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Effects of standardized allicin on production performance, welfare and meat quality of broiler chickens

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The aim of the study was to evaluate the standardized allicin effect on the health, productivity and meat quality of broilers. The antimicrobial properties of the preparation and its impact on the intestinal microbiota were evaluated. It was also attempted to determine the lowest effective dose of preparation. 960 Ross 308 chickens were divided into 4 groups (8 replications each). Birds were maintained in a litter pen with a stocking density of 33 kg/m² and constant access to feed and water. The groups were differentiated by the preparation used: group 1 (control), without supplementation, then standardized allicin doses of 150, 200 and 250 µg/kg body weight were applied in group 2, 3 and 4, respectively at 3-day intervals. The birds' productivity was recorded over 42 days, then 16 birds/group were slaughtered and dissected. Samples of meat were collected. There were no differences in the final body weight of the birds, but significantly the lowest feed conversion ratio and highest

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livability rate in group 4 were found. The allicin-supplemented groups showed a lower incidence of foot pad dermatitis, as well as a quantitative reduction in the aerobic bacteria in the intestinal contents. No effect on the quality of the meat obtained was noticed.

KEYWORDS: *Allium sativum /* foot pad dermatitis / garlic / intestinal microbiota

Garlic (*Alium sativum*) is one of the most popular plants used as a source of natural medicinal substances, with a wide spectrum of action. Its antimicrobial properties are well established, which has led to its utilization in various formulations within traditional medicine as well as in veterinary livestock management. In the case of poultry, these works focus on the use of garlic as an alternative to antibiotics [Navidshad *et al.* 2018], an internal parasite-fighting substance [Alnassan *et al*. 2015, Velkers *et al*. 2011] or as a feed additive [Reddy *et al*. 1991, Fadlalla *et al*. 2010] in the form of an aqueous extract or powder. It has been observed to have a significant effect on enhancing the production performance of birds [Aji *et al.* 2011, Ashayerizadeh *et al*. 2009]. The use of garlic extract, among others, confirmed its stimulating effect on cellular immunity in laying hens by increasing the percentage of macrophages [Dorhoi *et al.* 2006]. The increase in the production of antibodies against *Salmonella enteritidis*, *Pasteurella multocida* and *Leptospira pomona* following the administration of a product containing garlic to the chickens' diet was reported [Szigeti *et al*. 1998]. The antiviral properties of garlic were confirmed [Haq *et al.* 1999] using an addition of 20 g garlic/kg feed, which significantly increased antibody titres against Newcastle Disease Virus (NDV) and infectious bursal disease (IBD) in broiler chickens. One of the two main antimicrobial mechanisms of action of herbal extracts is the inhibition of the synthesis of flagellin, a bacterial filament-building protein, which prevents the movement of bacteria, and their cells exhibit a diminished capacity to adhere to epithelial cells, thereby rendering them non-infectious. Among others, essential oils extracted from fresh garlic bulbs exhibit such an activity, which was confirmed in vitro studies against *Tetratrichomonas gallinarum* and *Histomonas meleagridis* [Zenner *et al*. 2003].

The indicated works, although confirming the properties of garlic both in terms of antimicrobial properties and in the formation of the lipid profile of the obtained products, use garlic in different forms (extracts, isolates, powdered). Such treatments allow us to determine the overall effect of garlic, without specifying the effect of a single biologically active substance, the amount of which in garlic is considerable [Martins *et al*. 2016]. Allicin is regarded as the primary compound, with its properties frequently attributed to the synergistic effects resulting from supplementation. However, it should be noted that some of the biologically active substances are not persistent in nature and their presence is only recorded under specific conditions. An example of such a substance is allicin. In nature, it is formed after plant tissue is damaged by an enzymatic reaction [Cavallito *et al.* 1945]. The precursor of allicin is the non-proteogenic amino acid alliin (S-allyl- l-cysteine sulphoxide). In practice, it is unstable and undergoes rapid redox reactions, which limits its antimicrobial properties. For instance, it was determined [Koch i Lawson 1996] that approximately 35 times more DADS (diallyl disulphide - a compound formed by the decomposition of allicin) is required for its inhibitory effect on *Escherichia coli* and *Staphylococcus aureus* compared to allicin.

The relatively low oxidative stability of allicin results in diminished efficacy of the preparation, often causing the allicin content to decrease to trace levels well before the completion of its use. Thus, conclusions about its properties and efficacy regarding poultry health or production performance are not always justified. The use of standardized allicin may contribute not only to systematize the knowledge on its use in poultry husbandry but may also influence the development of the lowest effective doses possible for use in poultry production.

The aim of the study was to examine the effect of standardized allicin on the health, production effects and meat quality of broiler chickens. An attempt was also made to determine the lowest effective dose of the preparation.

Material and methods

Preliminary determination of the antimicrobial properties of standardized allicin

The *in vitro* antimicrobial activity of allicin was tested against reference strains from the American Type Culture Collection (ATCC): *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 13883 and *Salmonella typhimurium* ATCC 13076. Fresh 24-hour cultures of Tripticase Soy Agar (TSA) were used in the study. Tests were performed using the microdilution method in MHB broth (Mueller-Hinton Broth) in 96-well microtiter plates. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were assessed. Using the previously prepared stock solution, serial twofold dilutions were made in MHB, obtaining extract concentrations from 40 to 0.02 mg/mL. Bacterial inoculum (with an optical density of 0.5 McFarland) was prepared in sterile saline from each strain. Bacterial suspensions of 2 µl each were then transferred to the wells, obtaining a concentration of approximately 10^6 CFU/mL.

In addition, in the last two wells, a positive control (inoculated with bacterial suspension only) was performed - a control for strain viability - and a negative control (medium alone, without inoculum added) - a control for medium sterility. The MIC value was determined by identifying the lowest concentration at which no bacterial growth was apparent in the well following incubation at 36-37°C for 24 hours. For this, a spectrophotometric method was used by reading the absorbance at 570 nm using an ELx808 microplate reader (Bio-Tek Instruments, Inc., USA). For the determination of MBC, 5 µl of the contents of each well were transferred onto the MHA medium (Mueller-Hinton Agar). The media were incubated at 36-37°C for 18-24 hours. After incubation, MBC concentrations at which no bacterial growth occurred were determined. MBC/MIC≤4 was taken as the bactericidal effect and MBC/MIC>4 as the bacteriostatic effect. Tests were performed in triplicate.

Main experiment

The animal study protocol was approved by the Local Ethical Commission for Animal Experiments at the University of Life Sciences in Lublin, Poland (approval no. 35/2021). All methods were carried out in accordance with relevant guidelines and regulations reported in accordance with ARRIVE guidelines (https://arriveguidelines.org).

The material for the main study consisted of 960 unsexed broiler chickens of the Ross 308 set, purchased from the certified local hatchery from a reproductive stock with a health certificate and preventive vaccination program that complies with current regulations. Birds were kept for 42 days and divided into four experimental groups (8 replications of 30 birds each). Birds were individually wing-marked. The birds were maintained in a litter system, in pens, with a stocking density of 33 kg/m^2 at the end of the production cycle.

Broiler chickens were kept on the experimental farm under the housing conditions indicated in Council Directive 2007/43/EC [2007]. Microclimatic conditions and lighting have been adapted to the requirements of the birds as recommended by the manufacturer of the Ross 308 commodity hybrids [Aviagen 2025]. The birds were provided with *ad libitum* access to complete feed and water. The manufacturer's declared feed value of the compound feed is shown in Table 1.

Table 1. Composition of feed mixtures used in chicken feeding regardless of experimental group

Item	Starter (1-19 day)	Grower (21-35 day)	Finisher (5-7 days before slaughter)
Metabolic energy (kcal/kg)	3000	3100	3180
Crude protein (%)	21.00	19.50	18.00
Crude oils and fat (%)	4.80	6.40	6.60
Crude fiber (%)	3.40	3.40	3.60
Crude ash (%)	12.00	12.00	8.00
Assimilable phosphorus (%)	0.43	0.42	0.38
Total calcium (%)	1.00	0.92	0.80
Salt (%)	0.16	0.16	0.16
Lysine (%)	1.31	1.22	1.12
Methionine (%)	0.59	0.55	0.50
Vitamin A (IU/kg)	14000	10000	9000
Vitamin D3 (IU/kg)	5000	5000	2000
Vitamin E (mg/kg)	75	40.0	35.0
6-phytase / EC 3.1.2.26 (FTU/kg)	400	400	400
Endo-1,4-β-xylanase / EC 3.2.1.8 (FXU/kg)	140	140	140
Coccidiostat – salinomycin (mg/kg)	70	70	-
Material composition	wheat, soybean meal, maize, calcium carbonate, monocalcium phosphate, vegetable oils and fats, sodium chloride, sodium bicarbonate		

The differentiating factor between groups was the addition of an aqueous solution of standardized allicin at the following doses: Group 1 (control) - maintained without supplementation, Group 2 - supplemented with an aqueous solution of allicin at a dose of 150 µg/kg body weight, Group 3 - 200 µg/kg BW and Group 4 - 250 µg/kg BW. The preparation of allicin derived from Garlimed Ltd. (Łódź, Poland) in the form of spray-dried alliin and freeze-dried alliinase enzyme, manufactured according to the Polish patent No P.442206. Prior to the administration of the allicin solution to the avian subjects, water quality assessments were conducted to evaluate the impact of water parameters on the product's efficacy. Allicin was prepared according to the producer's instructions. Briefly, both alliin (2.4% w/v) and alliinase enzyme (0.2% w/v) were dissolved in water. Afterwards, both solutions were mixed (1:1) and incubated at room temperature for 20 minutes. An analysis of the formation of allicin in the tested water and its content after 4 hours was performed. Results are presented in Table 1S and on Figure 1S.

Table 1S. Gradient elution parameters

Eluant	Time (min.)						
	0	5	25	26	28	30	40
A	0	15	27	50	50	0	0
B	100	85	73	50	50	100	100

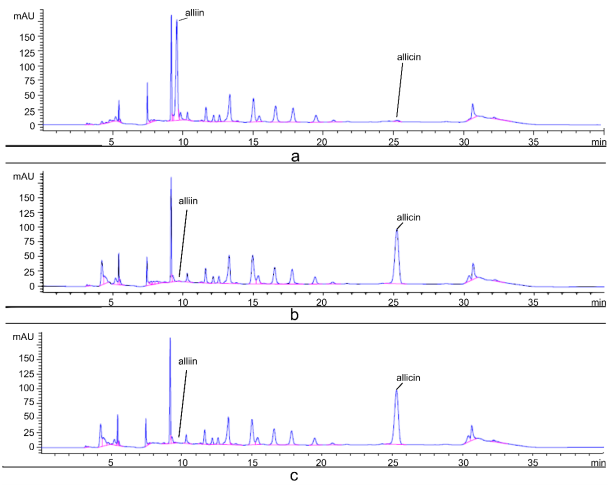

Fig. 1S. Analysis of alliin and allicin content in aqueous extract solutions. (a) – before preparation of solution used, (b) - 20 minutes after substrate and enzyme are mixed, (c) – 4 hrs after applying to birds.

The solution was prepared immediately prior to administration (m/v) and then dosed directly into hand-filled drinkers, with the amount of test substance and the amount of water formulated based on the birds' current body weight and water intake, so that the birds took the required dose while ensuring they received the optimum amount of drinking water as recommended by the manufacturer. The allicin solution was administered in quantities that the birds took no longer than 3 hours after its application. Allicin was applied at 3-day intervals. In order to obtain the correct dose, the preparation was administered in a volume according to the current body weight of the chicks. In a nutshell: the chick manufacturer's data were used as a starting point, and once the rate of increase in the body weight of the chicks was noted at the time of weekly weighing, the % at which the birds over- or under-weighed the average body weight indicated in the instructions was determined. Throughout the rearing period, birds were individually weighed at 7-day intervals to determine body weight gains (BWG) (10 per pen). In addition, the feed remains in feeders were measured at the same time, which, together with the weight of the feed given to the birds, made it possible to determine their real feed intake (FI) and production indicators, including those affecting the economics of rearing (FCR - feed conversion ratio, kg/kg BWG). Bird survival rates were also recorded.

After 42 days of rearing, 42 broilers from each experimental group (21 males and 21 females) were selected for slaughter. After the birds were selected for slaughter, the condition of the foot pad and ankle joint was assessed for the presence and/or severity of foot pad dermatitis (FPD) according to the scale [Hocking *et al*. 2008] to indirectly assess the welfare of the birds during rearing. The visual assessment was always performed by the same member of the research team, with the samples coded by number instead of group assignment (blind trial). The birds were stunned electrically (electric current 45 mA), slaughtered by decapitation, plucked and eviscerated under laboratory conditions. The stunning procedure was in line with Council Regulation No 1099/2009 [2009] on the protection of animals at the time of killing. The carcasses were cooled using cold air (0°C, 4h).

Immediately after slaughter, the digestive tract of the birds was collected for microbiological analysis. Samples of the intestinal contents were diluted in a dilution series (10^3 - 10^8 times), and cultures were performed on media specific to the bacterial Group tested. In addition, species analyses were performed based on API tests (Biomerieux) [Rozempolska-Rucińska *et al*. 2023].

A simplified dissection analysis of the chilled carcasses was performer [Hahn and Spindler 2002]. The following elements of the carcasses were separated: breast muscles, legs (thigh and drumstick), wings, and trunk. On this basis, the slaughter yield (ratio of eviscerated carcass to pre-slaughter weight), the proportion of giblets (heart, liver, gizzard) in the BW and the percentage of elements in the carcasses were evaluated.

Analysis of the technological characteristics of meat was also carried out. The pH value of the meat was determined in the pectoral (left) and thigh (left) muscle (15 and 60 minutes, as well as 24 hours after slaughter) using a CP-251 pH meter [PN-ISO 2917:2001]. The colour of both muscles was measured using the reflectance method with a spherical spectrophotometer (X-Rite Series 8200) with X-Rite Color Master software, using a measuring aperture with a diameter of 25.4 mm. The measurement was carried out in the range of 360-740 nm. A standard illuminant D65 with a standard colourimetric observer with a field of view (observation angle) of 10° was used as the light source. The thickness of the meat samples assessed was approximately 20 mm. A white standard showing the parameters: L* = 95.87, a* = -0.49, b*=2.39. Results were expressed in units of the CIE [1978] system L*a*b* for which the values reflect respectively: L* - lightness of colour (from 0 for a perfectly black body to 100 for a perfectly white one); a* - chromaticity in the red (positive values) - green range (negative values); b* - chromaticity in the yellow (positive values) - blue range (negative values). Meat colour was assessed before and after heat treatment, and the change in meat colour parameters (ΔE) was calculated [Clydesdale 1976].

Samples of 100 g were separated from each right muscle (pectoral and thigh) to determine natural and thermal leakage (loss). Natural drip loss was estimated after storing the samples for 24 h at 4°C and expressed as a percentage of the initial meat sample weight. Thermal leakage was expressed as a percentage of the weight loss of the meat after heat treatment until a temperature of 70°C was reached at the central point of the sample. The Grau and Hamm [Grau i Hamm 1953] method was used to analyse the water holding capacity of the own water. Samples of 0.300 g of minced muscle were placed on Whatman No. 1 filter paper, and the paper was placed between two glass plates and pressure of 2 kg was applied for 5 minutes. The resulting leakage was contoured and planimetric. The result was expressed as the percentage ratio of the leakage area relative to the total area of the meat sample. After cooling, the samples were subjected to a cutting test using a TA.XT.plus texturometer from Stable Micro Systems and a triangular knife. The measurement was made on meat samples cut in the shape of a cube with a side of 20 mm. The cut was made across the muscle fibers. The speed of the knife during the test was set at 2 mm/sec.

The distribution of the data was assessed using the Kolmogorov-Smirnov test, followed by a one-way analysis of variance with Tukey's multiple comparisons tests. For data with a distribution other than normal, the Kruskal-Wallis test was used with the pairwise comparisons. The χ^2 test was used to verify whether particular groups differ in the frequency of FPD within a given severity of the disorder. The FPD frequency was analysed separately for each severity level (0 to 4 scale) depending on the group (preparation dose). The probability level of 0.05 was considered significant. These computations were performed using the SPSS 24.0 package [IBM 2017].

Results and discussion

Preliminary determination of the antimicrobial properties of standardised allicin

Table 2 shows the results of allicin against bacterial reference strains. The MIC values against Gram-positive bacteria were 0.025 mg/mL. The analysis showed bactericidal activity against *S. epidermidis* (MIC=0.025 mg/mL, MBC=0.025 mg/mL). The MBC (>0.2 mg/mL) could not be determined for *S. aureus*, and therefore the bacteriostatic effect of allicin against this microorganism can be reported. The analyzed preparation showed bactericidal activity against all Gram-negative bacterial strains. MIC values ranged from 0.025 to 0.1 mg/mL. The most sensitive Gram-negative bacterium was the *S. typhimurium* species (MIC=0.025 mg/mL, MBC=0.025 mg/mL), while the least sensitive was the *P. aeruginosa* species (MIC=0.1 mg/mL, MBC=0.1 mg/mL).

Table 2. Minimum inhibitory concentration (MIC, mg/mL) and minimum bactericidal concentration (MBC, mg/mL) of allicin against the reference strains

Microorganisms	Allicin		
	MIC	MBC	MBC/MIC
Gram-positive bacteria			
Staphylococcus aureus ATCC 25923	0.025	>0.2	>8
Staphylococcus epidermidis ATCC 12228	0.025	0.025	1
Gram-negative bacteria			
Escherichia coli ATCC 25922	0.025	0.05	2
Salmonella typhimurium ATCC 13076	0.025	0.025	1
Klebsiella pneumoniae ATCC 13883	0.05	0.05	1
Pseudomonas aeruginosa ATCC 27853	0.1	0.1	1

Main experiment

The allicin supplementation had no effect on broiler BW (Fig. 1). Analyzing feed intake in subsequent periods (Tab. 3), significant differences were found for almost all measurement intervals except for 3-4 weeks of birds' age. The total feed intake was significantly higher in the control Group than in the other experimental ones. The highest total BWG was observed in birds from the control and allicin-supplemented Group at a dose of 250 µg/kg body weight. The lowest values of birds' BWG were recorded for the 200 µg dose. Differences in feed intake and body weight gains contributed to significant differences being recorded in terms of feed conversion ratio, the highest in the control Group. The lowest FCR was recorded in groups supplemented with allicin at doses of 150 µg and 250 µg. No significant differences were found in the birds' mortality, but numerically the birds supplemented with the highest dose of allicin had the highest survival rate (Tab. 3).

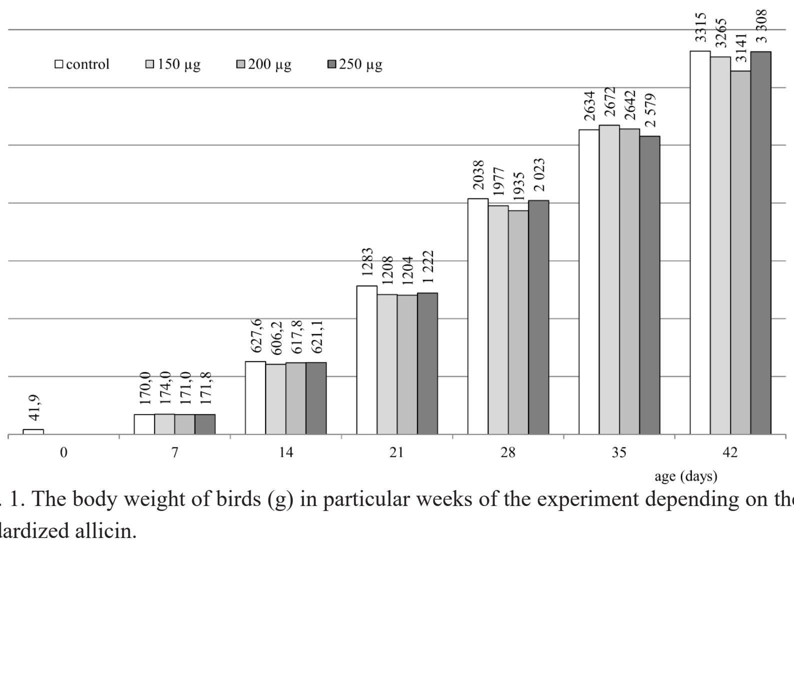

Fig. 1. The body weight of birds (g) in particular weeks of the experiment depending on the dose of the standardized allicin.

Table 3. Productivity of broiler chickens depending on the dose of the standardized allicin

Trait	Time (days)	Group				SEM	*P-value*
		Control	150 μg	200 μg	250 μg		
FI (kg/bird)	7-14	0.615[ab]	0.620[b]	0.611[ab]	0.610[a]	0.001	*0.029*
	15-21	0.739[b]	0.700[a]	0.692[a]	0.693[a]	0.003	*0.044*
	22-28	1.399	1.413	1.424	1.408	0.003	*0.649*
	29-35	1.391[b]	1.179[a]	1.210[a]	1.180[a]	0.013	*0.022*
	35-42	0.788[b]	0.579[a]	0.557[a]	0.584[a]	0.014	*0.021*
Total	FI (kg/bird)	4.93[b]	4.49[a]	4.49[a]	4.47[a]	0.028	*0.018*
	BWG (kg)	3.14[c]	3.09[b]	2.97[a]	3.14[c]	0.009	*>0.001*
	FCR (kg/kg BWG)	1.57[c]	1.45[a]	1.51[b]	1.43[a]	0.008	*0.007*
Liveability (%)		92.92	92.92	93.33	96.25	0.620	*0.497*

[ab]Means between groups differ significantly at P≤0.05; FI – feed intake, BWG – body weight gain, FCR – feed conversion ratio.

The severity of inflammation (number of points scored) was found to be significantly dependent on the Group from which the birds originated (Tab. 4), in case of zero (P=0.008) and third (P=0.000) FPD grade on feet and almost all grades (except 2[nd] stage) of ankle joint lesions (P≤0.001). There was a significant change in the rating scale towards the best (0) ratings for the foot pad of birds supplemented with allicin at 150 and 250 μg/kg BW. Similar observations were also made concerning lesions on the surface of the ankle joint. In this case, the use of allicin, regardless of the dose, reduced the occurrence of the highest grades (3 and 4).

Table 4. Proportion (%) of each stage of foot pad and ankle joint inflammation depending on the dose of the standardized allicin

Item	Foot Pad				
Scale (pts) Group (dose)	4	3	2	1	0
control	20.0	15.0	30.0	30.0	5.0
150 μg	4.5	18.2	27.3	22.7	27.3
200 μg	19.0	33.3	38.1	9.5	0.0
250 μg	10.0	5.0	30.0	25.0	30.0
χ^2 (p-value)	*0.069*	*0.008*	*0.803*	*0.129*	*>0.001*
	Ankle joint				
Scale (pts) Group (dose)	4	3	2	1	0
control	20.0	30.0	15.0	15.0	20.0
150 μg	0.0	0.0	40.9	45.5	13.6
200 μg	14.3	4.8	33.3	47.6	0.0
250 μg	10.0	5.0	30.0	50.0	5.0
χ^2 (p-value)	*0.006*	*>0.001*	*0.062*	*0.006*	*0.002*

FPD scores are based on the scale proposed by Hocking [2008] where 0 means no change and 5 means deep necrotic lesions of the foot pad.

Table 5 shows the structure of the chickens' intestinal bacterial population in relation to the dose of allicin used. It was found to have an inhibitory effect on the growth of the total number of mesophilic aerobic bacteria, with the effect tending to increase with a rising dose of the preparation. Similar observations were made for

Table 5. Results of the analysis of the intestinal microbiota composition (cfu/g) depending on the dose of the standardized allicin

Trait	Group				*P-value*
	control	150 µg	200 µg	250 µg	
Total number of aerobic mesophilic bacteria	$1.2 \times 10^{7\,b}$	$1.3 \times 10^{7\,b}$	$7.4 \times 10^{6\,a}$	$6.0 \times 10^{6\,a}$	*0.001*
Lactobacillus	$2.7 \times 10^{6\,c}$	$1.8 \times 10^{6\,b}$	$1.7 \times 10^{6\,b}$	$1.4 \times 10^{6\,a}$	*>0.001*
Coliform bacteria*	3.4×10^{5}	1.4×10^{7}	2.2×10^{2}	9.0×10^{5}	*0.075*
*E. coli**	6.0×10^{5}	6.8×10^{5}	7.3×10^{5}	5.0×10^{5}	*0.648*
Anaerobic bacteria*	$9.5 \times 10^{4\,b}$	$3.8 \times 10^{4\,a}$	$7.1 \times 10^{4\,a}$	$1.3 \times 10^{5\,c}$	*>0.001*

Bacteria of the genus Salmonella were not identified.
[ab]Means in groups differ significantly at P≤0.05; cfu – colony forming unit.
*Significant differences between groups were verified using Kruskal-Wallis test.

the total number of lactobacilli. Interesting observations as regards coliforms were made. The lowest total number of this Group of microorganisms was found in the digestive tract of chickens supplemented with allicin at a dose of 200 µg, the highest in those receiving the preparation at a dose of 150 µg/kg BW. In the case of *E. coli*, the results remained similar for all groups included in the study regardless of the use of the supplement. For anaerobic bacteria, an increase in the total number of anaerobic bacteria was found in the Group receiving the highest dose of allicin (250 µg/kg BW).

The results of the dissection analysis are presented in Table 6. Carcass weight differed significantly between groups, with the highest carcass weight characterized by birds from groups receiving allicin at doses of 150 and 250 µg with the lowest values of this trait recorded for birds from the Group supplemented with 200 µg/kg BW. Similarly, in the case of slaughter performance, the highest value of this trait was characteristic of birds from Group 2, with no significant differences found in the

Table 6. The results of dissection analyses of broiler chickens depending on the dose of the standardized allicin

Trait		Group				SEM	*P-value*
		control	150 µg	200 µg	250 µg		
Body weight (g)		3313.3	3271.1	3141.3	3306.7	48.51	*0.606*
Carcass weight (g)		2575.0^{ab}	2608.6^{b}	2454.1^{a}	2591.3^{b}	37.53	*0.016*
Breast muscle (g)		930.4^{b}	926.3^{b}	856.1^{a}	921.6^{b}	13.52	*0.001*
Thighs (g)		370.0	384.6	422.1	378.9	16.60	*0.734*
Drumsticks (g)		299.0	319.8	302.3	323.8	7.01	*0.517*
Wings (g)		231.7	240.4	225.9	235.4	4.38	*0.719*
Trunk (g)		687.3	707.3	679.7	694.7	12.84	*0.901*
Carcass yield (%)		77.81^{a}	79.73^{b}	78.13^{a}	78.41^{a}	0.348	*0.001*
Breast muscles		36.49	35.57	34.94	35.60	0.383	*0.585*
Proportion in carcass (%)	thighs	14.45	14.70	17.08	14.62	0.613	*0.417*
	drumsticks	11.64	12.25	12.30	12.53	0.196	*0.426*
	wings	9.19	9.30	9.13	9.22	0.098	*0.948*
	trunk	27.28	27.43	27.49	27.09	0.292	*0.968*
Proportion in body weight (%)	gizzard	1.331^{b}	1.168^{a}	1.386^{b}	1.288^{ab}	0.042	*0.002*
	liver	1.739^{ab}	1.661^{a}	1.921^{b}	1.784^{ab}	0.057	*0.012*
	heart	0.378^{ab}	0.403^{b}	0.361^{a}	0.391^{b}	0.008	*0.003*

[ab]Means between groups differ significantly at P≤0.05.

other groups. Significant differences were also observed in breast muscle weight. It was found to be significantly lowest in birds supplemented with allicin at a dose of 200 μg. The other groups did not differ significantly. No differences were found for the weight of the other carcass cuts or their contribution to the carcass weight. For edible offal, birds in Group 2, receiving 150 μg of preparation per kg of BW, had the lowest proportion of gizzard. Birds in this Group were also characterized by the lowest proportion of the liver relative to the BW. The highest proportion of liver was found in birds supplemented with allicin at 200 μg/ kg BW. The proportion of heart in the BW of birds was lowest for birds in Group 3, with the highest value recorded for birds supplemented with allicin at doses of 150 and 250 μg/kg BW.

Analyzing the technological attributes of chicken broiler meat (Tab. 7 and 8), it was found that in the case of non-heat-treated breast muscle, the meat of birds receiving 150 μg allicin per kilogram BW was characterized by higher colour saturation towards red, while supplementation at 250 μg showed a change in colour space towards green. Differences were also present for coordinate b* with the lowest yellow saturation characterizing the muscles of Group 3 birds (200 μg allicin/kg BW). Although there were no significant differences in colour change after heat treatment (ΔE), slightly different relationships were found in the colour of breast muscles after cooking. The muscles of birds receiving higher doses of allicin (200 and 250 μg) were characterized by higher lightness compared to the Group supplemented with the lowest dose of the tested preparation. It is also noteworthy that the control Group was not significantly different from any of the experimental groups. In the case of thigh muscles, significantly the highest red saturation occurred in the muscles of birds in Group 4, with the lowest values recorded for birds in the control Group.

Table 7. Results of colour assessment of breast and thigh muscles of broiler chickens depending on the dose of the standardized allicin

Muscle	Trait		Group				SEM	*P-value*
			control	150 μg	200 μg	250 μg		
Breast	raw	L*	59.31	58.61	59.53	59.59	0.276	*0.574*
		a*	0.41^{ab}	0.86^{b}	0.03^{ab}	-0.20^{a}	0.126	*0.015*
		b*	13.45^{b}	13.44^{b}	11.84^{a}	13.56^{b}	0.188	*0.003*
	thermally treated	L*	85.24^{ab}	83.85^{a}	86.25^{b}	85.79^{b}	0.236	*0.002*
		a*	1.01^{a}	1.68^{b}	1.12^{a}	1.18^{a}	0.060	*0.020*
		b*	15.31	15.76	15.28	15.60	0.073	*0.062*
	ΔE		26.10	25.53	27.04	26.38	0.322	*0.434*
Thigh	raw	L*	51.03	49.95	50.74	49.97	0.266	*0.365*
		a*	6.10^{a}	7.25^{ab}	6.85^{ab}	7.53^{b}	0.162	*0.009*
		b*	14.68	14.51	14.98	15.24	0.154	*0.336*
	thermally treated	L*	79.71	78.90	79.87	78.46	0.357	*0.459*
		a*	2.61	2.37	2.20	2.65	0.082	*0.177*
		b*	17.47	17.70	17.19	17.69	0.113	*0.348*
	ΔE		29.14	29.68	29.69	29.25	0.438	*0.857*

[ab]Means between groups differ significantly at P≤0.05.

For the technological traits of meat (Tab. 8), no differences were found in terms of the pH of both muscles analyzed. In the case of the pectoral muscle, no differences

Table 8. Results of the technological properties of breast and thigh muscles of broiler chickens depending on the dose of the standardized allicin

Muscle	Trait	Group				SEM	*P-value*
		control	150 µg	200 µg	250 µg		
Breast	pH15	5.79	6.00	5.89	5.76	0.028	*0.466*
	pH60	5.33	5.45	5.47	5.41	0.017	*0.420*
	pH24	5.48	5.40	5.56	5.47	0.013	*0.208*
	water holding capacity (%)	53.13	55.86	51.76	53.71	0.710	*0.237*
	natural leakage (%)	2.22	2.90	2.53	2.80	0.128	*0.411*
	thermal leakage (%)	25.91	27.22	27.76	26.47	0.410	*0.227*
	tenderness (N)	30.29	27.81	28.11	28.93	0.674	*0.565*
Thigh	pH15	5.85	5.78	5.87	5.94	0.020	*0.488*
	pH60	5.57	5.69	5.63	5.86	0.022	*0.102*
	pH24	5.84	5.65	5.80	5.81	0.019	*0.226*
	water holding capacity (%)	46.63[a]	53.64[b]	46.26[a]	44.39[a]	0.920	*0.001*
	natural leakage (%)	1.68	1.73	1.53	1.27	0.079	*0.153*
	thermal leakage (%)	29.01[a]	30.52[ab]	32.98[b]	27.72[a]	0.530	*0.003*
	tenderness (N)	27.93	25.79	30.67	27.90	0.838	*0.251*

[ab]Means between groups differ significantly at $P \leq 0.05$.

were found between the groups for leakage, tenderness or water holding capacity. The results for the thigh muscles are slightly different. The thigh muscles of birds supplemented with allicin at a dose of 150 µg/ kg BW were found to have significantly the highest water holding capacity compared to the other groups. Significant differences were also found for thermal leakage. The highest weight loss after heat treatment was found for birds receiving the preparation at a dose of 200 µg/kg, the lowest for the muscles of birds in Group 1 (control) and Group 4 (250 µg/ kg BW). There were no statistically significant differences in thigh muscle tenderness between the groups.

Our preliminary study showed that already very small amounts of allicin exhibit strong antibacterial activity against the main groups of bacteria, confirming the results of other authors, who also found that the very low concentrations obtained for antimicrobial and antifungal activity suggest a high activity of allicin and its transformation products, which may be comparable to commercially available antibiotics and become a prime candidate for therapeutic use [Ilić 2012]. The antimicrobial properties of garlic, comparable to common antibiotics (erythromycin, ampicillin, tetracycline), vary depending on whether the study was performed *in vitro* or *in vivo* [Fatima *et al.* 2011]. Fresh garlic shows significantly higher activity, which is probably due to the short-lived presence of allicin and its subsequent transformation into other compounds. Another study [Li *et al*. 2015] showed inhibitory properties of fresh garlic against *C. albicans* and methicillin-resistant *Staphylococcus aureus* (MRSA), but weak inhibitory properties against *P. aeruginosa*, while it had the potential to improve antibiotic activity against antibiotic-resistant pathogens. Fresh garlic extract can be used to help treat infections caused by multidrug-resistant strains.

Garlic supplementation is relatively well established, but the vast majority of preparations made from garlic are based on extract or powder form, which, due to the low stability of allicin [Borlinghaus *et al.* 2014] allicin is a reactive sulfur species

(RSS, makes it impossible to mention its content in these preparations. Moreover, as a substance of natural origin, allicin levels in the plants themselves fluctuate considerably depending on the variety of growing conditions [Khar *et al*. 2011, Marsic *et al*. 2019]. It is therefore necessary to standardize these preparations to know their real impact on bird productivity effects. The lack of standardization already at the stage of raw material used for garlic preparations makes the literature data in this regard inconsistent. For example, the addition of garlic powder (0.5%) improved the production effects of broiler chickens [Suriya *et al.* 2012]. Similarly, it was concluded [Kumar *et al.* 2010] that 250 ppm dietary addition of garlic in broilers significantly increased the BW after 42 days. Also, analyzing the addition of an aqueous extract of medicinal plants containing garlic in drinking water of broilers (10 ml/L) [Javed *et al.* 2009], it was stated that FI and FCR, BW, dressing percentage, breast and leg weight were improved. Other authors made the opposite observation in this regard, indicating that garlic preparations did not affect most productive traits. The dietary garlic addition (0.125 and 0.25%) increased the FI of broilers during the starter stage, but not during the growing one, while no difference was found in body weight and carcass characteristics (proportion of carcass, small intestines, proventriculus, gizzard, liver and abdominal fat) [Javandel *et al.* 2008]. The powdered garlic addition to the broiler diet did not affect the BW, feed intake and its conversion, but led to a significant impact on the carcass yield [Ashayerizadeh *et al.* 2009]. Reports can also be found that indicate the effect of garlic on selected productive results in birds. Broiler chicks supplemented with 100 mg of garlic had improved body weight gain at 7, 14 and 21 days of treatment, but FI, FCR and carcass yields were not affected [Aji *et al.* 2011]. Similarly, it was reported [Mahmood *et al*. 2009] that 0.5% garlic in the broiler basal diet led to improved BWG and FCR, without affecting carcass yield (dressing proportion, relative weight of heart, gizzard, liver, spleen and pancreas). In our study, no significant differences were found for final BWs and particular measurement periods. Although they were higher than the BWs indicated as typical by the chick producer (Aviagen®), they were not differentiated by experimental groups.

Body weight, although it is the most visible production result, does not have the greatest impact on overall production efficiency and profitability. The results obtained in the study indicate a significant improvement in the FCR. This is a factor that has a significant impact on poultry production efficiency due to its high contribution (55-60%) in structuring the cost of overall production [Singh *et al*. 2015]. Over the past decades, breeding work has managed to significantly reduce FCR while maintaining the high productivity of birds [Prakash *et al.* 2020]. Changes in this rate will depend on the health of the birds and especially the development of the digestive tract. It was found that there is a negative correlation between FCR and relative gizzard weight and a positive correlation between FCR and liver weight [Huang *et al.* 2022]. In addition to the functioning of the gastrointestinal tract, a large role in shaping FCR is also attributed to the intestinal microbiota actively influencing the digestion and nutrient absorption processes [Liu *et al*. 2021]. Effective methods to improve its composition are being sought to maximize microbial efficiency in the digestive process [Yadav and Jha 2019]. Allicin seems to have such an effect at the doses tested (150 and 250 μg), as confirmed by the upward trend in the total number of anaerobic bacteria in the intestinal microbiome (Tab. 5), among which those of the genus *Clostridium* are found in chickens. Thanks to the production of short-chain fatty acids, bacteria of this genus can positively impact the digestive processes and, consequently, the FCR [Stanley *et al*. 2016, Guo *et al.* 2020].

Footpad dermatitis (FPD) is the most common in fast-growing broiler chickens and slaughtered turkeys [Shepherd i Fairchild 2010]. Of several factors, ranging from genetic predisposition to zoohygienic conditions in the poultry house to the feeding regime of the birds, can influence its intensity [Swiatkiewicz *et al.* 2017]. Inflammatory lesions in the foot pad can also spread to the ankle joint, as well as other parts of the body [Piller *et al.* 2020]. However, the most common cause of FPD is elevated litter moisture. To minimize the risk of inflammation of the foot pad, litter materials with different water-binding capacities [Shepherd *et al*. 2017], as well as components that dry the litter, are used [Banaszak *et al.* 2022]. In our study, the climatic conditions, genotype and housing system of the birds were the same, so the source of variation was the use of allicin. This effect may be partly explained by an improvement in feed utilization and the formation of an equilibrium between different groups of microorganisms in the digestive tract. The synergistic effect of both may reduce the degree of fecal hydration and further influence the occurrence of FPD. The observed effect of allicin on the composition of the intestinal bacterial populations and their balance [Makuch *et al*. 2025] will also be important in this respect. Studies indicate that the use of symbiotics and probiotics is effective in reducing the incidence of FPD in broilers [Brugaletta *et al.* 2020]. Furthermore, it is suggested [Al-Massad et al. 2018] that the incidence of FPD is related to the cecal microbiome, which may also have occurred in our study due to the antimicrobial effect of allicin found.

One of the most important elements of dissection evaluation is the proportion of particular elements in the carcass weight. The use the addition of dried garlic to the diet of broiler chickens resulted in lower results than those obtained in our study [Al-Massad et al. 2018]. However, the authors found increasing carcass weight and significant differences in this trait with increasing dosage of garlic used, which is not supported by our results. In terms of slaughter performance, the results obtained are satisfactory and similar to the general trends in the literature for broiler chickens. However, it should be noted that a great variability can be observed in other works on the use of garlic or garlic-derived products. Some authors [Borgohain *et al.* 2019] obtained significantly lower slaughter yield results. This difference may be due both to the form of the preparation used and the lack of standardization, which translates into the content of active substances, but also to the choice of a various hybrid for rearing, which also affects slaughter performance [Kokoszyński *et al*. 2022].

The weight of the giblets (gizzard, liver and heart) is directly related to the utility type of the birds and their growth characteristics [Karthika *et al*. 2019]. These proportions also vary within the available lines of broiler chickens [Kokoszyński *et al.* 2017]. In the context of the use of garlic-based raw materials alone, it was found no differences between groups for the proportion of organs in the BW of birds supplemented with different doses of garlic [Odunowo i Olumide 2019]. Similar conclusions were also reached in work studying the effects of garlic and ginger on the production performance of broiler chickens [Olagoke *et al*. 2019]. Interestingly, FCR and gizzard proportion are negatively correlated, although this trend was not confirmed in our study [Huang *et al*. 2022]. It should be noted, however, that a solution standardized for allicin activity was used in our study, which may explain these differences. Allicin exhibits hepatoprotective activity and promotes liver regeneration by reducing oxidative stress and inhibiting apoptosis pathways [Samra *et al*. 2020] apoptosis and liver regeneration play an important role in liver injury. Therefore, we assessed allicin's protective effect on APAP-induced hepatotoxicity and studied its effect on NLRP3 inflammasome and apoptosis. Mice in the APAP group were injected by APAP (250 mg/kg, intraperitoneal). Studies also show allicin's ability to regulate the body's energy homeostasis [Zhang *et al*. 2020], which contributes to reducing or improving the condition of fatty liver disease [Soleimani *et al.* 2020]. Studies in mice also show the effect of allicin in regulating lipid metabolism and the potential for fat reduction following its use [Shi *et al.* 2019].

For the myocardium and cardiovascular system, cardioprotective nature of allicin was confirmed [Ma *et al.* 2017]. By reducing the incidence of dyslipidaemia, atherosclerosis and affecting endothelial characteristics and signaling pathways, it shows high potential in protecting against cardiovascular disease [Sánchez-Gloria *et al.* 2022]. These observations may explain the increased survivability of birds in allicin-supplemented groups, as one of the causes of mortality in fast-growing birds is so-called sudden cardiac death [Sosnówka-Czajka and Skomorucha 2022].

However, from the final point of view, the most important aspect is meat quality. We did not find disturbances in post-slaughter glycolysis, which could indicate the occurrence of dark firm dry (DFD) and pale soft exudative (PSE) type of meat [Adzitey i Nurul 2011], which are related to water-holding capacity. The absence of their occurrence also explains the lack of significant differences in natural leakage and water holding capacity.

The first element of carcass choice assessed by consumers is its colour. It is influenced by several factors related to both the bird's housing and feeding regime, as well as its haem pigment content [Wideman *et al.* 2016]. In the case of the garlic influence, few works address meat colour as a determinant of meat quality. Similarly to our results differences in a* and b* coordinates in bird meat were showed [Kim *et al*. 2009]. In addition, according to their study dietary supplementation with garlic led to decreased the shear force of meat. In our study, there was no statistically significant effect of allicin supplementation on the tenderness of the breast and thigh muscles measured after heat treatment. Meat colour is related to the concentration and chemical state of haem pigments, mainly myoglobin. Myoglobin oxidized to the brown metmyoglobin form and its accumulation is highly correlated with progress of lipid peroxidation [Shleikin i Medvedev 2014]. The changes in color parameters observed in our study may be the result of the antioxidant properties of allicin. As an antioxidant, allicin can interact to reduce the cellular levels of different types of reactive oxygen species produced by a variety of peroxidases, thereby inhibiting the peroxidation process [Nadeem *et al*. 2022].

Conclusions

Standardized allicin shows a very strong biocidal effect against reference bacterial strains. Its effect on field strains of microorganisms needs to be studied. The positive effect of standardized allicin on the production efficiency of chicken broilers was demonstrated in the lowered feed intake, feed conversion ratio and mortality. Variation in the results of dissection analysis due to allicin dose was recorded with no negative effect of the allicin on the meat's technological characteristics. Allicin contributed to a positive modification of the chickens' intestinal microbiota, which translated into a reduction in the incidence of foot pad dermatitis, thereby improving bird welfare. It has been shown that allicin at a dose of 250 µg/kg body weight can be an effective non-antibiotic agent for the health and production effects of broiler chickens. Standardization of allicin allows full control over the dosage used, as well as uniformity and certainty of dosage of this bioactive substance in a poultry flock.

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