



EFFECTS OF DIETARY HALLOYSITE SUPPLEMENTATION ON BROILER CHICKEN'S BLOOD PARAMETERS, CARCASS AND MEAT QUALITY, AND BONE CHARACTERISTICS: A PRELIMINARY STUDY*

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Abstract

The aim of this study was to determine the effect of halloysite supplementation to the complete diets for broiler chickens on selected blood parameters, carcass and organ characteristics, as well as bone and meat traits. The trial was conducted under experimental conditions in a large-scale poultry farm on 18,000 broiler chickens. The one-day broiler Ross 308 chicks were randomly divided into two groups: control (C) and experimental (E) in an amount of 9,000 birds per group. In each group, chicks were separated into five pens (100 m²) with 1800 chicks per pen. The birds were fed granulated complete mixtures without (C group) or with halloysite (E group) distributed in an amount of 1% (based on fresh matter) from the 11 d of life. Analysis revealed that halloysite supplementation to the broiler diet at the level of 1% showed a trend ($P = 0.056$) towards higher final body weight, significantly improved carcass yield ($P = 0.048$), and reduced the relative weights of the liver and gizzard ($P < 0.05$). No differences ($P > 0.05$) were observed either in terms of the physicochemical parameters of the broiler chickens' breast muscles, except a significantly lower b^* value of the colour parameter ($P = 0.048$). In turn, in the blood serum, the addition of 1% halloysite significantly decreased ($P < 0.05$) the content of triglycerides and the total cholesterol and low-density lipoprotein fraction, but did not affect ($P > 0.05$) the high-density lipoprotein fraction, calcium, phosphorus or alkaline phosphate content. Tibia and femur characteristics (weight, length, relative bone weight, bone mineral density, bone mineral content, and breaking strength) were not affected ($P > 0.05$) by the usage of halloysite. In conclusion, supplementation of broiler diet with halloysite in the amount of 1% had beneficial effects on the carcass yield and health status of the animals, without any adverse effect on the bone parameters or meat quality of the broiler chickens.

Key words: broiler chickens, carcass characteristics, meat quality, serum parameters, bone

Halloysite is one of the aluminosilicates in the group of clay minerals (kaolinite group). It consists of halloysite nanotubes (HNTs) which form in multiple rolled layers composed by a sheet of corner-sharing SiO₄ tetrahedral molecules bonded to edge-sharing AlO₆ octahedral molecules (Barton and Karathanasis, 2002). Between the aluminosilicate layers, there are crystallographic water molecules and kaolinite OH groups. Halloysite occurs mainly in two polymorphs – the hydrated form with an interlayer spacing of 10 Å and the anhydrous form with an interlayer spacing of 7 Å. In systematic mineralogy, kaolin group minerals are classified as phyllosilicate clay. One of the largest halloysite deposits in the world is in Dunino (Krotoszyce administrative district, Legnica county, Lower Silesian Voivodeship, south-western Poland). It has reserves estimated at 10 million

tons of material. This halloysite is characterised by a layered-tubular and platy structure with the ratio of HNTs (7 Å) to kaolinite of 66:34 and the specific surface area of 65–85 m²/g. Kaolinite minerals, including halloysite, are natural clay minerals with many potentially important uses in different industrial fields. They are ubiquitous in soils and weathered rocks, where they occur in a variety of particle shapes and hydration states, which in turn influence kaolin end uses (Massaro et al., 2020).

Trckova et al. (2004) showed that the structure of kaolinite easily adsorbs water and forms a plastic, paste-like substance. Therefore, due to its adsorption qualities and absence of primary toxicity, clays and clay minerals are used as non-conventional feed additives in broiler chickens' diet, used as a simple and effective preventive measure against the effects exerted by a number of toxic

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substances in the living organism as well as in the environment (Owen and Nodu, 2012; Ouachem et al., 2015 a; Nadziakiewicz et al., 2019). Some studies show that the use of silicate minerals in broiler chickens' diet would mitigate intestinal infection; however, higher doses can lead to local inflammatory response, which may cause intestinal barrier damage (Jarosz et al., 2017). It is well established that the excess moisture of excreta is the main reason for numerous pathogen-transmitting micro-organisms present in the breeding environment of birds. One of the ways to control litter moisture is the application of water-adsorbing substances to the feed, which form a complex with water. Hence, adding the clays or the clay minerals to the feed, which are characterised by their capacity to adsorb water in amounts many times greater than their weight, can regulate the content of intestinal fluid and thus improve the consistency of chicken faeces (Lemos et al., 2015; Schneider et al. 2017; Yalçın et al., 2017). Subramaniam and Kim (2015) noted that clays – as a feed additive – have the ability to bind and immobilise toxic substances in the gastrointestinal tract of animals, reducing their toxicity and positively affecting the metabolism. Moreover, it has been shown that clay minerals, including silicate minerals, can slow the passage of food through the digestive tract and also improve the digestibility of the diet as well as broiler performance by stimulating the gastrointestinal tract due to an improved mucosal morphology and increased absorption in the small intestine (Tatar et al. 2012; Wawrzyniak et al., 2017; Banaszak et al., 2020). On the other hand, importantly, clay and clay minerals may bind not only noxious substances but also some nutrients such as vitamins and growth promoter additives or trace elements, causing a nutritional imbalance for animals by forming non-absorbable complexes (Slamova et al., 2011; Subramaniam and Kim, 2015).

The use of clays and clay minerals in poultry nutrition is not new. Many studies have demonstrated that the inclusion of silicate minerals, such as kaolin, zeolite, bentonite, sepiolite and perlite, to the diet of animals significantly improves body weight gain and feed conversion ratio (Prvulovic et al., 2008; Shi et al., 2009; Zhang et al., 2017; Banaszak et al., 2020; Chen et al., 2020). Moreover, the addition of silicate minerals to broiler diet has a beneficial effect on performance and carcass yield whilst, conversely, decreasing abdominal fat (Eser et al., 2012; Ouachem et al., 2015 a; Yalçın et al., 2017). They may also affect meat quality parameters, muscular oxidative status and muscular mineral composition (Cheng et al., 2016; Li et al., 2017; Banaszak et al., 2020). Some evidence indicates that silicate minerals in broiler diet are also associated with blood serum parameters and thus affect birds' health (Park et al., 2002; Eser et al., 2012).

It is well known that many decades of intensive selection in poultry production has improved muscle growth. However, a high rate of growth may cause some anatomohistological and metabolic disorders. Rath et al. (2000) demonstrated that poultry bone development fails

to keep pace with overall growth, thereby generating excess physical load and predisposing bones to deformity and fragility. Therefore, one of the critical factors impacting skeletal growth and bone strength is nutrition. Some studies indicate that the inclusion of silicate minerals to the broiler diet would increase calcium absorption and medullary bone development, but decrease phosphorus utilisation (Watkins and Southern, 1992; Yalçın et al., 1995). Moreover, the addition of silicate minerals to the diet of broiler chickens may have a beneficial effect on bone characteristics (Safaei Katouli et al., 2012; Ouachem et al., 2015 a). On the other hand, some studies did not observe a significant effect of dietary silicate minerals or other minerals supplementation on bone parameters (Safari et al., 2015; Yalçın et al., 2017; Durna Aydın et al., 2020). In this respect, it is worth noting that in the available literature there are no studies on the use of halloysite in poultry nutrition, especially from the Dunino deposit.

The aim of this study was to determine the effect of halloysite supplementation to the complete diets of broiler chickens on the selected blood parameters, carcass and organ characteristics, as well as bone and meat traits.

Material and methods

Ethical approval and veterinary treatments

The animals were treated according to the regulations laid out in Directive 2010/63/EU on the protection of animals used for scientific purposes, enforced in Poland by Legislative Decree 266/2015. According to the EU/PL laws, approval from an animal ethics committee was not required, and the birds were not subjected to any invasive procedures.

Throughout the experimental period the birds were regularly monitored by a veterinarian. The chicks were vaccinated by the coarse drop method on the 1 and 7 d of life against the Ib virus of the Coronavirus genus causing infectious bronchitis. A vaccine against the Gumboro virus was given with water on the 16 and 23 d of life. In accordance with the requirements of the National Programme for the Suppression of some *Salmonella* Serotypes in Broiler Flocks, samples were tested at the Veterinary Hygiene Department in Kraków (Poland) and no *Salmonella* was found.

Birds and experimental diets

Detailed information on the course of the experiment can be found in the work of Nadziakiewicz et al. (2021). In brief, the trial was conducted during wintertime (January/February) on an experimental poultry farm in Zalesiany (Małopolska Voivodeship, Poland, 49°52'14.1"N 20°12'40.4"E) in a broiler house with an area of 1,000 m². Prior to the experiment, the building and equipment were thoroughly cleaned and disinfected in accordance with the principles of veterinary biosecurity. Before the chicks were delivered, the hall was lined with bedding (wheat

straw) and heated to an air temperature of 34°C and a floor temperature of 28°C. The one-day broiler Ross 308 chicks (Aviagen EPI Ltd, Poland) were randomly divided into two groups: control (C) and experimental (E) in an amount of 9,000 birds per group. In each group there were five pens (100 m²), with 1800 chicks per pen. On this day, the chicks were weighed, the general state of their health and condition was checked and they were fed *ad libitum* with the same starter type diet without halloysite addition for 7 days. From the 8 d of life, the birds were fed complete diets (compound mixtures in a granulated form, distributed automatically to the feeders) prepared according to Cargill Feed & Nutrition standards (Minneapolis, USA) without (C group) or with 1% addition (10 kg per 1 t of the mixture on a fresh matter basis) of halloysite from the Dunino deposit (E group). Halloysite mineral in a powdered form, naturally free of asbestos, dioxins and heavy metals, was mixed with compound feeds (calculated into

the diet) prior to granulation. The heating and lighting programmes were in accordance with Ross 308 broiler stock management (Aviagen, 2020).

During the fattening period, nutritionally balanced complete diets (Table 1 and 2) were used in 3 types according to the age of the broilers: grower I (8–19), grower II (20–32) and finisher (33–43 d of life), all without (C group) or with halloysite addition (E group). Broilers in C group were fed complete feed mixtures adapted to the rearing period while in E group, the birds received the same feeds as those used in the C group, but with 1% addition of halloysite (a dilution effect). The feed and water were given *ad libitum* throughout the entire rearing period, continuously controlling the amount of the feed given and, periodically, the water consumption. The methods used for chemical analysis of feed samples have been described in Nadziakiewicz et al. (2021).

Table 1. Composition of experimental feeds with or without added halloysite

Feed ingredients (g/kg)	Starter d1 – d7	Grower I d8 – d19		Grower II d20 – d32		Finisher d33 – d43	
		C ¹	E ²	C	E	C	E
Maize	331.6	340.8	337.3	336.9	333.5	352.4	348.8
Wheat	104.8	98.4	97.4	91.5	90.6	96.1	95.1
Triticale	33.2	41.6	41.2	44.5	44.1	50.9	50.4
Wheat flour	70.0	37.8	37.4	30.0	29.7	25.2	25.0
Soybean meal	221.2	263.0	260.4	245.9	243.4	258.8	256.2
Canola meal	39.8	45.2	44.8	–	–	–	–
Sunflower meal (dehulled)	–	32.1	31.8	30.0	29.7	–	–
Canola seed (full fat)	–	–	–	44.9	44.5	45.7	45.2
DDGS (maize)	–	–	–	38.1	37.7	42.3	41.9
Spray-dried pig blood cells	38.0	36.0	35.6	30.0	29.7	21.0	20.8
Fish meal	65.0	–	–	–	–	–	–
Soybean oil	54.0	64.9	64.3	68.1	67.4	69.0	68.3
Limestone	13.5	14.0	13.9	14.0	13.9	14.0	13.9
Sodium chloride	1.9	1.8	1.8	1.8	1.8	1.8	1.8
Sodium bicarbonate	2.0	1.5	1.5	1.5	1.5	1.5	1.5
Monocalcium phosphate	15.0	14.0	13.9	14.0	13.9	13.0	12.9
Mineral and vitamins premix	4.0 ³	3.5 ⁴	3.5 ⁴	3.5 ⁴	3.5 ⁴	3.0 ⁵	3.0 ⁵
L-Lysine	2.1	1.9	1.9	1.8	1.8	2.0	2.0
DL-Methionine	2.6	2.3	2.3	2.3	2.3	2.1	2.1
L-Threonine	1.3	1.2	1.2	1.2	1.2	1.2	1.2
Halloysite	–	–	10.0	–	10.0	–	10.0

¹Control group fed complete mixtures without halloysite.

²Experimental group fed complete mixtures with halloysite addition. ³The premix provided per 1 kg of starter diet: vitamin A, 12,500 IU (retinol); vitamin D₃, 1.26 mg (cholecalciferol); vitamin E, 52.5 IU (all-rac- α -tocopherol acetate); vitamin K₃, 2.8 mg (menadione); vitamin B₁, 2.8 mg (thiamine); vitamin B₂, 8 mg (riboflavin); vitamin B₆, 4 mg (pyridoxine); vitamin B₁₂, 0.02 mg (cyanocobalamin); d-biotin, 0.19 mg; folic acid, 1.2 mg; niacin, 47 mg; d-pantothenic acid, 12.7 mg; choline chloride, 370 mg; ethoxyquin, 0.28 mg; Mn, 100 mg; Zn, 100 mg; Fe, 70 mg; Cu, 20 mg; I, 1.5 mg; Se, 0.30 mg; coccidiostat, 70 mg (salinomycin sodium); endo-1,4-beta-xylanase, 1500 EPU; 6-phytase, 265 OTU. ⁴The premix provided per 1 kg of grower diet: vitamin A, 11,000 IU (retinol); vitamin D₃, 1.10 mg (cholecalciferol); vitamin E, 42.5 IU (all-rac- α -tocopherol acetate); vitamin K₃, 2.5 mg (menadione); vitamin B₁, 2.5 mg (thiamine); vitamin B₂, 5 mg (riboflavin); vitamin B₆, 3.8 mg (pyridoxine); vitamin B₁₂, 0.02 mg (cyanocobalamin); d-biotin, 0.19 mg; folic acid, 1.1 mg; niacin, 40 mg; d-pantothenic acid, 12.5 mg; choline chloride, 330 mg; ethoxyquin, 0.28 mg; Mn, 90 mg; Zn, 90 mg; Fe, 60 mg; Cu, 20 mg; I, 1.5 mg; Se, 0.25 mg; coccidiostat, 70 mg (salinomycin sodium); endo-1,4-beta-xylanase, 1500 EPU; 6-phytase, 660 OTU. ⁵The premix provided per 1 kg of finisher diet: vitamin A, 10,000 IU (retinol); vitamin D₃, 0.80 mg (cholecalciferol); vitamin E, 42.0 IU (all-rac- α -tocopherol acetate); vitamin K₃, 2.0 mg (menadione); vitamin B₁, 2.0 mg (thiamine); vitamin B₂, 4 mg (riboflavin); vitamin B₆, 3.5 mg (pyridoxine); vitamin B₁₂, 0.02 mg (cyanocobalamin); d-biotin, 0.17 mg; folic acid, 1.0 mg; niacin, 35 mg; d-pantothenic acid, 12.0 mg; choline chloride, 300 mg; ethoxyquin, 0.28 mg; Mn, 90 mg; Zn, 80 mg; Fe, 45 mg; Cu, 15 mg; I, 1.5 mg; Se, 0.20 mg; endo-1,4-beta-xylanase, 1500 EPU; 6-phytase, 660 OTU.

Table 2. Chemical composition of experimental feeds (% of dry matter)

Item	Starter d1 – d7	Grower I d8 – d19		Grower II d20 – d32		Finisher d33 – d43	
		C ¹	E ²	C	E	C	E
Dry matter (%)	91.0	89.7	90.0	88.1	88.4	90.0	89.7
Crude ash	6.4	5.0	6.0	4.9	6.0	4.3	5.2
Crude protein	24.3	23.6	23.4	22.8	22.2	21.6	21.3
Crude fat	4.9	4.5	3.9	6.7	6.2	9.6	9.1
Crude fibre	2.9	3.9	3.4	3.7	3.4	3.2	2.7
NDF	8.5	11.3	12.0	10.7	11.3	14.3	15.5
ADF	4.5	6.3	5.6	5.3	5.1	6.8	6.1
ADL	1.2	1.6	1.4	1.5	0.7	2.1	1.4
Starch	45.3	47.4	45.9	50.0	48.7	51.3	49.2
NFC ³	55.9	55.5	54.7	55.0	54.4	50.9	48.4
Calculated composition							
ME (MJ/kg) ⁴	12.35	12.50	12.38	13.01	12.88	13.23	13.10
calcium	1.01	0.71	0.70	0.62	0.61	0.50	0.50
phosphorus	0.69	0.61	0.60	0.53	0.52	0.47	0.47
sodium	0.18	0.17	0.17	0.16	0.16	0.16	0.16
lysine	1.38	1.30	1.29	1.26	1.25	1.20	1.19
methionine	0.64	0.60	0.59	0.58	0.57	0.56	0.55

¹Control group fed complete mixtures without halloysite.²Experimental group fed complete mixtures with 1.0% of halloysite addition.³Non-fibre carbohydrates; NFC = 100 – (NDF + crude protein + crude fat + crude ash).⁴Metabolizable energy calculated according to the European Table (Janssen, 1989) as a sum of the ME content of components.

At the end of the experiment (d 43), 20 Ross 308 broiler chickens from C group and 20 broiler chickens from E group were selected randomly for slaughter and their blood parameters, carcass and organ characteristics, as well as bone and meat traits were checked. The selection method was as follows: in the first step, 50 chickens from each pen (5 pens per group) were randomly selected, and then 4 male individuals from each reduced pen were randomly chosen, making up a representative sample of 20 birds (4 × 5) for each group.

Carcass quality measurements

Birds were individually weighed (after a fasting period of 12 h) and transported (including careful catching and loading) to a commercial poultry slaughterhouse. After careful unloading and hanging in randomized order, all birds were electrically stunned and slaughtered by decapitation. After evisceration, the hot carcass weight was recorded, and carcass yield was calculated [(carcass weight/live body weight) × 100%]. At slaughter, the breast muscle, gizzard and liver were removed from all carcasses and its percentage based on hot carcass weight was calculated [(weight of element/weight of carcass) × 100%]. All breast muscles were vacuum packaged and chilled by placing them in a cold store at 4°C. After 24 hours of chilling, the breast muscles (*m. pectoralis major*) were sampled to determine physicochemical parameters, except for drip loss, where the sample was tak-

en 15 min after slaughter. All measurements were made using an analytical OHAUS PR 2202 balance (Newark, NJ, USA, e = 0.01).

Physicochemical measurements

The samples for meat quality assessment were taken from the right carcass side, from the breast muscle. The ultimate pH (pH₂₄) of the breast muscle was determined 24 h post-mortem using a Matthäus (R. Matthäus, Eckelsheim, Germany) pH meter with a glass electrode standardised for pH 4.0 and 7.0 according to Polish Standard (PN-77/A-82058) with automatic correction for muscle temperature. Prior to the evaluation, the electrode was calibrated using pH 4.0 and 7.0 calibration buffers at a temperature similar to the measurement's temperature. The meat colour was assessed 24 h post-mortem by the L* (lightness), a* (redness), and b* (yellowness) system (Commission International de l'Éclairage, CIE) (CIE, 1978) using a Minolta CR-310 Chroma Meter (Minolta Co., Ltd., Osaka, Japan), with a 50 mm diameter measuring head on the inner side of the breast muscle immediately after it had been detached from the bone. Colour parameters were analysed on the inner surface of the breast muscle. Drip loss was determined from meat weight loss after 24 h of cold storage at 4°C. To this end, samples weighing 50 g (e = 0.001 g) were collected from the breast muscle 15 min post-mortem, placed in air-tight containers and cold stored for 24 h. The samples

were then weighed again, and drip loss was determined as a percentage loss of initial weight. Meat samples for Warner-Bratzler shear force (WB) were taken after 24 h of cooling at 4°C. Breast muscle fragments weighing around 90 g (± 0.001 g) were cooked in individual plastic bags in a water bath at 100°C until a core temperature of 76°C was obtained in the thickest part of the sample. At the end of cooking, the samples were cooled at room temperature for 30 min and kept in cold storage at 4°C for 45 min. After cooling, the samples were prepared for Warner-Bratzler shear force tests. A core that was 1.27 cm in diameter and approximately 3 cm in length was removed from each sample parallel to the fibre orientation through the thickest portion of the cooked muscle. The shear force was determined by cutting it perpendicular to the direction of the muscle fibre using a TA-XT plus texture analyser (Stable Micro Systems, Godalming, UK) equipped with a Warner-Bratzler blade.

Microstructural analysis

For microstructural analysis, the breast muscle samples were taken immediately after slaughtering the birds (15 min) from the left side of each carcass. Muscle samples were cut into 1 cm³ pieces (parallel to the muscle fibres) and frozen in isopentane that was cooled using liquid nitrogen and stored at -80°C until subsequent analyses. Samples were mounted on a cryostat chuck with a few drops of tissue-freezing medium (Tissue-Tek; Sakura Finetek Europe, Zoeterwoude, The Netherlands). Serial transverse sections (10- μ m thick) were cut at -20°C in a cryostat (SLEE Medical GmbH, Mainz, Germany) and stained with haematoxylin and eosin. Finally, all sections were dehydrated in a graded series of ethyl alcohol, cleared in xylene and mounted in DPX mounting medium (Fluka, Buchs, Switzerland). A minimum of 300 muscle fibres per sample were counted to determine muscle fibre diameter.

The liver samples – taken immediately after slaughter – were fixed in 4% paraformaldehyde (PFA). The fixed samples were dehydrated in increasing concentrations of alcohol and embedded in Paraplast®. Paraffin blocks were cut using a Leica RM2145 (Leica Biosystems Nussloch GmbH, Germany) rotary microtome into 6- μ m-thick sections. All the sections were stained with haematoxylin and eosin, dehydrated through an increased series of ethanol and mounted under glass with DPX.

The sections were analysed under a NikonE600 light microscope (Nikon Solutions Co., Ltd., Tokyo, Japan), and the measurements were made using the MultiScan v.14.02 image analysis system (Warsaw, Poland).

Blood serum biochemical analysis

Blood samples were collected during slaughter from 20 birds per group which were used for carcass quality measurements. In the blood serum, the content of triglycerides, total cholesterol, high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL),

calcium, phosphorus, and alkaline phosphate (ALP) were determined with a spectrophotometer SP-830 plus (Mettler Inc., Taipei City, Taiwan) using the original Alpha Diagnostics kits (Warsaw, Poland). The content of HDL and LDL lipoprotein fractions in the blood serum was calculated on the basis of the Friedewald equation (Friedewald et al., 1972).

Bone analysis

Immediately after slaughter, the right femur and right tibia from each bird were manually defleshed of adhering tissues and frozen at -20°C. After thawing overnight at 7°C, the tibia was weighed and measured for length, after which the relative bone weight (bone weight to body weight ratio) was calculated. The measurements of whole-bone mineral density (BMD), and bone mineral content (BMC) were performed using the dual-energy X-ray absorptiometry (DXA) method on a Discovery W (Hologic, Marlborough, MA, USA). The breaking strength of the bones were determined with a TA XT plus texture analyser (Stable Micro Systems, Godalming, UK) fitted with a 3-point-bend rig with a load cell capacity of 500 N, a crosshead speed of 10 mm/min, and a span of 40 mm, over which the bone was set.

Statistical analysis

The normality of data distribution was tested using the Shapiro-Wilk test and the homogeneity of variance was tested by the Levene's test. For comparison of the means of C and E groups, the Student's t-test was applied. In the case of abnormal distribution and/or unequal variance of data, the Mann-Whitney U-test was applied to determine significant differences between the C and the E groups. The statistical model included the random effect of bird and the fixed effect of treatment (halloysite addition). Data were analysed in a randomised complete block design, in which the pen was considered a block. Statistical analysis was performed using Statistica 13 (Dell Software Inc., Aliso Viejo, CA, USA) and expressed as means and standard error of the mean (SEM). A statistically significant difference between means was assumed when the probability value was less than 0.05 ($P < 0.05$), and the tendency was considered to be $0.05 < P \leq 0.10$, unless otherwise stated.

Results

Carcass quality parameters and relative organ weight

The data on the growth performance parameters of broiler chicken are described in the paper of Nadziakiewicz et al. (2021). The effect of dietary supplementation of halloysite on body weight, carcass yield and relative breast muscle and organ weights in broiler chickens is shown in Table 3. It was shown that a diet with 1% halloysite supplementation had no significant effect on the body weight and breast muscle percentage ($P > 0.05$), but

significantly affected the carcass yield and the relative weights of the gizzard and liver. Accordingly, broiler chickens from E group were characterised by a significantly higher carcass yield ($P = 0.048$) and a lower relative weight of the gizzard ($P = 0.034$) and liver ($P = 0.023$) in comparison with C group.

Table 3. The effect of dietary supplementation of halloysite on body weight, carcass parameters and relative organ weights of broiler chickens

Item	C ¹		E ²		P-value
	mean	SEM	mean	SEM	
Body weight (g)	2690	21.30	2750	18.83	0.056
Carcass yield (%)	71.16	0.32	73.95	0.28	0.048
RW ³ Breast muscle (%)	24.94	0.53	25.71	0.42	0.162
RW Gizzard (%)	1.52	0.02	1.15	0.03	0.034
RW Liver (%)	3.21	0.06	2.58	0.07	0.023

¹Control group fed complete mixtures without halloysite.

²Experimental group fed complete mixtures with 1.0% of halloysite addition.

³Relative weight.

Physicochemical parameters

The effect of dietary supplementation of halloysite on the physicochemical parameters, including the pH₂₄, meat colour, drip loss and shear force of breast meat from broiler chickens Ross 308 is presented in Table 4.

Table 4. The effect of dietary supplementation of halloysite on physicochemical parameters and muscle fibre diameter of breast meat from broiler chickens

Item	C ¹		E ²		P-value
	mean	SEM	mean	SEM	
pH ₂₄	5.93	0.02	5.89	0.02	0.342
L*	52.95	0.53	52.69	0.70	0.173
a*	13.44	0.27	13.72	0.31	0.086
b*	5.84	0.15	4.12	0.19	0.048
Drip loss (%)	1.95	0.17	1.77	0.19	0.168
Shear force (N)	16.87	0.32	16.35	0.34	0.096
Muscle fibre diameter (μm)	57.53	1.69	57.51	1.42	0.487

¹Control group fed complete mixtures without halloysite.

²Experimental group fed complete mixtures with 1.0% of halloysite addition.

The physicochemical analysis showed that both the values of meat acidity (pH₂₄) and drip loss, as well as the shear force of the breast muscles were similar in both the C and the E group ($P > 0.05$). In the case of meat colour, significantly lower b* values ($P = 0.048$) were observed for the E group compared to the C group. On the other hand, there were no significant differences in L* and a* values following halloysite supplementation ($P > 0.05$).

Breast muscle and liver microstructure

No significant changes ($P > 0.05$) in the muscle fibre diameter of the breast muscle of the broiler chickens were observed following halloysite supplementation (Table 4). In the case of the liver, microscopic analysis showed no changes of the pathological character in the C and E groups (Figure 1 a–b). The livers of both examined groups showed the regular hepatocyte system forming hepatic lobules with pronounced central veins. Hepatocytes were arranged in linear cords between a capillary network. The lobular connective tissue was poorly marked without any features of proliferation.

Blood serum biochemical parameters

The addition of 1% halloysite to the broiler chickens fed a basal diet significantly reduced ($P < 0.05$) the levels of serum triglycerides, total cholesterol and LDL fraction (Table 5). No other significant changes in serum biochemical parameters (HDL fraction, calcium, phosphorus and alkaline phosphate) were observed ($P > 0.05$).

Bone analysis

The femur and tibia general, densitometric, and mechanical properties of broiler chickens from C and E group are given in Table 6. Both for the femur and the tibia, the diet with 1% halloysite supplementation has no significant effect on the bone weight and length, or on the relative bone weight values ($P > 0.05$). Similarly, in the case of the bone BMD, BMC, and the breaking strength of the femur and the tibia, no statistically significant differences were found between C and E groups ($P > 0.05$).

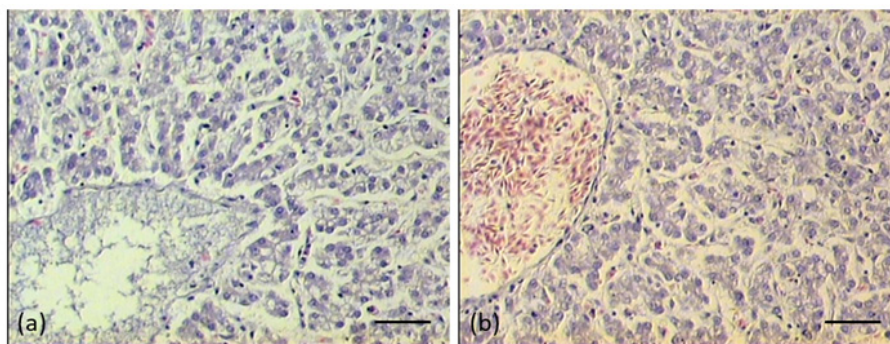


Figure 1. Representative photomicrographs of haematoxylin and eosin staining of the liver from the following: (a) C group fed complete mixtures without halloysite; (b) E group fed complete mixtures with 1.0% of halloysite addition. The histological examination showed no structural differences between the control and experimental group. Scale bar 50μm

Table 5. The effect of dietary supplementation of halloysite on blood serum biochemical parameters of broiler chickens

Item	C ¹		E ²		P-value
	mean	SEM	mean	SEM	
TGs (mg/dl)	92.84	1.02	75.68	0.93	0.022
TCHL (mg/dl)	156.61	1.02	143.12	0.63	0.045
HDL (mg/dl)	83.89	0.37	84.75	0.30	0.118
LDL (mg/dl)	51.79	0.63	46.31	0.33	0.031
Ca (mg/dl)	11.48	0.15	12.55	0.19	0.356
P (mg/dl)	6.23	0.06	7.03	0.14	0.768
ALP (U/L)	4.24	0.04	4.47	0.06	0.491

¹Control group fed complete mixtures without halloysite.²Experimental group fed complete mixtures with 1.0% of halloysite addition.

TGs – triglycerides; TCHL – total cholesterol; HDL – high-density lipoprotein cholesterol fraction; LDL – low-density lipoprotein cholesterol fraction; Ca – calcium; P – phosphorus; ALP – alkaline phosphate.

Table 6. The effect of dietary supplementation of halloysite on the femur and tibia parameters of broiler chickens

Item	Femur				P-value	Tibia				P-value
	C ¹		E ²			C		E		
	mean	SEM	mean	SEM		mean	SEM	mean	SEM	
BW ³ (g)	13.16	0.30	13.57	0.36	0.516	18.89	0.41	20.20	0.50	0.479
RBW ⁴ (%)	0.489	0.01	0.493	0.01	0.083	0.702	0.01	0.734	0.02	0.062
BL ⁵ (mm)	81.08	0.55	82.33	0.82	0.546	110.52	0.80	112.56	1.21	0.496
BMD ⁶ (g/cm) ²	0.148	0.01	0.145	0.00	0.252	0.123	0.01	0.118	0.01	0.312
BMC ⁷ (g)	0.396	0.01	0.403	0.02	0.487	0.512	0.02	0.536	0.02	0.746
BS ⁸ (N)	375.97	6.55	380.07	7.99	0.618	294.72	6.94	308.41	6.41	0.357

¹Control group fed complete mixtures without halloysite.²Experimental group fed complete mixtures with 1.0% of halloysite addition.³BW – bone weight.⁴RBW – relative bone weight.⁵BL – bone length.⁶BMD – bone mineral density.⁷BMC – bone mineral content.⁸BS – breaking strength.

Discussion

Nutrition has a significant impact on poultry meat quality and safety. The response of the bird to its feed is closely related to changes in the growth of the skeleton, muscle and fat deposits. One promising way to decrease the cost of production and increase the nutritional effectiveness of feed is the use of feed additives such as aluminosilicate minerals (Safaei et al., 2014). It has been reported that the inclusion of kaolin clay to the broiler diet had no effect on the body weight (Owen and Nodu, 2012). Similarly, Ouachem et al. (2015 b) showed that the addition of kaolin up to 30% of dry matter intake had no significant effect on body weight. However, the carcass yield increased with increasing proportions of kaolin supplementation. These observations are consistent with our results, in which the body weight and relative weights of breast muscle were not affected by dietary halloysite supplementation. However, carcass yield increased significantly following halloysite supplementation, which may be the result of increased digesta retention time, allowing more effective endogenous digestive enzyme

activity in the digestion and absorption of fats, proteins and carbohydrates (Ouhida et al., 2000). According to Safaei Katouli et al. (2012) inclusion of silicate minerals in broiler diets improves weight gain without adverse effects on feed intake and feed conversion ratio. In turn, Wawrzyniak et al. (2017) suggest that dietary supplementation of zeolite increases feed consumption, and enzyme activity of the gastrointestinal tract in broilers, which improves growth performance.

The results of studies regarding the effect of clay and clay minerals on carcass parameters are inconclusive. Some researchers have shown that the addition of zeolite (Lotfollahian et al., 2004; Tatar et al., 2012), perlite (Tatar et al., 2008; 2012) or sepiolite (Eser et al., 2012; Yalçın et al., 2017; Uzunoğlu and Yalçın, 2019) does not have a significant effect on the carcass characteristics of broiler chickens. On the other hand, others (Safaei Katouli et al., 2012) have reported that dietary zeolite increased meat production and thigh weight percentages. Banaszak et al. (2020) state that this improvement can be due to the effect of aluminosilicates in improving gastrointestinal tract health and the feed conversion ratio. It is clear that

numerous other factors, such as water quality, mineral quality, weather, and management conditions may also affect the response of poultry to dietary halloysite supplementation. Minerals differ in structural, physical and chemical properties, which influence the results of their use in animal production (Nadziakiewicz et al., 2019). In this context, the effect of clays' use as a feed additive may depend not only on their origin or level of incorporation in the diet, but also on other properties related to their deposits and their pollutant content.

In agreement with the earlier study of Owen and Nodu (2012), the present study shows that the relative gizzard and liver weights were lower for broilers fed diets containing 1% halloysite, but no pathological changes in the liver have been observed. The liver is the main site of phagocytosis by Kupffer cells, which destroy aged blood cells and pathogens that may enter the blood via the hepatic portal (Zaefarian et al., 2019). According to Subramaniam and Kim (2015) clays have the ability to bind and immobilise toxic substances in the gastrointestinal tract of animals, reducing their toxicity and positively affecting the metabolism. Therefore, it can be assumed that the size of the liver could depend on the addition of halloysite, which affects both the availability of nutrients and the detoxification function (Biesek et al., 2021). In contrast to our findings, some researchers have reported that relative liver and gizzard weights were not affected by dietary silicate minerals (Saçakli et al., 2015; Uzunoğlu and Yalçın, 2019). These were related mostly to the function of the gizzard, which is an organ that shows considerable adaptability to diet composition and quantity. Among other things, it is responsible for the rate of flow of digestive contents, feed maceration, storage, protection from potentially pathogenic bacteria, pellet-casting, as well changing the centre of gravity. Yalçın et al. (2017), who observed a similar reduction on the gizzard weight percentage, explained this effect as having been caused by the slight reduction of feed intake of groups fed diets with sepiolite. Furthermore, inclusion of silicate minerals in broiler diets can improve the digestibility of certain nutrients and reduce the gastrointestinal passage rate, thus lengthening the time nutrients undergo digestion (Safaei Katouli et al., 2012).

Muscles form an important part of carcass composition and are usually the main source of meat products for humans. Thus, their quality is very important for food safety and human health. In poultry meat, the rate and extent of post-mortem pH decline are important factors affecting meat quality (water holding capacity, tenderness and colour; Połtowicz, 2000). In our study, there was no significant effect of halloysite on the acidity of meat. The pH_{24} values for breast meat of both groups ranged from 5.93 to 5.83 which allow the classification of this meat as normal. The results are in accordance with previous studies, which have shown that the addition of silicate minerals such as palygorskite (Prvulovic et al., 2008; Yang et al., 2016; Li et al., 2017) or opoka (Makarski et

al., 2020), to the diet does not have an effect on the pH_{24} value of the breast muscles of broiler chickens.

The colour of the meat is one of the main determinants of meat quality since any discoloration has a negative impact on customer choice. Meat colour parameters such as lightness (L^*), redness (a^*) and yellowness (b^*) are mostly related to the haem pigment content, myoglobin forms, and internal reflectance (Lindahl et al., 2001). Earlier studies have shown that the content of haem pigments depends largely on bird genotype, age, sex, muscle type and activity, as well as nutrition (Mancini and Hunt, 2005; Augustyńska-Prejsnar and Sokołowicz, 2014; Makarski et al., 2020). Meat colour also correlates with other qualitative meat traits and is equated with meat freshness. Yang et al. (2016) and Li et al. (2017) reported that the addition of silicate minerals (palygorskite) to the diet of broiler chickens had no significant effect on the L^* , a^* , or b^* colour values of the breast muscles. Similarly, in our study, adding halloysite did not significantly affect colour parameters L^* and a^* of the breast muscles. However, the value of parameter b^* was significantly lower following halloysite supplementation, which may indicate an increase in the fraction of deoxymyoglobin (Mb) and a decrease of the fraction of oxymyoglobin (MbO), compared with the control group. The lower value of colour parameter b^* is due mainly to a lower ratio of MbO/Mb and a lower internal reflectance (FOP) value. According to Lindahl et al. (2001), the yellowness (b^* value) is influenced most by the myoglobin forms, less by the internal reflectance, and almost not at all by the pigment content. This is in agreement with earlier studies (Feldhusen, 1994), where very low correlations between yellowness and pigment content were reported. In contrast to the present study, Cheng et al. (2016) reported that breast redness (a^* value) was decreased by the supplementation of 10 g/kg palygorskite. In turn, Makarski et al. (2020) found that opoka supplementation to the broiler chickens diet resulted in a lighter colour of breast meat (higher L^* value) in contrast to legs, where a decrease in L^* and a^* values was noted. Differences in the values of the colour parameters of the breast and leg muscles of broiler chickens are probably related not only to animal nutrition but also to muscle fibre composition. It is well known that broiler chickens breast muscle is typical homogeneous white muscle formed from muscle fibre type IIB, whereas leg muscles are heterogeneous structures formed from several types of muscle fibres.

The biochemical parameters of blood serum are important indicators of animal health. In our study, supplementation of 1% halloysite caused a significant decrease in the level of blood serum triglycerides. Such lipids are deposited within the body in the form of adipose tissue, so our results suggest that lipolysis processes outweighed lipogenesis processes in broilers fed halloysite. In turn, the lower total cholesterol serum levels noted in the E group evidences acceleration of synthesis or plasma blood uptake of the lipoprotein fractions by the liver cells. Moreover, halloysite supplementation resulted in

a significant decrease in the LDL fraction of the serum, but had no effect on the HDL fraction. These results are comparable with those of Eser et al. (2012), who demonstrated that the inclusion of sepiolite in the diet of broilers reduced the levels of serum cholesterol and triglycerides. In turn, Park et al. (2002) indicated that blood cholesterol concentration was significantly lower in broilers fed a diet with 3.0% natural zeolite supplementation. Similarly, Yalçın et al. (2017) demonstrated that triglycerides and cholesterol serum levels decreased linearly with sepiolite concentration in the diet. Boudroua et al. (2016) reported that the LDL cholesterol fraction was lower in the bentonite group compared to the control. In contrast to our study, these authors observed that raw bentonite supplementation increased cholesterol and triglyceride serum concentration. Al-Beitawi et al. (2017) suggested that changes of serum cholesterol fraction concentration may be related to the fact that nanoparticle-sized ingredients might increase the functionality or bioavailability of ingredients and nutrients, which could possibly increase absorption and utilization of these nutrients, leading to improved general performance of broiler chickens, including blood biochemistry. On the other hand, many earlier studies have indicated that the level of cholesterol and triglycerides in the blood serum were not affected by dietary silicate mineral supplementation (Prvulovic et al., 2008; Eleroglu et al., 2011; Uzunoğlu and Yalçın, 2019).

A very important problem in modern poultry farming, especially in the case of rapidly growing broilers, is that of leg abnormalities, which lead to economic losses and reduced welfare (Shim et al., 2012; Kierończyk et al., 2017). It is widely accepted that calcium and phosphorus play an important role in bone mineralisation. Therefore, their level in the blood serum is a good indicator of bone development. As shown by Boudroua et al. (2016), serum calcium concentration increases significantly in broiler chickens receiving bentonite compared to those in the control group. In contrast, Eraslan et al. (2005) indicated that the calcium and phosphorus serum levels decreased in broilers fed diets containing bentonite when compared to the control group. On the other hand, Prvulovic et al. (2008) reported that aluminosilicates did not affect calcium and phosphorus serum levels. Inclusion of 1.5 or 3% sepiolite to the diet had no effect on the calcium and phosphorus blood serum levels in laying hens (Mızrak et al., 2014). These observations are consistent with our results, wherein the levels of calcium and phosphorus in the blood serum were not affected by dietary halloysite supplementation. Similarly, Eleroglu et al. (2011) did not observe any significant differences in the levels of calcium or phosphorus concentration in the blood serum following zeolite supplementation. In turn, Safaei Katouli et al. (2012), who analysed the effect of kaolin, bentonite and zeolite supplementation in the broiler diet on the blood calcium and phosphorus levels, reported that the blood calcium level increased significantly in the treatment containing 3% zeolite compared

to the control group. In the case of kaolin and bentonite, they did not observe any significant differences in the blood calcium concentration. Also, the level of phosphorus did not change significantly after the supplementation with kaolin, bentonite, or zeolite. However, Eraslan et al. (2005) indicated that the serum level of calcium and phosphorus decreased when broilers were fed diets containing bentonite.

An important marker of bone formation in birds is alkaline phosphate. It can be detected in the liver, lungs, skeletal muscle, and hearts of birds, contrary to mammals. In avian species, enhanced bone alkaline phosphate activity correlates to increased osteoblastic activity. For this reason it is used as a marker for the evaluation of skeletal health and bone disease in birds. In the present study, the addition of halloysite showed no significant effects on the level of alkaline phosphate in blood serum. These results are in agreement with the previous findings of Boudroua et al. (2016) and Uzunoğlu and Yalçın (2019), who reported that the use of bentonite or sepiolite in the diet did not have a significant effect on alkaline phosphate levels in broiler chickens' blood serum.

The results of our study are consistent with the findings of Safaei Katouli et al. (2012), who reported that supplementation of the mineral kaolin to broiler diets has no effect on the tibiotarsal weight and length indices. In contrast, Ouachem et al. (2015 b) had shown that relative tibia weight increases following kaolin supplementation. For other silicates, such as sepiolite, it was reported that inclusion of this clay in the broiler diet had no effect on the bone characteristics (length, thickness, breaking strength) (Yalçın et al., 2017). Durna Aydın et al. (2020) reported that the biomechanical properties of the femur and tibia bones were not affected by the addition of glauconite to the diet. On the other hand, Safaei Katouli et al. (2012) noted that the addition of 3% of bentonite or zeolite significantly improved the tibiotarsal weight/length, robusticity, and tibiotarsal indices. According to Durna Aydın et al. (2020), the conflicting results concerning the effect of clay minerals on bone parameters may be related to the type and dose of the clay mineral used as well as their chemical composition and environmental factors.

The results of this study indicate that supplementing the broiler chicken diet with 1% halloysite improved carcass yield, reduced the relative liver and gizzard weights, and decreased the level of the colour parameter b^* of the breast muscles. Moreover, adding halloysite to the diet had no negative effect on the bone characteristics or the physicochemical parameters of the breast muscles, but did have some positive effects on broiler chickens' health. The levels of triglycerides, total cholesterol, and LDL fraction in blood serum were reduced with halloysite inclusion in the diet. In conclusion, dietary halloysite supplementation could be beneficial for broiler chicken health, while potentially increasing the health promoting properties of chicken meat for humans.

Conflict of Interests Statement

The authors declare that they have no conflict of interests regarding the publication of this article.

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