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ORIGINAL PAPER

Effect of zeolite and halloysite addition on the microstructure of breast muscle and small intestine in broiler chickens

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ABSTRACT. The aim of this study was to assess the impact of aluminosilicates in feed and litter on the microstructure of broiler breast muscle and small intestine. The research was carried out on 3 farms with 500 broilers divided into five groups per farm. Groups II–V received halloysite and zeolite additives in feed and litter, while Group I served as control. In the duodenum, aluminosilicate supplementation improved villus height on Farms 1 and 3, increased surface area on Farm 3, and enhanced crypt depth and muscularis thickness on Farm 2 (Groups II and III). Jejunal morphology showed farm-specific variations: Farm 1 animals developed significant differences in villus width (Group I vs. III, IV, V), absorptive surface (Groups I, II vs. IV, V), and muscularis thickness (Group I vs. III, V). Farm 2 birds demonstrated improved crypt depth and muscularis thickness in Group IV, while Farm 3 broilers showed proportion-dependent effects on villus height, width and surface area, and crypt depth. Ileal improvements were limited to Farm 2 broilers, showing increased villus width and absorptive surface. Muscle microstructure alterations were observed exclusively on Farm 3, with changes in capillary density per muscle fiber and intramuscular fat distribution. Overall, the addition of aluminosilicates had the most pronounced effect on broilers from Farm 3, influencing both intestinal morphology and muscle microstructure.

Introduction

Poultry production plays a fundamental role in global food security, providing a major source of affordable, high-quality animal protein. During the first ten months of 2024, domestic hatcheries incubated approximately 1583.2 mln hatching eggs for broiler chick production. This intensive production scale increases the risk of parasitic and microbial disease outbreaks, often requiring the use of antibiotics, which carries the associated risk of antibiotic resistance. To promote sustainable poultry production, natural solutions that support animal health and welfare are necessary. In this context, aluminosilicates – a class of naturally occurring minerals – have emerged as a promising alternative. These substances can absorb ammonia and bind mycotoxins, thereby potentially conferring beneficial effects on animal health (Banaszak et al., 2021a). In addition, aluminosilicates may improve the bioavailability of essential minerals such as calcium and phosphorus, as well as mitigate the adverse effects of aflatoxins in poultry diets (Prvulovic et al., 2008). Literature generally indicates that supplementing broiler diets with 0.2–1% aluminosilicates exerts a positive effect on production results (Gilani et al., 2016). Research conducted by Banaszak et al. (2021b) showed that the application of aluminosilicates both in poultry feed and litter beneficially affected growth performance, certain carcass traits,

and meat quality. However, before implementing aluminosilicates as feed or bedding additives, key factors must be considered, including their chemical composition, origin, dosage, and potential interactions with other dietary components (Gilani et al., 2016).

Zeolite is extensively utilised in veterinary medicine for both disease prevention and treatment (Wawrzyniak et al., 2017). Its unique crystalline structure includes a system of channels that confer specific properties, such as ion exchange capacity, water adsorption (Pavlak et al., 2022), and detoxification through binding of toxic substances (Wawrzyniak et al., 2017). These properties contribute to improved animal growth by maintaining ionic balance in the digestive system, improving nutrient absorption, and reducing oxidative stress (Wawrzyniak et al., 2017). Studies have shown that the inclusion of zeolite in broiler chicken diets positively influences broiler performance and intestinal morphology. Consistent improvements have been observed in villus height, width, and surface area in the small intestine (Wawrzyniak et al., 2017), with similar findings also reported by Khambualai et al. (2009), Wu et al. (2013) and An et al. (2023). However, the effect of zeolite on the microstructure of broiler muscle has not yet been described in the literature, validating the significance of the present research.

Halloysite, a mineral of volcanic origin, is characterized by lack of swelling in water, which distinguishes it from other clay minerals. One of the largest known halloysite deposits in the world is located in Poland, with estimated reserves exceeding 10 million tonnes, providing significant potential for animal nutrition and husbandry applications (Nadziekiewicz et al., 2022). Although research on the use of halloysite in animal production remains limited, available studies suggest its potential beneficial effects. According to Banaszak et al. (2020), halloysite improved jejunal morphology, increasing villus height and absorptive surface area, In a subsequent study (Banaszak et al., 2021b), the same group documented positive effects of halloysite on growth performance and meat quality when supplemented in broiler feed and litter. Similar to zeolite, no studies have investigated the impact of halloysite on the muscle microstructure of broiler chickens.

Therefore, the aim of the present study was to assess the impact of the aluminosilicates, zeolite and halloysite, administered through feed and litter, on the microstructure of the superficial pectoral muscle and small intestine in broiler chickens.

Material and methods

In accordance with Directive 2010/63/EU and Decision of the Local Ethics Committee No. 13/2016 dated June 17, 2016, the experiment did not require the approval of the Local Ethics Committee.

The study was conducted on three farms. On each farm, 500 1-day-old Ross 308 broilers, randomly assigned to 5 groups (10 replicates/group), were utilised. Birds in the control group (Group I) were fed a complete mixture, without any feed or litter additives. Groups II–V were supplemented with varying proportions of halloysite and zeolite in feed (Table 1) and litter (Table 2), depending on the farm. Birds were reared according to Ross 308 standards with *ad libitum* access to pelleted feed and water. Aluminosilicate bedding supplementation was performed five times during rearing. The chemical composition of zeolite, halloysite, and phase-fed broiler diets was previously detailed by Banaszak et al. (2021b). The diets provided to the broilers were isocaloric and isoprotein, with nutrient content and energy levels (12.50–13.50 MJ/kg)

Table 1. Inclusion rates of aluminosilicates in feed across experimental farms and growth phases

Farm	Zeolite to halloysite ratio in feed	Percentage of mixture in feed in groups II–V, %			
		Starter	Grower 1	Grower 2/Finisher	Finisher
1	50:50	0.5 (1–10 days)	0.5 (11–22)	1 (23–35)	1.5 (36–42)
2	0:100	0.5 (1–13 days)	0.5 (14–21)	1 (22–42)	–
3	100:0	0.5 (1–13 days)	0.5 (14–21)	1 (22–42)	–

Table 2. Amount of aluminosilicates added to litter on individual farms depending on the feeding phase

Farm	Litter addition per 1 m^2				
	I	II	III	IV	V
1	–	650 g halloysite	325 g halloysite and 325 g zeolite	650 g zeolite	160 g halloysite and 490 g zeolite
2	–	650 g halloysite	325 g halloysite and 325 g zeolite	650 g zeolite	160 g halloysite and 490 g zeolite
3	–	650 g halloysite	325 g halloysite and 325 g zeolite	650 g zeolite	160 g halloysite and 490 g zeolite

formulated in accordance with the nutritional standards for broiler chickens (Smulikowska and Rutkowski, 2018). The birds were maintained under controlled thermal conditions: 30 °C from day 1, 27 °C from day 7, and 21 °C from day 21 until the end of the rearing period. Relative air humidity was maintained between 60–65%, and ventilation was set at 1 m^3/kg body weight/hour (Biesek et al., 2022). No specific lighting program was implemented, and the birds were exposed to continuous artificial lighting (24 hours per day). On day 42 of rearing, 50 broilers were randomly selected for slaughter. The procedure was carried out in accordance with Council Regulation (EC) (No. 1099/2009 of 24 September 2009) by qualified personnel through atlanto-occipital decapitation for rapid exsanguination, preceded by electrical stunning.

To evaluate the pectoral muscle microstructure, 10 samples (0.5 × 1.5 cm) from each group were collected and flash-frozen in liquid nitrogen (−196 °C). The frozen samples were sectioned into 10-µm slices using a cryostat (Thermo Shandon, London, UK) and mounted on glass slides for staining. Three staining protocols were applied: oil red O staining to quantify intramuscular lipids (red staining; Dubovitz et al., 1973), haematoxylin and eosin (H&E) for fibre diameter and count analysis, and alkaline phosphatase staining to assess capillary density per muscle fibre (Bogucka et al., 2022).

The microstructure of the duodenum, jejunum and ileum was evaluated histologically. Tissue samples (approx. 2 cm) were collected from the middle portion of each section of the small intestine (10 samples per group). The samples were fixed in Bouin's solution, followed by dehydration, clearing, and paraffin embedding using a Microm STP 120 tissue processor (Thermo Shandon). Subsequently, the samples were embedded into paraffin blocks using a TES 99 embedding station (Medite, Burgdorf, Germany) and sectioned into 10-µm-thick slices using a Finesse ME + rotary microtome (Thermo Shandon). The tissue sections were stained using the Periodic Acid-Schiff (PAS) method for histological evaluation (Dubowitz et al., 1973).

Microscopic images of both pectoral muscle and small intestine samples were captured using a UB203i microscope with ToupCamTM digital camera, followed by quantitative analysis with Multiscan 18.03 software (Computer Scanning Systems II, Warsaw, Poland). For the superficial pectoral muscle evaluation, four parameters were measured: (1) muscle fibre diameter determined according to the shortest diameter method (Brooke, 1970); fibre density within a 1.5 mm^2 area; intramuscular fat content across 3 mm^2 and capillary density per 1.5 mm^2. Small intestinal morphology was assessed by measuring 10 randomly selected villi per specimen, recording villus height and width, crypt depth, and muscularis thickness. Villus surface area was calculated using the formula:

$$(2\pi) \times (VW / 2) \times (VH),$$

where; VW – villus width, VH – villus height (Sakamoto et al., 2000).

Statistical analyses were performed using the STATISTICA software package. Variance homogeneity was verified using Levene's test, confirming homogeneous variance across all groups. Normality of distribution for each group was assessed with the Shapiro-Wilk test, revealing near-normal distributions of dependent variables. Random sampling ensured proper experimental randomisation. Statistical comparisons were performed using one-way ANOVA followed by Tukey's post-hoc test for intergroup differences, with statistical significance set at $P < 0.05$.

Results

The microstructural characteristics of the pectoral muscle in broiler chickens from Farm 1 showed no significant effects of zeolite and halloysite supplementation. No statistically significant differences were observed between the control and experimental groups in any of the parameters measured (Table 3).

The results of histological analysis of the small intestine of broiler chickens from Farm 1 revealed

Table 3. Microstructure of the superficial pectoral muscle of broiler chickens (Farm 1)

Group	Muscle fibre diameter, µm	Number of muscle fibres/1.5 mm^2	Capillary number/muscle fibre	Fat percentage, %
I	52.79 ± 3.85	251.20 ± 36.33	1.2 ± 0.4	2.8 ± 1.6
II	53.09 ± 6.57	236.20 ± 38.78	1.1 ± 0.2	4.4 ± 3.0
III	51.55 ± 5.55	240.40 ± 50.83	1.0 ± 0.2	3.5 ± 1.0
IV	52.64 ± 6.27	230.00 ± 55.91	1.3 ± 0.3	2.7 ± 0.9
V	50.34 ± 6.27	258.20 ± 44.74	1.1 ± 0.3	2.8 ± 1.8

I – control group, II – 650 g halloysite, III – 325 g halloysite and 325 g zeolite, IV – 650 g zeolite, V – 160 g halloysite and 490 g zeolite; data are presented as mean values ± SEM; $P > 0.05$ (not statistically significant)

Table 4. Histomorphology of the small intestine in broiler chickens (Farm 1)

Traits	Duodenum	Jejunum	Ileum
Villus height, µm			
I	1367.28^{b} ± 96.95	1182.76 ± 180.28	848.31 ± 128.11
II	1641.32^{a} ± 163.83	1287.66 ± 147.97	676.73 ± 174.57
III	1653.18^{a} ± 153.98	1323.15 ± 179.81	798.04 ± 121.82
IV	1421.06^{b} ± 171.07	1150.38 ± 162.36	723.72 ± 138.48
V	1520.59^{ab} ± 130.95	1230.88 ± 132.42	718.43 ± 165.07
Villus width, µm			
I	210.17 ± 26.31	230.52^{a} ± 28.93	171.73 ± 21.27
II	216.51 ± 23.38	208.23^{ab} ± 21.16	157.28 ± 20.92
III	223.12 ± 30.12	190.27^{bc} ± 26.95	182.83 ± 35.38
IV	239.01 ± 58.36	172.23^{c} ± 22.69	158.77 ± 40.94
V	209.83 ± 9.44	177.10^{bc} ± 22.52	175.35 ± 23.71
Villus surface area, µm^2			
I	901575.57 ± 136923.80	852396.04^{a} ± 144551.22	456043.72 ± 58857.25
II	1124914.83 ± 211178.84	840218.34^{a} ± 95003.06	327082.42 ± 51212.77
III	1165637.30 ± 224843.86	781515.74^{ab} ± 118147.65	465725.56 ± 156445.46
IV	1077139.78 ± 329260.65	623814.35^{b} ± 134236.42	364202.51 ± 135965.95
V	1000305.39 ± 88390.76	679825.30^{b} ± 61968.28	393869.97 ± 95555.53
Crypt depth, µm			
I	132.67 ± 5.16	123.18 ± 24.16	107.10 ± 12.20
II	140.53 ± 9.87	132.91 ± 7.98	101.82 ± 13.98
III	142.21 ± 12.38	128.94 ± 11.61	99.59 ± 7.61
IV	138.30 ± 12.48	126.96 ± 6.73	106.20 ± 13.92
V	127.09 ± 14.98	132.58 ± 9.21	105.34 ± 18.88
Muscle thickness, µm			
I	153.22 ± 21.30	130.66^{b} ± 20.32	172.76 ± 31.69
II	181.91 ± 32.02	151.57^{ab} ± 24.83	158.93 ± 58.26
III	172.55 ± 18.65	178.58^{a} ± 41.22	139.10 ± 23.25
IV	158.54 ± 21.50	154.26^{ab} ± 38.57	131.45 ± 13.71
V	170.15 ± 14.15	184.68^{a} ± 29.52	148.66 ± 32.55

I – control group, II – 650 g halloysite, III – 325 g halloysite and 325 g zeolite, IV – 650 g zeolite, V – 160 g halloysite and 490 g zeolite; data are presented as mean values ± SEM; ^{ab} – means within a column with different superscripts are significantly different at $P < 0.05$

that aluminosilicate supplementation positively influenced villus height in the duodenum, with Groups II and III showing significantly greater villus height compared to the control (Group I) and Group IV ($P < 0.05$) (Table 4). Significant differences ($P < 0.05$) between the studied groups of broiler chickens were observed mainly in the jejunum, particularly in villus width, absorptive surface area and muscularis mucosa thickness. In terms of villus width, statistically significant differences were recorded between Groups I and Groups III, IV and V, as well as between Groups II and IV ($P < 0.05$). The villus surface area was significantly greater in Groups IV and V compared to Groups I and II. Additionally, significant differences in muscularis mucosa thickness were observed between Group I and Groups III and V ($P < 0.05$). In contrast, no significant differences were found between groups for any of the analyzed histological parameters in the ileum.

The evaluation of pectoral muscle microstructure in birds from Farm 2 also showed no statistically significant changes between groups in fibre diameter, fibre number, capillary count per muscle fibre, or intramuscular fat content (Table 5).

Table 5. Microstructure of the superficial pectoral muscle in broiler chickens (Farm 2)

Group	Muscle fibre diameter, µm	Number of muscle fibres/1.5 mm^2	Capillary number/muscle fibre	Fat percentage, %
I	47.69 ± 8.90	350.80 ± 162.70	1.0 ± 0.3	2.0 ± 1.4
II	52.37 ± 5.16	256.40 ± 41.20	1.1 ± 0.3	1.9 ± 0.8
III	51.51 ± 8.32	280.20 ± 106.30	1.1 ± 0.4	3.1 ± 2.1
IV	52.09 ± 7.28	280.80 ± 78.27	1.1 ± 0.2	1.5 ± 1.7
V	48.17 ± 3.59	314.80 ± 22.67	1.0 ± 0.2	1.9 ± 1.3

I – control group, II – 650 g halloysite, III – 325 g halloysite and 325 g zeolite, IV – 650 g zeolite, V – 160 g halloysite and 490 g zeolite; data are presented as mean values ± SEM; $P > 0.05$ (not statistically significant)

Table 6. Histomorphology of the small intestine in broiler chickens (Farm 2)

Traits	Duodenum	Jejunum	Ileum
Villus height, µm			
I	1481.24 ± 212.97	922.05 ± 181.34	731.83 ± 77.77
II	1502.37 ± 174.60	1084.51 ± 202.14	757.67 ± 135.77
III	1679.44 ± 135.85	1141.41 ± 133.99	841.99 ± 124.81
IV	1645.44 ± 127.37	1119.75 ± 85.51	869.44 ± 47.67
V	1612.96 ± 169.57	1044.79 ± 150.07	737.34 ± 109.47
Villus width, µm			
I	210.52 ± 18.14	159.22 ± 15.55	133.21^{b} ± 21.12
II	218.08 ± 42.42	178.81 ± 40.03	160.75^{a} ± 15.73
III	213.06 ± 27.83	162.31 ± 18.60	160.84^{a} ± 25.31
IV	222.95 ± 57.86	147.18 ± 23.16	162.55^{a} ± 18.79
V	192.05 ± 9.13	161.45 ± 23.19	165.57^{a} ± 11.14
Villus surface area, µm^2			
I	980857.17 ± 168074.82	458268.08 ± 71784.88	302727.32^{b} ± 29322.09
II	1032876.60 ± 251667.11	612219.19 ± 211746.37	388034.48^{ab} ± 97265.95
III	1127602.57 ± 212627.70	578398.20 ± 38028.93	429110.99^{a} ± 113806.25
IV	1147665.69 ± 286070.29	522193.22 ± 109552.16	445013.84^{a} ± 69183.38
V	972010.77 ± 127569.31	529152.00 ± 102236.68	384923.74^{ab} ± 74808.37
Crypt depth, µm			
I	127.76^{ab} ± 8.59	106.19^{b} ± 9.60	100.37 ± 5.50
II	140.57^{a} ± 18.33	106.62^{b} ± 4.04	100.29 ± 11.73
III	132.91^{ab} ± 8.47	113.15^{ab} ± 5.23	97.99 ± 3.88
IV	119.80^{b} ± 5.49	117.98^{a} ± 11.90	106.50 ± 13.88
V	124.73^{b} ± 7.58	107.33^{ab} ± 7.07	96.72 ± 4.86
Muscle thickness, µm			
I	158.79^{b} ± 26.82	153.05^{ab} ± 33.65	179.40 ± 41.78
II	196.67^{ab} ± 32.48	125.63^{b} ± 42.19	148.86 ± 25.23
III	200.25^{a} ± 35.79	156.26^{ab} ± 20.28	167.61 ± 21.70
IV	169.81^{ab} ± 12.24	180.88^{a} ± 42.66	168.16 ± 54.14
V	170.41^{ab} ± 28.49	174.07^{ab} ± 25.63	176.70 ± 55.27

I – control group, II – 650 g halloysite, III – 325 g halloysite and 325 g zeolite, IV – 650 g zeolite, V – 160 g halloysite and 490 g zeolite; data are presented as mean values ± SEM; ^{ab} – means within a column with different superscripts are significantly different at $P < 0.05$

The microstructural features of the small intestine in broiler chickens from Farm 2 are summarised in Table 6. The results showed no significant effect of the tested substances on villus height, width and surface area in either the duodenum or jejunum. However, in the ileum, a beneficial effect of aluminosilicates on villus width was observed. All experimental groups (II, III, IV and V) had significantly greater villus width compared to Group I ($P < 0.05$). This increase in width also influenced villus surface area, with the highest values recorded in Groups III and IV compared to Group I. Significant differences in crypt depth in the duodenum were found between Group II versus Groups IV and V ($P < 0.05$), and between Group IV versus Groups I and II in the jejunum ($P < 0.05$). The muscularis mucosa thickness differed significantly between Group I

Table 7. Microstructure of the superficial pectoral muscle in broiler chickens (Farm 3)

Group	Diameter of muscle fibres, µm	Number of muscle fibres/1.5 mm^2	Capillary number/muscle fibre	Fat percentage, %
I	49.46 ± 6.64	231.40 ± 47.27	0.8^{b} ± 0.1	3.0^{b} ± 1.0
II	46.20 ± 2.55	270.40 ± 36.32	1.3^{a} ± 0.3	2.9^{b} ± 0.4
III	46.35 ± 6.19	259.20 ± 40.31	0.9^{b} ± 0.2	3.5^{b} ± 1.9
IV	48.76 ± 5.75	239.40 ± 51.37	1.3^{a} ± 0.1	3.3^{b} ± 1.3
V	51.15 ± 4.77	237.40 ± 37.79	0.9^{b} ± 0.1	5.5^{a} ± 1.8

I – control group, II – 650 g halloysite, III – 325 g halloysite and 325 g zeolite, IV – 650 g zeolite, V – 160 g halloysite and 490 g zeolite; data are presented as mean values ± SEM; ^{ab} – means within a column with different superscripts are significantly different at $P < 0.05$

Table 8. Histomorphology of the small intestine in broiler chickens (Farm 3)

Traits	Duodenum	Jejunum	Ileum
Villus height, µm			
I	1554.61^{ab} ± 163.43	1275.63^{ab} ± 136.19	853.94 ± 114.11
II	1341.21^{c} ± 100.77	1393.84^{a} ± 103.60	814.31 ± 136.35
III	1640.07^{a} ± 83.01	1288.68^{ab} ± 160.05	832.93 ± 106.34
IV	1441.44^{bc} ± 154.20	1321.09^{ab} ± 273.50	823.04 ± 174.48
V	1436.89^{bc} ± 152.56	1082.37^{b} ± 113.43	974.13 ± 176.74
Villus width, µm			
I	190.45^{ab} ± 25.78	148.78^{c} ± 15.57	168.07 ± 26.40
II	181.57^{b} ± 11.60	165.83^{bc} ± 19.02	181.67 ± 59.82
III	216.37^{a} ± 14.41	195.08^{a} ± 23.10	175.28 ± 24.01
IV	187.42^{ab} ± 22.58	170.28^{ab} ± 22.70	167.78 ± 16.85
V	200.26^{ab} ± 30.11	185.17^{ab} ± 20.61	166.50 ± 23.08
Villus surface area, µm^2			
I	922401.49^{b} ± 135916.49	592761.51^{b} ± 24089.92	453834.33 ± 111587.97
II	766755.77^{b} ± 90279.53	723163.32^{ab} ± 80233.13	446936.18 ± 84375.83
III	1114516.15^{a} ± 89482.78	785279.78^{a} ± 109895.37	461845.35 ± 112539.11
IV	843863.91^{b} ± 114377.17	698921.33^{ab} ± 148144.12	429695.78 ± 78899.46
V	913201.89^{b} ± 206920.35	631163.96^{b} ± 105928.77	501179.93 ± 63870.02
Crypt depth, µm			
I	144.81^{ab} ± 29.29	117.66^{b} ± 10.38	110.02 ± 16.88
II	129.89^{b} ± 11.15	120.64^{ab} ± 7.24	102.15 ± 7.69
III	136.78^{ab} ± 15.94	117.53^{b} ± 12.16	99.73 ± 7.89
IV	151.22^{ab} ± 5.27	128.18^{ab} ± 4.85	95.11 ± 13.32
V	158.36^{a} ± 18.67	132.66^{a} ± 10.43	99.53 ± 8.44
Muscle thickness, µm			
I	180.06^{ab} ± 32.94	185.53 ± 36.51	174.32 ± 27.73
II	147.43^{b} ± 30.79	191.00 ± 53.88	168.90 ± 56.38
III	150.35^{b} ± 13.11	174.46 ± 37.93	188.17 ± 41.40
IV	197.78^{a} ± 33.06	197.26 ± 34.34	160.38 ± 36.44
V	171.69^{ab} ± 24.63	216.93 ± 53.89	175.06 ± 37.10

I – control group, II – 650 g halloysite, III – 325 g halloysite and 325 g zeolite, IV – 650 g zeolite, V – 160 g halloysite and 490 g zeolite; data are presented as mean values ± SEM; ^{ab} – means within a column with different superscripts are significantly different at $P < 0.05$

and Group III in the duodenum ($P < 0.05$), and between Group II and Group IV in the jejunum ($P < 0.05$).

The microstructure characteristics of the pectoral muscle in broiler chickens from Farm 3 are summarised in Table 7. No statistically significant differences were observed between groups in terms of muscle fibre diameter and fibre number. However, significantly greater microvasculature was found in Groups II and IV compared to other groups ($P < 0.05$). The highest intramuscular fat content was recorded in Group V (160 g halloysite and 490 g zeolite in litter, and 100% zeolite in feed), which differed significantly from all other groups ($P < 0.05$).

In the duodenum, statistically significant differences in villus height were observed between Group III and Groups II, IV, and V ($P < 0.05$; Table 8), while villus width differed only between Groups II and III ($P < 0.05$). Group III had the largest villus surface area compared to all other groups ($P < 0.05$). Crypt depth in the duodenum differed significantly between Groups II and V, and muscularis thickness varied between Group IV versus Groups II and III ($P < 0.05$). In the jejunum, aluminosilicate supplementation significantly affected villus height, villus width, villus surface area, and crypt depth. Specifically, villus height differed between Groups II and V, while villus width varied between Group III versus Groups I and II ($P < 0.05$). The villus surface area was significantly different between Group III versus Groups I and V, and crypt depth differed between Group V versus Groups I and III. No significant differences were found for any analysed parameters in the ileum of Farm 3 broilers (Table 8).

Discussion

The intensive production methods used in modern poultry farming, which aim to maximize growth rates, significantly impact muscle fibre characteristics and ultimately determine final meat yield. Muscle fibre size is also influenced by various factors, such as nutrition, age, sex, muscle type and genetic background or breed (Koomkrong et al., 2015). In the present study, no significant effects of zeolite and halloysite supplementation in feed or litter were observed on the diameter or number of muscle fibres per 1.5 mm² on any of the farms. These findings are consistent with those reported by Nadziakiewicz (2023), who also found no changes in muscle fibre diameter in Ross 308 broilers following the inclusion of 1% halloysite in the feed starting from day 11 of rearing.

Adequate vascularisation is a critical determinant of proper muscle development, as capillary density directly correlates with muscle functional activity and oxygen requirements, with more metabolically active muscles developing greater vascular networks (Bogucka et al., 2022). The current study showed a significantly higher number of capillaries per muscle fibre on Farm 3 (100% zeolite in feed) in Group II (650 g of halloysite in litter) and Group IV (650 g of zeolite in litter) ($P < 0.05$). These findings support previous work demonstrating that strategic dietary supplementation can promote muscle angiogenesis, potentially mitigating risks of ischemic damage during rapid growth periods (Bogucka et al., 2022).

Intramuscular fat content (IMF) is one of the key factors influencing meat quality. Modern intensive poultry production prioritises maximising muscle yield, which is often accompanied by a reduction in IMF. This, in turn, can negatively affect the technological and sensory characteristics of meat, particularly its tenderness, juiciness and palatability (Reszka et al., 2020). The current investigation revealed that aluminosilicate supplementation significantly affected IMF exclusively on Farm 3 (100% zeolite in feed), where Group V (160 g halloysite and 490 g zeolite in litter) had markedly higher IMF levels compared to other treatments ($P < 0.05$). These findings are consistent with results reported by Banaszak et al. (2021b), who also demonstrated a significant effect of zeolite and halloysite on IMF content.

Poultry intestines represent approximately 3.5% of total body weight that undergo rapid post-hatch development, with the duodenum showing particularly dynamic surface area expansion during growth. Research demonstrates that both zeolite and halloysite exert a stabilising effect on intestinal physiology, promoting epithelial regeneration and improving overall gut function. These aluminosilicates accelerate intestinal digesta passage, leading to reduced feed intake and improved growth performance in broiler chickens (Wu et al. 2013; Banaszak et al. 2020; Banaszak et al., 2022). After hatching, development follows a precise temporal pattern, with epithelial maturation occurring in distinct phases. During the first week post-hatching, intestinal villi develop primarily at their base and mid-region. After approximately seven days, this growth becomes restricted to the basal region only, marking a critical transition in gut development (Bogucka et al., 2016; Bogucka et al., 2017).

In the present study, the addition of zeolite and halloysite had a significant effect ($P < 0.05$) on individual parameters of all small intestine sections of broiler chickens. Characteristics such as villus height and villus height-to-crypt depth ratio are closely related to the efficiency of nutrient absorption and the rate of intestinal epithelium renewal (Wu et al., 2004; Wang et al., 2008).

In chickens from Farm 2, halloysite supplementation in both litter and feed significantly influenced crypt depth and muscularis thickness in the duodenum. In contrast, findings by Banaszak et al. (2020) demonstrated that 1% halloysite added to feed increased villus height in the jejunum. Moreover, according to Wu et al. (2013), halloysite may stimulate epithelial regeneration and promote villus growth, thereby supporting intestinal health and overall gut function.

The inclusion of halloysite in feed alone, or a combination of 325 g halloysite and 325 g zeolite in litter significantly increased duodenal muscularis thickness in Farm 2 ($P < 0.05$). On the other hand, the combined application of zeolite and halloysite in feed and litter on Farm 1 reduced jejunal villus surface area while increasing muscularis thickness ($P < 0.05$). The muscular layer is responsible for controlling intestinal motility and intestinal transit, and thus these modifications likely influence absorption processes (Verdal et al., 2010).

While aluminosilicates showed no effect on villus dimensions in the duodenum and jejunum of Farm 2, distinct proportion-dependent effects were observed in the jejunum of birds from Farm 3, affecting villus height, width, surface area and crypt depth. Similar outcomes were reported in previous studies by Banaszak et al. (2020) and Incharoen

et al. (2009), who also showed the dose-dependent morphological response of the intestinal mucosa to clay-based additives.

Other study has reported that a 2% zeolite supplementation increased duodenal villus height, absorptive surface area, and crypt depth (Warzywniak et al., 2017). Here, the addition of zeolite alone to the litter contributed to crypt deepening in the jejunum of birds from Farm 2 ($P < 0.05$). An increase in crypt depth is generally considered an indicator of intestinal health, as it is associated with increased cell proliferation and epithelial turnover (Verdal et al., 2010; Sobolewska et al., 2017). Within the crypts, intestinal stem cells undergo continuous division to replenish and maintain the integrity of the intestinal epithelium (Samanya and Yamauchi, 2002).

The inclusion of aluminosilicates to poultry litter contributes to the creation of a more favourable microclimate in poultry houses. Broilers are naturally inquisitive animals that exhibit scratching and pecking behaviours, often ingesting small amounts of litter material as part of their normal activity. Consequently, aluminosilicates may exert both an indirect environmental effect and a direct physiological effect when consumed. The direct effects may include modulation of the gut microbiota, improvement in intestinal morphology, and support of systemic detoxification and antioxidant balance. These mechanisms collectively help explain the observed histological differences in the gastrointestinal tract and muscles tissues of birds receiving aluminosilicate supplementation.

Conclusions

In summary, the effects of aluminosilicate supplementation on the histological features of the small intestine and muscles in broiler chickens varied depending on the farm. The minerals contributed to improvements in intestinal structure, including villus elongation and deepening of the crypts, thereby expanding the intestinal absorptive surface and enhancing epithelial regenerative capacity. Specifically, the Farm 3 results provide clear evidence that aluminosilicates significantly affect both intestinal development (villus morphology) and muscle characteristics (vascularisation and intramuscular fat deposition). These findings indicate that while the magnitude of effects may vary by production environment, strategic aluminosilicate application can effectively modulate key histological parameters responsible for nutrient absorption efficiency and muscle quality in broiler chickens.

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Conflict of interest

The Authors declare that there is no conflict of interest.

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