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Effect of diatomite-containing mineral sprinkles on litter properties and air pollutants in broiler houses*

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Modern broiler chicken production needs to consider limits on air pollutant emissions and rigorous welfare standards. Therefore, the investigation of mineral litter additives and/or sprinkles - combining diatomite with bentonite (DTBN) and/or dolomite (DTDL) - for their impact on litter properties and air quality is crucial. In an experiment conducted on broiler chickens, one-day-old Ross 308 chicks (6 coops x 80 birds/group) were assigned to four groups: C - no additives to litter, DTBN (75/25; wt/wt) - diatomite and bentonite mixture applied at 280 g/m² before facility settling (D0), DTDL - diatomite and dolomite applied on the 10th (D10) and 26th (D26) rearing day at 100 g/m², and DTBN+DTDL - both mixtures applied on the same dates. During the 42-day rearing

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period, the chicks were fed a complete ration of compound feed. The concentration of noxious gas admixtures in the air (NH_3, H_2S, CO_2, CH_4) as well as litter samples from under the animals were collected on D0, D10, and D35. Mineral mixtures increased dry matter content ($p\leq0.05$) and decreased the concentration of ammonium nitrogen (N-NH_4, $p\leq0.05$) in the litter. Combined DTBN + DTDL reduced electrical conductivity (EC) but at the same time increased dehydrogenase (DhA) activity on D35. Minor, although statistically significant changes ($p\leq0.05$) in CO_2, NH_3, CH_4, H_2S, and dust concentration in the chicken house air were observed. In conclusion, diatomite with bentonite and/or dolomite mineral mixtures could help reduce the emission of air pollutants such as ammonia and may be useful in maintaining high environmental quality in broiler houses.

KEYWORDS: broiler chicken / litter / mineral additives / diatomite / air quality / electrical conductivity / litter dehydrogenase

The health of birds and, consequently, the quality of their final products, depend essentially on the quality of the rearing environment [Bessei 2006, Baracho *et al*. 2018, Tainika *et al*. 2023, Şen *et al*. 2023]. In addition to the sanitary condition of the litter, lighting, temperature, and humidity in the livestock building, monitored parameters include the content of dust and gaseous pollutants in the air, such as carbon dioxide (CO_2), hydrogen sulfide (H_2S), methane (CH_4), and ammonia (NH_3) [Homidan *et al.* 2003, Carey *et al*. 2004, Lacey *et al*. 2004, Baracho *et al*. 2018, Naseem and King 2018, Diarra *et al.* 2021]. Apart from rearing conditions, several other factors are of great importance, including animal species, stocking density, nutrition, and intestinal fermentation. Regarding dust and gas emissions, the type of litter and its humidity and temperature also play a vital role. These parameters determine the activity of biochemical processes occurring in the litter during animal rearing [Brink *et al*. 2023, Bist *et al*. 2023, Şen *et al*. 2023]. Proteins or uric acid excreted by animals influence the intensity of biochemical processes in the litter [Dias *et al*. 2010]. Notably, the resulting odorant mixture can contain over 100 gaseous compounds [Lacey *et al*. 2004], with NH_3 and H_2S making up the largest share [Carey *et al*. 2004]While ventilation and animal feeding systems are being optimized, an intensive research is being conducted to modify the properties of litter to enhance the microclimate within livestock buildings and to reduce the emissions from rearing [Dias *et al*. 2010]. A suitable litter material should have high absorbency, anti-caking properties, be low cost and easily available [Garcia *et al*. 2012], as well as soft and provide good thermal insulation [Souza *et al.* 2012]. An additional advantage is an adequate odor-binding capacity [Lacey 2004].

Research is ongoing to find additives that reduce biochemical activity in litter and improve its sorption capacity. However, certain rearing conditions make the effort challenging. In some cases, the modification of the composition of litter can stimulate microbial activity [Dias *et al.* 2010, Brink *et al*. 2022]. This phenomenon is due to the litter's water absorption, which helps reduce excess moisture. Unfortunately, the same quality also provides a more favorable environment for the growth of numerous microorganisms [Dunlop *et al.* 2016, Diarra *et al.* 2021]. As reported by Nowakowski *et al.* [2011], the use of a nanotechnology-mineral preparation based on nanosilver and vermiculite resulted in reduction in the ammonia content above the litter of from 5 to 73%, and in the upper layer of the litter of between 5 and 47%. The applied additive was also observed to affect the physical indices of the litter, including its temperature, humidity, and pH. According to Biesek *et al.* [2023], the incorporation of coffee husks into wheat straw (both components were pelleted) had a favorable impact on reducing sole dermatitis in broiler chickens and boosted their slaughter performance. Oketch *et al*. [2023] investigated the utilization of coconut peat, sawdust, and rice husks as litter materials in Pekin duck production. As shown by the cited authors, rice husks are an effective litter material with beneficial properties. The cited studies indicate that litter components are selected for their availability largely determined by the regional production of the raw material. However, naturally occurring minerals also offer numerous benefits in poultry production, including productivity, hygiene, health, environmental safety, and broiler meat quality [Banaszak *et al*. 2021, Emam *et al.* 2023].

Litter component selection is assessed by the quality of the animals' environment, including the concentration of harmful gaseous admixtures in the air during their rearing. Nevertheless, measuring harmful admixture concentrations identifies the effect and does not remove the cause of the problem. Gaseous pollutants arise from many biochemical factors including the contamination of the litter with feces. Chemical and biochemical indices that provide insight into microbial activity in the transformation of organic compounds markedly facilitate prompt decision making regarding the management of air quality in a livestock facility [Sargeant *et al*. 2020].

In light of mounting pressure to reduce atmospheric emissions and increasingly rigorous animal welfare standards, Gondek *et al.* conducted a study to assess the impact of litter supplementation with the addition of diatomite, a natural mineral material exhibiting exceptional sorptive properties and proven effectiveness in binding toxic substances as well as in improving the fertilizing properties of plant biomass [Gondek *et al*. 2022]. Nevertheless, further research on the suitability of diatomite as a functional additive has demonstrated that its efficacy can be enhanced by employing properly formulated mixtures with bentonite or dolomite [Gondek *et al*. 2023, Redding 2013]. This study aimed to assess the impact of adding a combination of diatomite and bentonite, as well as diatomite and dolomite, to wood shavings and peat moss litter on the chemical and biochemical properties of waste biomass from broiler chicken rearing

Material and methods

Animal care and welfare

The animals were treated in accordance with Directive 2010/63/EU, which regulates the protection of animals used for experimental and other scientific purposes, enforced in Poland through Legislative Decrees 266/2015 and 638/2020. The practices employed on animals were analogous to recognized animal husbandry methods and were not likely to cause pain, suffering, distress or lasting harm equivalent to, or higher than the introduction of a needle in good veterinary practice. For this reason, an ethics approval by an institutional review board was not required under Directive 2010/63/EU and the Act 2015/266/RP. The study reported in the manuscript adheres to the guidelines in

Percie du Sert *et al*. [2020] and was registered under the number BZ/4240/22/WHBZ. The heating and lighting programs were in accordance with Ross 308 broiler stock management [Aviagen 2020].

Experimental design

The experiment was carried out at the Experimental Poultry Farm in Potok, owned by Ekoplon S.A. in Poland. Prior to the commencement of the experiment, the building and equipment were thoroughly cleaned and disinfected in accordance with veterinary safety regulations. Before placing the chicks, the hall was lined with wood shavings and peat moss bedding (AMFLife, Poland) and heated to an air temperature of 34°C and a floor temperature of 28°C. One-day-old Ross 308 chicken broiler chicks (Aviagen EPI LTD, Poland) were randomly divided into four groups (480 chicks/group): C – control without litter additives, DTBN – diatomaceous earth (diatomite) and bentonite litter additive, DTDL – diatomite and dolomite litter additive, and DTBN+DTDL – litter additive of both mixtures, i.e. diatomite with bentonite and diatomite with dolomite. There were 6 coops in each experimental group, with 80 chicks in each coop. During the rearing period, the chicks were fed complete ration compound feeds. The starter was provided up to day 10 (D10), the grower from day 11 to 35 (D11-D35), and the finisher from day 36 to 42 (D36-D42) (Tab. 1). The compound feeds used in all experimental groups were formulated based on recipes employed under production conditions, in accordance with the recommendations of the CVB [2018]. Consequently, their composition included typical additives utilized by the feed industry during the various rearing periods (coccidiostats, feed enzymes, mineral and vitamin additives, etc.). Feed and water were administered ad libitum throughout the rearing period. Throughout the entire period of bird rearing, typical production parameters were collected and analyzed. However, due to their large number and specificity, they will be presented in a separate scientific publication.

Litter additives

In this study, a mixture of diatomite with bentonite (DTBN) and a mixture of diatomite with dolomite (DTDL) were employed as litter additives. A detailed description of the methodology employed for the preparation of these mixtures, along with their respective physical, chemical, and ecotoxicological properties, can be found in Gondek *et al*. [2023]. Prior to the introduction of the chicks into the chicken house, a mixture of diatomite (75%) and bentonite (25%) was added to the litter in the experimental groups DTBN and DTBN+DTDL at a rate of 280 g/m^2 in accordance with the recommendations of Oliveira *et al*. [2017] and then mixed with the substrate. The height of the applied substrate (litter) was 2 cm. In the DTDL and DTBN+DTDL groups, a mixture of diatomite (75%) and dolomite (25%) was applied as a sprinkle on the litter at a rate of 100 g/m^2 on the D10 (last day of the starter period) and 26th day of the chicken rearing.

Table 1. Composition (%) and nutritive value of compound feeds used in the experiment

Specification	Starter D1-D10	Grower D11-D35	Finisher D36-D42
Wheat grain	30.000	35.013	35.917
Maize grain	28.300	27.850	32.500
Post-extraction soybean meal	31.986	26.964	20.620
Canola cake	3.000	4.000	5.000
Soybean oil	3.250	3.550	3.700
Phosphate 1Ca	0.625	0.275	-
NaCl	0.275	0.285	0.265
Sodium bicarbonate	0.175	0.125	0.130
Fodder chalk	1.300	1.000	0.950
Choline chloride 60%	0.096	0.065	0.043
Sacox 120[1]	0.058	0.058	-
L-lisine HCl	0.220	0.205	0.265
DL-methionine 990	0.295	0.250	0.235
L-threonine	0.075	0.055	0.070
L-valine	0.050	0.010	0.010
Max-Vit 0.25% Prestiż[2]	0.250	0.250	0.250
Ravabio Advance P[3]	0.005	0.005	0.005
Axtra PHY[4]	0.040	0.040	0.040
Dry matter (%)	89.2	89.4	87.8
Crude ash (g)	55.0	52.4	42.3
Total protein (g)	208.5	177.2	180.2
Crude fat (g)	40.1	63.8	53.3
Crude fiber (g)	25.5	27.3	27.7
NDF (g)	97.1	94.4	95.2
ADF (g)	54.6	59.4	58.0
ADL (g)	7.5	8.5	7.6
Starch (g)	369.2	429.5	454.5
NFC[5] (g)	505.5	506.2	556.4
Lysine[6] (g)	13.01	11.72	10.84
Methionine[6] (g)	6.16	5.51	5.15
ME[7] (kcal/kg)	2954	3029	3129
ME[7] (MJ/kg)	12.37	12.68	13.10
Nitrogen (N), g	39.02	31.33	33.19
Calcium (Ca), g	11.62	11.84	7.28
Phosphorus (P), g	5.49	6.60	3.33
Potassium (K), g	9.35	9.15	7.08
Magnesium (Mg), g	2.09	2.85	1.55
Copper (Cu), mg	28.26	19.49	19.64
Iron (Fe), mg	240.5	234.9	134.0
Manganese (Mn), mg	158.9	178.7	194.3
Zinc (Zn), mg	146.8	146.7	153.0

[1]Sacox® 120 microgranulate (salinomycin sodium, 120g/kg).
[2]Mineral and vitamin additive.
[3]Enzyme product containing xylanases and β-glucanases obtained by fermentation with *Talaromyces versatilis*.
[4]Phytase enzyme produced from *Trichoderma reesei* (ATCC SD-6528).
[5]Non-fiber carbohydrate NFC = 100 – (NDF + total protein + crude fat + crude ash).
[6]Estimated from the composition of the compound feed.
[7]Metabolic energy estimated according to the European Table [Janssen 1989] as the sum of EM of feed components.

Litter sampling

Fresh bedding (peat moss with wood shavings) was sampled prior to the introduction of the experimental additive to the substrate (D0). Litter from under the animals was collected twice: on the last day of feeding the chicks with the starter (D10, prior to the application of the DTDL sprinkle) and on the last day of feeding the chicks with the grower (D35). Subsequently, material was obtained from each coop in accordance with the experimental design. Following collection, representative samples from each coop were immediately placed in a chilled container (~4°C) and transported to the laboratory.

Chemical and biochemical analyses in litter

The dry matter content (DM) of the litter samples, separately for each coop, was determined by drying the samples at 105°C for 24 hours. The pH was then determined potentiometrically in a suspension of litter and water (litter : water = 1 : 10) with a pH meter (CP-505), and the electrical conductivity (EC, litter : water = 1 : 10) was measured with a CCO-501 conductivity meter [Gondek *et al.* 2022]. The $N\text{-}NH_4$ content was determined following the extraction of the litter with a 0.05 mol/dm^3 HCl solution, which was then colorimetrically analyzed using a Backman DU 640 spectrophotometer at 470 nm [Gondek 2009]. Dehydrogenase (DhA) activity was quantified by the conversion of colorless, water-soluble 2,3,5-triphenyltetrazolium chloride (TTC) to water-insoluble 1,3,5-triphenylformazan (TPF) in red Samples (0.5 g fresh weight of material) were incubated at 25±2°C for 16 hours. The enzymatic activity of dehydrogenases was determined by colorimetric method using Backman DU 640 spectrophotometer at 485 nm [Gondek *et al*. 2022]. Basal respiration (BR) of the litter from under the animals was determined after 24 h by measuring the amount of CO2 released, which was absorbed in a 0.05 mol/dm NaOH solution and precipitated as barium carbonate by adding a 0.5 mol/dm $BaCl_2$ solution. Unused sodium hydroxide solution was titrated with 0.1 mol/dm HCl in the presence of phenolphthalein as an indicator. The results were used to calculate the amount of CO_2 released [ISO 16072: 2002]. Substrate-induced respiration (SIR) was determined based on CO_2 secretion 6 hours after the addition of glucose (10% solution) in accordance with ISO 14240-1:1997. The respiratory-activation quotient (QR) was calculated by dividing BR values by SIR values in accordance with ISO 17155 [2012].

Analysis of noxious gas admixtures and dust measurement

The concentration of noxious gas admixtures in the air inside the chicken house (NH_3, H_2S, CO_2, CH_4) was measured on days D10, D28 (two days after the second dose of DTDL was applied), and D35. The test was conducted in the afternoon, at the height of the animals' heads, using a GFG MICROTECTOR II G450/4 device (GfG – Gesellschaft für Gerätebau mbH, GfG Polska Ltd.) that meets the requirements of PN-EN IEC 62990-1:2023-03/A11:2023-07 (toxic gases). Measurements were taken after the meter had been in the measuring point for 15 minutes. A Fluke 985 particle counter (Fluke Corporation, Fluke Europe B.V. Eindhoven, The Netherlands) was used for dust analysis, including different dust grain fractions. The particle sizes were measured at 0.3, 0.5, 1.0, 2.0, 5.0 and 10.0 μm. The results were then related to the Kovacs'classification of dust in a livestock building [Kołacz and Dobrzański 2019].

Statistical analysis

The experiment was conducted using a completely randomized design with three replicates, where the coop was considered as a block. The normality of data distribution was assessed using the Shapiro-Wilk test. The data were subjected to one-way analysis of variance (ANOVA). The statistical model used for the analysis included the random effect of the block (coop) and the fixed effect of the treatment (addition of experimental mixtures):

$$Yijk=\mu+Ti+Rj+Eij$$

where:

μ – the general mean;

Ti – the treatment effect (mixture addition);

Rj – the repetition (replicate) effect;

Eij – experimental error.

Individual means were compared using the Duncan test. Differences between means were considered statistically significant at $p\leq0.05$, and trends were considered at $0.05<p\leq0.10$, unless otherwise stated. Statistical calculations were performed in two stages: The first stage analyzed data from day D10, when only the DTBN litter additive was applied. The second stage analyzed data from day D35, which evaluated the impact of adding two distinct mineral mixtures (DTBN and DTDL). Additionally, to determine the relationships between selected chemical and biochemical parameters, Spearman rank correlation coefficients were calculated. The choice of this test was justified by the non-normal distribution of the traits. All statistical computations were performed using Statistica 12.0 (TIBCO Software Inc., Palo Alto, CA, USA). The results are presented in tables as means and standard error of the mean (SEM).

Reasults and discussion

Physical and chemical parameters of litter

Dry matter (DM) of the wood shavings and peat moss bedding prior to the introduction of animals was 84.3%±2.27, the pH value was 4.90±0.69, and EC was 0.17 mS/cm±0.02. The crude ash content was 9.70%±1.08, while the total carbon and total nitrogen contents were 755 g/kg±21 and 26.2 g/kg±0.75, respectively (data not included in tables).

The dry matter of the litter on D10 ranged from 76 to 81%, both for the C and DT+DL groups (Tab. 2). As the animals were reared, the moisture content of the litter

Table 2. Contents of dry matter (DM) and ammonia nitrogen (N-NH4), pH value and electrolytic conductivity (EC) in litter on days 10 (D10) and 35 (D35) of broiler chicken rearing

Term	Group[1]	DM[2] (%)	N-NH4[3] (g/kg)	pH	EC[4] (mS/cm)
D10	C	78.53	1.31a	5.23	4.36
	DTBN	78.69	0.35b	5.27	4.34
P-value		0.9607	<0.0001	0.3899	0.8371
SEM		0.70	0.18	0.02	0.05
D35	C	57.80^{b}	4.94^{a}	6.34	5.76^{ab}
	DTDL	64.40^{ab}	4.54^{ab}	6.34	5.84^{ab}
	DTBN	66.96^{ab}	3.95^{b}	6.41	6.20^{b}
	DTBN+DTDL	68.31^{a}	3.52^{b}	6.46	4.52^{b}
P-value		0.0007	0.0151	0.3716	<0.0001
SEM		1.21	0.19	0.03	0.18

[1]DTBN – addition of a diatomite-bentonite mixture to the litter before the birds were inserted (280 g/m^2); DTDL – addition of a diatomite-dolomite mixture to the litter on day 10 and day 26 of bird rearing (100 g/m^2 each).
[2]Dry matter.
[3]Ammonium nitrogen.
[4]Electrical conductivity.
[ab…]Within a column means bearing different superscripts differ significantly at $p \leq 0.05$, according to Duncan's multiple range tests.

increased in all groups. However, while DM content of the litter decreased by 23.3 percent points (pp) in group C between D10 and D35, the addition of DTDL and/or DTBN reduced this trend to approximately 11 pp.

The difference in N-NH_4 content between C and DTBN was confirmed in the litter taken from under the animals on D10 (Tab. 2). N-NH_4 content in the litter with a mixture of DTBN was almost 4 times lower. On D35, lower N-NH_4 was observed in the litter of the experimental groups in which a mixture of DTBN and DTDL+DTBN was used as an additive persisted, and the differences compared to the control group were significant ($p \leq 0.05$).

The pH values measured in the litter were not significantly different within the sampling days (Tab. 2). Regardless of the experimental group, higher pH values for the litter were recorded on D35. In general, however, the mineral additives had no effect on the litter's pH values.

The EC of the litter on D35 in groups C and DTBN was found to be significantly higher than on D10 (Tab. 2). The lowest EC were observed in the litter to which a mixture of DTDL and DTBN was added.

Selected biochemical parameters of litter

The lowest DhA in the litter was determined on D10, without difference between C and DTBN group (Tab. 3). Significantly higher DhA values were observed in the litter from under the animals t on D35, regardless of the group. The greatest increase in DhA activity was observed for the litter taken from group C. There was no significant variation in the litter with both DTDL and DTBN mixtures supplemented separately.

However, higher DhA values were found after DTBN application and the highest after the joint application of DTDL+DTBN mixtures.

The BR values differed significantly between the sampling days (Tab. 3). On both D10 and D35, higher BR were recorded in litter supplemented with the DTBN mixture. Significantly, the highest BR was observed for litter collected on D35 in the DTBN and DTDL+DTBN groups. The addition of glucose to the analyzed litter samples resulted in a notable increase in the respiration rate (SIR), likely due to the presence of a readily available carbon source in microbial habitat. The highest SIR value was observed in the litter collected from under the animals in the control variant C on D35 (Tab. 3). Among the litter that was supplemented with mineral mixtures, the highest SIR value was recorded in the DTDL variant.

Table 3. Dehydrogenase activity (DhA), basal respiration (BR), and substrate-induced respiration (SIR) in litter on days 10 (D10) and 35 (D35) of broiler chicken rearing

Term	Group[1]	DhA[2] (g TPF/g DM/h)	BR[3] (g CO_2/g DM/h)	SIR[4] (g CO_2/g DM/h	QR[5]
D10	C	9.63	5.10^{b}	45.57	0.12^{b}
	DTBN	8.10	10.68^{a}	45.71	0.24^{a}
P-value		0.1061	0.0004	0.9791	0,0489
SEM		0.47	1.12	2.37	0.05
D35	C	118.97^{a}	67.48^{b}	1041.46^{a}	0.06^{b}
	DTDL	76.82^{c}	91.53^{ab}	917.89^{b}	0.10^{ab}
	DTBN	81.95^{bc}	115.08^{a}	871.16^{b}	0.13^{a}
	DTBN+DTDL	114.68^{ab}	115.49^{a}	860.16^{b}	0.13^{a}
P-value		0.0175	0.0006	0.0032	0.0192
SEM		6.53	5.89	22.68	0.03

[1]DTBN – addition of a diatomite-bentonite mixture to the litter before the birds were inserted (280 g/m^2); DTDL – diatomite-dolomite litter addition on days 10 and day 26 of bird rearing (100 g/m^2 each).
[2]Dehydrogenase activity.
[3]Basal respiration.
[4]Substrate-induced respiration.
[5]Respiratory-activation quotient.
[ab…]Within a column means bearing different superscripts differ significantly at $p \leq 0.05$, according to Duncan's multiple range tests.

The respiratory-activation quotient (QR) of the litter, reflecting the number of microorganisms present in either a dormant or active state, ranged from 0.06 to 0.24 (Tab. 3). On D35, the QR ranged from 0.06 for C group to 0.13 for both the DTBN and the DTDL+DTBN variants. The data presented indicate that the DTDL variant exhibited the most stable value of the QR coefficient.

Concentrations of CO_2, NH_3, CH_4 and H_2S

On D10, the concentration of CO_2 in the air of the chicken house was approximately twice that observed on D35 ($p \leq 0.05$), while the concentration of CH_4 was 2-3 times higher ($p \leq 0.05$) (Tab. 4). Conversely, the concentration of NH_3 in the air of the control group increased by 1 ppm between D10 and D35. The NH_3 concentrations in the group with DTBN+DTDL mixtures on D35 were approximately 2-2.5 ppm higher than in

Table 4. Concentration of noxious gases in the air on days D10 and D35 of broiler chick rearing

Term	Group[1]	CO_2 (%)	NH_3 (ppm)	CH_4(ppm)	H_2S(ppm)
D10	C	0.11	3.8^b	16.6^a	<1
	DTBN	0.12	4.8^a	10.9^b	<1
P-value		0.4520	0.0070	< 0.0010	-
SEM		0.01	0.18	0.52	-
D35	C	0.05^b	4.8^b	5.0^{ab}	<1
	DTDL	0.05^b	4,0^{bc}	4.2^b	<1
	DTBN	0.07^a	3,2^c	3.0^b	<1
	DTBN+DTDL	0.06^{ab}	5.7^a	9.0^a	<1
P-value		0.0170	< 0.0010	0.0010	-
SEM		0.01	0.21	0.26	-

[1]DTBN – addition of a diatomite-bentonite mixture to the litter before the birds were inserted (280 g/m^2); DTDL – addition of a diatomite-dolomite mixture to the litter on day 10 and day 26 of bird rearing (100 g/m^2 each).

[ab...]Within a column means bearing different superscripts differ significantly at $p \leq 0.05$, according to Duncan's multiple range tests.

the other groups ($p \leq 0.05$). The concentrations of H2S were below the sensitivity of the measuring devices regardless of the test date. It is important to note that despite the observed discrepancies ($p \leq 0.05$) in the concentrations of CO_2, NH_3, and CH_4 between groups and rearing stages, they remained consistently below the harmful levels.

The measurements indicate that the dustiness of the air in the coops of all groups at the evaluated stages of rearing can be considered average pollution according to Kovacs' classification (Tab. 5). There were no differences in the concentration of dust in the air, both the total fraction and the individual grain fractions, depending on the stage of rearing and the composition of the mineral additive used for litter.

The dry matter content of litter during animal rearing is one of the most important parameters affecting animal health [Bilgili *et al*. 2009, Toledo *et al.* 2019]. However, it is also crucial to consider the impact of biochemical processes that occur in litter already mixed with animal feces [Tasistro *et al*. 2004]. As anticipated, the dry matter content declined between D10 and D35, regardless of the experimental group. The water content in litter is influenced by a number of factors, including the health of the animals, the season, the type and location of drinkers and the ventilation system [Świątkiewicz *et al*. 2009], as well as species and the litter material used [Benabdeljelil and Ayachi 1996, Bilgili *et al*. 2009, Shepherd *et al*. 2017, Munir *et al*. 2019, Toledo *et al*. 2019, Brink *et al*. 2022]. Wood shavings and, above all, peat moss are considered to have superior absorptive properties compared to straw, as they allow for the maintenance of a moisture content of approximately 40 to 50% in the last week of the rearing period [Shepherd 2017, Brink *et al*. 2022]. This value aligns with that observed in the control group. The reduction of litter sogginess is also facilitated by the addition of mineral substances, primarily aluminosilicates, to both the litter material [Banaszak *et al*. 2021, Sholikin *et al*. 2023] and the feed [Prasai *et al*. 2017, Światkiewicz *et al.* 2017, Nadziakiewicz *et al*. 2019, 2021]. A similar outcome was observed in our study, where the litter was enriched with DTDL and DTBN mixtures, both individually and in combination. In a study by Rogeri *et al*. [2016], the contribution of ammonia nitrogen ($N\text{-}NH_4$) to total N content averaged 9.6%. Mowrer *et al.* [2013] demonstrated that $N\text{-}NH_4$ accounted for more than 17% of total N. This significant data illustrates the amount of nitrogen in the form of $N\text{-}NH_4$ that can be released under rearing conditions. In our study, the content of $N\text{-}NH_4$ in the litter exhibited a significant dependence on the time of animal rearing, with a notable increase observed on D35 compared to D10 [Toppel *et al*. 2019]. On average, the proportion of $N\text{-}NH_4$ in the total N content of the litter was 2.15% on D10 and 6.58% on D35However, a positive effect of litter supplementation with mineral mixtures was observed in the experimental groups with DTBN and DTDL+DTBN. A strong positive correlation (r = 0.77; $p \leq 0.05$) was found between litter pH and $N\text{-}NH_4$ content (Tab. 6).

Lower litter pH values may improve litter quality due to reduced enzymatic activity and an unfavorable environment for the growth of bacteria that convert nitrogen compounds to ammonia (NH_3), which is one of the factors affecting bird welfare [Toppel *et al.* 2019]. On D10, the pH values of the litter under the animals were only slightly higher than those measured before their introduction (C). Blake and Hess [2001] demonstrated that bacterial uric acid decomposition intensifies when litter pH exceeds 7, with uricase activity being greatest at pH 9. In our study, pH values in the litter collected on D35, regardless of the experimental group, exhibited a significant ($p \leq 0.05$) increase in average from 5.25 on D10 to 6.39 on D35. However, the pH values did not reach a level indicating increased biochemical transformation intensity.

Table 6. Spearman rank correlation coefficient values between selected chemical and biochemical parameters in poultry litter

Parameter	H-NH4	DhA	BR	SIR
pH	0.77*	0.65*	0.77*	0.70*
EC	0.69*	0.60*	0.58*	0.67*

*Value significant at p<0.05

Even though the electrical conductivity (EC) can serve as an important indicator of the intensity of biochemical processes occurring in the litter, the existing literature lacks studies on the evaluation of the EC of litter during animal rearing. The available

Table 5. Concentration of different dust fractions in the air of the chicken house on D10 and D35 of chick rearing

Term	Group[1]	Grain size, grains/cm^3 (%)						
		0.3 µm	0.5 µm	1 µm	2.5 µm	5 µm	10 µm	Total
D10	C	118.7 (70)	25.4 (15)	11.3 (7)	8.7 (5)	2.6 (2)	0.9 (1)	168 (100)
	DTBN	121.2 (68)	27.7 (16)	13.0 (7)	10.1 (6)	3.0 (2)	1.2 (1)	176 (100)
P-value		0.0630	0.0610	0.0590	0.1040	0.0780	0.0540	0.0640
SEM		0.58	0.20	0.11	0.71	0.24	0.030	0.82
D35	C	119.0 (72)	24.6 (15)	10.5 (6)	8.0 (5)	2.3 (1)	0.8 (1)	165 (100)
	DTDL	117.2 (71)	24.0 (15)	10.3 (6)	8.0 (5)	2.4 (2)	0.9 (1)	163 (100)
	DTBN	118.3 (69)	26.3 (15)	12.2 (7)	9.6 (6)	3.0 (2)	1.1 (1)	171 (100)
	DTBN+DTDL	115.5 (67)	27.5 (16)	13.8 (8)	10.9 (6)	3.5 (2)	1.4 (1)	173 (100)
P-value		0.0670	0.0610	0.1020	0.0840	0.1260	0.0590	0.1840
SEM		1.00	0.61	0.66	0.52	0.15	0.09	1.27

[1]DTBN – addition of a diatomite-bentonite mixture to the litter before the birds were inserted (280 g/m^2); DTDL – addition of a diatomite-dolomite mixture to the litter on day 10 and day 26 of bird rearing (100 g/m^2 each).

researchreveals that this parameter exerts a profound influence on the populations of microorganisms responsible for the circulation of nutrients in nature [Dong *et al.* 2022]. It is also an important element determining the natural use of many organic materials, including poultry litter compost [Gondek *et al.* 2022]. The EC value in the litter results from the introduction of soluble salts or biochemical processes that release ions, such as calcium or magnesium, from the litter's organic structures, thus increasing conductivity[Jalalipour *et al*. 2020]. According to Alsar *et al*. [2020] DT contains various inorganic salts that can be released into solutions as ions. A study by Alsar *et al*. [2020] on the kinetics of ion release from DT into a solution indicates that the desorption process lasts 4-5 h and involves ions such as Cl^{-}, SO_2^{2-}, Na^{+}, Ca^{2+}, Mg^{2+}, and K^{+}. This process may be important for both EC and pH values of the litter. In the present study, the EC differed significantly between litter collection dates, regardless of the experimental group. However, the lowest EC values were found on D35 for the variant in which DTDL+DTBN was used. This phenomenon may be attributed to the mesoporous structure of the developed mixtures and their capacity to bind certain ions [Gondek *et al*. 2023].

The existing literature on the quality of litter in broiler chicken rearing typically concerns the chemical and microbiological properties of the substrate [Utama and Christiyanto 2021], or the suitability of the substrate for use as fertilizer, feed or solid fuel [Jeon *et al*. 2013]. In general, there are no studies on the dehydrogenase activity or respiratory activity of broiler litter. It is widely acknowledged that the composition of the litter can be modified by the introduction of various organic or mineral additives, which in turn affects its colonization by microbial communities. Under specific conditions of broiler rearing, temperature, humidity, and the availability of components used by microorganisms are important in modifying the activity of biochemical processes in the litter. The biochemical indices determined for the litter taken from under the animals provide evidence of the differing influence of the applied mineral additives on microbial communities. It is challenging to provide a clear indication of the course and the effect of the transformations taking place in the litter. The applied mixtures of diatomite with dolomite and diatomite with bentonite, due to their properties, mainly mesoporous structure, can increase microbial activity by affecting humidity and air availability. However, it is important to note that the study demonstrated a strong positive correlation (Tab. 6) between pH values and DhA ($r = 0.65$; $p \leq 0.05$), BR ($r = 0.77$; $p \leq 0.05$), and SIR ($r = 0.70$; $p \leq 0.05$) and EC with DhA ($r = 0.60$; $p \leq 0.05$), BR ($r = 0.58$; $p \leq 0.05$), and SIR ($r = 0.67$; $p \leq 0.05$). Mineral litter additives, such as mixtures of diatomite with dolomite or bentonite, can, for example, bind or neutralize contaminants. However, their beneficial effect on the microbial activity of the litter in terms of nutrient supply may be limited. The additives investigated may also play an important role in zoohygienic aspects [Gondek *et al*. 2022].

Poultry facilities are a source of numerous gaseous emissions, which are contingent upon a multitude of variables, including the density of animals in the building and air movement caused by the movement of birds and the ventilation employed. Furthermore, the decomposition of organic matter in the litter and feces of poultry facilities results in the production of harmful gaseous admixtures, including ammonia, carbon dioxide, and volatile organic compounds [Yasmeen *et al.* 2019]. The accumulation of these air pollutants in chicken houses has been shown to negatively impact the health and welfare of animals, as well as their performance and productivity. The impact of pollutants on broilers is contingent upon the concentration and duration of the agent in question [Costantino *et al*. 2020, Tahamtani *et al.* 2020]. Modification of the chemical composition of the litter can indirectly affect those parameters in the chicken house [Carey *et al.* 2002, Lacey *et al.* 2004, Anderson *et al*. 2020, Brink *et al.* 2022; Bist *et al*. 2023]. The results of our experiment indicated that there were no differences in the concentration of noxious gas admixtures between the experimental groups. The concentration of CO_2 was found to be within the normal range, well below the standard value of 0.3% (3,000 ppm). The recorded changes in the concentration of this gas were related not only to its content in the exhaled air, but primarily to the intensity of the operation of the ventilation equipment, as indicated by readings from the measuring devices with which the chicken house was equipped. Similarly, the concentrations of NH_3 and CH_4 differ slightly between the experimental groups and remain well below the normal range. In contrast, the concentration of H_2S remained below the sensitivity of the measuring devices.

Improved litter conditions can reduce the incidence of diseases such as footpad dermatitis and other infections that are often associated with poor litter quality. Implementing these additives aligns with modern welfare standards, ensuring that the chickens are raised in more humane conditions, which is increasingly demanded by consumers and regulatory bodies.

Further research is recommended to elucidate the mechanisms by which the additives used affect biochemical processes in the litter and air quality in poultry facilities. Additionally, consideration of different rearing conditions or different bird species would assist in the evaluation of the potential benefits and risks of using these mixtures in rearing practice.

Conclusions

1. The addition of designed mineral mixtures (diatomite with dolomite, diatomite with bentonite) into the litter, particularly when used in conjunction with one another, elevated the DM content and reduced the $N\text{-}NH_4$ content, particularly during the final phase of animal rearing.
2. The designed mineral mixtures had no effect on the pH values of the litter under the animals. In contrast, the combined use of DTDL and DTBN mixtures reduced the EC value, particularly on day 35 of rearing
3. There was small variation in dehydrogenase activity in the litter from under the animals when supplemented with DTDL or DTBN separately. However, a the highest value of DhA was observed after the joint application of DTDL+DTBN.
4. The determined BR and SIR values demonstrated no clear directionality.

However, a strong positive correlation was observed between pH and DhA, BR, and SIR, as well as between EC and DhA, BR, and SIR.

5. In the experiment conducted, measurements of the concentration of noxious gaseous admixtures at the evaluated stages of rearing demonstrated only small differences between the experimental groups. Furthermore, the dustiness of the air in the coops of all groups can be considered average pollution.

Disclosures

The authors listed on this paper declare no conflicts of interest.

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