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Role of effective microorganisms on broiler performance and odor emission from litter in broiler chicken production

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ABSTRACT

Poultry production is a reliable animal protein source used in different communities. Malodor emitted from poultry houses limits their production and significantly contributes to air pollution. Mitigations are thus required to make broiler chicken production more environmentally friendly and productive. This study investigated the role of effective microorganism (EM) on broiler performance and odor emission from litter in broiler chicken production. A total of 180-day-old chicks were divided into four treatments: T_0 (without EM-supplementation), T_1 (EM-supplemented in water), T_2 (EM-supplemented in feed), and T_3 (EM-supplemented in water+feed), with three replicates of 15 birds each. Data on broiler performance, including water intake (WI), feed intake (FI), average weight gain (ADG), feed conversion ratio, and carcass characteristics, were measured and analyzed using one-way ANOVA in SPSS (version 25). Malodor agents were characterized by using gas chromatography. Significant ($p<0.05$) increase in WI and FI was delayed among chickens with EM treatments until 4th- 6th and 5th – 6th weeks, where T_0 had lower ($p<0.05$) FI and WI, respectively. T_3 had the highest ($p\leq0.05$) increased ADG at the 5th and 6th weeks. Heart, wing, thigh, girth, and live weight did not differ ($p>0.05$) among treatments, while T_0 had higher ($p<0.05$) liver and gizzard weight compared to the estimates from other groups. T_0 recorded higher ($p<0.05$) gases, emitted from grower to finisher, compared with starter. EM treatment decreased ($p<0.05$) the number of gases emitted, with T_3 recorded as the lowest. Thus, EM applications may alter the emission of odor-causing compounds in the broiler chicken litter without compromising its performance.

Keywords:
Broiler, layers, microbes, offensive odor, growth.

1. Introduction

The exponential growth of the global population has increased the demand for protein from animal sources, particularly poultry products, such as meat and eggs. In sub-Saharan Africa, where economic hardships are unabated, the demand for poultry products is projected to increase by 70% between 2005 and 2050 (Erdaw and Beyene, 2022). However, the poultry industry faces critical challenges in meeting this growing demand, principally due to the negative impact of malodorous emissions from poultry droppings. Malodor emissions, which result from the decomposition of manure, feces, and other waste materials, have become a significant environmental and social concern, often leading to conflicts with neighborhoods and restrictions on farm expansion, particularly in urban areas (Janni, 2020; Thomas and Sunil, 2023).

Malodor emissions from poultry waste are a complex mixture of volatile compounds, including methane, ammonia, sulfur dioxide, nitrous oxides, and dimethyl disulfide, which significantly contribute to both environmental pollution and health challenges for farmers, birds, and nearby residents (Haider et al., 2024). However, progress has been made in understanding these emissions. Calvet et al. (2011) reported ammonia, carbon dioxide, methane, and nitrous oxide in a commercial broiler farm. Broučeck and Čermák (2015) evaluated the concentrations and emissions of ammonia, methane, nitrous oxide, and carbon dioxide in poultry barns. However, these studies lacked a comprehensive characterization of the gases emitted from broiler litter, underscoring the need for further investigation. In addition to the effects of gases emitted from poultry droppings on the environment, emissions adversely affect the efficiency of bird growth through their influence on birds' metabolism and overall health (Serrano et al., 2012). Although chemical additives are commonly used to mask odors (McCrory and Hobbs, 2001), they can adversely affect bird productivity, farm sustainability, and environmental health (Naseem and King, 2018). As a result, natural alternatives, such as supplementation with effective microorganisms (EM), have gained increasing attention as a sustainable strategy to mitigate malodor emissions (Yousaf et al., 2022).

Effective microorganisms, including Saccharomyces cerevisiae, Bacillus subtilis, and Lactobacillus species, are known to improve waste decomposition (Fan et al. 2018) and reduce ammonia levels. However, earlier studies have predominantly focused on ammonia mitigation (Calvet et al., 2011; Broučeck and Čermák, 2015), neglecting other harmful gases such as sulfur compounds and volatile organic compounds, which also pose significant risks (Swelum et al., 2021; Bist et al., 2023). Moreover, there is limited understanding of the simultaneous effects of EM on broiler performance, bird health, and litter gas emissions. Thus, this study addresses these gaps by studying the effect of effective microorganisms in reducing a broader spectrum of malodor compounds and enhancing broiler performance and productivity. By integrating gas characterization with performance metrics, this study provides a more holistic understanding of the benefits of EM in broiler production, paving the way for environmentally friendly and economically viable poultry farming practices.

2. Materials and Methods

2.1 Ethical statement

The study received approval and ethical clearance from the University of Zululand Research Ethics Committee (UZREC171110030PGM2017/445)

2.2 Experimental site

A six-week experimental study was conducted at the Poultry Farm of the University of Zululand, KwaDlangezwa, KwaZulu-Natal, South Africa. The Farm is located at a latitude of 28.85° S and a longitude of 31.85° E. A subtropical climate with average temperatures of 28.4 °C and 14.5 °C in the summer and winter, respectively. The average annual rainfall is approximately 944 mm (Ndlovu et al., 2021).

2.3 Experimental animals, diet, design, and management

A total of 180-day-old Cobb chicks were sourced from a reputable hatchery and randomly assigned to four treatments: T_0 (without EM), T_1 (with EM in drinking water at 1 L of EM/100 L of water), T_2 (with EM in feed at 1 L of EM/100 kg of feed), and T_3 (with EM in feed and drinking water at equal concentrations used in T_1 and T_2, respectively) (Table 1). According to the experimental unit, a commercially produced effective microorganism (EM) (Prowell L) was added to the water or feed. Prowell L contains *Saccharomyces cerevisiae*, *Bacillus subtilis*, *Bifidobacterium bifidum*, *Bifidobacterium animalis*, *Lactobacillus acidophilus*, *Lactobacillus casei*, *Bifidobacterium longum*, *Lactobacillus fermentum*, *Lactococcus lactis*, *Lactobacillus plantarum*, *Streptococcus thermophilus*, and *Lactobacillus bulgaricus*. Clean, cool water and feed (Table 2) were provided ad libitum. The experiment was divided into starters (0-14 days), grower (15-28 days), and finisher (29-42 days) stages (Table 2). Chicks were housed in a concrete house with 12 pens and a density of 15 birds per square meter, which was later increased from 2 weeks to 15 birds per 3 square meters until the end of the experiment. Broiler bedding consisted of wood chips that were approximately 4 cm thick. A standard biosecurity protocol was followed throughout the experimental period. The chicks were only vaccinated against Newcastle disease when they were day-old.

Table 1. Treatment allocation to experimental birds.

Treatment	Rep/Tre	Chicken/rep	Total
T_0	3	15	45
T_1	3	15	45
T_2	3	15	45
T_3	3	15	45
Total	12	60	180

Remark: T_0 (No EM), T_1 (EM in water), T_2 (EM in feed), T_3 (EM in water and feed)

Table 2. Percentage composition of experimental broiler maize-based commercial diets

Composition (%)	Starter	Grower	Finisher
	0-2 weeks	3-4 weeks	5-6 weeks
Protein	20	19	16
Moisture	12	12	12
Fat	2.5	2.5	2.5
Calcium	0.8	0.7	0.6
Phosphorus	0.6	0.5	0.5
Fibre	5	6	5

2.4 Measurement of Broiler Performance

Feed and water intake were determined by subtracting the daily leftovers from the quantity offered on the previous day (Manyelo et al., 2022). Weight gain was measured once a week (Schomburg et al., 2023). The feed conversion ratio was determined by

dividing the feed quantity consumed per unit weight gain by that of the chicks (Yitbarek et al., 2016).

2.5 Evaluation of carcass characteristics

A total of 60 birds (15 per treatment and 5 per replicate) were randomly selected for carcass characteristic determination (Zhang et al., 2023). The selected birds were weighed, stunned, bled, scalded, and de-feathered using a rotary drum picker at an abattoir (Khalil et al., 2021). The internal organs were detached, pooled, and weighed. The whole chicken, wing, thigh, liver, and heart weights were measured using PGW 4502e d= 0.01 g Precision Balance, while wing length, chest girth, and thigh length were measured using graduated measuring fiber tape.

2.6 Odor sampling

Odor emissions were collected weekly, trapped using pumps and polyacetate bags, and analyzed using gas chromatography as described by Miano et al. (2022). To trap the odor, polyacetate bags (Nalo Bratfolie Kalle GmbH, Germany) were placed over the entire cone immediately before sampling to concentrate the volatile compounds. Each sample, weighing 50 g, was placed in a laboratory weighing boat with either clean, unused wood chips (control) or littered wood chips, and connected to a suction pump with traps. The system was sealed for 30 minutes before suction to concentrate the gas. The air inside the bags was drawn through an adsorbent trap for 1 h using a portable battery-operated pump (Spectrex Personal Air Sampler PAS 500, USA) calibrated to 200 ml per 20 min. Simultaneously, air samples from empty polyacetate bags were collected and stored at -20°C in sealed vials until analysis. The traps contained 2 mg of a 50:50 mixture of Tenax TA (Alltech Associates, USA) and graphitized carbon (Carbotrap™, Supelco, USA) inside glass tubes sealed at both ends with glass wool. Gas samples were collected every seventh morning after the wood chips were replaced. Odor samples were taken from treated feed, untreated feed, clean wood chips, used wood chips, and two feces samples per treatment.

2.7 GC-MS analysis of prepared samples and compound identification

Volatile samples from poultry were analyzed using a coupled Varian 3800 gas chromatograph (Varian, Palo Alto, CA, USA) and Varian 1200 mass spectrometer, as Cunha et al. (2023) described. The gas chromatograph was supported with an Alltech EC–WAX column of 30 m × 0.32 mm internal diameter × 0.25 mm film thickness (Alltech Associates Inc., Deerfield, IL, USA). Helium was used as the carrier gas at a flow rate of 1 mL min-1 as described by Mokgehle et al. (2018). The traps were placed in a Varian 1079 injector employing a Chromatoprobe fitting with thermal desorption by heating the injector at 40 °C for 2 min with a 20:1 split ratio, then increasing to 200 °C and holding at 200 °C min^{-1} in split-less mode for thermal desorption. After a 3-minute hold at 40 °C, the gas chromatograph oven was ramped up to 240 °C at 10 °C min-1 and held there for 12 minutes. Compound identification was carried out using Varian Workstation software with the NIST05 mass spectral library, comparisons with retention times of chemical standards, where available, and comparisons between calculated Kovats retention indices.

A homologous series of alkanes (C8–C20) was used to determine Kovats retention indices. Compounds were verified using the retention times of authentic standards (97–99.5%, Sigma Aldrich Inc. GmbH, Germany), (3E)-1, 3-octadiene (98 %, ChemSampco, USA), and published Kovats indices. Compounds present at higher or similar

percentages in the controls were considered contaminants and ignored in the analysis. To determine the emission rates, known standards of dominant compounds were injected into the cartridges and thermally desorbed under identical conditions as the samples (Helin et al., 2020). The peak areas of compounds in the samples were compared with the standards and used to calculate the total emission rates of compounds per fecal sample per hour and the emission rate per compound per fecal sample per hour. Individual compounds comprising ≥10% of the average relative amount emitted at all growth stages and during different feeding rations were considered dominant.

2.8 Statistical analysis

Data on broiler performance, carcass characteristics, and odor emissions from litter were analyzed using one-way analysis of variance (ANOVA) in SPSS version 25. Differences among means were tested for significance using the LSD test. The means were considered statistically significant at $p<0.05$.

3. Results and Discussion

3.1 Results

3.1.1. Water and Feed Intake

Water intake showed no significant differences ($p>0.05$) among the treatments during the early stages (weeks 1–4). However, at week 5, birds in T_1, T_2, and T_3 exhibited significantly higher water intake ($p\leq0.05$) than birds in the control group (T_0). This trend persisted until week 6, when birds in T_3 had the highest water intake (Table 3). Similarly, feed intake was not significantly different among treatments ($p>0.05$) until week 3, when birds in T_3 showed a significantly lower feed intake than the control ($p<0.01$). The control group consistently had the highest feed intake, whereas the T_3 birds had the lowest feed intake ($p<0.05$).

Table 3. Effect of effective microorganism supplementation on broiler water and feed intake

	Water intake (L)						Dry matter intake (kg)					
Week	T_0	T_1	T_2	T_3	SEM	p-value	T_0	T_1	T_2	T_3	SEM	p-value
1	0.79^{a}	0.90^{a}	0.93^{a}	0.93^{a}	0.12	0.977	0.29^{a}	0.30^{a}	0.30^{a}	0.30^{a}	0.02	0.997
2	0.99^{a}	1.93^{a}	1.92^{a}	1.84^{a}	0.23	0.938	0.89^{a}	0.84^{a}	0.85^{a}	0.84^{a}	0.03	0.733
3	1.42^{a}	2.64^{a}	2.88^{a}	2.66^{a}	0.22	0.66	1.42^{a}	1.21^{ab}	1.24^{ab}	1.15^{b}	0.12	0.016
4	1.64^{a}	1.69^{a}	1.71^{a}	1.70^{a}	0.21	0.55	2.04^{a}	1.69^{b}	1.71^{b}	1.70^{b}	0.11	0.05
5	2.15^{b}	4.73^{a}	4.58^{a}	5.76^{a}	0.12	0.05	2.15^{a}	1.76^{b}	1.76^{b}	1.67^{b}	0.22	0.001
6	2.82^{a}	6.84^{b}	7.25^{b}	7.66^{a}	0.32	0.05	2.82^{a}	2.31^{b}	2.22^{b}	2.14^{b}	0.20	0.05

Remark: T_0 (No EM), T_1 (EM in water), T_2 (EM in feed), and T_3 (EM in both feed and water). Water (L) and dry matter (kg) intake /week/treatment. ^{abc} Parameters with different superscripts are different at $p<0.05$ in the same row

3.1.2. Growth Performance and Feed Conversion Ratio

The average daily weight gain (ADG) did not differ significantly ($p>0.05$) among treatments during weeks 1–4. However, significant differences emerged at weeks 5 and 6, with T_3 birds achieving the highest ADG ($p\leq0.05$). The feed conversion ratio (FCR)

also showed no significant differences until week 5, when birds in T_2 had the highest FCR, whereas birds in T3 maintained a superior FCR during week 6 (Table 4).

Table 4. Feed conversion ratio and daily gain for broilers under different treatments at different weeks

	ADG (kg)						FCR					
Week	T_0	T_1	T_2	T_3	SEM	p-value	T_0	T_1	T_2	T_3	SEM	p-value
1	0.11[a]	0.10[a]	0.08[a]	0.11[a]	0.01	0.572	3.00[a]	3.22[a]	3.74[a]	2.71[a]	0.01	0.57
2	0.43[a]	0.31[a]	0.30[a]	0.29[a]	0.02	0.77	2.13[a]	2.88[a]	2.91[a]	2.91[a]	0.20	0.18
3	0.77[a]	0.74[a]	0.77[a]	0.73[a]	0.03	0.940	1.88[a]	1.63[a]	1.64[a]	1.58[a]	0.12	0.35
4	3.04[a]	2.60[a]	2.79[a]	2.95[a]	0.01	0.764	0.71[a]	0.65[a]	0.62[a]	0.58[a]	0.03	0.49
5	0.38[b]	0.30[c]	0.27[c]	0.46[a]	0.02	0.05	2.94[c]	2.30[c]	6.57[a]	3.20[b]	0.12	0.05
6	0.53[b]	0.45[b]	0.34[c]	0.58[a]	0.03	0.05	3.20[ab]	4.10[a]	2.20[b]	3.10[a]	0.04	0.05

Remark: T_0 (No EM), T_1 (EM in water), T_2 (EM in feed), T_3 (EM in both feed and water). Average daily weight gain (kg) and feed conversion ratio (FCR) /week/treatment. [abc] Parameters with different superscripts are different at $p<0.05$.

3.1.3. Carcass Characteristics

The EM treatment significantly affected some visceral organs ($p\leq0.05$). Liver and gizzard weights were significantly lower in the T_1, T_2, and T_3 groups than in T_0 (control), while heart weight showed no differences ($p>0.05$). Wing and thigh lengths were significantly longer in the control group ($p<0.05$), while carcass weight (CW) was highest in T_1 ($p<0.001$), as indicated in Table 5.

Table 5. Carcass characteristics in EM treated (T_1, T_2, T_3) and non-treated (T_0) birds

	Treatments					
Body part	T_0	T_1	T_2	T_3	SEM	P-value
$Heart_g$	12.33[a]	12.20[a]	12.07[a]	12.00[a]	0.25	0.74
$Liver_g$	43.33[c]	38.67[b]	34.53[a]	34.33[a]	1.01	0.050
$Gizzard_g$	32.67[b]	29.20[a]	28.87[a]	29.07[a]	2.10	0.010
$WingL_{cm}$	13.40[a]	12.53[b]	13.00[ab]	12.27[b]	1.01	0.011
$WingW_g$	85.13[a]	87.53[a]	83.60[a]	80.93[a]	4.32	0.185
$ThighL_{cm}$	16.27[a]	15.73[ab]	15.70[ab]	15.27[b]	0.41	0.049
$ThighW_g$	234.80[a]	234.13[a]	226.07[a]	207.87[a]	7.26	0.059
$Girth_{cm}$	28.28[a]	28.80[a]	28.13[a]	28.73[a]	2.10	0.810
LW_{kg}	2.09[a]	2.22[a]	2.07[a]	2.13[a]	0.01	0.675
CW_{kg}	1.97[b]	2.14[a]	2.01[b]	1.88[b]	0.02	0.001
$LNoG_{kg}$	1.71[a]	1.65[a]	1.60[a]	1.76[a]	0.01	0.131
$NoLnG_{kg}$	1.62[a]	1.60[a]	1.60[a]	1.42[b]	0.01	0.010

Wing L (wing length), W (wing weight), L (thigh length), LW (live weight), CW (carcass weight), LNOG (legs with no guts), and NoLnG (no legs and guts). Different body parts were measured after 6weeks of age. [abc] Parameters in rows with different superscripts are significantly different at $P<0.05$.

3.1.4. Odor emission

A total of 48 gases were extracted from nine major groups of compounds: alcohols (6), aldehydes (2), aliphatic acids (10), furans (4), terpenes (8), ketones (3), phenolics (9), sulfur compounds (3), and nitrogen compounds (3) (Table 6). Aliphatic compounds,

phenols, and ketones were the highest compounds produced. 1-octen-3-ol was the most abundant and common emission in the alcohol category, which was found in the feces throughout the experimental period (Table 7). Decanal was the most common and abundant sub-compound in the aldehyde category, and its distribution among the treatments and across the weeks did not differ among the treatments (Table 8). Abundant emission of aliphatic acid compounds was observed in broilers aged (4-6 weeks) compared to that during the early phase (week 1-3) (Table 9). However, the concentrations of these compounds showed an impressive reduction in birds feces in T_3. Furfural and 5-methylfurfural were the most abundant sub-compounds of the Furan category, which were emitted in weeks 2 and 4. The emissions were subdued in week two and later in weeks five and six (Table 10). Pinocarvone, Pinocarveol, Verbenol, Verbenone, and Myrtenol are the most abundant terpenes (Table 11). However, the birds in T_3 showed low levels of these sub-compounds compared with other treatments. Octanone and Acetoin are the most abundant substances emitted as ketones; however, they are not different in birds' feces in different treatments (Table 12). Two sub-phenolic compounds (2-Hydroxy-4-methylbenzaldehyde, Phenol) were found to increase in concentration in the birds' feces regardless of treatment as the birds aged (Table 13). Benzaldehyde and 3-methyl-phenol were the most abundant phenolic compounds emitted in the feces of broilers and were not influenced by the treatments (Table 13). Dimethyl sulfone was primarily identified as a sulfur compound found in birds' feces from all treatments (Table 14). Indole maintained the most abundant concentration among the nitrogenous sub-compounds throughout the experiment (Table 15), with the occurrence of Isovaleramide concentration in all birds at week 4, which was later not emitted in the subsequent weeks, except its emission was found at week 6 for the birds from T_0 (Table 15).

Table 6. Gas characterization in the fecal sample of the experimental birds

Compounds/Gases S/N	Alcohols	Aldehydes	Aliphatic acids	Furans	Terpenes	Ketones	Phenolics	Sulphur compounds	Nitrogen compounds
1	3-methyl-1-butanol	Heptanal	Acetic acid	2-pentyl-furan	(1S)-4,6,6-trimethyl-bicyclo [3.1.1] hept-3-en-2-one	3-Octanone	Benzaldehyde	Dimethyl disulfide	2,5-dimethyl pyrazine
2	1-Pentanol	Decanal	Propanoic acid	Fufural	Pinocarvone	3-hydroxy-2-butanone	1,2-dimethoxybenzene	Dimethyl Sulfoxide	Isovaleramide
3	1-hexanol		Isobutyric acid	5-methyl-fufural	Myrtenal	2-butanone	2-methoxy phenol	Dimethyl sulfone	Indole
4	3-Octanol		Butanoic acid	Butyrolactone	Pinocarveol		2-Hydroxy-4-methylbenzaldehyde		
5	1-octen-3-ol		Isovaleric acid		Verbenol		Benzyl alcohol		
6	1-Octanol		Pentanoic acid		Verbenone		2-Phenylethyl alcohol		
7			Hexanoic acid		Carvone		Phenol		
8			Octanoic acid		Myrtenol		3-methyl-phenol		
9			Nonanoic acid				p-Ethyl phenol		
10			4-Oxo-pentanoic acid						

Table 7. Alcohol compounds in the faecal sample

Compounds	Week1				Week 2				Week 3				Week 4				Week 5				Week 6			
	T_0	T_1	T_2	T_3	T_0	T_1	T_2	T_3	T_0	T_1	T_2	T_3	T_0	T_1	T_2	T_3	T_0	T_1	T_2	T_3	T_0	T_1	T_2	T_3
Alcohols																								
3-methyl-1-butanol	-	-	-	-	-	2.89	-	6.92	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1-Pentanol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1-hexanol	-	3.45	0.78	2.71	2.36	0.61	-	1.36	1.05	2.69	2.76	1.85	-	-	-	-	4.58	2.55	-	-	0.25	0.80	3.24	-
3-Octanol	-	-	-	-	-	-	-	-	-	-	3.38	1.59	-	-	-	-	-	0.82	0.58	5.02	0.64	0.79	3.71	0.24
1-octen-3-ol	10.94	12.16	9.58	20.94	19.79	17.20	19.13	26.26	24.33	29.89	23.29	20.03	8.40	2.54	5.31	8.71	9.33	16.66	24.83	14.82	23.18	10.91	19.39	15.42
1-Octanol	-	-	-	-	-	-	-	-	-	-	2.012	1.591	-	-	0.40	-	-	0.78	2.24	1.27	0.50	0.37	-	-

Table 8. Aldehyde compounds in the faecal sample

Compounds	Week1				Week 2				Week 3				Week 4				Week 5				Week 6			
	T_0	T_1	T_2	T_3	T_0	T_1	T_2	T_3	T_0	T_1	T_2	T_3	T_0	T_1	T_2	T_3	T_0	T_1	T_2	T_3	T_0	T_1	T_2	T_3
Alcohols																								
3-methyl-1-butanol	-	-	-	-	-	2.89	-	6.92	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1-Pentanol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1-hexanol	-	3.45	0.78	2.71	2.36	0.61	-	1.36	1.05	2.69	2.76	1.85	-	-	-	-	4.58	2.55	-	-	0.25	0.80	3.24	-
3-Octanol	-	-	-	-	-	-	-	-	-	-	3.38	1.59	-	-	-	-	-	0.82	0.58	5.02	0.64	0.79	3.71	0.24
1-octen-3-ol	10.94	12.16	9.58	20.94	19.79	17.20	19.13	26.26	24.33	29.89	23.29	20.03	8.40	2.54	5.31	8.71	9.33	16.66	24.83	14.82	23.18	10.91	19.39	15.42
1-Octanol	-	-	-	-	-	-	-	-	-	-	2.012	1.591	-	-	0.40	-	-	0.78	2.24	1.27	0.50	0.37	-	-

Table 9. Aliphatic acid compounds in the faecal sample

Compounds	Week1				Week 2				Week 3				Week 4				Week 5				Week 6			
	T_0	T_1	T_1	T_2	T_0	T_1	T_1	T_2	T_0	T_1	T_1	T_2	T_0	T_1	T_1	T_2	T_0	T_1	T_1	T_2	T_0	T_1	T_1	T_2
Acetic acid	9.85	8.15	11.48	10.06	1.82	3.64	2.28	2.46	2.76	1.07	3.07	1.196	24.99	28.42	23.19	5.42	19.48	19.48	22.17	22.73	20.02	22.63	9.48	1.72
Propanoic acid	-	-	-	-	-	-	-	-	-	-	0.30	-	1.45	0.53	1.06	0	1.79	2.64	4.14	2.04	10.94	12.38	0.60	0.05
Isobutyric acid	-	-	-	-	-	-	0.10	-	-	-	0.33	-	4.49	0.93	0.87	0.18	0.50	0.52	0.68	0.29	1.81	1.57	0.05	-
Butanoic acid	9.01	3.16	1.66	0.92	-	-	-	0.79	-	-	0.92	-	1.59	0.44	0.47	0.34	1.67	1.47	1.92	1.24	5.44	6.35	1.60	-
Isovaleric acid	1.44	-	-	-	-	-	0.39	-	-	-	0.49	-	4.96	6.59	3.24	0.64	0.67	1.03	1.18	0.89	1.62	6.82	0.30	-
Pentanoic acid	0.94	0.15	-	-	-	-	-	-	-	-	0.15	-	0.22	0	0	-	0.20	1.35	-	-	0.67	0.79	-	-
Hexanoic acid	-	-	-	-	-	-	5.52	-	-	-	-	-	0.55	1.35	0.54	0.24	0.13	-	-	-	0.44	0.49	0.67	-
Octanoic acid	-	-	-	-	1.10	-	-	-	-	-	-	-	2.38	0.20	0.05	-	-	-	-	-	0.00	-	-	-
Nonanoic acid	-	-	-	-	-	-	-	-	-	-	-	-	-	0.20	0.22	-	0.07	-	-	-	-	0.13	-	-
4-Oxo-pentanoic acid	-	-	-	-	-	-	-	-	-	-	-	-	0.66	-	-	-	-	-	-	-	-	-	-	-

Table 10. Furan compounds in the faecal sample

Compounds	Week1				Week 2				Week 3				Week 4				Week 5				Week 6			
	T_0	T_1	T_2	T_3	T_0	T_1	T_2	T_3	T_0	T_1	T_2	T_3	T_0	T_1	T_2	T_3	T_0	T_1	T_2	T_3	T_0	T_1	T_2	T_3
2-pentyl-furan	-	-	-	-	0.84	-	-	0.23	1.56	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Fufural	-	2.13	-	-	5.82	3.41	1.77	0.48	-	-	-	-	0.57	6.18	2.13	3.28	-	-	-	-	-	-	-	-
5-methyl-fufural	-	0.05	-	-	1.27	1.03	0.51	0.54	-	-	-	-	-	2.53	0.77	2.45	-	-	-	-	-	-	-	0.05
Butyrolactone	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 11. Terpenes compounds in the faecal sample

Compounds	Week1				Week 2				Week 3				Week 4				Week 5				Week 6			
	T_0	T_1	T_2	T_3	T_0	T_1	T_2	T_3	T_0	T_1	T_2	T_3	T_0	T_1	T_2	T_3	T_0	T_1	T_2	T_3	T_0	T_1	T_2	T_3
(1S)-4,6,6-trimethyl-bicyclo [3.1.1] hept-3-en-2-one	-	-	-	-	-	-	-	-	-	-	-	-	9.38	27.01	15.76	13.16	-	-	-	-	-	-	-	-
Pinocarvone	0.17	-	-	-	0.37	0.11	-	1.70	-	1.48	-	-	-	-	1.23	-	-	-	-	-	-	-	-	0.57
Myrtenal	-	-	0.09	-	-	-	2.28	0.08	-	-	-	-	-	-	-	2.25	-	-	-	-	0.06	-	0.80	1.42
Pinocarveol	0.58	0.00	0.512	-	0.92	-	0.24	0.71	0.90	2.68	0.98	1.14	-	-	-	-	-	-	-	-	-	0.01	-	-
Verbenol	0.44	-	-	-	0.82	-	0.37	0.85	0.73	0.50	-	-	-	-	0.30	-	-	-	-	-	-	-	-	-
Verbenone	0.09	-	-	-	1.76	0.86	0.48	0.86	0.35	1.07	-	-	4.76	0.93	-	2.91	-	-	-	-	-	-	-	0.29
Carvone	-	-	-	-	-	-	-	-	-	-	-	1.00	-	-	-	-	-	-	-	-	-	-	-	-
Myrtenol	2.56	0.99	1.39	2.90	2.25	1.38	2.82	6.98	5.73	11.04	3.19	2.68	-	-	0.38	-	2.29	-	-	-	0.48	0.64	6.28	0.44

Table 12. Ketone compounds in the faecal sample

Compounds	Week1				Week 2				Week 3				Week 4				Week 5				Week 6			
	T_0	T_1	T_2	T_3	T_0	T_1	T_2	T_3	T_0	T_1	T_2	T_3	T_0	T_1	T_2	T_3	T_0	T_1	T_2	T_3	T_0	T_1	T_2	T_3
3-Octanone	11.98	4.28	13.13	1.17	27.19	16.03	22.50	25.72	20.54	14.62	23.62	29.32	11.95	4.37	15.18	5.86	10.07	4.92	-	-	-	3.95	5.05	22.52
Acetoin	29.11	37.61	32.38	25.61	6.06	14.98	2.49	1.22	-	-	4.94	2.85	12.59	-	1.65	2.36	4.90	1.90	1.84	10.24	4.12	4.17	1.30	0.72
2-butanone	6.82	-	-	-	-	-	-	-	-	-	0.43	-	-	-	-	-	1.57	0.53	0.50	0.50	-	-	-	-

Table 13. Phenolic compounds in the fecal sample

Compounds	Week1				Week 2				Week 3				Week 4				Week 5				Week 6			
	T_0	T_1	T_2	T_3	T_0	T_1	T_2	T_3	T_0	T_1	T_2	T_3	T_0	T_1	T_2	T_3	T_0	T_1	T_2	T_3	T_0	T_1	T_2	T_3
Benzaldehyde	0.13	1.27	0.95	12.30	2.00	3.10	1.46	-	0.19	0.28	-	-	1.54	5.47	3.03	17.44	1.67	1.89	2.35	3.52	0.76	0.19	16.63	0.77
1,2-dimethoxybenzene	0.07	-	0.17	-	-	-	-	0.05	-	-	-	0.55	-	-	-	-	-	-	-	-	-	-	-	-
2-methoxy phenol	-	-	-	-	-	0.13	-	-	-	0.14	-	0.60	-	-	-	-	-	-	-	-	0.44	-	0.02	1.41
2-Hydroxy-4-methylbenzaldehyde	-	-	-	-	-	-	-	-	2.16	0.39	1.67	1.43	1.75	1.59	3.15	3.73	-	0.73	-	3.85	-	0.54	-	1.28
Benzyl alcohol	0.16	0.99	0.15	0.53	1.57	0.82	4.02	1.66	1.10	1.66	-	2.13	0.12	-	-	1.64	-	-	-	-	-	-	-	0.97
2-Phenylethyl alcohol	0.50	0.18	0.62	0.18	-	-	-	-	0.09	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Phenol	-	-	3.27	-	-	-	-	-	-	0.22	-	0.59	0.24	0.48	0.51	-	0.07	4.68	2.61	2.40	1.91	2.33	2.84	3.74
3-methyl-phenol	0.11	0.28	0.03	0.42	3.08	3.50	6.73	1.48	4.84	13.71	5.87	4.61	-	0.94	7.17	9.81	18.67	22.92	14.53	6.33	7.13	7.76	5.97	27.29
p-Ethyl phenol	0.04	-	-	-	-	0.51	1.23	0.83	0.81	0.34	-	0.64	-	-	0.37	0.55	-	0.90	-	-	0.68	0.62	2.24	10.86

Table 14. Sulphur compounds in the fecal sample

Compounds	Week1				Week 2				Week 3				Week 4				Week 5				Week 6			
	T_0	T_1	T_2	T_3	T_0	T_1	T_2	T_3	T_0	T_1	T_2	T_3	T_0	T_1	T_2	T_3	T_0	T_1	T_2	T_3	T_0	T_1	T_2	T_3
Dimethyl disulfide	-	-	-	-	-	-	7.96	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Dimethyl Sulfoxide	-	-	-	-	-	-	-	-	-	-	-	0.08	-	0.54	-	-	-	-	-	-	-	0.42	-	-
Dimethyl sulfone	5.54	3.83	2.26	3.04	0.58	2.19	4.87	2.07	0.15	2.48	0.21	0.64	0.35	0.02	0.61	0.52	0.30	0.16	0.31	0.11	0.13	0.17	0.08	0.28

Table 15. Nitrogen compounds in the fecal sample

Compounds	Week1				Week 2				Week 3				Week 4				Week 5				Week 6			
	T0	T1	T2	T3	T0	T1	T2	T3	T0	T1	T2	T3	T0	T1	T2	T3	T0	T1	T2	T3	T0	T1	T2	T3
2,5-dimethyl pyrazine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9.40	-	-	-	-
Isovaleramide	-	-	-	-	-	-	-	-	-	-	-	-	3.90	0.50	0.71	0.43	-	-	-	-	0.04	-	-	-
Indole	0.47	0.90	0.83	0.25	1.04	1.57	4.54	1.17	0.27	0.73	0.52	1.01	0.12	0.50	0.11	0.72	0.66	2.33	1.22	0.73	1.61	1.69	0.50	2.38

Table 16. Effect of diet and Effective Microorganism treatment on types of gas produced from broiler litter

	Gas Production type (average number)					
	T_0	**T_1**	**T_2**	**T_3**	**STD**	**P-Value**
Starter	14.75[b]	15.7[b]	12.3[a]	11.2[a]	1.5	0.05
Grower	16.00[a]	18.0[a]	21.0[b]	17.0[b]	1.2	0.05
Finisher	16.00[a]	16.0[a]	13.0[b]	13.0[b]	2.3	0.05
STD	1.3	1.3	4.3	2.7	-	-
P-value	0.05	0.05	0.05	0.05	-	-

T_0 = Control; T_1 = EM in water; T_2 = EM in feed; T_3 = EM in feed and water combination

3.1.6. Effect of diet phase on gas emissions

Dietary phase significantly affected the number of gases emitted ($p \leq 0.05$). The starter phase recorded the highest emissions at T_0 and T_1, whereas T_3 consistently had the lowest emissions across all the phases (Table 16).

3.2 Discussion

Water intake by the broilers was not affected by effective microbes (EM) supplementation at the early stage of the study (week 1-4); however, this increased with age (weeks 5 to 6). The delayed increase in water intake in EM-treated birds, particularly at T_3, highlights the potential role of EM in stimulating thirst through microbial activity. Although studies on poultry are scarce, similar effects of probiotics have been reported in humans (Reuben et al., 2021). Increased water intake could benefit poultry, enhance physiological functions, and potentially aid in toxin clearance (Ahmed et al., 2014). The absence of wet litter in this study confirmed that water intake levels remained within a beneficial range.

At the early stage of the birds (weeks 1 and 2), feed intake was similar for all groups. This suggests that the developing gut of birds is yet to react to these treatments. However, from weeks 3 to 5, the control group showed a higher feed intake than the EM-treated birds. This implies that the inclusion of EM as a feed additive might have a decreasing effect on the feed intake of broiler chickens but does not necessarily affect feed efficiency and utilization, as daily weight gain and feed conversion ratio did not differ. The reduction in feed intake observed in EM-treated birds from week 3 onwards suggests improved feed utilization efficiency. EM may promote gut health by eliminating pathogenic microbes, leading to improved nutrient absorption. This aligns with a study by Alhotan (2021), who reported that EM can reduce feed costs while maintaining or improving performance. The variability in results across studies may stem from differences in EM strains or administration methods.

Average daily weight gain (ADG) was not affected by EM supplementation in the first 4 weeks; however, there was an increase in broiler chicken ADG at the later stage (week 5 to 6). The lack of effect of supplementation at the early stage may be attributed to the immaturity of the microflora, which needed more time to develop and acclimatize. The results of this study are in agreement with those of Olnood et al. (2015), who suggested that EM sometimes requires time for adaptation. At the advanced stage of broiler production (week 6), T3 birds had higher ADG and feed conversion ratios. The observed improvement in ADG and FCR in T_3 during weeks 5 and 6 highlights the role of EM in enhancing nutrient assimilation and growth efficiency, particularly as the gut microbiome stabilizes (Olnood et al., 2015). These results align with previous reports that EM can improve growth performance by enhancing nutrient absorption (Ashraf et al. 2005). Some visceral organs (liver and gizzard) were affected by EM treatments. Broiler chickens in T_1, T_2, and T_3 had lighter liver and gizzard weights than the birds from T_0. This may be associated with hard work by the gizzard, grinding the relatively high fiber intake with little moistening in T_0 compared to T_1, T_2, and T_3. Increased gizzard size has been associated with a greater density of muscle tissue, promoted by relatively higher activity (Willis and Reid, 2008). In addition, the muscular liver was linked to high pressure and activity from continued detoxification of unwanted chemical components (by-products), probably absorbed from harmful microbes due to less flushing.

Live weight was expected to be higher in the control group than in the treatment group since feed intake was relatively higher in the control group. In contrast, broiler chickens from the different groups showed no differences in weight. The indifference in live weight may be associated with the efficient utilization of feed by the broiler chickens in the EM treatment groups, despite consuming less feed compared to broiler chickens in the control group. A similar study by Mafiri (2014) showed that EM did not improve dietary intake or growth performance. However, chicken wing length, thigh length, and no legs or guts (NoLnG) were significantly longer in the control group than in the treatment group. Its effect on wings was also complicated to explain, but it was associated with high gut flushing, with the potential to lose a large amount of protein in the aqueous solution. It was observed that as the chicken grew, the amount and type of odor emitted increased. These results were similar to those obtained by Naseem and King (2018), who reported in their review paper on studies monitoring ammonia concentrations from 1950 to 2018 that ammonia concentration increased with age and size of broilers. In vivo studies using probiotics (EM) have shown decreased ammonia levels in poultry (Upadhaya et al., 2019) and methane levels in lambs (Gemiyo et al., 2017). Ammonia, Carbon dioxide, and methane, which are traditional gases often measured, were not determined in this experiment, as they have been extensively reported (Madsen et al., 2010; Kupper et al., 2020). However, nine groups of gases were identified, among which aliphatic acids, phenolics, and terpenes were the dominant gases. To our knowledge, this is the first report of these compounds in broiler chicken litter. The higher abundance of phenolic and aliphatic acids found in this study may be linked to the plant-based feed used.

Among different types of gases produced, sulfur compounds, Dimethyl disulfide, Dimethyl Sulfoxide, and Dimethyl sulfone were also emitted. Only Dimethyl disulfide had a bad smell (decaying fish odor), while Dimethyl Sulfoxide and dimethyl sulfone were friendlier with no odor. (Jeon et al., 2022). For the nitrogenous compounds (2,5-dimethyl pyrazine, Isovaleramide, and Indole), Indole was the only one that smells offensive (feces) and has been reported in many other studies (Tesso and Liu, 2019, Nowak et al. 2016). Treatment with EM tended to reduce sulfur compounds, but strangely, it did not affect the indole concentration. The reduction of sulfur compounds was also observed in the study by Kalus et al. (2017), but did not last. A report by Kalus et al. (2017) confirmed that most of the sulfur and phenolic compounds were detected in this study. Therefore, it was not surprising that alcohols, phenols, indoles, and ketones were detected in this experiment because they have been previously detected in animal waste (Ngwabie et al., 2008; Filipy et al., 2006; Hanajima et al., 2010; Mackie et al., 1998), although EM depresses some of these gases. Sulfur compound emissions are known to correlate with temperature and are produced during the decomposition of nitrogen-based compounds, such as proteins (Higgins et al., 2008; Hanajima et al., 2010) and amino acids that contain sulfur. Broiler feed is richer in protein, especially starter feed; hence, the reason for the higher sulfur compounds in early feeding. Comprehensive profiling of 48 gases, including phenolics, aliphatic acids, and terpenes, provides a novel contribution to our understanding of broiler litter emissions. EM supplementation significantly reduced the concentrations of key odorous compounds such as dimethyl disulfide and indole in T_3, showcasing its potential to mitigate a broad spectrum of malodors. Unlike previous studies that focused solely on ammonia and methane (Madsen et al., 2010; Kupper et al., 2020), this study highlights the complex nature of poultry emissions and the effectiveness of EM in reducing diverse volatile compounds.

EM can suppress the malodors of animal manure either through direct or indirect mechanisms. According to Li and Ni (2024), EM works in one of the following mechanisms: as a mixed culture of many species of naturally occurring, beneficial microorganisms, some of which can transform NH_4 +-N to NO_3-N, thereby decreasing the potential for N volatilization and increasing the potential for nitrogen fixation (through photosynthetic bacteria), and contains beneficial microorganisms that reside in the animals' intestines as feed and drinking water are utilized. Effective microorganisms suppress the growth and activity of the indigenous putrefactive types that cause malodors in the manure and transform proteins and amino acids into NH3-N and NH4+-N. Therefore, EM in the intestines has the potential to reduce ammonia levels in the manure and blood (Li and Ni, 2024). These three combined actions of EM may transfer proteins in the feed into effective and available nutrients, thereby increasing the feed utilization rate and suppressing malodor production by indigenous intestinal microorganisms. The significant interaction between diet composition and EM treatment demonstrated the potential for synergistic effects in managing emissions. The consistent reduction in gases in T_3 across all dietary phases emphasizes the efficacy of combining EM with tailored diets to minimize environmental impacts.

4. Conclusion

The use of EM in broilers revealed minor performance improvements, as seen by a relative increase in thigh and wing length and carcass weight, but no difference in live weight and average daily gain. EM showed the potential to decrease feed intake but increase water intake in broilers in weeks 5 and 6, thereby reducing feed production costs. A total of 48 gases classified into nine groups (alcohols (6), aldehydes (2), aliphatic acids (10), furans (4), terpenes (8), ketones (3), phenolics (9), sulphur compounds (3), and nitrogen compounds (3)) were identified. Although it was difficult to fully describe the total number of gases, the general trend was that it tended to decrease with an increase in EM treatment. The number of odorous compounds, such as indole, dimethyl disulfide, Dimethyl Sulfoxide, and Dimethyl sulfone, tended to decrease with EM treatment. It has also been established that diet type and composition influence the kind of gases produced. It must be noted that the robustness of the gas data did not make it easy to analyze, especially mass composition; hence, there is a need for further studies.

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