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Age-Dependent Physical and Chemical Attributes of Chicken Droppings in Health Monitoring

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ABSTRACT

This study aimed to characterize the physical and chemical properties of chicken droppings (n = 73), collected at various age periods and classified visually as normal (N) or abnormal (A). Significant differences were observed between the two groups in terms of texture, pH, dry matter (DM), fatty acids (FAs), short-chain fatty acids (SCFAs), and volatile compounds (VCs) ($p \leq 0.05$). Chicken age significantly influenced color parameters, texture, pH, DM, and SCFA content in both N and A droppings, as well as FA content in N droppings ($p \leq 0.05$). Compared with abnormal droppings, normal droppings consistently had a firmer texture, lower a* and b* color values, higher DM, higher linoleic acid, and lower α-linolenic acid across all age periods ($p \leq 0.05$). Acetic acid was the predominant SCFA, with lower concentrations in N droppings than in A droppings. Alcohol and organic acids were more abundant in most A droppings, whereas ketones were the dominant volatile compounds in both groups. Overall, the majority of dropping characteristics were affected by age. While several traits differentiated N from A droppings, a larger sample size is needed to better establish characteristic distribution patterns across different droppings.

Keywords: Color parameters, Broilers, Droppings, Short-chain fatty acids, Volatile compounds

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Introduction

Broiler production represents a major component of the global poultry industry, supplying a significant portion of meat consumed worldwide [1]. As demand for animal-derived products grows, intensified broiler production makes disease management increasingly challenging [2]. Since poultry contributes substantially to global protein intake, the rising incidence of chicken diseases has become a pressing concern [3]. Rapid and accurate assessment of chicken health can support producers in making informed decisions, limiting disease spread, improving animal welfare, and safeguarding economic resources.

The chicken intestinal barrier plays a critical role in protecting against pathogens, supporting commensal microbial populations, and facilitating nutrient digestion and absorption [3,4]. This barrier is composed of microbial, chemical, physical, and immunological components distributed across multiple layers [5]. In poultry, gut bacteria ferment non-digestible carbohydrates to produce short-chain fatty acids (SCFAs) as an energy source, with acetate, propionate, and butyrate being the primary microbial metabolites [6]. Gastrointestinal disorders, which significantly affect poultry health and productivity, are a major category of diseases that increase morbidity and mortality while imposing economic losses [7]. Disruptions to intestinal microbiota can increase susceptibility to infections, compromising both animal health and the safety of poultry products [8]. Ensuring proper nutrition

is essential for maintaining chicken growth and health in farming systems, as nutrition and gut health are closely linked; gut health itself encompasses immunology, microbiology, and physiology [9].

Farm management practices also play a substantial role in chicken performance and welfare [10]. Conventional health assessment methods typically involve on-site sample collection followed by laboratory diagnosis [11]. This process is time-consuming and requires skilled veterinarians and laboratory personnel. Rapid on-site detection kits offer an alternative but may have limited sensitivity for certain infections [12].

Chicken droppings provide a direct reflection of their health status and are valuable indicators of digestive health and disease. Abnormalities in droppings can signal intestinal disorders caused by bacterial, viral, or parasitic infections, as well as nutritional deficiencies, allowing for early detection [13,14]. Currently, veterinarians rely on manual observation of droppings, a process that is labor-intensive and time-consuming. The integration of advanced technologies such as automated sensors or computer vision systems into poultry production remains limited [14,15]. While some non-invasive approaches, such as visually scoring fecal consistency in pig farms, offer useful insights, these subjective methods often lack consistency and objectivity [16]. Consequently, research into specific biomarkers for monitoring chicken health is necessary. For instance, volatile organic compounds produced by bacteria can serve as biological indicators for diagnostic purposes [17], whereas SCFAs may reflect the presence of a healthy gut microbiota [18]. Despite this potential, the literature currently provides limited information on the physical and chemical properties of normal and abnormal chicken droppings, which could be instrumental in biomarker research and in developing reliable diagnostic tools for monitoring poultry welfare.

This study aims to characterize the physical and chemical attributes of chicken droppings collected across different age periods and categorized as normal or abnormal through visual inspection. The objective is to investigate how age influences dropping characteristics and whether these characteristics reliably differentiate between normal and abnormal droppings.

Materials and Methods

Sample collection

Cecal droppings (n = 73) were obtained from broiler chickens aged 3 to 37 days. All birds were Ross 308 broilers, maintained under standardized housing and dietary conditions in compliance with Council Directive 2007/43/EC (28 June 2007) [19–22].

Samples were collected from the deep litter across different poultry houses and organized by age intervals: 0–5 days (Group I), 6–10 days (Group II), 11–20 days (Group III), 21–30 days (Group IV), and 31–40 days (Group V) **(Table 1)**. The droppings were visually assessed by a poultry veterinarian, who classified them as normal or abnormal based on the Biomin-Feces catalog criteria. Images illustrating the appearance of droppings are shown in **Figure 1**. After collection, each sample was placed in a labeled plastic tube and stored at −20 °C until further chemical analyses.

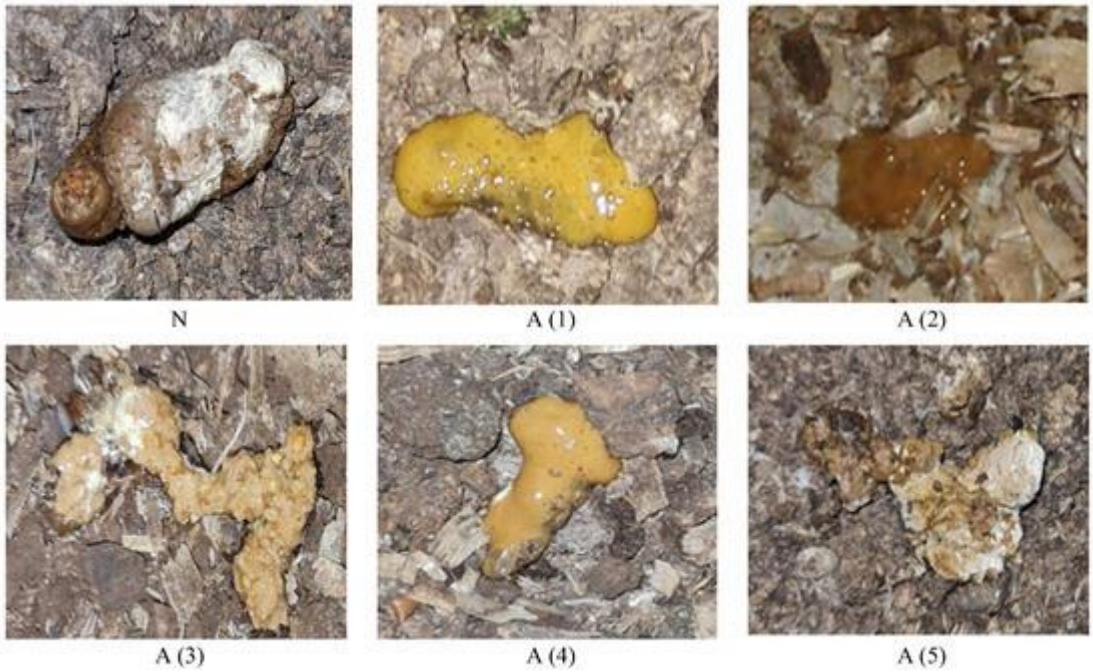

Figure 1. Images of droppings of broiler chickens. N—Normal; A—Abnormal; (1)—foamy; (2)—liquid, foamy; (3)—with feed residues; (4)—liquid; (5)—pathology

Table 1. Description of sample groups

Period	Group Name	Droppings Evaluation	Description	Average Chicken Age
V GR (31–40 days)	V GR A37	Abnormal	Suspected pathology; contains shed intestinal lining / mucosa	37 days
V GR (31–40 days)	V GR A36	Abnormal	Suspected pathology; mixed with undigested feed particles	36 days
IV GR (21–30 days)	IV GR A23	Abnormal	Contains feed residues; suspected coccidiosis (Eimeria acervulina)	23 days
IV GR (21–30 days)	IV GR A27	Abnormal	Suspected coccidiosis (Eimeria acervulina + Eimeria maxima)	27 days
IV GR (21–30 days)	IV GR A27_1	Abnormal	Contains feed residues; suspected coccidiosis (Eimeria maxima)	27 days
IV GR (21–30 days)	IV GR N22	Normal	Firm, brown or greyish-brown droppings with a small white uric acid cap	22 days
IV GR (21–30 days)	IV GR A21	Abnormal	Mixed with undigested feed particles	21 days
III GR (11–20 days)	III GR A16	Abnormal	Watery and foamy	16 days
III GR (11–20 days)	III GR A14	Abnormal	Watery / liquid consistency	14 days
III GR (11–20 days)	III GR A13	Abnormal	Contains visible undigested feed particles	13 days
III GR (11–20 days)	III GR N13	Normal	Firm, brown or greyish-brown droppings with a small white uric acid cap	13 days
II GR (6–10 days)	II GR A7	Abnormal	Watery / liquid consistency	7 days
II GR (6–10 days)	II GR N7	Normal	Firm, brown or greyish-brown droppings with a small white uric acid cap	7 days
I GR (0–5 days)	I GR N4	Normal	Firm, brown or greyish-brown droppings with a small white uric acid cap	4 days

Assessment of pH, dry matter, texture, and color

The acidity of the dropping samples was measured using a pH meter (Inolab 3, Hanna Instruments, Villafranca Padovana PD, Italy). Dry matter content was determined by oven-drying samples at 103 ± 2 °C until their weight stabilized. Color measurements were taken at three points on each sample's surface using the CIE L*a*b* system

(CromaMeter CR-400, Konica Minolta, Tokyo, Japan). Dropping texture was quantified as hardness, reflecting the force required to deform the sample, using a CT3 Texture Analyzer (Brookfield, Middleboro, MA, USA).

Short-chain fatty acid analysis

Concentrations of key SCFAs—including acetic, propanoic, isobutyric, butyric, isovaleric, valeric, and caproic acids—were determined following Zhao *et al.* [16] with minor modifications detailed in Supplementary File S1. Gas chromatography–mass spectrometry (GC–MS) was used for detection and quantification.

Volatile compound analysis

Volatile compounds were extracted using solid-phase microextraction (SPME) prior to GC–MS analysis. The full procedural details are provided in Supplementary File S1.

Fatty acid profiling

The fatty acid composition of the droppings was analyzed using gas chromatography coupled with flame ionization detection (GC–FID), following the methodology outlined in Supplementary File S1.

Statistical analysis

All measurements were performed in triplicate. Normality of the data was assessed using the Shapiro–Wilk test. The effects of chicken age (Groups I–V) and droppings type (normal vs. abnormal) on physical and chemical traits were evaluated using two-way ANOVA, with Tukey's HSD post hoc test for pairwise comparisons. Significance was set at $p < 0.05$. Statistical analyses were carried out using IBM SPSS Statistics 23.0 (SPSS Inc., Chicago, IL, USA).

Results and Discussion

PH, dry matter, texture, and color of droppings

Table 2 summarizes the texture, pH, and dry matter content measured across the sample groups. Normal droppings were observed in Groups I–IV, whereas abnormal droppings appeared in Groups II–IV, with some showing potential pathological characteristics in Groups IV and V. The analyzed parameters differed significantly among the groups ($p \leq 0.05$), highlighting variations associated with both age and droppings type.

Table 2. Texture hardness, pH, and dry matter content of broiler chicken droppings

Sample Group Name	Texture	Hardness (mJ)	pH
V GR A37	0.40 ± 0.02 c	28.88 ± 1.13 cdf	5.74 ± 0.23 bcdef
V GR A36	0.50 ± 0.02 d	25.64 ± 1.00 bce	5.21 ± 0.21 acg
IV GR A27	0.20 ± 0.01 a	19.80 ± 0.77 a	4.84 ± 0.19 a
IV GR A27_1	0.30 ± 0.01 b	22.93 ± 0.89 ab	5.70 ± 0.23 bceg
IV GR A23	0.30 ± 0.01 b	26.14 ± 1.02 bd	5.42 ± 0.22 afgh
IV GR N22	0.40 ± 0.02 c	31.89 ± 1.24 fi	5.27 ± 0.21 ae
IV GR A21	0.40 ± 0.02 c	26.79 ± 1.05 de	6.99 ± 0.28 i
III GR A16	0.30 ± 0.01 b	31.34 ± 1.22 fh	6.90 ± 0.28 i
III GR A14	0.20 ± 0.01 a	30.84 ± 1.20 fg	5.83 ± 0.23 cdef
III GR A13	0.20 ± 0.01 a	38.68 ± 1.51 k	6.63 ± 0.27 hi
III GR N13	0.50 ± 0.02 d	33.38 ± 1.30 ghij	5.96 ± 0.24 eh
II GR A7	0.20 ± 0.01 a	21.03 ± 0.82 a	5.25 ± 0.21 adg
II GR N7	0.70 ± 0.03 e	37.30 ± 1.46 k	5.83 ± 0.23 adef
I GR N4	0.20 ± 0.01 a	35.36 ± 1.38 jk	5.10 ± 0.21 ab

Data are represented as means (n = 3) ± SD. a–k Means with different letters in column are significantly different ($p \leq 0.05$). DM—dry matter; I, II, III, IV, V—age periods; GR—group; N—normal droppings; A—abnormal droppings; 4, 7, 13, 14, 16, 21, 22, 23, 27, 36, 37—average age (days) of broiler chickens.

Hardness testing showed that the toughest poop came from batch II GR N7. The softest ones were I GR N4, II GR A7, III GR A13 and A14, and IV GR A27. Healthy (normal) droppings had noticeably different firmness in every age group (always $p < 0.05$). In weeks II, III, and IV, normal poop was clearly firmer than the runny or abnormal ones. Among the abnormal samples, only weeks II and III felt about the same.

The most alkaline (highest) pH turned up in a few sick-looking samples: III GR A13, A16, and IV GR A21. For normal poop, pH stayed pretty much the same when comparing weeks I vs. IV, II vs. III, and II vs. IV. The abnormal ones only matched between weeks III and IV (and also IV vs. V in the possibly diseased birds).

Dry matter was highest in I GR N4, II GR N7, and III GR A13; the driest-looking normal samples. The wettest (lowest dry matter) were II GR A7 and the two IV GR A27 samples. Across all age groups, healthy poop was always drier than the abnormal stuff. Dry-matter levels in normal droppings changed noticeably between certain weeks (especially II vs. III and II vs. IV), while abnormal samples were alike in weeks II and IV but different in week III. The suspicious abnormal samples also shifted a lot between weeks IV and V.

Statistics (two-way ANOVA) confirmed that both the bird's age and whether the dropping was normal or abnormal (plus the combination of those two) had a big impact on firmness and dry-matter percentage ($p < 0.001$). Age by itself didn't affect pH much ($p = 0.28$).

What the birds ate clearly shapes the bacteria in their poop and changes things in the cecum. Our pH numbers line up with other studies—one group found around 6.5, and variations seem tied to the kinds of short-chain fatty acids in the feed. Acidic conditions in the gut (sometimes dropping to 5.1–5.2) help protein digestion, thanks in part to the very acidic true stomach (proventriculus) in chickens.

For color **(Table 3)**, the lightest dropping was IV GR A21, while the darkest were two abnormal ones from week III (A14 and A16). Normal poop from week III looked obviously lighter or darker than normal poop from the other weeks. In the abnormal samples, only weeks II and III had matching lightness.

Table 3. Color coordinates (L *, a *, b *) of chicken droppings

Sample Group Name	L* (Lightness)	a* (Red–Green)	b* (Yellow–Blue)
V GR A37	43.07 ± 1.83 bc	4.68 ± 0.18 g	18.75 ± 0.84 i
V GR A36	49.56 ± 2.10 df	3.17 ± 0.13 f	20.36 ± 0.91 i
IV GR A27	54.60 ± 2.32 f	1.46 ± 0.06 c	11.59 ± 0.52 fg
IV GR A27_1	45.46 ± 1.93 bd	3.14 ± 0.12 f	15.03 ± 0.67 h
IV GR A23	39.66 ± 1.68 b	2.56 ± 0.10 e	11.90 ± 0.53 fg
IV GR N22	52.65 ± 2.24 ef	0.75 ± 0.03 b	9.28 ± 0.41 bd
IV GR A21	68.00 ± 2.89 g	1.20 ± 0.05 c	8.11 ± 0.36 b
III GR A16	34.64 ± 1.47 ab	2.01 ± 0.08 d	9.40 ± 0.42 be
III GR A14	33.77 ± 1.43 ab	3.18 ± 0.13 f	11.43 ± 0.51 fg
III GR A13	41.10 ± 1.75 b	2.06 ± 0.08 d	11.61 ± 0.52 fg
III GR N13	42.46 ± 1.80 b	1.96 ± 0.08 d	11.92 ± 0.53 fg
II GR A7	41.89 ± 1.78 b	2.24 ± 0.09 d	9.00 ± 0.40 bc
II GR N7	48.58 ± 2.06 cde	0.63 ± 0.02 ab	10.06 ± 0.45 cdef
I GR N4	53.13 ± 2.26 ef	0.40 ± 0.02 a	5.75 ± 0.26 a

Data are represented as means ($n = 3$) ± SD. a–i Means with different letters in column are significantly different ($p \leq 0.05$).; I, II, III, IV, V—age periods; GR—group; N—normal droppings; A—abnormal droppings; 4, 7, 13, 14, 16, 21, 22, 23, 27, 36, 37—average age (days) of broiler chickens; L*, lightness; a*, redness or −a*, greenness; b*, yellowness or −b*, blueness; NBS, National Bureau of Standards units.

Among the droppings analyzed, V GR A37 exhibited the most pronounced red coloration (a*), whereas the least redness was observed in I GR N4 and II GR N7. Across all age groups, normal droppings consistently showed lower a* values than abnormal ones, and differences in redness between normal and abnormal droppings with potential pathological features were statistically significant across age periods ($p \leq 0.05$). For abnormal samples, the a* values in age period IV were notably higher than those in age periods II and III ($p \leq 0.05$).

Yellowness (b*) was highest in V GR A37 and V GR A36, while the lowest b* was recorded in I GR N4. Normal droppings showed lower b* values than abnormal samples in every age group. Among normal droppings, b* values were consistent only between age periods II and IV, whereas in abnormal droppings, age period III differed significantly from periods II and IV ($p \leq 0.05$). Abnormal droppings with potential pathological characteristics also demonstrated significant b* differences across age periods ($p \leq 0.05$). Two-way ANOVA indicated that L* and b* were significantly affected by age ($p = 0.06$ and $p < 0.001$, respectively), while a* was influenced by both age and droppings type ($p < 0.001$).

Droppings characteristics—including color, texture, and shape—are influenced by breed, season, and health status. Examination of droppings is a reliable method for identifying digestive system abnormalities [23]. Healthy birds typically excrete firm, mostly brown droppings with small white caps, as noted by Li Guoming *et al.* [15] and Machuve *et al.* [23, 24]. In contrast, birds affected by coccidiosis may produce yellow, watery, or dark brown flattened droppings [15,23], although certain foods such as corn, strawberries, tomatoes, and forsythia flowers can

also cause color changes [23]. Newcastle disease can lead to mixed light yellow and green fluid droppings. While white coloration may be present in both healthy and diseased birds, salmonella infection can be distinguished by its slimy texture [15,24]. Green droppings can also result from plant-based diets or infections such as intestinal worms, Marek's disease, or avian influenza. Black coloration may indicate internal bleeding or consumption of dark-colored foods like berries or charcoal, while orange or red droppings may arise from lead toxicity, coccidiosis, or intestinal inflammation [23].

In this study, normal droppings were characterized by firmer texture, higher dry matter, and lower a* and b* values than abnormal droppings across all age periods. The significant differences observed in these traits suggest that texture, dry matter, and color parameters could serve as effective biomarkers for monitoring chicken health.

Short-Chain Fatty Acid (SCFA) Composition

The SCFA content of the droppings is summarized in **Table 4**, with clear differences observed between samples ($p \leq 0.05$). Acetic acid was the dominant SCFA in all droppings, reaching its peak in II GR A7 and V GR A37, while the lowest concentrations were found in normal droppings III GR N13 and IV GR N22 and in abnormal samples III GR A13, III GR A16, and IV GR A21. Normal droppings contained significantly lower acetic acid than abnormal droppings in age periods II and IV ($p \leq 0.05$). For normal droppings, acetic acid content varied significantly across all age groups ($p \leq 0.05$), whereas in abnormal droppings, the values were comparable only between age periods III and IV.

Table 4. Short-chain fatty acids content (mmol/kg) in chicken droppings

Sample Group Name	Acetic Acid (µmol/g)	Butyric Acid (µmol/g)	Isovaleric Acid (µmol/g)	Valeric Acid (µmol/g)	Caproic Acid (µmol/g)	Propanoic Acid (µmol/g)	Isobutyric Acid (µmol/g)
V GR A37	93.22 ± 3.77 h	2.55 ± 0.10 c	nd	nd	nd	nd	nd
V GR A36	28.45 ± 1.15 cd	0.59 ± 0.02 a	nd	nd	nd	nd	nd
IV GR A27	45.10 ± 1.82 f	3.58 ± 0.14 d	nd	nd	nd	2.27 ± 0.10 c	nd
IV GR A27_1	32.65 ± 1.32 de	nd	nd	nd	nd	nd	nd
IV GR A23	37.64 ± 1.52 e	nd	nd	nd	nd	nd	nd
IV GR N22	5.99 ± 0.24 a	nd	nd	nd	nd	nd	nd
IV GR A21	5.46 ± 0.22 a	nd	0.066 ± 0.03 a	nd	nd	nd	nd
III GR A16	10.32 ± 0.42 a	2.09 ± 0.08 bc	0.28 ± 0.01 c	nd	nd	0.83 ± 0.04 a	nd
III GR A14	62.70 ± 2.54 g	16.24 ± 0.63 e	nd	0.28 ± 0.01 a	nd	1.74 ± 0.08 b	nd
III GR A13	5.96 ± 0.24 a	nd	0.14 ± 0.01 b	nd	nd	nd	nd
III GR N13	10.11 ± 0.41 a	nd	nd	nd	nd	nd	nd
II GR A7	93.39 ± 3.78 h	16.50 ± 0.64 e	0.56 ± 0.02 d	0.75 ± 0.03 b	nd	5.41 ± 0.24 d	0.44 ± 0.02
II GR N7	22.73 ± 0.92 b	nd	nd	nd	nd	nd	nd
I GR N4	26.07 ± 1.05 bc	1.71 ± 0.07 b	nd	nd	0.040 ± 0.002 a	nd	nd

Data are represented as means (n = 3) ± SD. a–h Means with different letters in column are significantly different ($p \leq 0.05$).; I, II, III, IV, V—age periods; GR—group; N—normal droppings; A—abnormal droppings; 4, 7, 13, 14, 16, 21, 22, 23, 27, 36, 37—average age (days) of broiler chickens.

In this study, short-chain fatty acid (SCFA) composition varied notably depending on droppings type and age. In droppings with potential pathological features, acetic, butyric, and propanoic acid levels showed minimal fluctuation across age periods, indicating a relative stability of these metabolites in compromised gut conditions. Butyric acid appeared sporadically, detected in one normal and six abnormal groups, with the highest levels observed in II GR A7 and III GR A14, and the lowest in V GR A36. Normal droppings from age period I exhibited

significantly higher butyric acid than other age periods ($p \leq 0.05$), whereas in abnormal samples, age period II differed significantly only from age period IV ($p \leq 0.05$).

Other SCFAs, including propanoic and isovaleric acids, were limited to four sample groups, while valeric acid was detected in only two. The peak concentrations for these acids were observed in II GR A7. For abnormal droppings, SCFA values were generally consistent only between age periods III and IV. Isobutyric and caproic acids were rare, appearing solely in II GR A7 and I GR N4, respectively. Two-way ANOVA confirmed that the content of all SCFAs measured—acetic, propanoic, isobutyric, butyric, isovaleric, and valeric acids—was significantly influenced by age, droppings type, and their interaction ($p < 0.001$).

The composition of SCFAs in poultry droppings reflects a complex interplay of diet, environmental conditions, health status, and the gut microbiome. Carbohydrate fermentation primarily generates acetic, propionic, and butyric acids, whereas protein and amino acid catabolism produces additional SCFAs and lactic acid. These metabolites serve as indicators of microbial activity and gut health [25].

SCFAs are central products of anaerobic digestion, encompassing acetic, propionic, butyric, isobutyric, valeric, isovaleric, and caproic acids, which have practical relevance in poultry production and other applications [26]. Consistent with Mahato *et al.* [27], acetic acid predominates in chicken droppings, exceeding the concentrations of propionic and butyric acids [27-29]. Typically, acetate, propionate, and butyrate comprise approximately 95% of all SCFAs, often in a 60/20/20 ratio, with acetate as the most abundant [30].

Odorous compounds in poultry droppings largely originate from anaerobic microbial degradation of proteins and amino acids, particularly generating acetic and propionic acids [12,31,32]. Tryptophan-derived metabolites, such as indole and skatole, are key contributors to the malodor of animal feces [33,34]. Acetate, propionate, and butyrate are produced predominantly by carbohydrate-fermenting gut microbes, whereas branched-chain fatty acids derive from undigested proteins in the lower intestine [35, 36]. SCFA concentrations are influenced by age and diet; older birds and those receiving enzyme-supplemented feed typically show higher levels [37]. These findings align with Palander *et al.* [38], who reported that acetic acid consistently accounted for the largest proportion of SCFAs, ranging from 58 to 76% across all diets and age groups.

Fatty acid composition of broiler chicken droppings

The detailed fatty acid (FA) composition of broiler chicken droppings is presented in **Table 5**. Statistical analysis using two-way ANOVA revealed that both the age of the chickens and the type of droppings significantly influenced the levels of oleic acid (C18:1), linoleic acid (C18:2 cis), and alpha-linolenic acid (C18:3 α) ($p < 0.05$). In addition, the age period alone had a notable effect on the concentrations of palmitic acid (C16:0), stearic acid (C18:0), and eicosenoic acid (C20:1) in the samples ($p = 0.03$; $p = 0.003$; $p < 0.001$, respectively).

Interestingly, a significant interaction was observed between age and droppings type specifically for palmitic acid (C16:0), indicating that the effect of droppings category on this fatty acid varied depending on the age period ($p = 0.04$). These results suggest that both developmental stage and health status, as reflected by droppings classification, play a crucial role in shaping the fatty acid profile of broiler chicken excreta.

Table 5. Fatty acid content (percentage from the total fat content) in the droppings of broiler chickens

Sample Group Name	C18:1 cis (%)	C20:1 (%)	C22:1 (%)	C18:2 cis (%)	C20:2 (%)	C18:3 γ (%)	C18:3 α (%)	C16:0 (%)	C18:0 (%)	C20:0 (%)
V GR A37	20.58 ± 0.88 bc	nd	nd	25.80 ± 0.90 b	nd	nd	35.35 ± 1.43 gh	11.64 ± 0.47 f	6.63 ± 0.30 fgh	nd
V GR A36	26.24 ± 1.13 bc	nd	nd	34.76 ± 1.21 ce	nd	nd	10.92 ± 0.44 b	20.56 ± 0.82 j	7.51 ± 0.34 jk	nd
IV GR A27	28.41 ± 1.22 e	nd	nd	34.72 ± 1.20 cd	nd	nd	14.59 ± 0.59 c	15.61 ± 0.62 i	6.66 ± 0.30 fgi	nd
IV GR A27_1	31.49 ± 1.35 f	nd	nd	49.57 ± 1.72 g	nd	nd	8.17 ± 0.33 b	8.11 ± 0.32 ac	2.66 ± 0.12 a	nd
IV GR A23	24.46 ± 1.05 d	nd	nd	27.76 ± 0.96 b	nd	nd	21.57 ± 0.87 e	19.05 ± 0.76 j	7.15 ± 0.33 hik	nd
IV GR N22	27.61 ± 1.18 e	nd	nd	55.60 ± 1.93 h	nd	nd	4.78 ± 0.19 a	9.05 ± 0.36 bce	2.95 ± 0.14 a	nd
IV GR A21	19.67 ± 0.84 ab	5.43 ± 0.25 c	nd	35.04 ± 1.22 cf	nd	nd	30.59 ± 1.24 f	6.86 ± 0.27 a	2.27 ± 0.10 a	0.14 ± 0.01

III GR A16	28.43 ± 1.22 e	nd	nd	20.66 ± 0.72 a	nd	nd	34.86 ± 1.41 g	9.97 ± 0.40 de	6.07 ± 0.28 eg	nd
III GR A14	17.14 ± 0.74 a	nd	nd	38.12 ± 1.32 def	nd	nd	31.18 ± 1.26 f	8.92 ± 0.36 bcd	4.64 ± 0.21 b	nd
III GR A13	23.84 ± 1.02 d	nd	nd	38.00 ± 1.32 def	nd	nd	18.50 ± 0.75 d	14.81 ± 0.59 gi	4.85 ± 0.22 bc	nd
III GR N13	17.25 ± 0.74 a	nd	nd	47.45 ± 1.65 g	nd	0.35 ± 0.01	14.05 ± 0.57 c	14.96 ± 0.60 hi	5.94 ± 0.27 ef	nd
II GR A7	24.35 ± 1.04 d	1.03 ± 0.05 a	nd	21.62 ± 0.75 a	nd	nd	38.23 ± 1.55 h	7.72 ± 0.31 ab	7.05 ± 0.32 hij	nd
II GR N7	17.59 ± 0.75 a	nd	nd	48.01 ± 1.67 g	nd	nd	15.00 ± 0.61 c	13.96 ± 0.56 gh	5.43 ± 0.25 cde	nd
I GR N4	19.93 ± 0.86 ac	4.97 ± 0.23 b	1.10 ± 0.04	31.94 ± 1.11 c	0.69 ± 0.03	nd	22.96 ± 0.93 e	13.39 ± 0.53 g	5.03 ± 0.23 bd	nd

Data are represented as means (n = 3) ± SD. a–k Means with different letters in column are significantly different ($p \leq 0.05$).; I, II, III, IV, V—age periods; GR—group; N—normal droppings; A—abnormal droppings; 4, 7, 13, 14, 16, 21, 22, 23, 27, 36, 37—average age (days) of broiler chickens; C16:0—palmitic acid; C18:0—stearic acid; C18:1—oleic acid; C18:2 cis—linoleic acid; C18:3 α—alfa linolenic acid; C18:3 γ—gama linolenic acid; C20:1—eicosenoic acid; C20:2—eicosadienoic acid; C22:1—erucic acid; C20:0—arachidic acid.

The dominant fatty acids detected in the droppings were C18:1, C18:2 cis, and C18:3α. The greatest proportions of oleic acid (C18:1) appeared in samples III GR A16, IV GR N22, and IV GR A27. Among normal droppings, C18:1 levels matched only between the II and III age categories, whereas in abnormal droppings its concentration showed no meaningful variation across ages ($p \geq 0.05$). In contrast, abnormal droppings associated with potential pathological changes displayed significant age-related differences in C18:1 ($p \leq 0.05$).

For linoleic acid (C18:2 cis), the largest values were recorded in II GR N7, III GR N13, and IV GR A27_1, while II GR A7 and III GR A16 contained the smallest amounts. Every age group showed a consistently higher C18:2 cis level in normal droppings compared with abnormal ones. The α-linolenic acid (C18:3α) peak values occurred in II GR A7 and V GR A37, and the lowest concentration appeared in IV GR N22. Opposite to C18:2 cis, α-linolenic acid tended to be lower in normal droppings than in abnormal droppings at all ages. In normal droppings, both C18:2 cis and C18:3α showed similar quantities only during the II–III age interval ($p \geq 0.05$), whereas in abnormal and potentially pathological samples their concentrations did not differ significantly across ages.

Stearic (C18:0) and palmitic (C16:0) acids appeared in all droppings but at substantially lower levels than the previously mentioned fatty acids. Sample IV GR A23 showed the highest C18:0 content, while V GR A36 contained the greatest amounts of both C18:0 and C16:0. In normal droppings, palmitic acid levels were alike between the I–II and II–III age categories ($p \geq 0.05$), a pattern also observed for stearic acid. Within abnormal droppings, C16:0 showed a significant shift between the III and IV ages ($p \leq 0.05$), and C18:0 varied significantly across **all** ages ($p \leq 0.05$). Samples classified as abnormal with possible pathology showed no significant age-related differences for either fatty acid.

Some fatty acids appeared sporadically. Erucic acid (C22:1) and eicosadienoic acid (C20:2) were detected only in the normal droppings of I GR N4. γ-Linolenic acid (C18:3) occurred exclusively in III GR N13 (normal), whereas arachidic acid (C20:0) appeared only in IV GR A21 (abnormal). Eicosenoic acid (C20:1) emerged in one normal sample group (I GR N4) and two abnormal groups (II GR A7 and IV GR A21).

Information regarding how fatty acids fluctuate in chicken droppings over growth stages is scarce. Research on Sprague Dawley rat feces noted that although the profiles differed among groups, palmitic (C16:0) and stearic (C18:0) acids were generally most abundant, followed by oleic (C18:1) and linoleic (C18:2), with α-linolenic acid (C18:3) present only in small quantities [39]. Studies on swine manure similarly reported that palmitic, oleic, and stearic acids represent the main free fatty acids [40]. In the current study, normal droppings consistently contained higher proportions of linoleic acid and lower proportions of α-linolenic acid compared with abnormal droppings across all ages. Because these two fatty acids (C18:2 cis and C18:3α) showed significant distinctions between normal and abnormal samples at every age interval, they may serve as reliable biochemical indicators for assessing broiler health.

Volatile compound profile of the broiler chicken droppings

Figure 2 summarizes the volatile compound distribution in broiler droppings using a heatmap. The coloration ranges from pale yellow to deep red, representing increasing abundance from low to high intensities.

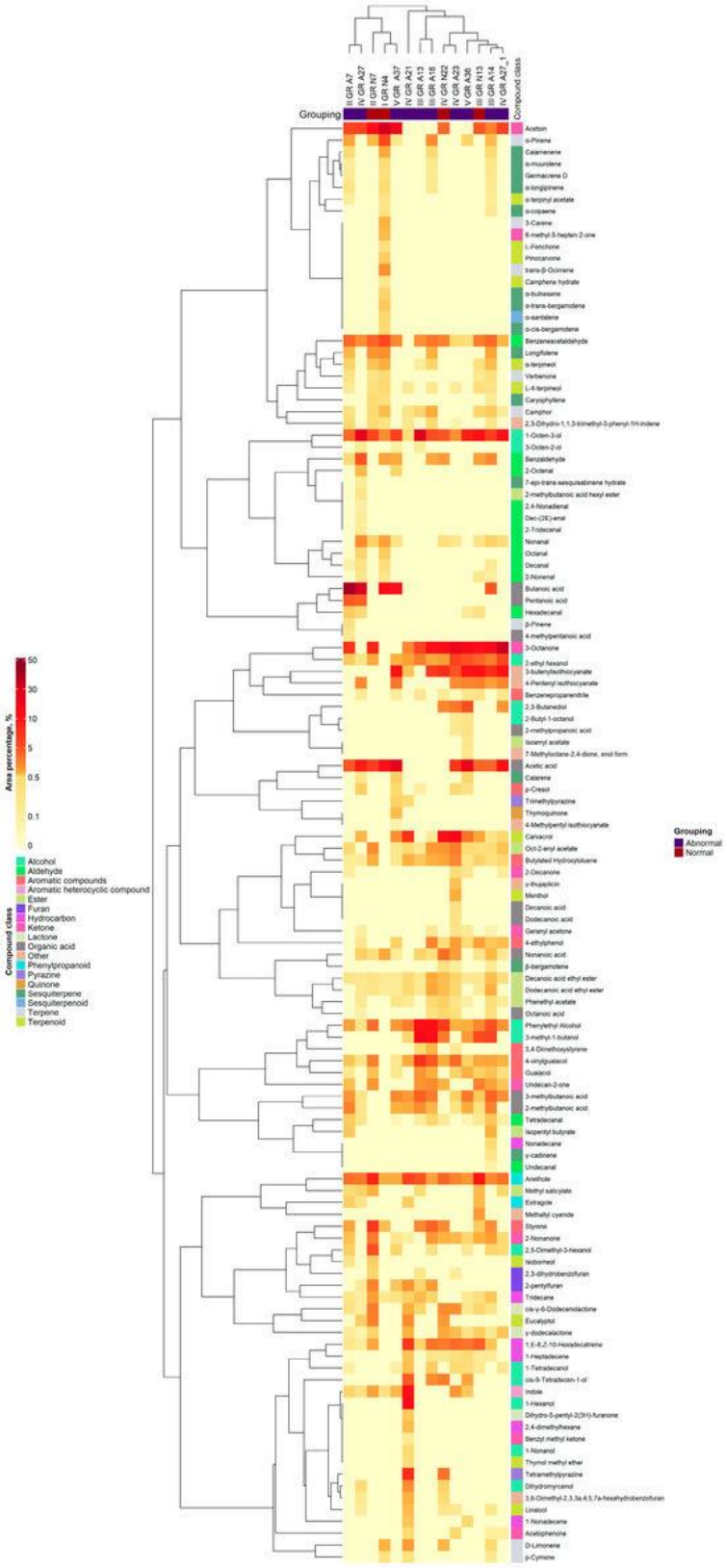

Figure 2. Volatile compound content (percentage from the total volatile compound content) in the droppings of broiler chickens

A broad spectrum of volatile compounds (VCs) was detected across the samples, and they represented many chemical families. The droppings contained alcohols, aldehydes, sesquiterpenes, terpenoids, organic acids, furans, ketones, aromatic substances, hydrocarbons, lactones, phenylpropanoids, pyrazines, and a few isolated molecules

such as a single quinone, one aromatic heterocycle, and a lone sesquiterpenoid. Among these categories, organic acids, alcohols, and ketones consistently appeared as the most abundant groups, whereas aldehydes, terpenoids, phenylpropanoids, and aromatic compounds were present at more moderate levels. Organic acids made up more than half of the VC profile in II GR A7 (56.2%) and were also strongly represented in IV GR A27 and IV GR A37 (45.1% and 40.6%). Several abnormal samples—including III GR A13, III GR A16, IV GR A21, and V GR A36—were dominated by alcohols, with values ranging from roughly one-quarter to over half of their VC content. Ketones were especially prominent in IV GR A27_1 (41.7%) and were the major class in every normal-dropping group across all age stages as well as in some abnormal groups (e.g., III GR A14, IV GR A23).

Across all detected molecules, a limited set of compounds displayed particularly high concentrations: acetic acid, butanoic acid, 3-octanone, 3-methyl-1-butanol, phenylethyl alcohol, 3-butenyl isothiocyanate, 1-octen-3-ol, acetoin, indole, carvacrol, and—less frequently—1-hexanol. Most of these appeared in almost every sample, although their proportions varied widely from one group to another. Acetic acid accumulated steadily toward the later age periods, and each age period within the normal-dropping category differed significantly ($p \leq 0.05$). Acetoin showed a similar pattern except between age periods III and IV, reaching peak levels in I GR N4, II GR N7, and V GR A37. 3-Methyl-1-butanol and phenylethyl alcohol appeared in greatest amounts in III GR A13 and III GR A16. Butanoic acid was especially elevated in II GR A7 and IV GR A27, with the normal I-period droppings differing significantly from the rest ($p \leq 0.05$). High levels of 1-octen-3-ol were observed in III GR A13 and IV GR A27, whereas carvacrol reached its highest values in IV GR N22 and IV GR A23.

Two-way ANOVA revealed that both age and dropping type, as well as their interaction, had strong effects on the levels of butanoic acid and carvacrol ($p < 0.001$). Age and type (with the exception of type for 1-hexanol) significantly affected acetoin, 3-butenyl isothiocyanate, and 1-hexanol ($p < 0.001$). Meanwhile, acetic acid, 3-methyl-1-butanol, and phenylethyl alcohol were significantly shaped by both age period and the combined interaction of age with dropping type ($p < 0.001$).

Highly odorous molecules—such as indole and SCFAs—are known to dominate broiler excreta [25]. Their presence, alongside compounds like acetoin, acetic acid, 1-octen-3-ol, butanoic acid, 3-butenyl isothiocyanate, and indole, can be traced back to microbial fermentation and the breakdown of dietary organic components inside the gut [25]. Similar patterns of metabolites have been reported in poultry and swine waste by Yasuhara [41] and Bicudo *et al.* [42]. Acetoin, for example, is a decarboxylation by-product of pyruvate and is produced by many Bacillus species, which have been isolated from duck droppings [43]. Butanoic acid is associated with gut ecosystem balance and helps to create the characteristic odor of chicken waste, together with 3-butenyl isothiocyanate and indole [44]. 1-Octen-3-ol is known from emissions of terrestrial mammals, including the urine of aged cattle [45]; although it may be absent in freshly excreted material, microbial lipid degradation over time usually generates it [46]. Terpenoid compounds detected in the samples may originate from dietary ingredients or from environmental contamination encountered on the farm or during sample handling [47].

Compounds such as 3-methyl-1-butanol, carvacrol, 1-hexanol, phenylethyl alcohol, and 3-octanone are not typically associated with chicken excreta, and their appearance is likely linked to residual feed materials that were not fully digested. Among these, 3-methyl-1-butanol is known to originate from bacterial metabolism of the amino acids valine and leucine [48], and several reports have described it as a marker of microbial decline or spoilage in chickens [49]. This compound is also recognized as a common microbial volatile in moist agricultural settings, including compost systems [46]. Carvacrol, in contrast, is a characteristic constituent of oregano essential oil [50]. The remaining compounds—1-hexanol, phenylethyl alcohol, and 3-octanone—occur naturally in many botanical sources, including fruits, flowers, vegetables, and essential oils, and are also found in various beverages [51,52].

A number of other volatile compounds—methallyl cyanide, 6-methyl-5-hepten-2-one, 3-carene, trans-β-ocimene, L-fenchone, pinocarvone, α-bulnesene, α-bergamotene, and α-santalene—were detected only at trace levels (<2%), and solely in the normal droppings of I GR N4 and III GR N13, with each compound confined to a single sample group. Isopentyl butyrate appeared only in abnormal droppings from II GR A7 and III GR A14, and α-muurolene was present in these samples as well as in III GR A16.

A separate set of compounds—1-hexanol, isoamyl acetate, 4-methylpentyl isothiocyanate, 1-nonanol, menthol, 7-methyloctane-2,4-dione, 2,4-nonadienal, hexyl 2-methylbutanoate, thymol methyl ether, thymoquinone, dec-(2E)-enal, thujaplicin, and dihydro-5-pentyl-2(3H)-furanone—occurred exclusively in the abnormal samples from the IV and V groups. Their concentrations were minimal (<1.2%, apart from 1-hexanol), and each compound again appeared only in a single sample group.

Conclusions

The results of this investigation expand the currently limited information on the physical and chemical traits of normal versus abnormal chicken droppings. These insights may support future research into gut-microbiota-related health issues in poultry and may assist in the development of technologies for assessing flock welfare. Age period, dropping category, and their interaction significantly affected texture, dry matter percentage, and most short-chain fatty acid (SCFA) levels. Fatty acid composition was largely shaped by both age and dropping type, and age also played a prominent role in determining pH, lightness, and yellowness. In all age groups, normal droppings exhibited greater hardness, higher redness and yellowness, increased dry matter, and elevated linoleic and α-linolenic acid levels ($p < 0.05$) compared with abnormal ones.

Although the volatile compound profile was diverse and many compounds appeared only sporadically, no consistent distribution patterns could be drawn across the dropping categories. Overall, age period influenced most measured parameters, but distinguishing consistent chemical differences between normal and abnormal droppings may require examining a wider range of droppings to reveal clearer trends.

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