AGRICULTURAL AND FOOD SCIENCE

Agricultural and Food Science (2024) 33: 139–150

https://doi.org/10.23986/afsci.145308

Effect of distillers yeast in feed on texture, fatty acid profile and antioxidant properties of breast muscle of broiler chickens

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This research aimed to assess the impact of replacing a partially of post-extraction soybean meal in the diet with varying amounts of distillers yeast (3, 6 and 9%) on the composition and quality in the pectoral muscles of broiler chickens. Findings revealed that cockerels fed with 3% yeast exhibited elevated oleic acid levels and reduced n-6 fatty acids compared to those fed with 6% and 9% yeast. Furthermore, chickens consuming 3% yeast displayed higher antioxidant capacity (ABTS) and decreased levels of linoleic acid and its ratio to α-linolenic acid compared to the 9% yeast group. Moreover, muscles from cockerels on the 3% yeast diet and the control group demonstrated higher shear force, lower n-6/n-3 ratio and lipid peroxidation rate (TBARS) than those on the 9% yeast regimen. Conversely, cockerels on the 9% yeast diet exhibited reduced gumminess and ferric reducing antioxidant power (FRAP) compared to the control group. The study highlights yeast's role in altering broiler chicken meat's fatty acid profile, texture, and antioxidant properties.

Key words: cockerels, *Saccharomyces cerevisiae*, lipid quality, oxidative status, rheological tests

Introduction

To meet the nutritional needs of the ever-growing human population, poultry products are an essential source of complete protein. The need to meet the growing consumer demand for animal protein often requires the prophylactic use of antibiotics to achieve maximum production with the least possible losses (Fathima et al. 2023). However, due to the emergence of resistant strains of bacteria, restrictions have been placed on the use of antibiotics in animal production. Consequently, there is a strong need to find effective alternative antibiotics to maintain high herd health status while maintaining good production performance and quality meat with functional food characteristics (Huyghebaert et al. 2011, Sharma et al. 2014).

The most used yeast cultures in poultry production are fermentation-produced *Saccharomyces cerevisiae* (SC) (Feye et al. 2019). According to Fathima et al. (2022), SC fermentation products can be classified as paraprobiotics, which are non-viable microbial cells or fractions of cells that are immunologically active and thus have a beneficial effect on the host. Yeast paraprobiotics mainly contain inactivated or dried yeast cells and cell wall components. Yeast has biologically valuable proteins, a B-vitamin complex and essential trace elements that serve to produce extracellular enzymes such as amylases, p-galactosidase and phytase (Vohra et al. 2016, Sharma et al. 2020). Multi-strain yeast fractions contain polysaccharides, nucleotides and glycoproteins that can interact with host immune cells (Xie et al. 2022). The efficacy of yeast-derived para probiotics in poultry intestinal infections is being studied, and the results are promising (Zhao et al. 2010, Fathima et al. 2023). Previous studies indicate that yeast cell wall products modulate the host immune response and enzyme activity and will reduce the abundance of intestinal pathogens in poultry, including *E. coli* and *Salmonella* (Shanmugasundaram et al. 2014, Li et al. 2016, Markazi et al. 2017) and thus improve health and fattening efficiency (Xue et al. 2017). A small number of studies have also proven that supplementation with SC yeast and its products in the diet of broiler chickens improves the tenderness and oxidative stability of pectoral and thigh muscles (Zhang et al. 2005a, 2005b, Li et al. 2016, Li et al. 2017, Wang et al. 2022). In addition, Hussein and Selim (2018) also showed that a broiler chicken diet based on SC yeast and certain probiotics influences the fatty acid profile of muscles, increasing PUFA content, mainly n-3. Scientific literature lacks studies examining the utilization of yeast-fermented agricultural wheat distillery stillage cultures SC for feed purposes, particularly regarding their impact on meat quality and antioxidant status and fatty acid profile.

Received 26 April 2024 / Accepted 27 May 2024
The Scientific Agricultural Society of Finland
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The study aimed to determine the effect of partial replacement of post-extraction soybean meal in the feed with a different proportion of distillers yeast on the fatty acid profile, texture profile analysis (TPA), shear force and indicators of oxidative changes (TBARS) in the breast muscles of broiler chickens.

Materials and methods

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).

The study was conducted on 112 one-day-old cockerels of the Ross 308 line, with an average body weight of 40.72 ± 0.47 g, subjected to a 35–37-day fattening period. All broiler chickens were fed complete, balanced feed mixtures, *ad libitum* feed and water ration system. Table 1 presents the nutritional value and fatty acid profile, as well as the antioxidant activity of ABTS (2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) and FRAP (ferric reducing antioxidant power) for distillers yeast made from pure SC cultures on wheat distillers broth medium and feed mixes for each group of broiler chickens.

Table 1. Nutritional value, fatty acid profile, antioxidant activity (ABTS and FRAP) of feed mixtures for broiler chickens

Item	Distillers yeast	Starter				Grower				Finisher			
		A	B	C	D	A	B	C	D	A	B	C	D
Nutritional values (%)													
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^{-1})	20.46	19.65	19.66	19.65	19.62	19.91	19.86	19.92	19.82	20.07	20.06	19.90	19.94
Fatty acids (% of 100 g fat)													
C14:0	0.31	0.05	0.05	0.05	0.04	0.06	0.05	0.06	0.03	0.04	0.05	0.05	0.03
C16:0	17.75	6.02	6.44	6.21	5.62	6.07	5.82	6.17	5.82	4.93	6.17	5.90	5.14
C17:0	0.18	0.07	0.07	0.07	0.06	0.07	0.07	0.08	0.07	0.06	0.06	0.06	0.06
C18:0	1.94	1.92	2.06	1.85	1.85	1.92	1.83	1.80	1.74	1.82	1.87	1.78	1.73
C20:0	0.00	0.63	0.66	0.60	0.65	0.63	0.62	0.59	0.60	0.69	0.63	0.62	0.64
C22:0	0.00	0.35	0.36	0.32	0.37	0.34	0.33	0.33	0.32	0.41	0.33	0.32	0.36
C16:1	0.00	0.16	0.16	0.15	0.13	0.16	0.15	0.15	0.13	0.13	0.15	0.15	0.15
C18:1 n-9	17.80	58.02	56.12	56.81	58.72	57.84	58.15	57.59	56.11	60.60	56.46	57.91	59.38
C18:2 n-6	55.89	24.91	25.81	26.20	25.26	24.69	25.07	25.24	27.84	23.49	26.24	25.25	24.78
C18:3 n-3	4.47	6.30	6.69	6.22	5.64	6.64	6.30	6.51	5.84	6.03	6.49	6.40	6.04
C20:1 n-9	1.23	1.07	1.04	1.02	1.12	1.07	1.11	1.06	1.04	1.17	1.08	1.10	1.18
C20:5 n-3	0.00	0.18	0.19	0.16	0.19	0.17	0.17	0.14	0.17	0.23	0.15	0.16	0.20
C22:1 n-9	0.42	0.22	0.24	0.22	0.24	0.23	0.22	0.21	0.19	0.27	0.22	0.20	0.21
C24:1 n-9	0.00	0.09	0.10	0.10	0.12	0.11	0.11	0.09	0.10	0.14	0.10	0.10	0.11
SFA	20.19	9.04	9.65	9.12	8.58	9.09	8.72	9.01	8.58	7.95	9.10	8.73	7.97
MUFA	19.45	59.56	57.67	58.30	60.33	59.41	59.74	59.10	57.57	62.31	58.01	59.47	61.01
PUFA	60.37	31.40	32.68	32.58	31.08	31.49	31.54	31.89	33.85	29.74	32.88	31.80	31.02
ABTS (mmol TE/100 g dm)	1.21	0.82	0.67	0.87	0.69	0.68	0.66	0.69	0.62	0.68	0.52	0.53	0.53
FRAP (µmol TE/100 g dm)	139.72	71.46	72.72	78.00	84.61	72.16	70.52	71.75	75.36	65.64	58.18	60.70	63.08

A = control group; B: = diet with 3% addition of distillers yeast; C = diet with 6% addition of distillers yeast; D = diet with 9% addition of distillers yeast; dm = dry mass; GE = gross energy

A detailed characterization of the chemical and microbiological composition of the dried inactive distillers yeast, as well as the composition and nutritional value of the feed mixtures for each group and details of the maintenance conditions for broiler chickens, were presented in an earlier publication of this research (Rybarczyk et al. 2023).

Chicks were randomly assigned to one of four groups (28 cockerels in each, included 7 replicates), which differed in the amount of distillers dried yeast added to the feed. The control group (A) of broiler chickens was fed a mixed feed with no yeast; group B chickens received a mixed feed with 3% yeast addition; group C broiler chickens received a mixed feed with 6% yeast addition; group D broiler chickens received a mixed feed with 9% yeast addition. Broilers were fed complete feed mixtures of Starter (1–10 days of age), Grower (11–27 days of age) and Finisher (28–37 days of age). Regardless of the fattening period, all feed mixtures were prepared once for the entire time they were fed to the broilers.

On the 35th and 37th days of fattening, 26 birds from each group of broiler chickens were slaughtered. On each of these days half of the birds representing all experimental groups and replicates were slaughtered. During dissection, 15 min post mortem, three samples were taken from right breast muscles - one for determination of intramuscular fat content, another for determination of fatty acid profile, and the third for determination of antioxidant potential. The left pectoral muscles were used to determine the texture profile and shear force. The pectoral muscles collected during cockerel dissection were placed in sealed bags and labeled accordingly. Samples prepared this way were placed into thermoses (about 4 °C), transported to the laboratory (1 h) and stored in refrigerator at 4 °C for about 24 hours. Subsequently, pectoral muscle samples intended for determination of fatty acid profile were stored at –80 °C for about one month, and muscles for rheological tests and meat fat content were stored at –21 °C for about one month. In contrast, muscle samples intended to determine antioxidant activity were stored at 4 °C or –20 °C, depending on when the determinations were made.

Basic chemical composition

Reference methods by 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 basic chemical components were carried out:

• Dry matter was determined by drying 2 g samples at 102 °C for 12 hours to constant weight (950.46B, p. 39.1.02) in a SUP-4M laboratory dryer (Wawa-Med, Poland).

• Total protein content was determined by the Kjeldahl method (conversion factor 6.25) (992.15, p. 39.1.16) in a Kjeltec 2300 Foss Tecator distillers (Häganäs, Sweden).

• Crude fat was determined by petroleum ether extraction (960.39 (a), p. 39.1.05) using a Büchi Extraction System B-811 extraction apparatus (Büchi, Switzerland).

• Crude ash was determined by burning the samples at 550°C for 10 hours (920.153, p. 39.1.09) in an FCE 7SHM Czylok muffle furnace (Poland).

• Gross energy was determined using a KL-14 calorimeter (Precyzja-Bit PPHU Sp. z o.o. Bydgoszcz, Poland).

Fatty acid profile

Lipids were extracted from feed and meat using the Bligh and Dyer (1959) method. Single-phase lipid solubilization with a chloroform-methanol mixture (1:1) was used. Fatty acid methyl esters (FAME) were obtained from the tissue by alkaline hydrolysis of extract of lipids with 0.5 N sodium methylate (CH_3ONa) (AOAC 2016). Next, the FAMEs were separated using a gas chromatography apparatus coupled with a mass spectrometer (Agillent Technologies 7890A), and equipped with a split/splitless type injector. Conditions of FAME separation were as follows: column SPTM 2560, 100 m 0.25 mm ID, 0.20 lm film, catalogue no. 24056; carrier gas: helium at a constant flow rate of 1.2 ml min^{-1}; split 1:50; injector temperature: 220 °C; detector temperature: 220 °C; programmed furnace temperature: 140 °C (5 min) increased to 240 °C at a rate of 4 °C min^{-1}; analysis time: 45 min. The qualitative interpretation of chromatograms was based on comparing retention times and mass spectra of the particular FAMEs of the sample with those of analogous FAME standards by Sigma Company (Lipid Standard). As an internal standard, C 19:0 was used. The average of three parallel determinations was taken as the quantitative result. The fatty acid profile analysis results were presented as % per 100 g of feed or breast muscle lipids.

Lipid quality indices

Fatty acids were grouped as follows:

• ΣPUFA (Polyunsaturated Fatty Acids) = (C18:2 n-6 + C18:3 n-3 + C20:2 n-6 + C20:3 n-6 + C20:4 n-6 + C20:4 n-3 + C22:4 n-6 + C22:5 n-3 + C22:6 n-3);

• ΣMUFA (Monounsaturated Fatty Acids) = (C16:1 + C18:1 n-7 + C18:1 n-9 + C20:1 n-9);

• ΣSFA (Saturated Fatty Acids) = (C14:0 + C15:0 + C16:0 + C17:0 + C18:0);

• ΣUFA (Unsaturated Fatty Acids) = ΣMUFA + ΣPUFA.

Lipid quality indices, in relation to human health, were calculated as follows:

• ΣPUFA/ΣSFA;

• ΣMUFA/ΣSFA;

• ΣUFA/ΣSFA;

• ETA (Eicosatetraenoic acid) + DHA (Docosahexaenoic acid) = C20:4 n-3 + C22:6 n-3;

• LA (Linoleic acid)/ALA (α-Linolenic acid) = C18:2 n-6 / C18:3 n-3;

• Σn-3 = (C18:3 n-3 + C20:4 n-3 + C22:5 n-3 + C22:6 n-3);

• Σn-6 = (C18:2 n-6 + C20:2 n-6 + C20:3 n-6 + C20:4 n-6 + C22:4 n-6);

• Σn-6/Σn-3;

• DI (5) (Δ5 – Desaturase Index) = (C20:3 n-6 + C20:4 n-6);

• DI (18) (Δ9– Desaturase Index activity for 18:0) = (C18:0 + C18:1 n-9);

• EI (Elongation Index) = 100 [(C18:0 + C18:1 n-9) / (C16:0 + C16:1 + C18:0 + C18:1 n-9)];

• NVI (Nutritional Value Index) = (C18:0 + C18:1 n-9)/C16:0);

• DFA (Hypocholesterolemic effect) = (ΣUFA + C18:0);

• OFA (Hypercholesterolemic effect) = (C14:0 + C16:0).

Antioxidant activity determinations

Antioxidant activity in breast muscle was analyzed using ABTS and FRAP methods. For this purpose, 15 pectoral muscle samples were collected from each group of broiler chickens, which were divided into three periods of analysis (24 h, 7 and 90 days). The muscle samples for the first two periods were stored at 4 °C. For the third period (90 days), breast muscle samples were frozen and stored at –20 °C (LG, M600, Seoul, South Korea). After this time, the pectoral muscles were thawed for 24 hours at 4 °C. Then, the breast muscles were minced using an electric shredder (model MM/1000/887, Zelmer, Rzeszow, Poland) with a 2 mm sieve diameter. In addition, ABTS and FRAP were determined in Starter, Grower and Finisher feeds after prior grinding according to the abovementioned methodology.

Before determining the reduction of ABTS cation radical and FRAP iron ions, ground muscles were frozen at –80 °C (Arctiko, MUF 40, Esjberg, Denmark) and then subjected to a 24-hour freeze-drying process (Alpha 1-2 LD plus, Martin Christ GmbH, Osterode am Harz, Germany) under 0.220 mbar vacuum. The resulting freeze-dried products were vacuum-packed (Hendi, Profi Line 410, De Klomp, The Netherlands).

The extracts used for the determination of antioxidant activity (ABTS and FRAP) were prepared by pouring 4 ml of phosphate buffer with pH = 6.8 into lyophilized ground chicken breast muscles (about 0.2000 g), followed by sonification for 15 minutes (Sonic 6D, Polsonic, Warsaw, Poland) and left for 24 hours at 4 °C. After this time, the extracts were again subjected to ultrasound for 15 minutes. After sonification, the samples were centrifuged (MPW-380R; MPW Med. Instruments, Warsaw, Poland) at 10 000 rpm for 10 minutes at 4 °C. The supernatant was poured into 10 ml PET tubes. The resulting extracts were stored at –20 °C until the start of analyses (2 days).

ABTS determination was performed according to the method described by Re et al. (1999). The starting solution of ABTSº+ cation radical, containing 7 mmol of ABTSº+ and 2.45 mmol of potassium peroxodisulfate (VI), was diluted with redistilled water before analysis so that at λ=734 nm its absorbance was ~ 0.700. 0.03 ml of chicken breast muscle extracts were taken into spectrophotometer cuvettes, and the control sample contained redistilled water and the addition of 3 ml of ABTSº+ solution. After 6 minutes, the absorbance of the samples (λ=734 nm) was measured against redistilled water (Shimadzu, UV-1900 i, Kyoto, Japan). The analysis results were expressed in mmol Trolox (TE)/100 g of breast muscle dry weight.

FRAP potential was determined according to Benzie and Strain (1996). FRAP reagent was prepared by mixing acetate buffer pH = 3.6 (300 µmol), 10 µmol 2,4,6-Tripyridyl-S-triazine (TPTZ) in 40 µmol HCl and 20 µmol $FeCl_3$ in a ratio of 10:1:1 (v/v/v). To the spectrophotometer's cuvettes containing 0.8 ml of chicken breast muscle extracts, 0.2 ml of redistilled water and 3 ml of FRAP working solution were added and mixed thoroughly. After 10 min, the absorbance of the solution was measured at 593 nm against redistilled water (Shimadzu, UV-1900i, Kyoto, Japan). The analysis results were expressed as µmol Trolox (TE)/100 g of breast muscle dry weight.

Thiobarbituric acid reactive substances analysis (TBARS)

The procedure for determining TBARS was carried out according to the method of Salih et al. (1987) as modified by Pikul et al. (1983). Ten grams of ground pectoral muscle were homogenized in 35 ml of cold 4% perchloric acid with 0.01% alcoholic solution of butylhydroxytoluene (2,6-bis(1,1-dimethylethylbutyl)-4-methylphenol - BHT) in a homogenizer (T 25, Ika Ultra-Turrax Corp., Staufen, Germany) for 10 minutes at 4000 rpm. The homogenized sample was filtered through a Whatman No. 1 filter into a 50 ml volumetric flask and made up to the mark with perchloric acid. The filtrate (5 ml) was mixed with 5 ml of 0.02 M 2-thiobarbituric acid (TBA) solution in stoppered tubes. The tubes were incubated in boiling water for 1 hour and then cooled to room temperature. Absorbances were measured at 532 nm using a spectrophotometer (Specord 210, Analytic Jena AG, Jena, Germany) compared to a control sample containing 5 ml of perchloric acid and 5 ml of 0.02 M TBA solution. As Krzywicki (1982) recommended, TBARS values in units of mg of malondialdehyde in 1 kg of meat were calculated by multiplying the absorbance value of the samples by 5.5.

Texture Profile Analysis (TPA) and Share Force (SF)

After thawing at 4 °C for 24 hours, the breast muscles were placed in heat-resistant plastic bag. Next, they were immersed in a water bath at 80–81 °C until the internal muscle temperature reached 72 °C (model LWM 12/200 WSL Sp. z o.o. Świętochłowice, Poland). The samples were then allowed to cool in the air to 20 °C. The internal temperature was monitored with a handheld thermometer. The texture profile and shear force of the test samples were determined using an Instron universal testing machine, model 5543 (Canton, Norwood, MA).

Texture profile analysis was conducted utilizing techniques detailed by Bourne (1978, 2002). Two samples parallel to the longitudinal orientation of the muscular fibers were excised from each muscle. Meat samples for TPA assessment were cylindrical in form, 1 cm high, and 1.27 cm in width at the base. They were acquired using a handheld steel cork borer. Each specimen was compressed in 2 successive cycles of 70% compression with 5 s intervals between cycles, employing a cylindrical probe of 5.7 cm width. The sliding speed of the head was set at 50 mm/min. From the resultant force-time curve, the subsequent parameters were calculated (Bluehill 3 – examination Software Instron): hardness [N] (the maximum peak force during the first compression); springiness (the height that food recovers during the time between the end of the first compression and the beginning of the second compression); cohesiveness (ratio of the positive force area during the second compression to that during first compression); gumminess - the product of hardness and cohesiveness; and chewiness - the product of springiness and gumminess.

SF was determined using a Warner-Bratzler knife and expressed in Newtons (N). The speed of the knife travel was set at 50 mm min^{-1}. Two samples each (height 1 cm and diameter 2.54 cm) were cut from a portion of the pectoral muscle, placed parallel to the muscle fibers, and analyzed.

Statistical analysis

Statistica v.13.3 software was used to perform statistical calculations. The results obtained were tested for normal distribution using the Shapiro-Wilk test. Statistical analysis of the results for intramuscular fat content, fatty acid profile and texture, and shear force was performed using one-way analysis of variance (ANOVA). For the analysis of indices determining the antioxidant potential of breast muscle, a two-factor analysis of variance was conducted in a group of broiler chickens × storage time arrangement. The obtained data was analyzed statistically by using the least squares method of the GLM procedure according to the following linear model:

$Yijk = \mu + ai + bj + abij + eijk$

Yijk = trait value;

μ = overall mean;

ai = effect of broiler chicken group;

bj = effect of muscle storage time;

abij = interaction (chicken group × storage time);

eijk = random error.

The significance of differences between broiler chicken groups and muscle storage times was determined by Tukey's test. The tables show the mean values and their standard error.

Results

Fatty acid profile

Tables 1–3 show the results of determining the fat and fatty acid profile of distillers yeast and feeds, as well as breast muscle, and on this basis, the calculated indices. Concerning the control feeds (Starter, Grower and Finisher) containing wheat, corn and post-extraction soybean meal in their composition, distillers yeast was mainly characterized by a higher content of palmitic (C16:0) and linoleic (C18:2 n-6) acid, and a lower content of oleic (C18:1 n-9) acid. In addition, the yeast analyzed was characterized by a higher proportion of SFA and PUFA acids and a lower MUFA than the feeds for cockerels from the control groups (Table 1).

Cockerels that received 3% distillers yeast in feed were characterized by significantly higher oleic acid content (45.38) than cockerels that received 6 and 9% distillers yeast in feed (43.97 and 44.01, respectively). In addition, the muscles of broiler chickens that received 3% yeast in the feed contained a lower proportion of linoleic acid in intramuscular fat (16.32) in relation to broiler chickens that received 9% yeast in the feed (17.08). In the other fatty acids and fat content of the breast muscles, there were no significant differences between the analyzed groups of broiler chickens (Table 2).

Based on the results presented in Table 3, the pectoral muscles of cockerels in the group getting 9% distillers yeast in the feed were characterized by a significantly higher LA/ALA ratio (8.45) compared to cockerels that got 3% distillers yeast in the feed (7.56). Also, cockerels getting 9% yeast in the feed had a higher ratio of n-6 to n-3 fatty acids (5.88) in muscle than cockerels in the control group and those getting 3% distillers yeast (5.38 and 5.39, respectively). In addition, broiler chickens that received 6 and 9% yeast in their feed had significantly higher n-6 fatty acid content (21.17 and 21.37, respectively) compared to broiler chickens that received 3% yeast (20.31).

Table 2. Fat content and fatty acid profile in the broiler chickens' breast muscles

Fat and fatty acids (%)	Group of broiler chickens				SEM	*p*-value
	A	B	C	D		
Crude fat	1.41	1.49	1.39	1.33	0.03	0.403
C14:0	0.26	0.26	0.24	0.26	0.00	0.574
C15:0	1.47	1.39	1.54	1.55	0.03	0.191
C16:0	16.55	16.71	16.78	16.75	0.08	0.776
C17:0	0.53	0.56	0.53	0.53	0.01	0.304
C18:0	7.98	7.80	8.05	8.07	0.07	0.538
C16:1	2.48	2.49	2.62	2.48	0.05	0.766
C18:1 n-7	0.75	0.70	0.76	0.75	0.01	0.486
C18:1 n-9	44.74	45.38^{a}	43.97^{b}	44.01^{b}	0.21	0.048
C18:2 n-6	16.56	16.32^{B}	16.79	17.08^{A}	0.08	0.003
C18:3 n-3	2.14	2.18	2.08	2.04	0.02	0.190
C20:1 n-9	0.58	0.60	0.59	0.58	0.00	0.075
C20:2 n-6	0.26	0.27	0.2	0.28	0.01	0.501
C20:3 n-6	0.44	0.42	0.46	0.44	0.01	0.600
C20:4 n-6	3.07	2.88	3.16	3.09	0.06	0.434
C20:4 n-3	0.24	0.24	0.22	0.22	0.00	0.431
C22:4 n-6	0.45	0.43	0.47	0.47	0.01	0.285
C22:5 n-3	0.97	0.89	0.91	0.88	0.02	0.518
C22:6 n-3	0.54	0.48	0.52	0.50	0.10	0.610

A = control group; B = diet with 3% addition of distillers yeast; C = diet with 6% addition of distillers yeast; D = diet with 9% addition of distillers yeast; A, B = $p \leq 0.01$; a, b = $p \leq 0.05$; means in rows and denoted using different letters differ statistically significantly; SEM = standard error of the mean

Table 3. Values of lipid indices for the fatty acid profile in the breast muscles of broiler chickens

Indices	Group of broiler chickens				SEM	*p*-value
	A	B	C	D		
ΣPUFA	26.79	26.72	27.11	27.17	0.13	0.535
ΣMUFA	48.55	49.18	47.93	47.82	0.20	0.068
ΣSFA	24.66	24.11	24.96	25.01	0.14	0.071
ΣUFA	73.21	73.28	72.84	72.83	0.13	0.500
ΣPUFA/ΣSFA	0.92	0.90	0.92	0.92	0.01	0.643
ΣMUFA/ΣSFA	1.98	2.04	1.93	1.92	0.02	0.052
ΣUFA/ΣSFA	2.98	3.05	2.93	2.92	0.02	0.052
ETA+DHA	0.78	0.72	0.75	0.72	0.02	0.616
LA/ALA	7.83	7.56^{B}	8.18	8.45^{A}	0.09	0.001
Σn-3	3.89	3.79	3.74	3.65	0.03	0.092
Σn-6	20.77	20.31^{Bb}	21.17^{a}	21.37^{A}	0.11	0.004
Σn-6/Σn-3	5.38^{B}	5.39^{B}	5.69	5.88^{A}	0.11	0.000
DI (5)	7.11	6.85	6.99	7.06	0.12	0.987
DI (18)	84.80	85.31	84.48	84.48	0.17	0.261
EI	73.47	73.48	72.85	73.03	0.16	0.249
NVI	3.20	3.19	3.11	3.12	0.02	0.235
DFA	81.19	81.08	80.90	80.90	0.01	0.480
OFA	16.81	16.97	17.03	17.01	0.09	0.592

A = control group; B = diet with 3% addition of distillers yeast; C = diet with 6% addition of distillers yeast; D = diet with 9% addition of distillers yeast; A, B = $p \leq 0.01$; a, b = $p \leq 0.05$; means in rows and denoted using different letters differ statistically significantly; SEM = standard error of the mean

Antioxidant status

The antioxidant potential of feed and breast muscle of broiler chickens was determined by SET-type methods using the ferrous ion reduction ability test (FRAP) and ABTS cation radical scavenging capacity, as shown in Tables 1, 4 and 5.

Table 4. Antioxidant activity of chickens' breast muscles measured by ABTS method

Time of storage (T)	Group of broiler chickens (G)				Total	SEM	*p*-value		
	A	B	C	D			G	T	G × T
24 h, 4 °C	2.40	2.62	2.56	2.11	2.42	0.07			
7 days, 4 °C	2.39	2.56	2.56	2.26	2.44	0.05	0.025	0.155	0.159
3 months, –20 °C	2.37	2.34	2.17	2.32	2.30	0.06			
Total	2.39	2.52[a]	2.45	2.23[b]	mmol TE/100 g dry mass of meat				
SEM	0.09	0.05	0.07	0.05					

A = control group; B = diet with 3% addition of distillers yeast; C = diet with 6% addition of distillers yeast; D = diet with 9% addition of distillers yeast; a, b = $p \leq 0.05$; means in rows and denoted using different letters differ statistically significantly; SEM = standard error of the mean

Table 5. Antioxidant activity of chickens' breast muscles measured by FRAP method

Time of storage (T)	Group of broiler chickens (G)				Total	SEM	*p* - value		
	A	B	C	D			G	T	G × T
24 h, 4 °C	61.03	58.08	60.22	55.17	58.63[Y]	1.86			
7 days, 4 °C	72.93	52.46	53.90	57.56	58.26[Y]	2.74	0.034	0.000	0.071
3 months, –20 °C	82.84	93.56	77.11	64.25	79.44[X]	4.65			
Total	70.49[a]	67.27	63.24	58.99[b]	mmol TE/100 g dry mass of meat				
SEM	4.32	5.91	4.01	2.51					

A = control group; B = diet with 3% addition of distillers yeast; C = diet with 6% addition of distillers yeast; D = diet with 9% addition of distillers yeast. X, Y = $p \leq 0.01$; a, b = $p \leq 0.05$; means in columns and rows and denoted using different letters differ statistically significantly; SEM = standard error of the mean

Analysis of pectoral muscles showed that the average value of ABTS potential was significantly higher in chickens fed feed with 3% yeast addition (2.52 mmol TE) compared to the group of chickens that received 9% yeast in feed (2.23 mmol TE). Also, in the case of FRAP's reducing potential, their value was found to be significantly higher in the breast muscles of broiler chickens fed the control feed (70.49 mmol TE) compared to broilers that received 9% distillers yeast in their feed (58.99 mmol TE). In addition, regardless of the broiler chicken group, there was a significant increase in activity against FRAP after 3 months of storage of breast muscle (79.44 mmol TE) compared to 24 h and 7 days of storage (58.63 and 58.26 mmol TE, respectively).

TBARS value

The meat's malondialdehyde content (MDA) increases with the degree of lipid oxidation. The amount of distillers yeast significantly influenced the TBARS value added to the feed and storage time and the simultaneous interaction of both factors (Table 6). In the muscles of chickens that received 9% yeast in feed, a significantly higher - MDA content (0.59 mg) was recorded than in the muscles of cockerels from the control group and those receiving 3% yeast in feed (0.41 and 0.43 mg, respectively), and to a lesser extent to the group of cockerels receiving 6% yeast in feed (0.53 mg). Regardless of the broiler chicken group, breast muscles stored for 7 and 90 days had significantly higher MDA content (0.53 and 0.54 mg, respectively) than fresh muscles (0.40 mg). In addition, based on the significant interaction found (chicken group × storage time), it was shown that the muscle lipids of chickens from the control group and those receiving 3% distillers yeast in feed (0.37 and 0.39 mg, respectively) had significantly higher oxidation resistance (lower TBARS) during 3-month freezer storage than those of cockerels receiving 6 and 9% distillers yeast in feed (0.57 and 0.74 mg, respectively).

Table 6. TBARS status – degree of lipid peroxidation of breast muscles of broiler chickens

Time of storage (T)	Group of broiler chickens (G)				Total	SEM	*p*-value		
	A	B	C	D			G	T	G × T
24 h, 4 °C	0.39^{y}	0.39	0.42^{y}	0.43^{y}	0.40^{y}	0.01			
7 days, 4 °C	0.53^{x}	0.51	0.51^{y}	0.56^{y}	0.53^{x}	0.01	0.000	0.000	0.000
3 months, –20 °C	0.37^{by}	0.39^{b}	0.67^{ax}	0.74^{ax}	0.54^{x}	0.05			
Total	0.41^{B}	0.43^{B}	0.53^{Ab}	0.59^{Aa}	mmol TE/100 g dry mass of meat				
SEM	0.02	0.02	0.03	0.04					

A = control group; B = diet with 3% addition of distillers yeast; C = diet with 6% addition of distillers yeast; D = diet with 9% addition of distillers yeast. A, B = $p \leq 0.01$; a, b and x, y = $p \leq 0.05$; means in columns and rows and denoted using different letters differ statistically significantly; SEM = standard error of the mean

TPA and SF

The results of the Texture Profile Analysis and Shear Force of breast muscles are shown in Table 7. The breast muscles of chickens fed 9% distillers yeast supplement in the feed were characterized by significantly lower gumminess and SF (18.94 and 54.38 N, respectively) than broiler chickens from the control group (24.63 and 63.91 N, respectively). In addition, the muscles of cockerels that received 9% yeast in their feed had significantly lower SF (54.38 N) relative to those of cockerels that received 3% yeast in their feed (63.48 N) and from the control group (63.91 N). However, there were no significant differences in hardness, cohesiveness and springiness between the analyzed groups of broiler chickens.

Table 7. TPA parameters and shear force of broiler chickens' muscles

Parameter (N)	Group of broiler chickens				SEM	*p*-value
	A	B	C	D		
Hardness	56.46	54.09	50.03	48.29	1.61	0.129
Cohesiveness	0.42	0.42	0.41	0.41	0.00	0.519
Springiness	0.49	0.49	0.48	0.48	0.00	0.285
Gumminess	24.63^{a}	22.98	21.17	18.94^{b}	0.82	0.046
Chewiness	12.07	10.72	10.19	10.04	0.40	0.098
Shear force	63.91^{a}	63.48^{a}	58.41	54.38^{b}	1.50	0.027

A = control group; B = diet with 3% addition of distillers yeast; C = diet with 6% addition of distillers yeast; D = diet with 9% addition of distillers yeast. a, b - $p \leq 0.05$; means in columns and rows and denoted using different letters differ statistically significantly. SEM = standard error of the mean

Discussion

Based on the study, it can be indicated that with the increase in the proportion of distillers yeast in feed mixtures for cockerels, the proportion of oleic acid in the breast muscles decreased. In contrast, the proportion of linoleic acid and fatty acids of the n-6 family increased, and the ratio of n-6 to n-3 and LA to ALA fatty acids increased. In addition, based on the average values, a trend can be observed that as the proportion of yeast in broiler chicken feed increased, the values of MUFA/SFA and UFA/SFA fatty acid ratios decreased (p= 0.052), which is probably related to the decreasing proportion of MUFA (p=0.068) and increasing proportion of SFA (0.071). Also, Grabež et al. (2022) showed that replacing 10–30% of crude protein in the feed of broiler chickens with *Cyberlindnera jadinii* yeast increased the proportion of SFA and MUFA fatty acids and decreased PUFA. On the other hand, Hussein and Selim (2018) showed that the addition of 0.5% dried SC yeast and 0.5% probiotic (*Lactobacillus acidophilus, Bacillus subtilis and Aspergillus oryzae*) and their combined feeding in broiler chickens influenced higher PUFA content, n-3 fatty acids and PUFA/SFA ratio and lower n-6/n-3 ratio of breast muscle compared to the control diet. They conclude combining SC yeast and probiotics can be a functional feed additive for broiler chickens. It is confirmed by Saleh et al. (2013), who studied the effect of broiler chicken diets supplemented with 0.05% *Aspergillus avamori* and 0.10% SC or a combination of both. They showed that muscle fat content increased with both types of yeast, but there was a decrease in saturated fatty acids and an increase in unsaturated fatty acids in muscle. According to these authors, the changes in the fatty acid profile were partially related to the expression of muscle delta-6 fatty acid desaturase mRNA. In comparison, in a study by Min et al. (2012), an increase in the

proportion of distillers dried grains with solubles (DDGS) in the feed from 5 to 25% affected the fatty acid profile of broiler chicken breasts and thighs by increasing the proportion of polyunsaturated to saturated fatty acids, a finding confirmed by Corzo et al. (2009).

The results of our study showed that the breast muscle lipids of broiler chickens from the control group and those receiving 3% distillers yeast in feed showed greater resistance to oxidation as evidenced by lower TBARS, especially after 3 months of freezer storage, than those of receiving 6 and 9% yeast in feed. In addition, as the proportion of yeast increased, the antioxidant potential of pectoral muscles deteriorated, as evidenced by lower ABTS activity and lower FRAP reducing potential. Different results were obtained by Zhang et al. (2005b), who showed that regardless of the amount of SC yeast added to the feed of broiler chickens (0.3, 1.0 and 3.0%), TBARS values after 15 days of refrigerated storage of breast muscles and thighs were significantly lower than in the control group. Also, in a study by Attia et al. (2022), SC yeast at 0.02 and 0.04% improved the antioxidant parameters as evidenced by lower MDA content in the liver and total antioxidant capacity (TAC) in blood serum with the exception that MDA content was significantly higher at the higher SC yeast dose in the feed. In the study by Zhang et al. (2005a) using whole SC yeast and derived yeast extract (YE) and yeast cell wall (YCW) at 0.3, 1.0 and 3.0% in the feeding of broiler chickens, reduced malondialdehyde (MDA) content in breast and thigh muscles compared to the control group. Li et al. (2016) showed that supplementation of Arbor Acres chickens with 1 g kg^{-1} of feed with powdered YCW had an effect on reduced MDA accumulation in the ileal mucosa. Another study by Li et al. (2017) showed a beneficial effect of combined feeding of Arbor Acres chickens with YCW and palygorskite on meat quality and muscle oxidative status (lower MDA content). Another study found that adding dried SC yeast with a probiotic to feed broiler chickens increases total protein and intramuscular fat content and lowers MDA content in breast muscles (Hussein and Selim 2018). A study by Wang et al. (2022) showed that the addition of various doses of yeast hydrolysate (YH) derived from SC yeast to broiler chickens' diets had a beneficial effect on lowering serum and liver MDA content and meat quality by reducing pH drop and higher ultimate pH (24 h post mortem) and lower cooking loss of breast muscles. Also, Grabež et al. (2022) showed that oxidative stability after 19 days of refrigerated storage of leg muscles was higher (lower TBARS) in cockerels of the Ross 308 line fed a feed in which 30% of crude protein was replaced by *Cyberlindnera jadinii* yeast, compared to a control group fed a feed consisting of soybean, wheat and oat-meal. In contrast, feeding different levels of *Saccharomyces cerevisiae* fermentation product (SCFP) to broiler chickens did not affect color characteristics, water holding capacity, cooking losses and shear force. However, including SCFP at 750 g t^{-1} reduced breast muscle lipid oxidation as evidenced by lower TBARS, which was not statistically confirmed for the higher SCFP dose of 1 500 g t^{-1} (Aristides et al. 2018). In comparison, high doses of DDGS (5–25%) in broiler chicken feed resulted in a significant increase in MDA content in breast muscle at 20% DDGS addition (Min et al. 2012). Also, Corzo et al. (2009) proved that the thigh muscles of broiler chickens fed feed with 8% DDGS were more susceptible to oxidation (higher TBARS) than the thigh meat of broilers fed a control diet.

In our study, the results obtained from the analysis of the texture and shear force of pectoral muscles indicate that broiler chickens fed the highest dose of distillers yeast were characterized by the lowest gumminess and shear force, which may indicate their better tenderness. It is confirmed by the tendency, observed based on average values, for the hardness and chewiness of breast muscles to decrease with an increase in the proportion of yeast in cockerel feeds. Also, in a study by Zhang et al. (2005b), it was shown that supplementing broiler chickens' feed with SC yeast at 0.3; 1.0 and 3% had a lowering effect on shear force in raw breast and thigh muscles as well as in cooked drumstick compared to the control group. Another study by Zhang et al. (2005a) proved that supplementing broiler chickens with whole SC and YE yeast compared to the control group significantly affected the lower SF of cooked breast and drumstick muscles. In addition, the authors showed that only whole SC yeast in the birds' feed significantly affected lower SF in raw drumstick. On the other hand, in the study of Grabež et al. (2022), replacing part of the crude protein in the feed with *Cyberlindnera jadinii* yeast did not significantly affect the SF value of cooked leg muscles of Ross 308 broiler chickens compared to the control group. However, numerically, the SF of the muscles of chickens fed with the addition of yeast was higher.

In summary, the results of the study showed that an increase in the proportion of distillers yeast in the feed of broiler chickens influenced an increase in the proportion of linoleic acid and its ratio to α-linolenic acid (LA/ALA) and a higher ratio of n-6 to n-3 and a lower proportion of oleic acid and n-6. In addition, as the proportion of yeast increased, the antioxidant potential of breast muscles deteriorated, as evidenced by lower ABTS activity and FRAP reducing potential and a higher degree of lipid peroxidation TBARS. On the other hand, regarding TPA analysis of pectoral muscles, an increase in the proportion of distillers yeast in cockerel feed improved meat tenderness, as evidenced by a decrease in the shear force and gumminess of cooked pectoral muscles. In the same experiment, there was no significant effect of the level of distillers dried yeast in the diet of broiler chickens on carcass quality,

pH, water holding capacity, sensory characteristics, and macronutrient and micronutrient content 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 fed 6% and 9% distillers dried yeast in their diets (Rybarczyk et al. 2024). The obtained research results may indicate that supplementation of feed for broiler chickens with distiller's yeast, despite the increase in muscle susceptibility to oxidation, does not cause adverse effects on the sensory quality.

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