who reported in an in vivo trial using the same animals that Spirulina supplementation increased water intake, resulting in higher moisture content in the excreta. The combination of elevated pH and increased moisture likely promoted ammonia volatilization, enhancing the conversion of ammonium (NH_4^+) to NH_3. Furthermore, and according to the NH_4^+/NH_3 equilibrium, as pH increases, a greater proportion of nitrogen exists in the gaseous NH_3 form, which is subsequently readily volatilized (Hassouna et al., 2016).

Unexpectedly, dry matter (DM) content was consistently high across all treatments, ranging from 94 % to 95 %, suggesting very dry material. However, the thawing process may have affected these values.

The total nitrogen (TN) values also revealed notable differences. Both SPFF_TNZ and SPNN_TNZ showed higher TN concentrations compared to their respective controls, suggesting a possible link between Spirulina inclusion and increased ammonia emissions. Indeed, and as ammonia is one of the main nitrogen-based gaseous emissions from poultry manure (Sigurdarson et al., 2018), the higher ammoniacal nitrogen content ($N–NH_4^+$) in the SPFF_TNZ and SPFF_HS treatments—measured at 140 mg/L and 145 mg/L, respectively—supports such conclusion (Table S5). In contrast, the CFF_TNZ and CNN_TNZ

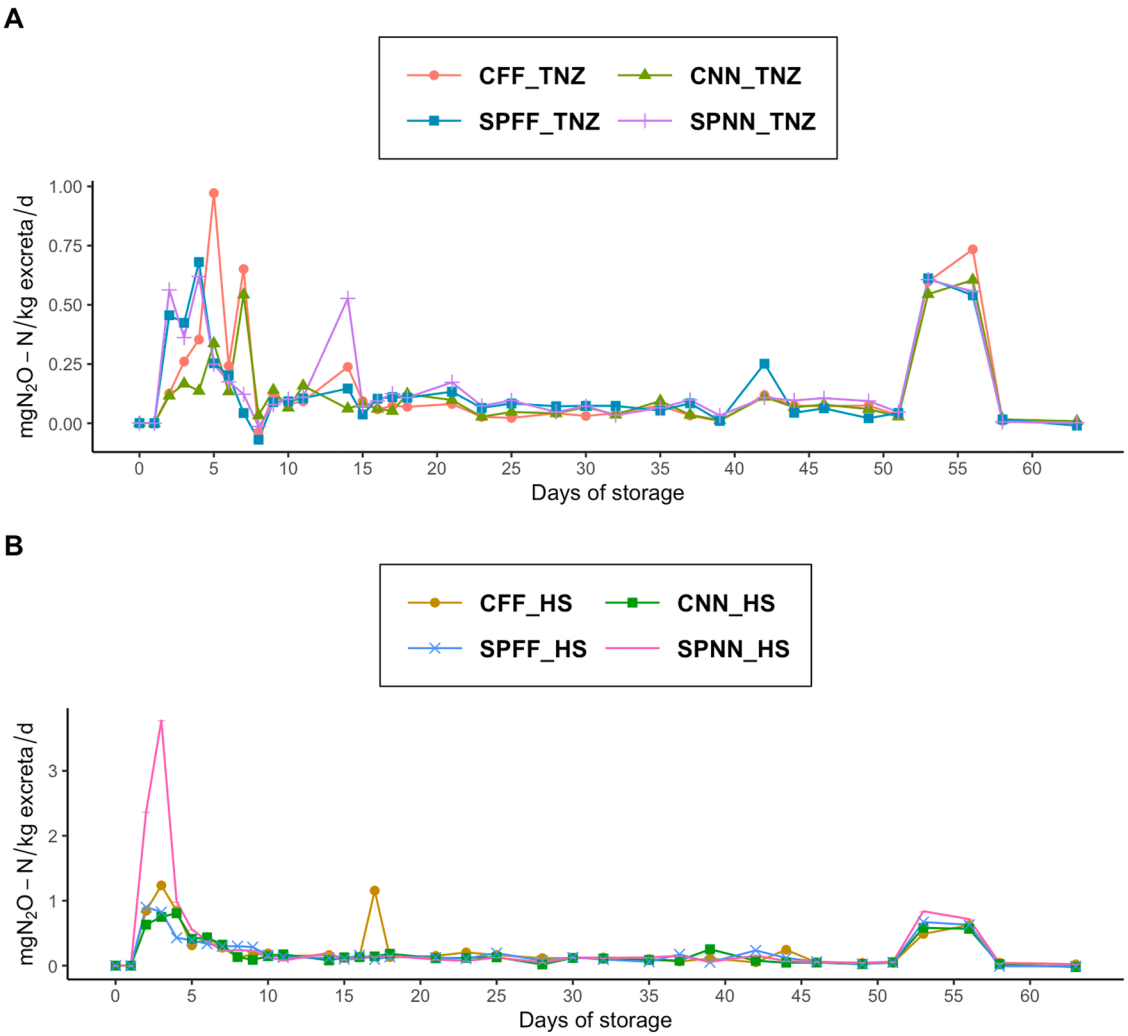

Fig. 3. Temporal dynamics of daily nitrous oxide emissions from excreta during storage. Values presented are arithmetic means of three replicates. (A) CFF_TNZ: Control diet + Fully Feathered + Thermoneutral Zone; CNN_TNZ: Control diet + Naked Neck + Thermoneutral Zone; SPFF_TNZ: 15% Spirulina Inclusion + Fully Feathered + Thermoneutral Zone and SPNN_TNZ: 15% Spirulina Inclusion + Naked Neck + Thermoneutral Zone. (B) CFF_HS: Control diet + Fully Feathered; CNN_HS: Control diet + Naked Neck; SPFF_ HS 15%: Spirulina Inclusion + Fully Feathered and SPNN_ HS: 15% Spirulina Inclusion + Naked Neck.

treatments showed lower values (e.g., 118 mg/L, Table S5), corroborating the link between dietary Spirulina and increased nitrogen excretion.

Interestingly, SPNN_HS showed a high TN content of 43.2 g/kg. However, this should not be interpreted as a sign of improved nitrogen utilization. Indeed, and as reported by Fernandes et al. (2023b), animals under heat stress had reduced feed intake and lower body weight, resulting in lower nitrogen ingestion and potentially altered excretion dynamics. To maintain an isonitrogenous diet with 15 % Spirulina inclusion, the amount of soybean meal was substantially reduced due to Spirulina's high protein content (60–70 %). However, as noted by Pestana et al. (2020) and Evans et al. (2015), Spirulina proteins may be less digestible due to protein gelation, which can increase excreta viscosity and nitrogen retention. Moreover, the cell wall of *L. platensis*, composed of peptidoglycans and phycobilisome protein complexes, may reduce digestibility and bioaccessibility (Costa et al., 2023; Spínola et al., 2023) further contributing to these results. Supporting these results, Zampiga et al. (2024) reported that broilers fed diets containing 3 % and 6 % Spirulina had lower ileal digestibility of essential and non-essential amino acids than those fed soybean-based diets. Reduced amino acid digestibility could further increase nitrogen excretion, thus contributing to higher ammonia emissions, as observed in this study. In our case, the use of Spirulina at 15 %, without the addition of exogenous enzymes or digestibility enhancers, likely exacerbated such effects. The resulting increase in digesta viscosity led to looser excreta and more intense excreta microbial activity, overall contributing to a marked and prolonged ammonia emission peak during the first month of storage. In addition, the microbial fermentation of undigested protein in the hindgut, particularly in the ceca, may have further increased ammonia production (Apajalahti and Vienola, 2016). In accordance, Fernandes et al. (2024) also observed an increased gastrointestinal tract length in Spirulina-fed birds, potentially due to slower passage rates caused by higher digesta viscosity. These findings may help explain the lower overall performance observed in the Spirulina-fed treatments.

Ammonium concentrations remained below 1.5 g/kg across all treatments, reinforcing the dry nature of the samples and suggesting that ammonia losses likely occurred during thawing. Ammonium, the predominant inorganic nitrogen form in fresh poultry manure, is highly susceptible to volatilization under aerobic and alkaline conditions, particularly when pH exceeds 7 (Nahm, 2003; Sommer et al., 2003). The combination of low ammonium levels and high dry matter (DM) content suggests, as already reported in Material and Methods, that part of the nitrogen may have been lost as ammonia (NH_3) prior to or during sample handling, potentially leading to an underestimation of gaseous emissions. The balance between total carbon (C) and ammonium is critical in determining nitrogen dynamics, especially during manure storage or

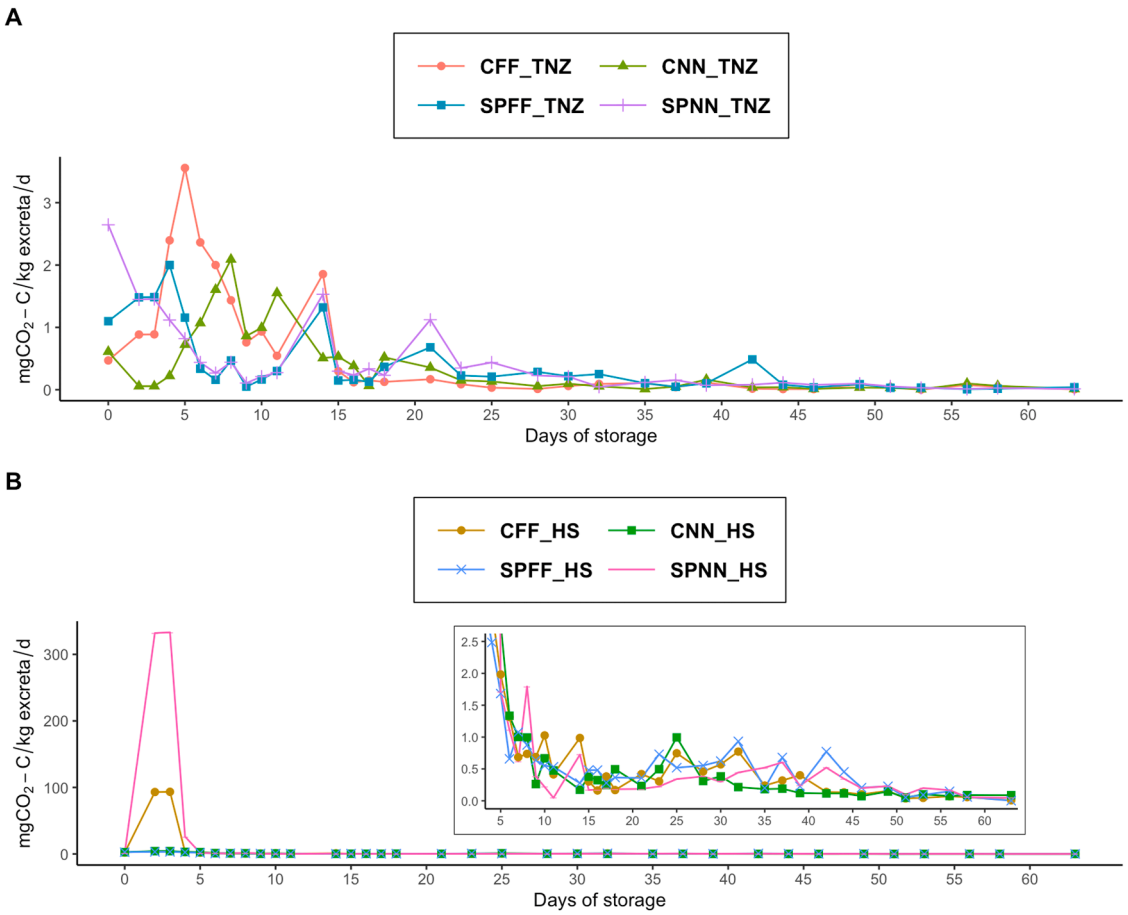

Fig. 4. Daily carbon dioxide emission rates from excreta during storage period. Values presented are arithmetic means of three replicates. (A) CFF_TNZ: Control diet + Fully Feathered + Thermoneutral Zone; CNN_TNZ: Control diet + Naked Neck + Thermoneutral Zone; SPFF_TNZ: 15% Spirulina Inclusion + Fully Feathered + Thermoneutral Zone and SPNN_TNZ: 15% Spirulina Inclusion + Naked Neck + Thermoneutral Zone. (B) CFF_HS: Control diet + Fully Feathered; CNN_ HS: Control diet + Naked Neck; SPFF_ HS 15%: Spirulina Inclusion + Fully Feathered and SPNN_ HS: 15% Spirulina Inclusion + Naked Neck. The main panel highlights the pronounced initial emission peak observed in the SPNN_HS treatment, whereas the inset provides an expanded scale from day 5 onward to facilitate comparison among treatments during the later storage phase.

composting. In the current study, the excreta showed C:N ratios between 8 and 10, typical of nitrogen-rich organic substrates such as poultry manure. Such a low C:N ratio indicates limited carbon availability for microbial immobilization of nitrogen, which promotes the conversion of NH_4^+ to NH_3 and subsequent volatilization losses (Bernal et al., 2009). Spirulina-fed treatments, in particular, showed slightly lower carbon values, likely linked to reduced body weight and feed intake, as observed (Fernandes et al., 2024). Moreover, the high-protein, low-carbohydrate composition of Spirulina (Becker, 2007; Pestana et al., 2020) may exacerbate nitrogen excess while providing insufficient fermentable carbon, further lowering the C:N ratio and enhancing the risk of ammonia emissions under more alkaline conditions (Zhao et al., 2008).

While changes in pH, nitrogen forms, organic matter, and carbon content offer critical insights into the underlying biochemical processes during excreta storage, gaseous emissions define the environmental footprint of broiler production systems. The genetic component was not further considered. Nonetheless, kindly refer to Supplementary material 1 for a complete set of details.

Nitrogen emissions. Cumulative ammonia (NH_3) emissions peaked within the first 20 days of storage and showed statistically significant differences among treatments ($p < 0.05$). The highest emissions were observed in the CHS and SPHS treatments, with values of 9.86 and 7.78 g/kg of manure, corresponding to 3.10 and 3.41 g/kg of live weight, respectively. Increased emissions were also found in the SPTNZ group (6.72 g/kg manure; 3.32 g/kg live weight), whereas the CTNZ group, showed the lowest ammonia emissions (3.17 g/kg manure; 0.94 g/kg live weight). These results indicate two key factors influencing ammonia emission profiles during storage: ambient temperature at the time of excretion (during the in vivo trial) and the dietary inclusion of Spirulina. Notably, the Spirulina inclusion rate was 15 %, a relatively high proportion, especially in the absence of additives or enzymes to aid digestibility. This, as previously explained, may have increased the viscosity of the gastrointestinal contents, leading to more watery excreta and enhanced microbial activity in the manure. Consequently, emissions peaked meaningfully and persistently during the first month of storage, likely due to the higher nitrogen content and exacerbated by heat stress conditions. Under heat stress conditions, broiler chickens experience a significant shift in nutrient metabolism that leads to increased protein catabolism (Brugaletta et al., 2022). Previous studies have shown that heat stress not only alters carcass composition by reducing breast muscle yield but also significantly affects protein metabolism (Howlider and Rose, 1989; Geraert et al., 1996; Lu et al., 2018). The increased catabolism of amino acids not only results in a loss of lean tissue but also leads to enhanced production of nitrogenous waste, including uric acid and urea (Rhoads et al., 2013). These nitrogenous compounds are excreted through manure and subsequently degraded by microbial urease activity during storage, producing NH_3. Moreover, Ma et al. (2021) demonstrated that HS enhances hepatic transaminase activity and increases the uptake of glucogenic amino acids by the liver, further promoting

deamination and nitrogen excretion. Therefore, the observed increase in ammonia emissions during manure storage under HS conditions can be reasonably explained by the increased nitrogen excretion resulting from intensified amino acid catabolism.

Conversely, nitrous oxide (N_2O) emissions were comparatively low across treatments, consistent with the low concentrations of nitric nitrogen ($N—NO_3 < 0.5$ mg/L in all treatments; Table S5). These emissions are more closely tied to microbial nitrification processes within the intestinal tract, rather than to the specific dietary ingredients used (Nahm, 2003). Emissions observed during the first 10 days are likely attributable to nitrification due to negligible initial nitrate content (Fangueiro et al., 2008). However, Spirulina inclusion may have altered gut microbiome (Abdelfatah et al., 2024), potentially modifying fermentation dynamics. In accordance, N_2O emissions followed a similar pattern to that of ammonia, with higher emissions in SPHS and CHS, followed by SPTNZ (14.52, 12.14, and 9.20 mg/kg, respectively), although these differences were nonetheless statistically significant.

Carbon emissions. Total carbon content was higher in the Control treatments (CFF_TNZ, CNN_TNZ, CFF_HS and CNN_HS) when compared to Spirulina groups (SPFF_TNZ, SPNN_TNZ, SPFF_HS and SPNN_HS). Despite this, CO_2 emissions differed significantly among heat stress treatments but showed no significant differences between CTNZ and SPTNZ. Calvet et al. (2011a) noted that CO_2 emissions are more meaningfully influenced by animal physiology, behavior, and nutrition than by manure composition alone, suggesting that the majority of emissions occur during the animal's life rather than post-excretion. Our measurements, conducted on excreta without litter, yielded values ranging from 20.57 to 457.63 mg CO_2/kg. This contrasts with most literature, which studied litter (e.g., mixed with straw or wood shavings), leading to different emission patterns due to altered microbial dynamics (Calvet et al., 2011b; Eugene et al., 2015; Anderson et al., 2021; Liu et al., 2022). Therefore, the comparison of the herein presented data with those available in literature should be done by taking into consideration such differences. Fangueiro et al. (2008) highlights that oxygen availability promotes aerobic microbial degradation, which raises material temperature and accelerates organic matter transformation. This aligns with our observation that excreta from groups CHS and SPHS was more watery, thus possibly containing more oxygen and promoting early emission peaks at the beginning of the storage period. Nonetheless, the high variability in CO_2 measurements likely confounded the detection of intergroup differences.

Methane (CH_4) emissions from stored manure are constrained by the lack of anaerobic fermentation substrates and the presence of oxygen. Nevertheless, Spirulina-fed birds exhibited slightly elevated methane emissions (CTNZ and CHS showed concentrations of 0.037 μg/kg and 0.059 μg/kg, respectively, whereas SPTNZ and SPHS exhibited levels of 0.046 μg/kg and 0.073 μg/kg, respectively), suggesting potential shifts in gut fermentation patterns due to altered diet composition as proposed by Tardiolo et al. (2025). Although these emissions remained overall low, no statistically significant differences were observed among treatments.

Global Warming Potential. Global Warming Potential (GWP) was used to integrate all greenhouse gas emissions and facilitate a holistic comparison between treatments. Significant differences were observed, with the CTNZ group showing the lowest GWP (3.57 g CO_2eq/kg) and the SPHS group the highest (7.89 g CO_2eq/kg). The inclusion of Spirulina in the diet, combined with higher rearing temperatures, has contributed to increased GHG emissions, as evidenced by the emission profiles of the SPTNZ, CHS, and SPHS treatments. In contrast, the CTNZ group consistently demonstrated the lowest emissions across all measured GHGs. These differences may be attributed to variations in metabolic activity, gut microbial fermentation, and nutrient digestibility between experimental treatments. Additionally, it is noteworthy that the SPHS group exhibited a significantly lower final body weight compared to the CTNZ group, as reported by Fernandes et al. (2024) and Fernandes et al. (2023b). This reduction in growth performance is likely associated to a poorer feed conversion ratio, potentially leading to higher excretion of undigested nutrients and metabolic by-products. Such increased excretion may, in turn, contribute to greater emissions during manure storage. On the other hand, SPHS's elevated GWP was primarily due to higher CO_2 emissions, though variability in sample measurements affected statistical clarity. These findings suggest that dietary Spirulina and heat stress interact to alter microbial and metabolic processes, ultimately influencing the emission profile and environmental impact of broiler production.

Finally, it also important to highlight that the present study was conducted using slow-growing broiler genotypes, which may differ from fast-growing commercial broilers in growth performance, nitrogen metabolism, and excreta characteristics. Therefore, caution should be exercised when extrapolating the present findings to conventional intensive production systems, and further studies including fast-growing genotypes are warranted to confirm the observed responses to Spirulina inclusion.

Conclusion

To conclude, the inclusion of Spirulina in broiler diets influenced the chemical composition of excreta and the emission of ammonia and greenhouse gases under both thermoneutral and heat stress conditions. Under thermoneutral conditions, Spirulina supplementation increased the crude protein content of the excreta, leading to higher ammonia emissions. Across both environmental conditions, dietary treatment had no significant effect on CO_2 and N_2O emissions, whereas temperature had a more pronounced impact. The Global Warming Potential (GWP) was not substantially affected under thermoneutral conditions; however, the SPHS group exhibited the highest GWP under elevated temperature. Overall, emissions were consistently higher under heat stress, likely due to impaired gastrointestinal function caused by elevated ambient temperatures. These findings highlight the importance of considering environmental conditions when evaluating the sustainability of alternative protein sources. Further research is needed to confirm these trends, especially considering that methodological factors such as excreta sampling, storage conditions, and sample processing may have influenced the outcomes.

CRediT authorship contribution statement

C. Zangoli: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **S. Chrysanthopoulos:** Writing – review & editing, Visualization, Resources, Methodology, Investigation, Formal analysis. **S. Pocheville:** Writing – review & editing, Validation, Resources, Methodology, Investigation, Formal analysis. **E.A. Fernandes:** Writing – review & editing, Visualization, Resources, Methodology, Investigation, Formal analysis. **M. Zappaterra:** Writing – review & editing, Visualization, Resources, Methodology, Investigation, Formal analysis. **A.M. de Almeida:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **D. Fangueiro:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Disclosures

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.psj.2026.106665.

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