



EFFECT OF DISTILLER'S DRIED YEAST ON SELECTED MICROBIOTIC FRACTIONS AND THE CARCASS AND MEAT QUALITY OF BROILER CHICKENS*

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Abstract

The study was conducted on 112 broiler chickens fed for 35 and 37 days with various levels of distiller's yeast in diets – 3% in starter, 6% in grower, and 9% in finisher feeds. The results showed that as the level of distiller's dried yeast in the broiler diets increased, the total number of bacteria in the mucosa of the small intestine, cecum, and colon decreased, including mainly *Enterobacteriaceae*, *Enterococcus*, and *E. coli*. The addition of 9% yeast to the diet significantly reduced the abundance of *Lactobacillus* sp. in the intestinal mucosa of broiler chickens. There was no significant effect of the level of distiller's dried yeast in the diet of broiler chickens on carcass quality, pH, water holding capacity, sensory characteristics, and macronutrient and micronutrient content in breast muscle. On the other hand, there was a significant effect of the analyzed distiller's yeast on color characteristics and basic chemical composition of breast muscle. The highest lightness (L^*), yellowness (b^*), and chroma (C^*) and the lowest dry matter content were found in the meat of chickens receiving 6% and 9% distiller's dried yeast in their diets. The results of the study indicate that for the best results in terms of carcass and meat quality, the optimal content of the analyzed yeast in the diet of broiler chickens should not exceed 3%.

Key words: chicken, microbiota, slaughter value, meat quality, chemical composition

The risks posed by the use of antibiotics in intensive animal production are associated with the development of bacterial resistance in both human and veterinary medicine, and also with the safety and quality of animal products (Costa et al., 2007; Yegani and Korver, 2008). As a result, public acceptance of the use of antibiotics in animal production is declining, and increasingly aware consumers are seeking products from antibiotic-free production systems that ensure animal welfare and the safety and quality of animal products (Sharma et al., 2014; Zavišić et al., 2023).

In recent years, rising corn-soybean meal prices have led to an increase in feed prices, posing serious challenges for the livestock industry. In addition, restrictions on the use of antibiotics in livestock production have prompted researchers to seek alternative sources of feed and supplements to improve health and production efficiency while maintaining high carcass and meat quality.

Yeast has been known for many years to have a positive effect on growth and performance in poultry. Feed yeasts, such as *Saccharomyces cerevisiae* (SC), are

a rich source of nutrients, particularly complete protein (40%–50%) and biologically active compounds, that can provide functional benefits to both the gastrointestinal tract and immune system, as well as hematobiochemical parameters (Elghandour et al., 2019; Ogbuewu et al., 2019; Attia et al., 2022).

The addition of yeast to diets contributes to the production of lactic acid, thereby lowering the pH in the gastrointestinal tract and reducing the population of pathogenic microorganisms (Ogbuewu et al., 2019; Saadia and Nagla, 2010). In addition, yeast contains mannan-oligosaccharides (MOS), fructooligosaccharides (FOS), and β -glucans – natural components of the yeast cell wall that stimulate the growth of beneficial bacteria in the intestine and reduce the proliferation of pathogenic bacteria (Jayasena et al., 2017; Yang et al., 2008). The yeast cell wall also contains chitin, mannan, and glucan, which are known immunostimulants, as evidenced by increased secretions of IgA and IgG immunoglobulins in the ileum of broiler chickens (Attia et al., 2017; Li et al., 2016; Rodriguez et al., 2003). The SC yeast also contains

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a complex of B vitamins, important trace elements and enzymes such as cellulase and phytase (Sharma et al., 2020).

Earlier studies indicate the positive effects of feeds with different levels of SC yeast, including higher daily gain (DG) and body weight (BW), lower feed intake (FI) and improved feed conversion ratio (FCR) (Buba et al., 2016; Shankar et al., 2017; Swamy and Upendra, 2013). A few studies have also demonstrated that SC yeast positively affects selected carcass and meat quality traits (Paryad and Mahmoudi, 2008; Zhang et al., 2005), which was not confirmed in Karaoglu and Durdag (2005) and Attia et al. (2020). The level of SC yeast in broiler diets influences the results obtained, and a higher dietary level is not always associated with more favorable results in terms of production efficiency and carcass quality (Ahmed et al., 2015).

Scientific literature lacks studies examining the utilization of yeast-fermented agricultural distillery stillage cultures for feed purposes, particularly regarding their impact on the quality and composition of meat, as well as their technological and sensory suitability, and their influence on the intestinal mucosa microbiome of broiler chickens.

This study aimed to analyze the microbial fractions of the mucosa in three intestinal sections and the quality of broiler carcasses and breast muscles with regard to the level of distiller's dried yeast in their diets.

Material and methods

Ethical statement

According to Polish law (Act of 15.01.2015 on the Protection of Animals Used for Scientific and Educational Purposes), ethical approval for this experiment was not required because the experiment was carried out under standard production conditions and the birds were not exposed to excessive pain, suffering, or stress. The experimental protocol was consulted and approved by the Animal Welfare Advisory Team of the Faculty of Biology and Animal Science of the Wrocław University of Environmental and Life Sciences (Decision no. 3/2023).

Experimental designs

The study was conducted in the vivarium of the Experimental Department of the University of Life Sciences in Wrocław. The treatment of the broiler chickens was performed according to the requirements of the European Union (1986). A group of ($n = 112$) one-day-old cockerels of the Ross 308 line with an average weight of 40.72 ± 0.47 g were fed until the 35th or 37th day of life. The chickens were purchased from the ModernHatch company hatchery (Magnuszowice, Poland) where they had been subjected to the hatchery sexing procedure.

The day-old male chicks were placed in 28 boxes of 1.1 m^2 each in a straw litter system. In each box there

was a bell-shaped drinker and a feeder. All the chicks were fed *ad libitum* with a complete balanced diet and water. The chicks were maintained under controlled microclimatic conditions in accordance with the manufacturer's recommendations for the Ross 308 line. The air temperature on the first day was 32°C , and was then lowered by approx. 0.5°C per day to approx. 22°C on the 21st day of life, and maintained at this level until the 37th day of life. Relative humidity throughout the experiment was in the range 50%–60%. The lighting program comprised 18 hours of light and 6 hours of darkness.

The chicks were randomly assigned to one of four groups (28 cockerels in each group) that differed in the amount of dried inactive distiller's yeast added to the diet. Inactive feed yeast is manufactured through microbiological synthesis using technically pure cultures of SC yeast strains, with wheat distillery stillage serving as a substrate. During the drying process, yeast cells disintegrate at temperatures of 100°C – 103°C , releasing amino acids.

The control group (A) was fed an unaltered diet while groups B, C and D received feeds containing 3%, 6% and 9% yeast, respectively. Each group of 28 chicks contained 7 replicates of 4 chicks per box. The chicks were fed individually prepared appropriate starter (1–10 days of age), grower (11–27 days of age) and finisher (28–37 days of age) diets.

The characteristics of the chemical composition of the SC distiller's yeast, the composition and nutritional value of the diets for each group, and details of the rearing conditions for the chicks were presented in an earlier publication of this research (Rybarczyk et al., 2023). The basic chemical and mineral composition in the feed mixtures for all the analyzed groups of broiler chickens is presented in Table 1.

Diets and chemical composition

Reference methods following the official analytical methods of EN ISO 9831:2004 and AOAC (2016) were used to analyze the basic chemical composition of feed and breast muscle. The following determinations of the basic chemical components were made:

- Dry weight was determined by drying 2 g samples at 102°C for 12 h to constant weight (950.46B, p. 39.1.02) in a SUP-4M laboratory dryer (Wawa-Med, Poland).
- Total protein was determined by the Kjeldahl method (conversion factor 6.25) (992.15, p. 39.1.16) in a Kjeltec 2300 Foss Tecator distiller (Häganäs, Sweden).
- Crude fat was determined by light petroleum extraction (960.39 (a), p. 39.1.05) using a Büchi Extraction System B-811 (Büchi, Switzerland).
- Crude ash (total mineral content) was determined by combustion of samples at 550°C (920.153, p. 39.1.09) for 10 hours in an FCE 7SHM Czylok muffle furnace (Poland).

Table 1. Nutritional value (%) and macro- (g/kg) and microelements (mg/kg) content in feed for broiler chickens depending on the fattening phase

Items*	Distillers' yeast	Starter				Grower				Finisher			
		A	B	C	D	A	B	C	D	A	B	C	D
Nutrient													
dry matter	91.24	92.68	93.19	93.42	92.86	92.57	92.26	92.24	92.75	92.60	92.06	92.13	92.51
crude ash	6.74	6.43	6.31	6.22	6.35	4.30	4.37	4.42	4.22	4.50	4.16	3.86	4.59
crude protein	44.18	24.87	24.88	24.61	25.53	20.47	19.53	20.52	19.62	21.03	19.53	18.49	21.08
crude fat	5.17	7.11	6.88	6.85	6.72	7.75	7.86	7.71	7.52	8.49	8.68	7.90	7.86
crude fiber	6.04	4.37	5.03	4.49	4.74	4.54	4.44	5.03	4.68	4.64	4.92	4.91	4.61
GE (MJ/kg)	20.50	19.65	19.66	19.65	19.62	19.91	19.86	19.92	19.82	20.07	20.06	19.90	19.94
Mineral composition													
Ca	20.58	13.07	14.61	14.14	13.71	7.63	11.98	11.56	6.18	8.18	8.90	7.59	10.69
Mg	2.25	1.61	1.39	1.56	1.53	1.41	1.65	1.52	1.09	1.12	1.10	1.10	1.21
Na	0.83	1.15	1.29	1.31	1.28	0.91	1.01	0.97	0.88	0.92	1.01	0.91	1.22
K	9.11	9.16	8.30	9.21	8.11	7.46	6.88	6.87	5.69	7.90	6.93	5.37	6.40
P	6.31	6.49	6.87	6.63	6.96	4.03	4.53	4.23	3.80	4.13	3.90	4.31	4.55
Cu	17.98	29.80	37.16	30.77	35.13	29.79	20.93	22.43	28.12	24.97	19.60	19.09	17.04
Mn	58.57	129.17	138.77	142.30	143.84	81.46	109.77	105.49	92.07	113.97	117.51	115.62	124.29
Fe	118.59	333.66	349.86	351.94	337.45	197.37	208.99	213.02	166.21	150.00	160.69	225.49	206.38
Zn	58.90	103.39	114.07	116.95	128.35	71.38	102.38	90.41	90.09	96.84	121.50	100.05	114.55
Se	0.09	0.29	0.45	0.27	0.25	0.27	0.35	0.27	0.21	0.45	0.33	0.24	0.50

A: control group, diet without addition of distillers' yeast, B: diet with 3% addition of distillers' yeast, C: diet with 6% addition of distillers' yeast, D: diet with 9% addition of distillers' yeast. GE – gross energy. * – values as a result of chemical analysis.

Mineral content

The ground feed and breast muscle samples were freeze-dried under vacuum at -55°C for approximately 48–72 h (depending on the sample size) using a Modulyo apparatus (Edwards, UK) to obtain a constant sample weight. The freeze-dried meat samples were then ground using a WŻ-1 laboratory grinder (Zakład Badawczy Przemysłu Piekarskiego, Poland). Breast muscle samples (0.3 g for Ca, Mg, K, and Na, and 1.0 g for Cu, Mn, Zn, and Fe) were wet digested in a 7 ml mixture of $\text{HNO}_3\text{-H}_2\text{O}_2$ (2:3, v/v) in a MarsX apparatus (CEM Corporation, Matthews, USA). The digestion process consisted of two stages: the first stage (10 minutes), during which the temperature was raised to 190°C , and the second stage (7 minutes), during which the temperature was maintained at 195°C . The digested samples were placed in polypropylene tubes and diluted to 50 ml with distilled water. Macronutrient (K, Na, Ca, Mg) and micronutrient (Zn, Fe, Mn, Cu) contents were determined by flame atomic absorption spectroscopy (FAAS, air-acetylene flame) using an AA 240FS SIPS20 spectrometer (Varian, Mulgrave, Australia) according to AOAC procedures (2016). The phosphorus (P) content of the samples was determined after prior digestion with HNO_3 (65%) and HClO_4 acids in a MarsX9 closed microwave extraction system (MARS 6 Microwave Reaction System, CEM Corporation, Matthews, USA). Analysis was performed spectrophotometrically by the ammonium vanadomolybdate method using a Specol 11 spectrophotometer (Carl Zeiss, Jena, Germany) at 470 nm. Selenium in the feed and meat was determined by hydride genera-

tion atomic absorption spectroscopy (HG-AAS). The selenium content of the samples was determined after prior digestion with HNO_3 . The residue was then dissolved in 25% H_2SO_4 and the selenium content was determined by atomic absorption using Vapor Generation Accessory 77 (Agilent Technologies, Inc., USA). The macronutrient and micronutrient contents of the samples were expressed relative to the fresh weight.

Carcass quality

Carcass quality traits were tested at 35 and 37 days of age. Slaughter was performed following EU Directive 2010/63/EE (2010) after a 12-hour pre-slaughter fast. On each of the two days, approximately 56 birds were slaughtered, with equal numbers from each group, representing all replicates. In total, for each group, 4 carcasses from each replicate were selected for testing. In addition, the body weight of the broilers was determined, which was used to estimate the weight of the carcasses according to Aviagen (2019) for broilers of the Ross 308 line. After evisceration of the birds, the weight of the eviscerated pectoral muscles including the fillet (without skin) and the weight of the thighs (without skin, leaving the bone) was determined 15 minutes *post-mortem*. The percentage of pectoral and thigh muscle in the estimated carcass weight was then calculated.

Meat quality

Twenty-six pectoral muscles (104 in total) were collected from each group immediately after slaughtering.

The right pectoral muscles were used for physicochemical and chemical analysis of the meat in the later stages of the study, and the left pectoral muscles were used for physicochemical and sensory analysis of the meat previously subjected to freezing. At 15 minutes *post-mortem*, the weight of the pectoral muscles was determined as a whole and, after removal of the tenderloin, of the right and left muscles individually. At the same time, the pH of the right pectoral muscle was measured. Then, the right and left pectoral muscles, properly labeled, were placed in sealed bags. The samples prepared in this way were transported and stored at approximately 4°C. Twenty-four hours after slaughtering, detailed meat quality tests were performed. The pH, color characteristics, and water holding capacity (WHC) were measured in the right pectoral muscles, as well as the basic chemical composition and the macro- and micronutrient content. Left pectoral muscles were frozen at -21°C for a period of one month. After thawing at 4°C for 24 hours, pH and water holding capacity were determined, and sensory evaluation of the heat-treated meat was performed.

- pH – measurements were made using a SevenGo™ SG2 pH meter (Mettler Toledo, Switzerland). A dagger electrode was inserted at three points in the pectoral muscle and the results were averaged.

- The color characteristics were determined 24 h *post-mortem* on the inside of the muscles at three locations, which were then averaged. The measurement was performed with a HunterLab Mini Scan XE Plus 45/0 spectrophotometer using a D65 light source and a 10° observer. During the measurement, the following color characteristics were determined: lightness (L^*), redness (a^*), yellowness (b^*), and chroma (C^*).

- Drip loss was determined by the weight of right pectoral muscles weighed 15 minutes after slaughter and the weight of chilled muscles at 4°C 24 hours *post-mortem*.

- Water holding capacity (WHC) in pectoral muscles was determined 24 h after slaughter using the method of Grau and Hamm (1952) as modified by Pohja and Niini-väara (1957). WHC was expressed as % free water and % bound water.

- Water loss during thawing and cooking – breast muscles were removed from the freezer, weighed (pre-thaw weight), thawed at 4°C for about 24 h, and reweighed (pre-cooking weight), which allowed determination of thawing loss. The samples were heated in water at 80–81°C to an internal temperature of 72°C and weighed after cooling to 20°C (post-cooking weight). The cooking loss was determined from the pre- and post-cooking weights. The next day, the samples were subjected to shear and sensory evaluation. Total fluid loss was determined based on the pre-thaw loss and post-cook weight.

- Sensory evaluation was performed under the conditions prescribed by PN-EN ISO 8589:2010. Breast samples were taken to determine their color, aroma, tenderness, juiciness, and flavor. Samples were of approximately equal size (about 25 g) and were placed in lidded one-ounce glass jars labeled with three-digit random

codes and kept in a water bath (54°C) until presented to the panelists. The analysis was conducted in daylight and at room temperature (20°C). To neutralize the flavor, each person was given hot tea without sugar between sample evaluations. This evaluation was performed in 10 sessions by a team of 5 trained persons using a 5-point scale (1 = unacceptable and 5 = very acceptable) according to PN-EN ISO 8586:2014-03. During the two sessions, which took place every day, all panelists received 10 coded samples at the same time.

Microbiological determinations

On the 37th day of life, nine intestinal sections were taken from each group of broilers during post-slaughter necropsy, including sections of the small intestine, cecum and large intestine. The abundance of the tested microorganisms was determined by the culture method according to the current PN-ISO and microbiological laboratory standard (ISO 7218:2008). The evaluation of microbiological variability included the total numbers of bacteria: TBC – total bacteria count, TAe – total aerobic bacteria count, TAn – total anaerobic bacteria count, *Enterobacteriaceae* family (TEb), *E. coli* (TEc), *Enterococcus* (TEcc), *Staphylococcus aureus* (TSt), and those belonging to the genera *Lactobacillus* (LAB), *Lactococcus* (LAC), and *Bifidobacterium* (TBif). The study also included the determination of yeast abundance (TYC). The presence of *Salmonella* sp. (Sal), and *Campylobacter* sp. (Camp) was also determined. Commercial culture media, namely PEMBA (TAe), PCA (TBC), RCA (TAn), Chromcoli (TEb and TEc), Slantex-Bartley and BEA (TEcc), Beard-Parker (TSt), DRBC (TYC), MRS agar (LAB), M-17 (LAC), SS (Sal) and COLUMBIA agar (Camp) were used for the determinations. According to Faleiro (2015), the number of *Bifidobacterium* sp. was determined using MRS agar with lithium chloride (3.0 g/l), sodium propionate (200 mg/l) and cysteine (0.5 g/l). All cultures were incubated under conditions appropriate to microbial group, i.e.: oxygen-limited and temperature/time conditions of 37°C/48 h for TAn, LAB, LAC, TBif and Camp; 37°C/48 h for TAe, TBC, TEb, TEc, TEcc, TSt and Sal; and 24°C/72 h for TYC.

Statistical analysis

Statistica v.13.3 software was used to perform statistical calculations, and the results obtained were tested for normal distribution using Shapiro-Wilk tests. The Tukey test was used to determine the significance of differences between means. If a non-normal distribution was found, the non-parametric Kruskal-Wallis test was used. Principal component analysis (PCA) was also performed on the variable factors in each fragment of the intestinal mucosa of the broiler chickens.

Statistical analysis was performed to compare carcass and meat quality traits, as well as microbiological data between the different groups, using the least squares method of the GLM procedure (Statistica 13.3 PL) according to the following linear model:

$$Y_{ijk} = \mu + ai + bj + eijk$$

where: Y_{ijk} – the trait measured; μ – the overall mean; ai – the effect of group ($i = 1, 2, 3, 4$); bj – the effect of the panelist on the sensory characteristics of meat ($k = 1, 2, 3, 4, 5$); $eijk$ – the random error.

Results

Microbiological tests of the mucosa microbiota

The microbial fractions of distiller's dried yeast, as well as the respective feed mixes and feces of the production phases (starter, grower, finisher) are presented in a previous publication based on the same research dataset (Rybarczyk et al., 2023).

For all the intestinal sections (small intestine, cecum, and large intestine), the addition of dried distiller's yeast to the diet was shown to have had a significant effect on the total abundance of the selected microorganisms. It was found that the addition of 3% yeast to the diet resulted in a slight increase (log 5.34) compared to the control group (log 5.13). On the other hand, the average value obtained for group B (6%) was significantly higher ($P=0.014$) than the results obtained for group C (9%) yeast in the feed (log 4.75) – Figure 1.

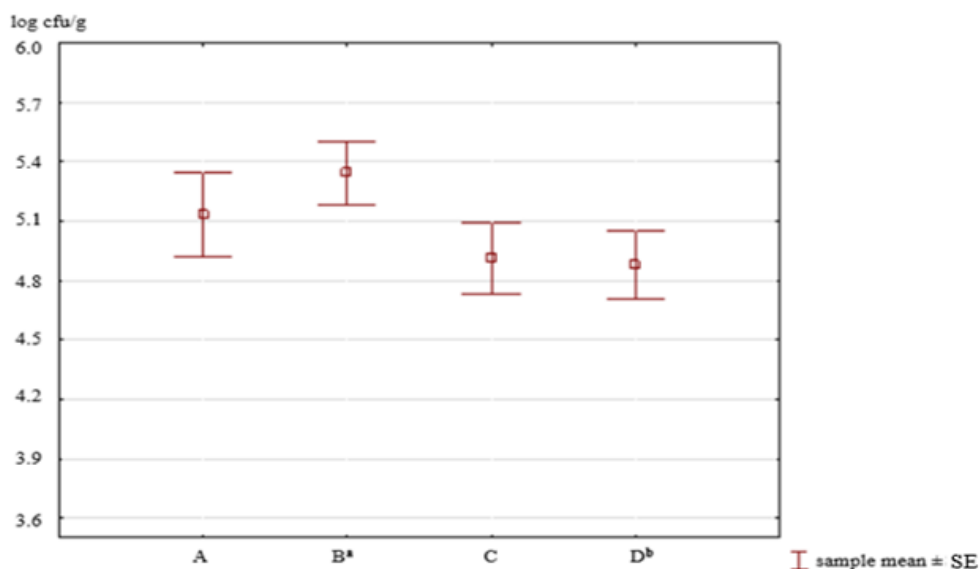
The statistical significance of the differences between the different microbial fractions in individual intestinal sections as well as for the whole is presented in Table 2. The microbial summary for the whole intestine (total) showed that 3% and 6% of inactive distiller's yeast added to the diet did not reduce the abundance of bacteria such as LAB, TEb or TEc. We observed only a tendency to reduce the number of TBC, TEc and TAn cells adhering to the chick intestinal mucosa. However, a statistically significant effect, as expressed in lower cfu counts, was obtained for all the above bacterial fractions in the group of broilers receiving 9% yeast in their diets.

For LAC, TBif and TYC, there was no effect from yeast supplementation in the diet.

The increase in the proportion of inactive yeast in the diet mainly affected a reduction in the number of bacteria in the small intestine and cecum (a low negative correlation), while remaining insignificant for the microorganisms inhabiting the large intestine (Figure 2).

Complementing the cluster analysis are the results for microbial fractions by broiler group and gut section (Table 2). All gut sections analyzed showed a significant reduction in the number of TBC with increasing levels of distiller's dried yeast in the diets. However, in the small intestine, a statistically significant effect (reduction of log 2.07 cfu) was already obtained in the group of broilers receiving 3% yeast in the diet compared to the control group. For the large intestine (reduction by log 1.32 cfu), the effect started at 6%, while for the cecum at 9%.

In the whole intestine, correlations were found between the level of distiller's yeast and the abundance of adherent TEcc (reduction by more than log 1) and LAB. However, in the case of LAB bacteria, the most pronounced effect of yeast was observed in the cecum (reduction by log 1.44 cfu) from the addition of 6% yeast to the broiler diet. A statistically significant effect of yeast supplementation on the tested microbiological parameters was also observed for *Enterobacteriaceae* (TEb) and *E. coli* (TEc). In the case of TEb, the labeled cfu of bacteria depended on the intestinal segment. In the small intestine, there was no significant variation (log 2.49–2.57), in the cecum it increased (log 3.24–4.40), and in the large intestine it decreased (log 5.26–4.45) with increasing yeast content in the broiler diets. Regardless of the total *Enterobacteriaceae* count determined, lower *E. coli* counts were found in all the groups receiving distiller's yeast, with the differences being most pronounced between the control group and those receiving 9% yeast in the diet.



A – control group; B – diet with 3% yeast; C – diet with 6% yeast; D – diet with 9% yeast; a, b – values with different letters differ significantly for $P \leq 0.05$. SE – standard error.

Figure 1. Average microbial counts determined in the guts according to the group of broiler chickens

Table 2. Colon microbiota composition depending on the group of broiler chickens

log cfu/g	TBC	TEb	TEc	TEcc	TSt	TAn	TAe	LAB	LAC	TBif	TYC
Small intestine											
A	6.65 A	2.49	4.45 A	3.71 A	0.53	6.84 A	4.67 A	5.01 A	4.41 A	5.64 A	4.44 A
B	4.85 B	2.57	4.30	3.34	0.00	5.52 B	4.39	5.59 A	4.66 A	5.29	4.06 A
C	4.58 B	2.55	4.12	2.89	0.00	4.69 B	3.87 B	4.89	3.94	4.48 B	3.69
D	4.53 B	2.50	3.91 B	2.54 B	0.00	4.73 B	3.57 B	4.23 B	3.09 B	4.18 B	3.12 B
SEM	0.311	0.222	0.112	0.158	0.166	0.096	0.134	0.137	0.205	0.174	0.452
P-value	0.002	0.495	0.001	0.010	0.910	0.001	0.002	0.001	0.001	0.002	0.001
Cecum											
A	6.89 A	3.24 B	6.78 A	3.14	1.00	7.23	5.84 A	7.33 a	6.67 A	6.00	4.49
B	6.52 A	4.00	6.36 A	3.89 A	0.50	7.31	6.44 A	7.18 a	6.16	6.31	4.71
C	6.04	4.15 A	5.84	3.71	0.51	6.89	4.85 B	5.89 b	4.94 B	5.48	4.69
D	5.82 B	4.40 A	5.32 B	2.81 B	0.00	6.54	5.14	6.21 b	5.61 B	6.51	4.40
SEM	0.120	0.169	0.133	0.240	0.163	0.158	0.453	0.126	0.214	0.300	0.307
P-value	0.001	0.010	0.001	0.005	0.139	0.307	0.001	0.012	0.001	0.111	0.080
Large intestine											
A	7.43 A	5.26 A	7.35 A	4.37 A	0.53	7.30	7.06 A	8.15 a	7.25	5.54	4.52
B	7.21	4.54	6.44 B	4.35	0.33	7.47	7.04 A	8.21 a	6.94	6.60	4.88
C	6.80 B	4.10 B	6.77 B	4.30	0.00	7.33	6.46 A	8.31 a	7.12	6.40	4.59
D	6.11 B	4.45 B	6.46 B	3.14 B	0.00	6.74	4.15 B	7.53 b	7.01	6.78	4.27
SEM	0.120	0.169	0.133	0.240	0.164	0.158	0.453	0.126	0.214	0.209	0.195
P-value	0.001	0.010	0.001	0.005	0.913	0.307	0.001	0.012	0.091	0.111	0.084
Total											
A	7.06 A	3.72	6.36 a	3.84 A	0.50	7.12 a	5.86 A	6.57 a	5.71	5.72	4.48
B	6.46	3.63	5.50	3.95 A	0.33	6.45	6.08 A	6.75 a	5.75	6.05	4.65
C	6.18	3.51	5.75	4.22 A	0.00	5.95	4.76 B	6.58 a	5.02	4.86	4.15
D	5.61 B	2.78	5.20 b	2.86 B	0.00	5.90 b	4.32 B	5.29 b	5.67	5.41	4.30
SEM	0.135	0.287	0.176	0.138	0.105	0.193	0.217	0.215	0.259	0.178	0.193
P-value	0.001	0.057	0.049	0.004	0.283	0.048	0.003	0.021	0.745	0.089	0.120

A – control group; B – diet with 3% yeast; C – diet with 6% yeast; D – diet with 9% yeast. TBC: total bacterial count; TAc: total aerobic bacterial count; TAn: total anaerobic bacterial count; TEb: *Enterobacteriaceae*; TEc: *E.coli*; TEcc: *Enterococcus sp.*; TSt: *Staphylococcus sp.*; LAB: *Lactobacillus sp.*; LAC: *Lactococcus sp.*; TBif: *Bifidobacterium sp.*; TYC: total yeast count. A, B – values in column with different letters differ significantly for $P \leq 0.01$; a, b – for $P \leq 0.05$. SEM – standard error of the mean.

Differences in the microbial response to yeast counts depending on the site of isolation were also observed for LAC and TBif. No changes in the abundance of LAC were found in the colon, in contrast to the cecum and small intestine environments. In these environments, significantly lower LAC counts were found in groups of cockerels receiving 6% and 9% yeast in the diet. Quantitative analysis of *Bifidobacterium sp.* (TBif) and yeast (TYC), considered in the context of the whole chicken intestine, showed no significant effect of yeast on mucosal adhesion capacity. However, different responses to yeast concentrations were observed depending on the intestinal segment. In the colon and cecum, the number of isolated TBif increased with increasing yeast in the diet by log 1.24 cfu and log 0.49 cfu, respectively. In the

small intestine, however, there was a statistically significant decrease in their abundance in groups of broilers receiving 6% and 9% yeast in the diet.

Analytical results from individual intestinal passages of broiler chickens did not show the presence of *Salmonella sp.*, *Campylobacter sp.*, or coagulase-positive *S. aureus*.

Carcass and meat quality

Based on the analysis of the results on the quality of broiler carcasses, there were no significant differences between the analyzed groups in the weight of pectoral muscles and thighs and their share in the estimated carcass weight, while maintaining similar body weight on the day of slaughter (Table 3).

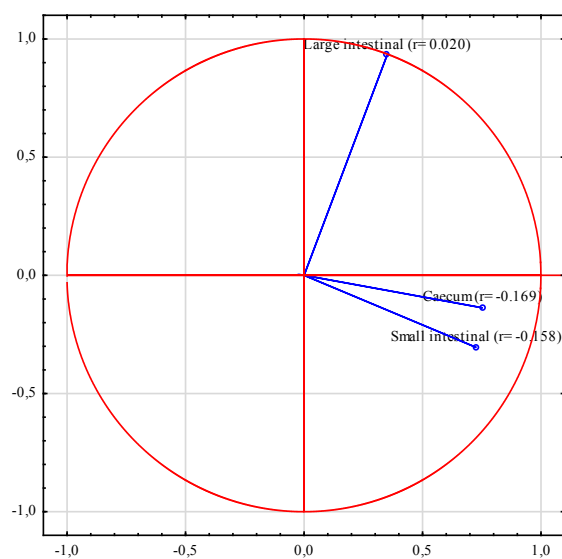


Figure 2. Cluster analysis (PCA) for variable factors in individual chicken guts fragments

Table 3. Slaughter value of broiler chicken carcasses

Traits	Groups of broiler chickens				SEM	P-value
	A	B	C	D		
BW (g)	2109.26	2172.60	2120.38	1996.67	37.55	0.407
Weight of breast muscle (g)	363.72	373.79	364.26	330.42	9.19	0.157
Weight of legs (g)	417.93	431.46	432.13	401.29	7.40	0.445
Estimated carcass weight (g)	1507.84	1578.98	1543.28	1472.74	28.90	0.511
Breast muscles (%)	17.15	17.35	16.94	16.34	0.18	0.109
Legs (%)	19.92	19.79	20.28	20.08	0.09	0.334

A – control group; B – diet with 3% yeast; C – diet with 6% yeast; D – diet with 9% yeast. BW – body weight of broiler chickens at slaughter on days 35 and 37 of life. SEM – standard error of the mean.

Table 4. Quality of fresh and frozen pectoral muscles

Traits	Groups of broiler chickens				SEM	P-value
	A	B	C	D		
Fresh muscles						
pH _{15min}	6.51	6.47	6.53	6.46	0.02	0.533
pH _{24h}	5.80	5.80	5.80	5.80	0.01	0.998
WHC (% of free water)	15.53	15.55	15.44	15.81	0.20	0.930
WHC (% of bound water)	79.17	79.06	79.25	78.86	0.27	0.963
Drip loss (%)	2.67	2.57	2.66	2.86	0.08	0.644
Lightness (L*)	61.45 b	62.53	62.92 a	62.66 a	0.20	0.046
Redness (a*)	5.31	5.21	5.11	5.63	0.09	0.244
Yellowness (b*)	17.50 b	18.30	18.61 a	18.69 a	0.15	0.014
Saturation (C*)	18.34 b	19.04	19.28	19.47 a	0.16	0.045
Frozen muscles						
Thawing loss (%)	8.36	7.74	7.23	8.09	0.25	0.408
Cooking loss (%)	19.83	20.52	19.88	20.28	0.33	0.858
Total fluid loss (%)	27.37	28.05	27.55	27.55	0.45	0.958
pH	5.79	5.78	5.80	5.77	0.01	0.784
Color (pts)	4.72	4.74	4.76	4.81	0.04	0.873
Aroma (pts)	4.14	4.12	4.13	4.04	0.03	0.669
Tenderness (pts)	3.69	3.76	3.76	3.97	0.06	0.439
Juiciness (pts)	3.55	3.39	3.65	3.55	0.06	0.429
Flavor (pts)	3.93	3.94	4.12	4.03	0.03	0.127

A – control group; B – diet with 3% yeast; C – diet with 6% yeast; D – diet with 9% yeast. a, b – values in rows with different letters differ significantly for $P \leq 0.05$. SEM – standard error of the mean. WHC – water holding capacity.

Table 5. Chemical composition and elemental content in pectoral muscles

Items	Groups of broiler chickens				SEM	P-value
	A	B	C	D		
Basic chemical composition (%)						
Dry matter	25.80 a	25.77 a	25.62	25.25 b	0.08	0.044
Total protein	22.70	22.42	22.58	22.27	0.08	0.251
Intramuscular fat	1.41	1.49	1.39	1.33	0.03	0.403
Ash	1.39	1.37	1.40	1.39	0.01	0.920
Macro- (g/kg) and microelements (mg/kg)						
Ca	0.06	0.06	0.07	0.06	0.00	0.125
Mg	0.31	0.31	0.33	0.31	0.01	0.534
Na	0.48	0.55	0.56	0.53	0.02	0.336
K	3.76	3.58	3.69	3.70	0.08	0.872
P	2.27	2.29	2.32	2.26	0.02	0.596
Cu	1.66	1.99	1.98	1.62	0.13	0.701
Mn	0.35	0.33	0.33	0.34	0.02	0.976
Fe	14.18	12.89	12.74	14.69	0.58	0.816
Zn	6.32	5.47	5.48	6.12	0.16	0.081
Se	0.07	0.06	0.06	0.06	1.01	0.854

A – control group; B – diet with 3% yeast; C – diet with 6% yeast; D – diet with 9% yeast. a, b – values in rows with different letters differ significantly for $P \leq 0.05$. SEM – standard error of the mean.

Based on the results presented in Table 4, there were no significant differences between the analyzed groups of broiler chickens in water binding capacity (WHC) of the meat, expressed as percentages of both free water and bound water, and in the amount of drip loss for fresh breast muscle. There were also no significant differences between the broiler groups in the water-binding properties of frozen meat for thaw loss, cooking loss, and total water loss. Based on the results of breast muscle pH measurements – 15 minutes and 24 hours after slaughter, pH of the frozen meat – there were no significant differences between the groups of broiler chickens. Also, in terms of the analysis of sensory evaluation characteristics, there were no significant differences between groups of broiler chickens in color, aroma, tenderness, juiciness, and flavor of breast muscles.

The study found significant differences between the analyzed groups of chickens in the characteristics of breast muscle color, i.e. lightness (L^*), yellowness (b^*), and chroma (C^*), but not for redness (a^*). Higher color lightness (L^*) was observed in the breast muscles of the chickens fed the diets supplemented with 6% and 9% yeast (62.92 and 62.66, respectively) compared to the control group of chickens (61.45). Significantly higher chroma (C^*) was characteristic of the breast muscles of broiler chickens receiving diets with 9% distiller's dried yeast (19.47) compared to the chickens in the control group (18.34). Higher yellowness (b^*) was characteristic of the meat of chickens receiving 6% and 9% distiller's dried yeast in their diets (18.61 and 18.69, respectively) compared to chickens in the control group (17.50).

Chemical composition and elemental content

Based on the analysis of the basic chemical composition of the breast muscle, significant differences in dry matter content were observed between the groups of broilers (Table 5). The control group and those with 3% yeast in the diet had significantly higher meat dry matter content (25.80% and 25.77%, respectively) than those receiving 9% yeast in the diet (25.25%). However, no significant differences were found between the groups of broilers in the content of ash, total protein and intramuscular fat in the breast muscle. Also, no significant differences were found between the analyzed groups of broilers in the content of the analyzed macronutrients (Ca, Mg, Na, P, and K) and micronutrients (Cu, Mn, Fe, Zn, and Se).

Discussion

Mucosal microbiome

The gastrointestinal tract (GIT) microbial community is a collection of commensal, symbiotic and pathogenic microorganisms that have a real impact on the metabolism, immunity and welfare of animals, consequently influencing production efficiency (Rybarczyk et al., 2023). Externally induced changes in the composition of the microbiota can have beneficial or adverse effects. The best tool for their regulation is proper diet selection, which optimizes the functionality of the digestive tract.

The primary function of the digestive tract is the digestion and absorption of nutrients. However, each sec-

tion of the digestive tract has different environmental conditions that affect the characteristics and functionality of the microbial community (Roto et al., 2015). The small intestine is the primary site of nutrient absorption from the ingested feed (Xiao et al., 2017). This results in the dominance of specific groups of bacteria, among which the most frequently isolated are representatives of *Lactobacillus*, *Enterococcus*, *Clostridium* and *Streptococcus* (Fathima et al., 2022). In our study, *Clostridium*, *Lactobacillus* and *Bifidobacterium* (group A) were also the dominant groups. We found no significant differences in the species profile in the cecum, except for the increased frequency of *E. coli* isolation, which was also found by Roto et al. (2015).

In our study, the high enzymatic activity and the presence of antimicrobial agents contributed to the significantly lower abundance of isolated microorganisms in the small intestine compared to the cecum. Microbial proliferation was also favored by the anatomical structure of this part of the intestine, which makes it the site of the largest microbial complex. The function of the cecum is essentially to absorb nutrients produced by microbial fermentation processes (Apajalahti and Vienola, 2016). This is especially true for increasing the availability of vitamins K and B and essential amino acids, and the digestion of non-starch polysaccharides (Sergeant et al., 2014; Attia et al., 2022). The metabolic activity of microorganisms in the cecum also influences the microbiota profile of the colon, where further nutrient absorption, microbial fermentation, and detoxification of harmful substances occur (Gong et al., 2002).

It is widely accepted that a greater diversity and species richness of gut bacteria has a beneficial effect on the microbiome and consequently produces health-promoting effects. The study by Ezema et al. (2012) showed improved protein and fiber digestibility in broiler chickens fed a yeast probiotic, which was related to the yeast's ability to increase the number of beneficial microorganisms and yeast enzymes in the GIT (Attia et al., 2020).

The introduction of probiotic microorganisms, including inactivated yeast (postbiotics), into poultry diets is closely related to the concept of competitive exclusion. We should emphasize here the ecological importance of *Enterobacteriaceae* in the gut. In our study, the analyses showed that in the cecal area, with an increase in the amount of yeast, the number of *Enterobacteriaceae* isolated increased, accompanied by a decrease in the labeled cfu of *E. coli* (Table 2). In other intestinal sections, no such relationship was found. However, an inhibitory effect of distiller's yeast on total bacterial count (TBC), *Enterobacteriaceae* (TEb), *E. coli* (TEc), and fecal coliforms (TEcc) was observed. In terms of statistically significant differences, this is most evident between the control group and the chickens receiving the highest content (9%) of distiller's dried yeast. Similar effects were obtained by Aguilar-Toalá et al. (2018), who showed a reduction in the number of pathogenic microorganisms, i.e. *E. coli*, *Salmonella typhimurium*, and vancomycin-

resistant *Enterococcus*. Aguilar-Toalá et al. concluded that the microbiological changes in the intestine were primarily due to a decrease in pH. This was accompanied by the presence of postbiotic antimicrobial compounds, including short-chain fatty acids, peptides, proteins, and organic acids. Similar results have also been found for the fecal microbiome of broiler chickens with diets containing yeast (from 3% to 9%), with higher levels of glucans, mannans, and lactic acid, and thus lowered pH, compared to control (Rybarczyk et al., 2023). These effects may be explained by the presence of yeast cell wall products that prevent or mitigate the effects of pathogen colonization of the poultry gut, thus reducing the competition for nutrients between pathogens and bacterial cells of the host gastrointestinal tract (Fathima et al., 2022). It is also important to highlight the mechanism of specific binding of mannose present in the yeast cell wall to bacteria. Yeast, especially postbiotics, do not permanently colonize the intestine and therefore, all molecules bound to their surface (cell wall), including bacteria, are expelled during digestion, thus reducing bacterial colonization (Line et al., 1997; Vohra et al., 2016; Aggarwal et al., 2022).

The results of our study confirm the findings of other researchers. Javadi et al. (2012) showed a significant reduction in total bacterial counts, *Streptococcus* sp., *E. coli*, and *Lactobacillus*, similar to the articles by Elghandour et al. (2019) and Chuang et al. (2019). However, they contradict the observations of Markazi et al. (2017) and Chuang et al. (2021) who found an increase in the abundance of endogenous *Lactobacillus* and *Bifidobacterium* or no significant effect on the presence of lactic fermentation bacteria, *E. coli* and *Salmonella* (Kang et al., 2015) as a result of the addition of prebiotic yeast to broiler chicken feed. In another study, the use of live SC yeast at 0.4% to 1.6% influenced a reduction of *E. coli*, *Klebsiella* sp., *Staphylococcus* sp., *Micrococcus* sp., *Campylobacter* sp., and *Clostridium perfringens* in layer hens, while the number of *Lactobacilli* increased (Hasanein and Soliman, 2010). According to those authors, the addition of live yeast at 0.4% or 0.8% to laying hen diets may improve productive performance and nutrient utilization via the inhibitory effect of yeast on pathogenic bacteria.

Our study showed that the use of the higher doses of distiller's yeast in the diet (especially 9%) had a negative effect on the abundance of endogenous LAB, LAC, and TBif bacteria in the small intestine, LAB and LAC in the cecum and LAB in the large intestine. Based on the analysis of the significance of the differences between the groups of broilers, it can be concluded that the abundance of LAB, LAC, and TBif, regardless of the intestinal section from which the mucosa was analyzed, was similar in broilers from the control group and the group receiving 3% distiller's dried yeast in the diet. It should also be mentioned that, according to the results of the same experiment (Rybarczyk et al., 2023), there were no significant differences in the abundance of LAB, LAC,

and TBif in the feces of broiler chickens, regardless of the fattening period.

An evaluation of the effect of postbiotic yeast on the overall microbial profile of the chicken digestive tract showed that as the yeast concentration increased (in addition to the effect obtained for 3% yeast), the abundance of isolated microorganisms decreased (Figure 1). One would expect a stimulation of bacterial growth, at least for LAB. However, it should be noted that too much growth of a particular group of microorganisms can cause a microbial imbalance in the stomach. In addition, too large an increase in microbial abundance can lead to competition for nutrients between the host and the microbes in the gut, since they use the same nutrients (carbohydrates, proteins, etc.) as a substrate for proliferation. The established effect of postbiotic yeast on the abundance, mainly of bacteria, in the GIT is part of the biocontrol that leads to the maintenance of rigor between the different groups of microorganisms and their host.

Carcass and meat quality

Based on our study, there were no significant differences in the carcass value of broiler chickens. However, based on the average values, it is possible to notice a tendency that broilers from the group receiving 3% distiller's yeast had higher body weight and, at the same time, higher estimated carcass weight and breast muscle weight and proportion compared to the groups of broilers receiving higher doses of distiller's yeast – 6% and 9% – in the feed, as well as to the control group. In the same experiment, the most favorable results in terms of feed efficiency were also obtained in the group of broilers receiving 3% distiller's dried yeast in the diet (Rybarczyk et al., 2023). In another study, Paryad and Mahmoudi (2008) found that the addition of SC yeast to the diets of broiler chickens at 1.5 and 2% improved carcass quality by increasing the proportion of breast muscle, leg, liver, heart, gizzard, and abdominal fat compared with the control group and those receiving 0.5% yeast in the diet. Karaoglu and Durdag (2005) showed that the addition of SC yeast (1 and 2%) to the diet of Ross 308 chickens did not affect final body weight and carcass characteristics. On the other hand, Ahmed et al. (2015) showed that the inclusion of 1% SC yeast in the diet of Hubbard chickens, compared to 2% and 3%, had a beneficial effect on carcass performance as expressed by higher breast and leg muscle weights. According to these authors, 1% SC yeast can serve as a natural substitute for antibiotics.

In our study, in terms of breast muscle quality and composition, we found that significant differences between groups of broiler chickens were in the characteristics of color (L^* , b^* , C^*) and dry matter content. The results indicate that chickens receiving the highest doses of distiller's dried yeast in their diets had the highest lightness (L^*), yellowness (b^*), and chroma (C^*), and the lowest dry matter content in meat. In a study by Zhang et al. (2005) in broiler chickens, the beneficial effects of SC whole yeast (WY), SC extract (YE), and

SC cell wall (CW) were shown to improve tenderness and have an antioxidant effect in breast muscle. In contrast, the results of a study in Arbor Acres broiler chicks showed that the addition of *Saccharomyces cerevisiae* at a dose of 0.04% versus 0.02% improved BW, FCR, and reduced FI in broiler chicks between 1 and 38 days of age, but had no effect on the basal chemical composition (total protein, intramuscular fat, dry matter, and ash) and physical properties (pH, tenderness, color, WHC) of breast and thigh muscles (Attia et al., 2020). Another study found that the addition of 0.5% dried SC yeast in combination with 0.5% multi-species probiotic preparation (*Lactobacillus acidophilus*, *Bacillus subtilis*, and *Aspergillus oryzae*) in the diet of broiler chickens improved the results of fattening efficiency and lower abdominal fat in carcasses, as well as higher content of total protein, globulin and glucose and lower content of total lipids and serum cholesterol compared to the control group. These authors demonstrated that the combination of SC yeast and the aforementioned probiotic in bird diets increased the content of total protein and intramuscular fat in breast muscle, decreased the content of malondialdehyde, and increased the content of polyunsaturated fatty acids (Hussein and Selim, 2018).

Conclusions

It was demonstrated that the addition of distiller's yeast at higher doses (6% and 9%) with respect to the control group, as well as the addition of 3% yeast, affects a significant reduction in the total microbial count including *Enterobacteriaceae*, *Enterococcus*, *E. coli*, and *Lactobacillus* of the intestinal mucosa, as well as lower dry matter content and higher lightness (L^*), yellowness (b^*), and chroma (C^*) of the breast muscle color. In summary, the results obtained indicate that the optimal proportion of the analyzed distiller's dried yeast in the diet of the broiler chickens, based on the results obtained in terms of carcass and meat quality and the results of fattening efficiency presented in another publication (Rybarczyk et al., 2023), should not exceed 3%. This may be because as the yeast content increases, the total abundance of bacteria in the GIT, including LAB, decreases, thus reducing the digestibility of protein and fiber.

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