Effects of probiotics and sex on physicochemical, sensory and microstructural characteristics of broiler chicken meat

Kamil Stęczny and Dariusz Kokoszynski
Katedra Hodowli Zwierząt, UTP University of Science and Technology, Bydgoszcz, Poland

ABSTRACT
The aim of the study was to determine the effects of probiotic preparations Pro-Biotyk Em-15 and EMFarma™ and sex on proximate chemical composition, physicochemical properties of breast and leg muscles, sensory properties (including texture) and microstructural characteristics of pectoralis major muscle in Ross 308 broiler chickens. The study used 48 carcases – 12 from males and 12 from females fed no probiotics, and 12 from males and 12 from females receiving the EM probiotics. At 42 days, the chickens receiving EM preparations had significantly higher WB-shear force of pectoralis major muscle, whereas the leg muscles contained significantly less water and fat and had higher pH24 value. Regardless of the treatment, males exhibited significantly higher springiness, chewiness, as well as significantly lower yellowness (b°), fibre perimeter, horizontal (H) fibre diameter and fibre cross section area of pectoralis major muscle when compared to females. In turn, male leg muscles had significantly higher fat content compared to female leg muscles. The genotype × sex interaction was significant for the content of water, protein and fat, and for electric conductivity (EC24) of leg muscles.

HIGHLIGHTS
• Probiotics did not improve the quality of chicken meat.
• The influence of sex on the quality of chicken meat was varied.

Introduction
In recent years, there has been a substantial increase in the world in the production of chicken meat. In 2000–2017 (FAOSTAT 2019), chicken meat production increased by 86% (from 58.7 to 109.0 million tons).

One of the essential factors affecting the quality of meat is the animals’ diet. Pietrzak et al. (2009) found higher protein content and lower fat content in the leg muscles of Ross 308 chickens fed with a probiotic (lactic acid bacteria Enterococcus faecium NCIMB 10415), which is beneficial to health. On the other hand, higher fat content was characteristic of the breast muscles of chickens receiving the above probiotic. The leg muscles were characterised by lower water holding capacity and higher cooking loss. In turn, Kim et al. (2016) reported no significant effect of the use of probiotic containing Enterococcus spp., Pediococcus spp., Bifidobacterium spp., and Lactobacillus spp. on the colour (attributes L°, a°, b°), pH, and cooking loss of breast muscles from broiler chickens aged 45 days. A significant impact of the probiotic addition was found only for the drip loss of breast muscle of the tested 45-day-old chickens. Other authors (Pelicano et al. 2003; Pelicia et al. 2004; Takahashi et al. 2005; Zhou et al. 2010) provided inconclusive results for the effect of feeding probiotics to animals on their meat quality.

Over the last decade, effective microorganisms (EM) have become increasingly popular in agricultural practice. They were investigated in broiler chickens (Wondmeneh et al. 2011; Jwher et al. 2013), laying hens (SimeamelaK et al. 2013; Gnanadesigan et al. 2014) and quails (Gesek et al. 2018). Showing the positive effects of the EM probiotics in broiler chicken production with regard to meat quality may encourage an increase in the biologisation of modern agriculture.

The objective of the study was to determine the effects of the probiotics Pro-Biotyk Em-15 and EMFarma™ and sex on proximate chemical composition, physicochemical properties of breast and leg muscles, sensory properties (including texture) and

CONTACT Assoc. Prof. Dariusz Kokoszynski kokoszynski@gmail.com Katedra Hodowli Zwierząt, Faculty of Animal Breeding and Biology, UTP University of Science and Technology, Bydgoszcz 85084, Poland

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microstructural characteristics of *pectoralis major* muscle in Ross 308 broiler chickens.

**Materials and methods**

**Birds and housing**

The experiment used 48 carcases from Ross 308 broiler chickens at the age of 42 days. Broiler carcases were purchased from a local poultry slaughterhouse. Before slaughter, broilers were kept on a commercial broiler farm in a windowless building, in which two production facilities (each with an area 500 m²) were separated by a technical room. Both facilities provided the same environmental parameters (temperature, humidity, air movement) depending on the age of birds. Air temperature in the poultry building was 33 °C on the first day of rearing and it was gradually reduced to 17 °C with the age of the birds. Relative humidity ranged from 55 to 70%, and air exchange varied between 0 and 4.2 m³/h/kg body weight. The poultry building was heated with gas-fired heaters. Stocking density on day 42 did not exceed the EU permitted level of 42 kg/m² floor area. The research was performed with the approval of the Local Ethics Committee for Experimental Animals (UTP University of Science and Technology in Bydgoszcz, Poland/Ministry of Science and Higher Education).

**Feeding programme**

Birds were fed *ad libitum* complete diets and had 24-h access to water throughout the rearing period (days 1–42). From days 1 to 8, chickens were fed a commercial complete broiler starter diet in crumble form. From days 9 to 42, birds received complete diets: grower 1 (days 9–24), grower 2 (days 25–32) and finisher (days 33–42). The finely ground diets were produced on the farm from purchased feed components (protein–fat concentrate, extracted soybean meal, soybean oil, feed wheat, mineral–vitamin mixture, ground limestone). Chemical composition of the experimental diets was analysed at the Feed Quality Laboratory of the UTP University of Science and Technology in Bydgoszcz, Poland, and the results are shown in Table 1.

**Effective microorganisms programme**

In the first facility (500 m²), Pro-Biotyk Em-15 was added to water (each time from 0.3 to 1.2 L depending on bird age) from day 1 of age, three times a week. Pro-Biotyk Em-15 mixed with EMFarma™ (1.25 L of each preparation) and water (2.5 L) were sprayed onto the feed and litter twice weekly from 2 weeks of age. In addition, when preparing the facility for the placement of day-old chicks, chemical disinfectants (sodium hypochlorite, Virocid F) and EMFarma™ (30 L plus 170 L water) were used to spray the ceiling, walls and poultry equipment. Furthermore, Pro-Biotyk Em-15 was used to spray feed on the paper and in the feeders (5 L of the preparation plus 5 L of water). In the second facility, no EM probiotics were used when preparing it for placement and during rearing.

**Microbial analysis**

The microbial profile of the Pro-Biotyk (Em-15) and EMFarma™ probiotics was determined using quantitative culture technique (Petri dish method). The determinations were made at the Department of Biotechnology and Food Microbiology of the Poznań University of Life Sciences in Poznań, Poland and the results are shown in Table 2. *Lactobacillus* spp. bacteria were enumerated in MRS Agar medium (OXOID), *Lactococcus* and *Streptococcus thermophilus* in M17 (OXOID), *Bifidobacterium* spp. in TOS-MUP (MERCK), *Bacillus subtilis* in TSB (OXOID), *Rhodopseudomonas* spp. in Van-Niel’s medium (ATCC medium 1676) and *Saccharomyces cerevisiae* yeast with chloramphenicol medium (YGC, BIOCORP).

**Evaluation of meat quality**

The pH of breast muscles (*pectoralis major* muscle) and leg muscles (drumstick muscle) was measured 24 h post-mortem on meat chilled at 4 °C for 18 h in a Hendi chill cabinet (Hendi, Gdaki, Poland). pH₂₄ was determined with a Star CPU pH metre (Ingenieurbüro R. Mattheus, Nobitz, Germany) designed for meat pH analysis. The determinations of pH values were accurate to 0.01. Before the measurement, the pH metre was calibrated with a standard solution (pH 5.5 and 7.0). Electric conductivity was measured also 24 h

| Chemical composition of diets for broiler chickens. | Chemical analysis | Starter | Grower 1 | Grower 2 | Finisher |
|-----------------------------------------------|------------------|---------|----------|----------|----------|
| DM, %                                          |                  | 89.58   | 87.93    | 89.79    | 89.96    |
| CP as fed basis, %                            |                  | 23.08   | 22.45    | 22.10    | 21.63    |
| Crude fat as fed basis, %                     |                  | 5.41    | 5.45     | 5.52     | 4.80     |
| Crude fibre as fed basis, %                   |                  | 2.21    | 1.60     | 2.00     | 2.24     |
| Crude ash as fed basis, %                     |                  | 3.75    | 4.67     | 4.45     | 4.59     |
| ADF as fed basis, %                           |                  | 5.66    | 4.74     | 4.44     | 5.36     |
| NDF as fed basis, %                           |                  | 10.19   | 9.26     | 9.60     | 10.27    |
| ME as fed basis, MJ/kg                        |                  | 12.60   | 13.20    | 13.33    | 13.09    |

DM: dry matter; CP: crude protein; ADF: acid detergent fibre; NDF: neutral detergent fibre; ME: metabolizable energy.
post-mortem ($EC_{24}$) on the carcasses chilled to 4°C. $EC_{24}$ was measured with an LF-Star CPU conductivity metre (Ingenieurbüro R. Matthäus, Nobitz, Germany). To determine $EC_{24}$ values, the stainless steel electrodes of the conductometer were placed in the drumstick or *pectoralis major* muscle at a 90-degree angle parallel with the muscle fibres. $EC_{24}$ was measured with an accuracy of 0.1 mS/cm.

After dissection, the breast and leg muscles were individually sampled from each carcase of 48 birds to determine basic chemical composition, drip loss, cooking loss, and meat colour attributes. The *pectoralis major* muscles were also sampled to determine their sensory properties (including textural traits) and micro-structural characteristics.

The basic chemical composition and the collagen content of breast and leg muscles from the compared broiler groups were determined by near-infrared transmission (NIT) spectroscopy. Calibration using artificial neural networks (ANN) was performed on a FoodScan device (FoodScan Laboratory, Foss, Cheshire, UK).

The colour of meat was determined on the *pectoralis major* muscle from the direction of breast-bone and on the thigh and drumstick (leg) surfaces, following dissection of the patella and tendons 24 h post-mortem. The meat colour coordinates: $L^*$ – lightness, $a^*$ – relative redness, on red-green axis, $b^*$ – relative yellowness, on yellow-blue axis were measured in the CIELab Colour System (1976). Meat colour was measured with a Konica Minolta CR410 chroma meter (Konica Minolta, Japan). Wide-area illumination was used (0° viewing angle, measurement area 50 mm in diameter, illuminant $D_{65}$). The chroma metre was calibrated against a CR410 white reference tile ($Y = 94.40$, $x = 0.3159$, $y = 0.3325$).

Drip loss was also determined for *pectoralis major* muscle and for thigh and drumstick muscles together. Each muscle was weighed on a Radwag PS 1000.R2 electronic scales (Radwag, Radom, Poland) to the nearest 0.01 g. Next, the meat samples of breast or leg from each bird were placed separately in a perforated bag (no. 1). In the next stage, the bag with the meat sample was placed into a second bag (no. 2) to prevent contact between dripping juice and the meat sample. The samples were suspended on racks and stored in a Hendi chill cabinet (Hendi, Gądki, Poland) at 4°C for 24 h. After this time, the samples of breast or leg meat were weighed again. Drip loss was calculated as the difference between sample weights before and after chilling. The loss in the meat sample weight was calculated as a percent of initial sample weight.

Cooking loss was determined in the samples of *pectoralis major* muscle and of thigh and drumstick muscles (together) weighing $20 \pm 0.2$ g. The meat samples were formed into balls, wrapped in absorbent gauze and placed in a 85°C water bath for 10 min. After removal from the water bath, heat-treated meat samples were cooled for 30 min at 4°C. Next, they were weighed again on a Radwag PS 1000.R2 electronic scales (Radwag, Radom, Poland) to the nearest 0.01 g. Meat weight loss was calculated as the difference in meat weight before and after heat treatment. The resulting cooking loss was expressed as percent loss of the initial meat sample weight.

The sensory properties were assessed on the *pectoralis major* muscles obtained from the 42-day-old Ross 308 broiler chickens. The breast meat samples were heat treated in 0.6% NaCl. 200-mL of water was added per 100 g of meat. After the heat treatment, the meat samples were chilled and assessed by a panel of six trained judges. The breast meat samples were assessed according to a 5-point scale provided by Barylko-Pikielna and Matuszewska (2009). The assessment scale for intensity of aroma and taste was as follows: 1 – imperceptible, 2 – perceptible, 3 – weakly distinct, 4 – distinct, 5 – very distinct. Aroma and taste desirability of the breast meat samples was assessed using the scale as follows: 1 – very undesirable, 2 – desirable, 3 – neutral, 4 – desirable, 5 – very desirable. Juiciness was scored as follows: 1 – clearly dry, 2 – slightly dry, 3 – weakly juicy, 4 – juicy, 5 – very juicy. Tenderness was assessed based on the following scale of assessment: 1 – very hard, 2 – hard, 3 – slightly tender, 4 – tender, 5 – very tender.

The meat texture (hardness, cohesiveness, springiness, chewiness, gumminess, WB shear force) and rheological properties (sum of elasticity moduli and sum of viscosity moduli) were determined with an Instron 1140 apparatus (Instron Corp., USA), using the TPA double compression test, the Warner-Bratzler (WB) test, and the stress-relaxation test. For each sample, each test was performed in 5–7 replications.

The test was performed with 48 samples of heat-treated *pectoralis major* muscle obtained from the chickens after dissection. The determinations were performed...
made at the Department of Meat Technology of the
West Pomeranian University of Technology.

The muscles were tightly packed into PE foil bags
and heated in water at 72 ± 2°C until the temperature
reached 70.2°C in the geometric centre. On reaching
the target temperature, the muscles were cooled in cold
running water until reaching around 12°C in the geometric
centre and, after removing the drip, they were packed
into food packaging film to prevent drying out and
placed for around 12 h in the refrigerator at 3 ± 1°C until
analyses. The meat texture analyses were performed with
the samples after bringing them to around 18°C.

A 20 ± 1 mm thick slice was cut out from different
muscles perpendicular to the muscle fibre orientation,
using a Siemens MS 6000 electric slicer. The so pre-
pared samples were subjected to instrumental assess-
ment of mechanical properties.

In the TPA test the plunger 0.62 cm in diameter was
driven twice into the sample (parallel to muscle fibre
orientation), with a 80% deformation and crosshead
speed of 50 mm/min. From the curve representing the
strength-deformation dependence, the following
speed of the crosshead was 50 mm/min.

In the relaxation test the plunger 0.96 cm in diam-
eter was driven into the sample 2 mm deep (a 10%
deformation) for 90 s to record the changes in stresses.

The Warner-Bratzler (WB) test involved cutting the
muscle samples across the muscle fibres, and deter-
mining shear force (Bourne 1982). The working speed
of the crosshead was 50 mm/min.

In the relaxation test the plunger 0.96 cm in diam-
eter was driven into the sample 2 mm deep (a 10%
deformation) for 90 s to record the changes in stresses.
To calculate the elasticity and viscosity moduli the
generalised Maxwell model was applied, made up of
three elements connected parallel: the Hooke body
and two viscous-elastic Maxwell bodies. The model
equation assumes the following form:

\[ \delta = \varepsilon \cdot \left[ E_0 \cdot \exp \left( -\frac{-E_1 \cdot t}{\mu_1} \right) + E_2 \cdot \exp \left( -\frac{-E_2 \cdot t}{\mu_2} \right) \right] \]

where \( \delta \) tension (kPa); \( \varepsilon \) deformation; \( E_0 \) elasticity
module for the Hooke body (kPa); \( E_1, E_2 \) elasticity
moduli for Hooke body 1 and 2 respectively (kPa); \( \mu_1, \mu_2 \), viscosity moduli for Maxwell body 1 and 2 respect-
ively (kPa × s); \( t \), time.

For a more reader-friendly interpretation of the
results for each sample there was calculated the sum
of elasticity moduli \( E_0 + E_1 + E_2 \) as well as the sum
of viscosity moduli \( \mu_1 + \mu_2 \).

For histological analysis, samples of pectoralis major
muscle were collected from 48 birds, 12 males and 12
females from each group of chickens slaughtered at
42 days. From each bird after slaughter, three sections
(0.5 × 0.5 × 1 cm each) were taken from the middle
part of the pectoralis major muscle. The samples were
collected fixed with Sannomiya solution, dehydrated in alcohol and benzene, and embedded in paraffin blocks. The blocks were sectioned with micro-
tome, and sections of 10 μm were placed on glass
slides and counterstained with haematoxylin and eosin
(Burck 1975) and embedded in Canada balm. The
microstructural traits of pectoralis major muscle were
measured using the MultiScanBase v. 13 image anal-
ysis system (Computer Scanning System Ltd, Warsaw,
Poland). Fibre cross-section area, fibre perimeter and
its horizontal (H) and vertical (V) diameter, and thick-
ness of perimysium and endomysium were measured.
The determinations were made on three pectoralis
superficialis muscle preparations per bird. A total of
144 preparations of pectoralis major muscle were used
to determine the microstructure. Around 200 muscle
fibres were measured in each preparation and
150–200 measurements of the connective tissue thick-
ness (perimysium and endomysium) were made. A
magnification of 100× was applied. Based on the data
for horizontal (H) and vertical (V) diameters of the
muscle fibre, the H:V diameter ratio was calculated.

The numerical data collected during the study on
basic chemical composition, physicochemical, sensory,
textural and microstructural characteristics of the meat
were statistically analysed. Arithmetic means and
standard error of mean (SEM) were calculated for each
trait (for both groups together). Two-way analysis of
variance was used to determine the effect of probiotic
and sex on the analysed meat quality traits of broiler
chickens at the age of 42 days. The following linear
model was used:

\[ Y_{ijk} = \mu + a_i + b_j + (a \cdot b)_{ij} + e_{ijk} \]

where \( Y_{ijk} \), value of the analysed trait; \( \mu \), overall mean;
\( a_i \), effect of \( i^{th} \) group; \( b_j \), effect of \( j^{th} \) sex; \( a \cdot b \), inter-
action of group with regard to sex; \( e_{ijk} \), random error.

The analysed traits were statistically characterised
using SAS software ver. 9.4 (SAS Institute Inc. 2014). Significant differences (at \( p < .05 \)) between the com-
pared groups and between males and females were
determined with Tukey’s test. For all the analysed
traits of meat quality, the individual bird was the
experimental unit.

**Results**

**Chemical composition**

The compared groups of Ross 308 broilers differed
\( p < .05 \) only in the water and fat content of leg
muscles. Control chickens contained more water and fat in leg muscles compared to chickens receiving EM probiotics. Males exhibited significantly \( p < .05 \) higher fat content in leg muscles than females. The group × sex interaction for the evaluated quality meat traits was significant \( p < .05 \) for the water, protein and fat content of leg muscles (Table 3).

**Meat physicochemical properties**

The administration of EM probiotics to Ross 308 broilers had no significant \( p > .05 \) effect on the analysed physicochemical traits of breast and leg meat except for \( \text{pH}_{24} \) of leg muscles. Regardless of group, male breast muscles had lower yellowness \( (b^*) \) compared to females. The group × sex interaction was significant \( p < .05 \) for electric conductivity \( (E_{C24}) \) of leg muscles (Table 4).

**Meat texture**

At 42 d of age, chickens receiving EM probiotics were characterised by significantly \( p < .05 \) poorer tenderness (higher WB shear force) of heat-treated *pectoralis major* muscle compared to control birds. Regardless of treatment, the heat-treated *pectoralis major* muscle of males had higher \( (p < .05) \) springiness and chewiness. The group × sex interactions for the texture of pectoralis major muscle were not significant \( p > .05 \) (Table 5).

**Meat sensory properties**

Group, sex and group × sex interaction were not significant \( p > .05 \) for aroma and taste intensity and

### Table 3. Effect of diet on chemical composition of breast and leg muscles in 42-day-old broiler chickens.

| Item       | Group (G) – sex (S) | p-Value |
|------------|---------------------|---------|
|            | Control \( (n=12) \) | Probiotics \( (n=12) \) |         |
|            | \( \delta \) | \( \varphi \) | \( \delta \) | \( \varphi \) | SEM | G | S | G × S |
| Water      | BM 72.30 | 72.10 | 72.20 | 72.10 | 0.70 | .32 | .30 | .30 |
|            | LM 70.80 | 71.20 | 71.60 | 69.60 | 0.30 | .00 | .67 | .04 |
| Protein    | BM 23.40 | 23.20 | 23.30 | 23.40 | 0.20 | .05 | .17 | .01 |
|            | LM 19.60 | 20.30 | 20.60 | 20.50 | 0.20 | .47 | .28 | .06 |
| Fat        | BM 1.60  | 1.80  | 1.90  | 1.60  | 0.40 | .00 | .00 | .03 |
|            | LM 6.40  | 4.50  | 4.20  | 4.80  | 0.40 | .00 | .00 | .03 |
| Collagen   | BM 1.60  | 1.60  | 1.50  | 1.70  | 0.10 | .62 | .13 | .45 |
|            | LM 1.70  | 1.60  | 1.60  | 1.70  | 0.10 | .61 | .22 | .90 |

BM: breast muscle; LM: leg muscle.

\( a,b \):Values within a row followed by different letter differ significantly \( p < .05 \).

### Table 4. Some physicochemical traits of breast and leg meat from 42-day-old Ross 308 broiler chickens.

| Trait         | Group (G) – sex (S) | p Value |
|---------------|---------------------|---------|
|               | Control \( (n=12) \) | Probiotics \( (n=12) \) |         |
| \( \text{pH}_{24} \) | BM 6.05 | 6.01 | 6.22 | 6.02 | 0.10 | .14 | .07 | .22 |
|               | LM 6.51<sup>b</sup> | 6.51<sup>b</sup> | 6.81<sup>*</sup> | 6.58<sup>b</sup> | 0.10 | .01 | .12 | .11 |
| \( E_{C24} \) (mS/cm) | BM 10.20 | 10.60 | 10.60 | 10.00 | 0.30 | .83 | .80 | .11 |
|               | LM 8.80 | 8.50 | 10.60 | 10.00 | 0.20 | .08 | .55 | .03 |
| Drip loss (%) | BM 2.10 | 2.00 | 2.30 | 2.30 | 0.10 | .56 | .14 | .31 |
|               | LM 0.70 | 0.90 | 0.80 | 0.80 | 0.10 | .14 | .14 | .94 |
| Cooking loss (%) | BM 24.20 | 22.80 | 23.50 | 23.80 | 0.30 | .31 | .13 | .73 |
|               | LM 22.10 | 22.00 | 22.00 | 22.50 | 0.50 | .20 | .99 | .11 |
| \( L^a \) – lightness | BM 59.00 | 58.70 | 58.70 | 59.30 | 1.00 | .78 | .76 | .46 |
|               | LM 52.60 | 53.50 | 55.00 | 51.90 | 0.40 | .73 | .35 | .11 |
| \( a^1 \) – redness | BM 17.60 | 18.00 | 17.50 | 17.40 | 0.60 | .42 | .53 | .12 |
|               | LM 18.40 | 18.00 | 17.50 | 17.40 | 0.60 | .27 | .43 | .10 |
| \( b^* \) – yellowness | BM 7.60 | 8.60 | 7.60 | 9.00 | 0.40 | .52 | .00 | .60 |
|               | LM 7.70 | 7.90 | 8.40 | 8.00 | 0.50 | .32 | .83 | .49 |

BM: breast muscles; LM: leg muscles.

\( a,b \):Values within a row followed by different letter differ significantly \( p < .05 \).
desirability (Table 6), and also for the juiciness and tenderness of heat-treated pectoralis major muscle in the assessment of trained judges. The breast muscles of chickens (male and female on average) fed the probiotics had lower scores for aroma intensity and desirability, juiciness, taste intensity and desirability and tenderness compared to the breast muscles of control birds.

**Microstructure of the meat**

The supplementation of EM probiotics did not have a significant (p > .05) effect on fibre cross section area, fibre diameter and perimeter, H:V diameter ratio, perimysium and endomysium thickness. Males had significantly (p < .05) lower fibre cross section area fibre perimeter and horizontal diameter compared to females. The group × sex interaction for the microstructure of *pectoralis major* muscle from 42-day-old broiler chickens was not significant (p > .05) (Table 7).

**Discussion**

The present study provides a scientific evaluation of the effectiveness of Pro-Biotyk Em-15 and EMFarma™ probiotics on the meat quality of broiler chickens raised on a commercial poultry farm as part of agricultural operations. Our results have shown that the EM probiotics containing *Lactobacillus* spp., *Bifidobacterium* spp., *Lactococcus* spp., *Streptococcus thermophilus*, *Bacillus subtilis*, *Rhodopseudomonas* spp. and *Saccharomyces cerevisiae* yeast do not have a significant effect on the water, protein and fat content of the muscles. However, chickens supplemented with EM probiotics had significantly less water and fat in

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**Table 5. Textural and rheological traits of pectoralis major muscle from 42-d-old Ross 308 broiler chickens.**

| Trait                     | Group (G) – sex (S) | Control | Probiotics | p Value |
|---------------------------|--------------------|---------|------------|---------|
|                          | Male (n = 12)      | Female (n = 12) | Male (n = 12) | Female (n = 12) | SEM | G | S | G × S |
| Hardness (N)              | 24.90              | 25.10   | 24.10      | 23.60   | 1.10 | .31 | .88 | .74   |
| Cohesiveness              | 0.30               | 0.30    | 0.30       | 0.30    | 0.10 | .62 | .12 | .51   |
| Springiness (cm)          | 1.40               | 1.10    | 1.20       | 1.10    | 0.10 | .65 | .01 | .41   |
| Chewiness (N × cm)        | 9.60               | 8.70    | 9.80       | 7.70    | 0.60 | .52 | .02 | .31   |
| Gumminess (N)             | 8.10               | 7.70    | 8.20       | 7.00    | 0.50 | .46 | .10 | .43   |
| WB shear force (N)        | 45.70              | 46.30   | 56.70      | 53.60   | 3.10 | .00 | .67 | .52   |
| Sum of elastic moduli (kPa) | 448       | 412     | 458        | 465     | 29.40 | .48 | .21 | .47   |
| Sum of viscous moduli (kPa × s) | 18466  | 17579     | 18635      | 18169   | 526  | .8   | .19 | .31   |
the leg muscles compared to control birds. The greater lengths of jejunum (by 4.9 cm), ileum (by 7.9 cm) and caecum (by 1.4 cm) and diameters of ileum (by 1.1 mm) and caecum (by 0.7 mm) in the experimental compared to control birds (unpublished data) could have contributed to the differences in the amount of absorbed nutrients and thus the chemical composition of meat. In the experimental facility, from 2 weeks of age, the probiotic solution was sprayed twice a week onto the feed and litter using a hand sprayer, which made the legs of experimental chickens more active and significantly reduced the fat content of leg muscles. The increased motor activity of the experimental birds is suggested by significantly lower leg muscle acidity (significantly higher pH24) resulting from the lower glycogen content of the leg muscles.

Inatomi (2015) stated that administration of probiotics containing Bacillus mesentericus, Clostridium butyricum, Streptococcus faecalis had no significant effect on the moisture, protein and ash content of breast and leg muscles in Cobb 500 chickens aged 49 days. However, the same study showed a decrease in the fat content of breast and leg muscles. Other experiments (Král et al. 2013; Abdulla et al. 2017) found a significant reduction in the fat content of breast muscles from broiler chickens when using probiotic containing Bacillus subtilis, but this was not observed in our study. A significant reduction in meat fat improves the dietetic value of the meat, which is desirable for consumers from most parts of the world. On the other hand, the higher content of intramuscular fat improves meat taste and tenderness (Chartrin et al. 2006). Zhou et al. (2010) reported no effect of probiotic on the moisture, protein, crude fat and crude ash content of breast and leg muscles from 90-day-old Guangxi Yellow chickens. Another experiment (Ivanovic et al. 2012) found a significant increase in moisture with a significant reduction in the fat and protein content of drumstick meat from 42-day-old Arbor Acres broilers in four groups receiving different feed probiotics containing Lactobacillus plantarum plus Streptococcus faecium or Streptococcus faecium or Bacillus cereus IP5832 or Bacillus CH200 plus Bacillus CH201.

In our study, the administration of EM probiotics containing Lactobacillus spp., Bifidobacterium spp., Lactococcus spp., Streptococcus thermophilus, Bacillus subtilis, Rhodospseudomonas spp. and Saccharomyces cerevisiae yeast had no significant effect on the physicochemical traits (pH24, EC24, drip loss, cooking loss, L*, a*, b*) of the breast and leg muscles, except for pH24 of the leg muscles. With regard to electrical conductivity of leg muscles measured 24 h (EC24) post-mortem, the compared groups of chickens tended to show significant differences (p = .053). The leg muscles of experimental chickens had greater electrical conductivity (EC24) than the leg muscles of control chickens, which was probably associated with the lower water and higher protein content of the leg muscles of chickens receiving probiotic preparations. Our results confirm the negative correlations between electrical conductivity measured 24 h post-mortem and the meat water content, as well as the positive correlations between EC24 of meat and protein content reported by Łyczynski et al. (2009). Pelicano et al. (2003) stated in their study a significant effect of supplementing drinking water with probiotic containing Lactobacillus reuteri and Lactobacillus johnsonii on lightness of breast meat from Cobb 500 broilers aged 45 days. However, the same experiment found that the dietary inclusion of probiotic containing Bacillus subtilis or Bacillus subtilis plus Bacillus licheniformis or Saccharomyces cerevisiae had no significant effect on the L* (lightness), a* (redness), b* (yellowness) colour coordinates of breast muscles in 45-day-old Cobb 500 chickens. In turn, Park and Kim (2014), as in our study, demonstrated no significant effect of probiotic containing Bacillus subtilis B2A on the L*, a*, b* coordinates of breast muscles. Zhou et al. (2010) observed no effect of dietary probiotic containing Bacillus coagulans ZJU0616 on pH of breast muscles in 90-day-old Guangxi Yellow chickens, whereas Pelica et al. (2004) reported that prebiotics and probiotics of bacterial and yeast origin containing Enterococcus sp. and mannan oligosaccharides obtained from the cellular wall of Saccharomyces cerevisiae had no significant effect on pH of breast and leg muscles in 84-day-old free-range broiler chickens. However, Abdulla et al. (2017) reported significantly higher L* (lightness), a* (redness) and b* (yellowness) values of breast muscles from Cobb 500 chickens at the age of 42 days, which had been supplemented with probiotic containing Bacillus subtilis during the rearing period, compared to control birds. The same authors reported a significant decrease in drip loss and cooking loss of breast muscles from 42-day-old Cobb 500 chickens receiving probiotic, but this was not the case in our study. The experiment of Park and Kim (2014) found a significant increase in water holding capacity (WHC) and a significant decrease in drip loss determined after 1-day storage of the samples with increasing concentration of probiotic containing Bacillus subtilis B2A. A significant decrease in drip loss from breast muscles of 90-day-old Guangxi Yellow chickens administered with Bacillus coagulans ZJU0616 was also noted by Zhou et al. (2010). In turn, Pelicano et al. (2003) failed to
observe a significant effect of probiotic containing *Bacillus subtilis*, *Bacillus subtilis* plus *Bacillus licheniformis*, and *Saccharomyces cerevisiae* on WHC and cooking loss, while Pelica et al. (2004) found no significant effect of supplementing prebiotics and probiotics of bacterial and yeast origin containing *Enterococcus* sp. and mannan oligosaccharides obtained from the cellular wall of *Saccharomyces cerevisiae* on cooking loss in 84-day-old free-range broiler chickens.

The results of our experiment indicate a significant deterioration in tenderness (expressed as significantly higher WB shear force) of the breast muscles from 42-day-old Ross 308 chickens supplemented with EM preparations added in water, feed, water line and air sprinklers. Another study (Abdulla et al. 2017) found no significant effect of probiotic application containing *Bacillus subtilis* on tenderness of breast muscles from 42-day-old Cobb 500 chickens. On the other hand, Zhou et al. (2010) reported a significant deterioration in meat tenderness in 90-day-old Guangxi Yellow chickens receiving *Bacillus coagulans* ZJU0616, which is consistent with our findings, whereas Yang et al. (2010) found improvements in meat tenderness (significantly lower shear force values) in 42-day-old male Arbor Acres broiler chickens fed *Clostridium butyricum*. Pelicano et al. (2003) demonstrated no significant effect of adding probiotics containing *Lactobacillus reuteri* and *Lactobacillus johnsonii* to water on the texture and preference of breast muscles from Cobb broiler chickens aged 45 days as well as significantly higher values for flavour and general aspect. The dietary inclusion of probiotics did not have any significant effect on flavour, texture, preference and general aspect of cooked breast muscles from Cobb broilers.

In our study, the application of EM probiotics containing *Lactobacillus* spp., *Bifidobacterium* spp., *Lactococcus* spp., *Streptococcus thermophilus*, *Bacillus subtilis*, *Rhodopseudomonas* spp. and *Saccharomyces cerevisiae* yeast had no significant effect on sensory properties evaluated by a panel of trained judges. In turn, Loddi et al. (2000) did not find any significant effect of probiotic containing *Enterococcus faecium* or avoparcin probiotic or probiotic mixed with antibiotic, on intensity of aroma, off-aroma, flavour, off-flavour, tenderness, juiciness, acceptability and overall aspect of breast muscles from 42-day-old Ross chickens compared to control chickens.

**Conclusions**

In summary, it is concluded that administration of the EM probiotics had no significant effect on most of the meat quality traits under analysis. Breast muscles of chickens receiving EM probiotics were characterised by poorer tenderness, whereas leg muscles had a higher water and fat content, as well as higher pH 24 h post-mortem. Sex of birds had a greater effect on meat quality. A significant effect was found for textural (springiness and chewiness) and microstructural traits (fibre cross section area, fibre perimeter, horizontal fibre diameter) of *pectoralis major* muscles, and for the fat content of the leg muscles.

**Disclosure statement**

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

**Funding**

This research was realised from statutory research funds BS-12/2012 and BSM 50/2012 assigned by the Ministry of Science and Higher Education, Republic of Poland.

**ORCID**

Kamil Stęczny [http://orcid.org/0000-0002-8352-8091](http://orcid.org/0000-0002-8352-8091)

Dariusz Kokoszynski [http://orcid.org/0000-0002-6642-1129](http://orcid.org/0000-0002-6642-1129)

**References**

Abdulla NR, Zamri ANM, Sabow AB, Kareem KY, Nurhazirah S, Ling FH, Szili AQ, Loh TC. 2017. Physicochemical properties of breast muscle in broiler chickens fed probiotics, antibiotics or antibiotic-probiotic mix. J Appl Anim Res. 45:64–70.

Barylko-Pikielna N, Matuszewska I. 2009. Sensoryczne badania żywności [Sensory food testing]. Kraków (PL): PTZZ. [Polish].

Bourne MC. 1982. Food texture and viscosity concept and measurement. New York (NY): Academic Press Inc.

Burck HC. 1975. Histological technology. Warszawa: Państwowy Zakład Wydawnictw Lekarskich.

Chartrain P, Méteau K, Juin H, Bernadet MD, Guy G, Larzul C, Rémingon H, Mourot J, Duclos MJ, Baëza E. 2006. Effects of intermuscular fat levels on sensory characteristics of duck breast meat. Poult Sci. 85:914–922.

CIELab Colour System. 1976. Commission Internationale de l’Eclairage. France: CIE Publication.

FAOSTAT. 2019. Livestock primary, production quantity, poultry meat, 2017. Rome: FAO Publisher; [accessed 2019 Apr 25] [http://www.fao.org/faostat/en/#data/QL](http://www.fao.org/faostat/en/#data/QL).

Gesek M, Sokól R, Lambert BD, Otrocka-Domagałła I. 2018. Effect of effective microorganisms on internal morphology and morphometry in Japanese quails. Turk J Vet Anim Sci. 42:285–291.

Gnanadesigan M, Sandhanasamy I, Ponnusamy S, Lakshmanan R, Natarajan M, Rajagopal R. 2014. Quality evaluation of egg compositions and productivity of layers...
in EM (effective microorganisms) treatments: a field report. Egypt J Basic Appl Sci. 1:161–166.
Ivanovic S, Baltic M, Popov-Raljic J, Pisinov B, Madlic-Strizak D, Stojanovic Z, Pavlovic I. 2012. The effect of different probiotics on broiler meat quality. Afr J Microbiol Res. 6: 937–943.
Inatomi T. 2015. Growth performance, gut mucosal immunity and carcass and intermuscular fat of broiler fed diets containing a combination of three probiotics. Sci Post. 1:1.
Jwher DMT, Abd SK, Mohammad AG. 2013. The study of using effective microorganism (EM) on health and performance of broiler chicks. Iraqi J Vet Sci. 27:73–78.
Kim HW, Yan FF, Hu JY, Cheng HW, Kim Y. 2016. Effects of probiotic feeding on meat quality of chicken breast during postmortem storage. Poult Sci. 95:1457–1464.
Král M, Angelovičová M, Alfaig E, Walczycka M. 2013. Meat quality of broiler chickens fed diets with Bacillus subtilis and malic acid additives. Sci Pap Anim Sci Biotech. 46: 375–378.
Lodzi MM, Gonzales E, Takita TS. 2000. Effect of the use of probiotic and antibiotic on the performance, yield and carcass quality of broilers. Braz J Anim Sci. 29:1124–1131.
Łyczynski A, Runowska G, Pospiech E, Kościński-Podziadła M, Wojczak J, Rzosińska E, Grześ B, Mikołajczak B, Iwańska E. 2009. Estimation of selected porcine meat quality indicators on the basis of electrical conductivity measured 24 hours post-slaughter. Anim Sci Pap Rep. 27:5158.
Park JH, Kim JH. 2014. Supplemental effect of probiotic Bacillus subtilis B2A on productivity, organ weight, intestinal Salmonella microflora, and breast meat quality of growing broiler chicks. Poult Sci. 93:2054–2059.
Pelicano ERL, de Souza PA, de Souza HBA, Obá A, Norkus EA, Kodawara LM, de Lima TMA. 2003. Effect of different prebiotics and probiotics on broiler carcass and meat quality. Rev Bras Cienc Avic. 5:207–214.
Pelicia K, Mendes A, Sadanha E, Pizzolante CC, Takahashi SE, Moreira J, Garcia RG, Quinteiro RR, Paz I, Komiyama CM. 2004. Use of prebiotics and probiotics of bacterial and yeast origin for free-range broiler chickens. Braz J Poult Sci. 6:163–169.
Pietrzak D, Mroczek J, Garbaczewska A, Florkowski T, Riedel J. 2009. Effects of selected antimicrobial feed additives on the quality of meat and fat of chicken. Med Vet. 65: 268–271.
SAS Institute Inc. 2014. SAS/STAT user's guide, version 9.4. Cary, NC: SAS Institute Inc.
Simeamela M, Solomon D, Taye T. 2013. The effect of effective microorganisms on production and quality performance of Rhode Island Red layers. Int J Livest Prod. 4: 22–29.
Takahashi SE, Mendes AA, Saldanha E, Pizzolante CC, Pelicia K, Quinteiro RR, Komiyama CM, Garcia RG, Almeida Paz ICL. 2005. Efficiency of prebiotics and probiotics on the performance, yield, meat quality and presence of Salmonella spp. in carcass of free-range broiler chickens. Rev Bras Cienc Avic. 7:151–157.
Wondmeneh E, Gattachew T, Dessie T. 2011. Effect of effective microorganisms (EM) on the growth performance of Fayouni and Horro chicken. Intern. J Poult Sci. 10:185–188.
Yang X, Zhang B, Guo Y, Jiao P, Long F. 2010. Effects of dietary lipids and Clostridium butyricum on fat deposition and meat quality of broiler chickens. Poult Sci. 89:254–260.
Zhou X, Wang Y, Gu Q, Li W. 2010. Effect of dietary probiotic, Bacillus coagulans, on growth performance, chemical composition, and meat quality of Guangxi Yellow chicken. Poult Sci. 89:588–593.