Effects of in ovo injection of prebiotics and synbiotics on the productive performance and microstructural features of the superficial pectoral muscle in broiler chickens

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ABSTRACT The aim of the study was to compare the effects of 2 prebiotics and 2 synbiotics injected in ovo on productivity parameters, quality, and microstructure of the superficial pectoral muscle in 35-day-old broiler chickens. On day 12 of incubation, 9,000 eggs Ross 308 were randomly divided into 5 experimental groups treated with different bioactives in ovo injected: C, control with physiological saline; PI, with 1.760 mg inulin; PB, with 0.528 mg of commercial prebiotic Bi^{2}tos; SI, with 1.760 mg inulin and 1,000 CFU *Lactococcus lactis spp. lactis* IBB SL1; SB, with 0.528 mg Bi^{2}tos and 1,000 CFU *Lactococcus lactis spp. cremoris* IBB SC1. The synbiotic solution contained 20 μl bacterial suspension and 180 μl prebiotic solution. For productive parameters and further tests ten male birds for each experimental group were used. The birds were slaughtered on day 35 of age. At slaughter, samples of the left pectoral muscles were taken and preserved by freezing in liquid nitrogen. The pH and color of the meat were evaluated at 45 min and 24 h *post-mortem*. Water holding capacity (**WHC**) was measured and expressed as the percentage of free water in meat. Microscopic specimens were analysed using MultiScan software for the measurement of the percentage of oxidative and glycolytic fibres and mean diameter of the muscle fibres. In ovo injection of prebiotics Bi^{2}tos had a positive effect on body weight. In prebiotic group (PI) a negative impact on hatchability was observed. Prebiotics and synbiotics had no influence on the yield of the carcass and pectoral muscle. Bioactive compounds had a significant effect on the quality of meat parameters such as: pH 24 h (PI and PB group), L* 45' (SI and SB group), and WHC (groups PB, SI, and SB). The analysis of the enzymatic profile showed a significant increase in the percentage of glycolytic fibres in the pectoral muscle from chicken treated with a synbiotic with the addition of inulin (group SI).

Key words: prebiotics, synbiotics, in ovo technology, meat quality, histology

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INTRODUCTION

Bioactive compounds are defined as physiologically active and safe components of foods or food supplements that meet the basic human nutritional requirements necessary for maintaining health. These compounds contain small amounts of chemicals that naturally occur in whole plants, fruits, vegetables, nuts, oils, and cereal grains (Guaadaoui et al., 2014). They are exempt from waiting period requirements, have an immunostimulating effect (Madej and Bednarczyk 2016), and allow for the control of pathogens in the animal gastrointestinal tract (Chaveerach et al., 2004). Bioactive substances stimulate the body to produce more efficiently, positively affect animal health, reduce intestinal diseases, and the risk of infection of poultry products (Apata, 2009; 2012). There are many mode of action of probiotics, prebiotics and synbiotics. Due to the diversity and complexity of these processes, which are not fully understood, these mechanisms become the source of many discussions (Nowak et al., 2010; Dankowiakowska et al., 2013; Khan and Naz, 2013; Bogucka et al.,2017; Khare et al., 2018).

Prebiotics are sugars, mainly polysaccharides, resistant to digestion in the stomach and intestines of monogastric animals. They selectively stimulate the growth and/or activity of beneficial intestinal microbial flora or reduce the count of coliforms (Gibson and Roberfroid, 1995). Prebiotics are utilized by gut bacteria which convert indigestible carbohydrates into a source of energy. Prebiotic substances can be used for the manipulation of endogenous bacterial flora in the gastrointestinal tract and to promote the role of beneficial intestinal microorganisms (Hajati and Rezaei, 2010).

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It is assumed that prebiotics can have a direct and indirect action on the structure and function of the intestines. Seifert and Watzl (2007) reported that the direct effect of prebiotics relies on their partial absorption in the intestine, and interaction with epithelial cells and immune cells. The indirect effect of prebiotics comes from the modulation of the host's gut microbiome. This mechanism is possible because, contrary to a recently invalidated paradigm, the bird's digestive tract is not sterile, and its colonization begins on embryonic day (Gantois et al., 2009; Kizerwetter-Swida and Binek, 2008; Pedroso et al., 2016). Prebiotics are utilized by bifidobacteria and LAB strains, and after fermentation short chain fatty acids are formed (Mista et al., 2017), which reduce pH, increase the acidity of the intestinal environment, and are also the main source of energy for intestinal epithelial cells. Synbiotic is a product containing prebiotic and probiotic substances. This is an optimal combination of suitably selected probiotic bacteria and prebiotics. The prebiotic is a source of energy and a stimulant for beneficial bacteria (Gibson and Roberfroid, 1995). The mode of action of synbiotics relies on synergies action. These substances improve the birds' resistance, significantly affect lymphatic tissue accumulation in the intestines, increase the diameter and thickness of the intestinal wall of tonsils and increase glucose absorption (Awad et al., 2008; Majd et. al., 2013). The use of synbiotic is more effective than administration of probiotics or prebiotics alone. Feeding birds with fructooligosaccharide in combination with a CE treatment had positive influence on reduction in both the number of Salmonella-positive birds and the number of Salmonella per gram of ceca, compared to diets contain only with fructooligosaccharide (Bailey et al.,1991; Awad et al., 2009 Fallah et. al., 2013).

Previous studies on the effectiveness of probiotics, prebiotics, and synbiotics supplied with feed or water are mainly focused on the evaluation of parameters of animal productivity. While, findings on the effects of these bioactive compounds on meat quality are few and inconsistent, and mainly have regarded features associated with texture and palatability, as well as pH, water holding capacity (**WHC**), and color (Park and Kim, 2014). On the other hand, there is a limited number of research dealing with the effects of bioactive compounds injected in ovo on the features of meat quality associated with the microstructure of birds' skeletal muscles (Maiorano et al., 2012 and 2017; Tavaniello et al., 2018). Therefore, this study is intended to be a contribution to the current state of knowledge on this subject. Verification of the hypothesis is based on the possibility of affecting the embryo in two ways. First option by using prebiotic, we can stimulate endogenous development beneficial microflora, and second one by introducing a synbiotic, we can supply exogenous beneficial microflora, along with selected prebiotic specific for its development.

The aim of our study was to determine the effects of prebiotics and synbiotics injected in ovo on day 12 of embryonic development on the hatchability, broilers' performance (growth, feed intake, and conversion rate, carcass traits), some meat quality traits (pH, color, WHC) and microstructural features of pectoral muscle in 35-day-old broiler chickens.

MATERIAL AND METHODS

Birds, Experimental Design and Rearing Measurements

Hatching eggs (∼60 g) collected from 32-wk-old breeder flock (Ross 308) were incubated at a commercial hatchery (Drobex, Solec Kujawski, Poland) in a Petersime incubator (Zulte, Belgium). On day 12 of incubation eggs were candled, and unfertilized eggs and dead embryos were discarded. Immediately afterwards, 9,000 eggs were randomly allotted into 5 experimental groups (1800 eggs/group) and treated with different bioactives (dissolved in 0.2 ml of physiological saline), automatically injected in ovo into the air chamber (Bednarczyk et al., 2011): C, control with physiological saline; PI, with 1.760 mg inulin (Sigma – Aldrich, St. Louis, MO); PB, with 0.528 mg of a commercial prebiotic Bi^{2}tos (Clasado Ltd., Sliema, Malta), a non-digestive trans galactooligosaccharides (GOS) from milk lactose digested with *Bifidobacterium bifidum* NCIMB 41171; SI, with 1.760 mg inulin and 1,000 CFU *Lactococcus lactis* spp. *lactis* IBB SL1; SB, with 0.528 mg Bi^{2}tos and 1,000 CFU *Lactococcus lactis* spp. *cremoris* IBB SC1. The synbiotic solution contained 20 μl of bacterial suspension and 180 μl of prebiotic solution (Pruszynska – Oszmalek et al., 2015). The synbiotics were selected from several combination of pre- and probiotics *via* in vitro tests, which were followed by validation with an animal model (Bednarczyk et al., 2013; Slawinska et al., 2016).

At hatching, number of healthy chicks was scored for each experimental group. The values were expressed as a percentage of the total number of injected eggs. Afterwards, chicks were sexed and 3,250 males (42.0 g average weight) were housed at the experimental farm of the University of Warmia and Mazury in Olsztyn (Poland). The animals were reared according to the Polish Local Ethical Commission (No 22/2012. 21.06.2012) and in accordance with the animal welfare recommendations of European Union directive 2010/63. Birds were reared in pens (3.75 m^2) on litter with a stocking density of 17.33 birds/m^2, obtaining 10 repetitions per group. All birds were fed *ad libitum* the standard commercial feed mixtures (Table 1): starter (day 1 to 14), grower (day 15 to 30), finisher (day 31 to 34). The birds had constant access to water and feed. Amounts of feed offered to each pen were recorded, and uneaten feed in each pen was weighed daily (from 1 to 34 d). Along the rearing period, the growth and mortality of the birds were recorded. Cumulative daily feed intake and feed conversion ratio (**FCR**) and

Table 1. Composition and nutritional value of experimental feed mixtures for Ross 308 broiler chickens.

	Starter	Grower	Finisher
Ingredient (%)			
Wheat	26.73	29.19	30.66
Maize	30.00	30.00	30.00
Extracted soybean meal	32.50	28.20	25.33
Canola	5.00	6.00	7.00
Soybean oil	2.10	1.33	1.80
Lard	-	2.00	2.50
NaCl	0.30	0.30	0.28
Mel stern	1.09	0.95	0.85
Phosphate 1-Calcium	1.15	0.94	0.63
DL-Methionine	0.25	0.18	0.13
L-Lysine	0.32	0.32	0.27
L-threonine	0.06	0.09	0.05
Vitamin-mineral premix[1]	0.50	0.50	0.50
Calculated composition			
ME (kcal/kg)	2 980	3 100	3 200
CP (%)	22.00	20.50	19.50
Lysine (%)	1.35	1.25	1.15
Methionine (%)	0.57	0.49	0.43
Methionine + Cystine (%)	0.95	0.85	0.78
Calcium (%)	0.90	0.80	0.70
Phosphorus (%)	0.40	0.35	0.28
Sodium (%)	0.14	0.14	0.13

[1]Provided per kilogram of diets: vitamin A – 5,000,000 IU, vitamin D_3 – 1,400,000 IU, vitamin E – 18,200 mg, vitamin K_3 – 1,200 mg, vitamin B_1 – 600 mg, vitamin B_2 – 2,000 mg, vitamin B_6 – 1,200 mg, vitamin B_{12} – 8,000 mg, biotin (H) – 80,000 mg, Fe – 20,000 mg, Mn – 40,000 mg, Zn – 36,000 mg, Cu – 6,000 mg, I – 400 mg, Se – 140 mg, calcium pantothenate – 4,800 g, nicotinic acid – 20,000 mg, folic acid – 400 mg, choline chloride – 380 g, phytase – 500 FTU.

European Broiler Index were calculated on a pen basis and were corrected for mortality, taking into account weight and life duration of dead birds.

Slaughter Surveys

At 35 d of age, 10 birds for each group were randomly chosen (50 birds per treatment), individually weighed (after a fasting period of 12 h) and transported within 1 h (including careful catching and loading) to a commercial poultry slaughterhouse. After careful unloading and hanging in randomized order, all birds were electrically stunned and slaughtered. The hot carcass weight was recorded, and carcass yield was calculated. In addition, the pectoral muscle was removed from all carcasses and its percentage based on hot carcass weight was calculated.

pH, Color, and WHC

On pectoral muscle the following determinations were carried out: i) pH was recorded at 45 min (pH_{45}) and 24 h (pH_{24}) *post-mortem* using a portable pH–meter (R. Matthäus, Pöttmes, Germany) fitted with an integrated glass blade electrode. The device was calibrated with buffers of pH 4.0 an d pH 7.0; ii) at 45 min and 24 h post – mortem tri – stimulus color coordinates (lightness, L^*; redness, a^*; yellowness, b^*) were detected using a Chroma Meter CR-300 (Italia s.r.l.,Milano). Reflectance measurements were performed after the samples had oxygenated in air for at least 30 min by which time measurements were stable, taking 3 readings for each sample; iii) at 24 h post mortem, the WHC was determined using filter paper (Whatman No. 1) press method and was expressed as free water in meat (Grau and Hamm, 1952).

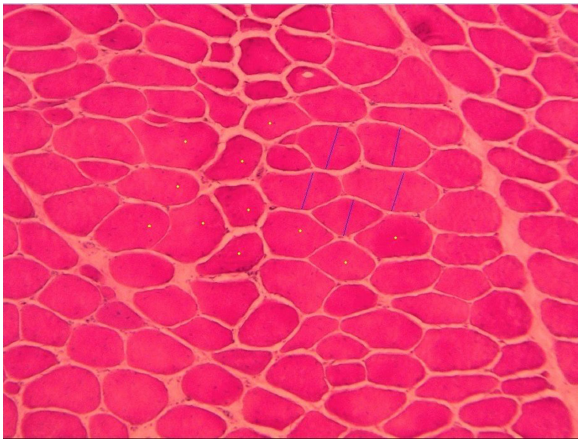

Figure 1. Cross-section of the *pectoralis superficialis* muscle of 35-day-old broiler chickens Ross 308, HE stain. The method of determining the number and diameter of muscle fibers (magnification × 100).

Microstructural Analysis of the Pectoral Muscle

Histological specimens for the microstructural analysis of pectoral muscles were prepared using the cryosection (freezing) technique. Immediately after slaughter, samples of the superficial pectoral muscle *(musculus pectoralis superficialis)* were taken, frozen in liquid nitrogen, then cut in a Leica cryostat into 10 μm slices. Slices were placed on microscope slides and stained using different histochemical reactions: haematoxylin and eosin staining (H+E) for the measurement of the diameter and density of muscle fibres. The NADH – tetrazolium reductase (NADH – R) to visualize two types of muscle fibres with different enzymatic activity: 1) fibres with high and moderated NADH – TR activity, slow- and fast – contracting oxidative fibres stained blue; and 2) fibres with low NADH – TR activity, white fast – contracting fibres stained a pale yellow color.

Microscopic Analysis

Prepared specimens were analysed microscopically using MultiScan v. 18.03 software (Computer Scanning System II Ltd, Warsaw, Poland). The mean diameter of muscle fibres (Figure 1) and percentage share of oxidative and glycolytic fibres per mm^2 were estimated.

Statistical Analysis

Descriptive statistics were applied, and data were presented as X ± SEM and standard error mean (SEM). Significance of differences between the experimental groups was estimated by one – way analysis of variance (ANOVA) and the Duncan test. All calculations were performed using computer software STATISTICA AXAP, version 10.0 MR1.

RESULTS AND DISCUSSION

Hatching rate and Productivity Parameters of Broiler Chickens

The hatching rate depends on many factors. Apart from hatching technology, it is determined directly by the quality of the shell (thickness, porosity, resistance to crushing) and the content of the egg, and indirectly by the conditions of rearing, feeding, and health of the stock (Krawczyk et al., 2012; Siwek et al., 2018). A high hatching rate was observed, ranging from 89.58 to 92.72%. However, the hatching rate in the PI group (inulin) was lower ($P < 0.05$) than in the C group; while, intermediate values were found for PB and S1 groups ($P > 0.05$). In hatcheries providing optimal conditions, the hatching rate of broiler chicken reaches 88–90% (Krawczyk et al., 2012). Correctly performed in ovo injection has no negative effect on hatching rates. Synbiotics were delivered to Cobb broiler chicken embryos on day 12 of incubation into the egg air chamber. Hatchability was 89.1, 91.6, and 91.9% in the S1, S2, and C groups, respectively. (Dunislawska et al., 2017). However, some authors have indicated both positive and negative impacts of certain bioactive compounds on chicks hatching. Authors found that probotics which contain bacteria like *Lactobacillus fermentum, Lactobacillus helveticus, Lactobacillus paracasei, Lactobacillus salivarius, Pediococcus parvulus* (dose 6 mg/egg), and *Bacillus subtilis* (in doses: 16×10^9 cfu and 32×10^9 cfu/egg) injected into the amniotic fluid in 17.5 d of incubation in significantly decreased hatchability (De Oliveira et al., 2014).

Broiler feed accounts for over 70% of the total production costs, and effective use of feed by birds is linked to the profitability of production. In our research, daily weight gain (ranging from 57.76 to 59.83), daily feed intake (ranging from 89.71 to 93.22), and FCR (ranging from 1.54 to 1.56) were not affected ($P > 0.05$) by the prebiotics and synbiotics treatments. In the same trial, EBI was it was higher in prebiotic (PB) and synbiotics (SI and SB) groups compared with the control (Table 2).

The injection of synbiotics and prebiotics significantly affected the average body weight of birds (table 3). Compared with C group, chickens from PB (Bi^2tos) and SI (*L. lactis* + inulin) groups were heavier ($P < 0.05$). While, the in ovo injection of inulin (PI) and probiotic enriched with Bi^2tos (SB) had no positive effect on chickens' BW compared to C group ($P > 0.05$). In addition, when we compered influence of prebiotics and synbiotics, SB birds had lower BW ($P < 0.05$) compared to PB ones, and chickens from PI group had lower BW compared with those of PB and SI groups. Pruszynska – Oszmalek et. al. (2015) found that the in ovo injection of prebiotics (inulin and Bi^2tos) and synbiotics (inulin + *Lactococcus lactis* subsp. *lactis* and Bi^2tos + *Lactococcus lactis* subsp. *cremoris*) caused an elevation of the activity of pancreatic enzymes, which can explain the observed higher BW of treated chickens. Sobolewska et al. (2017) also found that the use of the above mentioned synbiotics significantly affect gut structure which should contribute to improvement in nutrient absorption with the gut. Maiorano et al. (2012) found that chickens acquiring in ovo injection of solution containing 1.9 mg of raffinose family oligosaccharides (**RFO**) or synbiotics (homemade synbiotics: 1.9 mg of RFO + 1,000 cfu of *Lactococcus lactis* ssp. *cremoris* IBB SC1; commercial symbiotic: Duolac) had slightly higher final BW compared with control birds. In a recent study conducted on 275,000 birds, Maiorano et al. (2017) found that an administration in ovo with different commercial prebiotics (Bi^2tos or DiNovo) determined a small increase in average BW compared to the control. On the contrary, Slawinska et al. (2014), testing different kind of in-house and commercial synbiotic combinations (RFO + *Lactobacillus lactis* or lactose + *Lactobacillus acidophilus* + *Streptococcus faecium*) did not state any influence of synbiotics on chicken BW. Similar results were found by Tavaniello et al. (2019) that in ovo delivery of 2 synbiotics (*Lactobacillus salivarius* + GOS and *Lactobacillus plantarum* + Lupin RFO). Considering the feeding trials, Awad et al. (2009) and Swamy and Upendra (2013) observed that prebiotics or synbiotics significantly increased the BW of 35-day-old chickens. Aziz Mousavi et al. (2015) observed a positive and significant correlations ($P < 0.01$) between the synbiotic Biomin®IMBO and broilers' BW gain during the starter phase; although during the grower period, the synbiotic seemed to increase feed consumption of broilers but the differences in BW gain were not significant. These reports indicate that the differences in the types and doses of prebiotics or synbiotics, mode of their administration and environmental factors can produce varying responses in performance. Carcass yield (ranging from 63.76 to 67.95%) and breast muscle yield (ranging from 29.02 to 29.71%) were not affected ($P > 0.05$) by prebiotics and synbiotics treatment. Similar findings were reported by Tavaniello et al. (2018) and Maiorano et al. (2012), except for Duolac group that showed lower carcass yield. In contrast, in a feeding trial, Awad et al. (2009) found a significantly higher carcass yield in synbiotic treated broilers compared to control and prebiotic-fed broilers. Cheng et al. (2017) in a study conducted on 96-day-old male broiler chicks (Arbor Acres Plus) found that the dietary inclusion of synbiotic, consisting of probiotics (*Bacillus subtilis*, *Bacillus licheniformis*,

Table 2. Effect of in ovo prebiotic and synbiotics administration on productive traits of broilers at 35 d of age.

	Group[1]						
Item[2]	C	PI	PB	SI	SB	SEM	*P* -value
DWG (g/bird/d)	58.08	57.76	59.83	59.61	59.01	0.451	0.535
FI (g/bird/d)	91.08	89.71	93.07	93.22	92.04	0,489	0.120
FCR*(kg/kg)	1.56	1.54	1.55	1.56	1.55	0.01	0.928
EBI*	378	379	389	387	380	0.927	7.711
Chicken mortality* (%)	2.86	3.74	4.18	3.52	4.40	-	-
Hatchability* (%)	97.72[a]	89.58[b]	91.82[a,b]	92.42[a,b]	91.43[a,b]	-	0.811

[a–c]Means within a row with different letters are significantly different ($P < 0.05$);
[1]Group: C = Control, in ovo injection of physiological saline; PI = Prebiotic 1 (inulin); PB = Prebiotic 2 (Bi^{2}tos);
SI = Synbiotic 1 (inulin + *Lactococcus lactis* ssp. *lactis*); SB = Synbiotic 2 (Bi^{2}tos + *Lactococcus lactis* ssp. *cremoris*);
[2]DWG = Daily Weight Gain; FI = Feed Intake; FCR = Feed Conversion Ratio;
*data presented in Pruszynska – Oszmalek et. al. (2015) and Bogucka et. al (2017).

Table 3. Effect of in ovo prebiotic and synbiotics administration on carcass traits of broiler chickens at 35 d of age.

	Group[1]						
	C	PI	PB	SI	SB	SEM	*P* -value
Final body weight*(g)	2 064[c]	2 061[c]	2 140[a]	2 120[a,b]	2 093[b,c]	6.000	$P < 0.05$
Carcass yield (%)	67.95	66.59	63.76	65.37	65.43	1.019	0.772
Breast muscle yield (%)	29.48	28.97	29.18	29.02	29.71	0.200	0.750

[a–c]Means within a row with different letters are significantly different ($P < 0.05$);
[1]Group: C = Control, in ovo injection of physiological saline; PI = Prebiotic 1 (inulin); PB = Prebiotic 2 (Bi^{2}tos);
SI = Synbiotic 1 (inulin + *Lactococcus lactis* ssp. *lactis*); SB = Synbiotic 2 (Bi^{2}tos + *Lactococcus lactis* ssp. *cremoris*);
*data presented in Pruszynska – Oszmalek et. al. (2015) and Bogucka et. al (2017).

and *Clostridium butyricum*) and prebiotics (yeast cell wall and xylooligosaccharide) significantly increased breast yield compared to control group.

Meat Quality Parameters

Results of pH, color, and WHC are presented in Table 4. pH indicates the level of glycolytic transformations in the muscle, and is the basic parameter used for the assessment of meat quality, processing suitability and hardness. In our study, pH_{45} (ranging from 6.57 to 6.62) was found to be similar ($P > 0.05$) among groups. The intergroup differences in the pH of meat were recorded not earlier than 24 h *post-mortem*. In fact, pH_{24} was significantly higher in PI group than in PB group ($P < 0.05$). Intermediate values were found for C, SI and SB groups ($P > 0.05$). The pH values obtained in our experiment were comparable to those reported by Maiorano et al. (2012) and Tavaniello et al. (2018). However, Jakubowska et al. (2014) revealed that probiotics and prebiotics supplied with feed and water had no significant effect on the pH of broiler chicken meat. Similar findings were made by Park and Kim (2014), who found similar pH values in the group treated with the highest dose of probiotic and in the group treated with Bit^{2}tos prebiotic. Conversely, Cheng et al. (2017) found that the breast muscle pH value at 24 h postmortem in broilers was elevated with the incorporation of synbiotic in comparison to control group.

Ultimate meat pH influences directly other meat attributes such as color which is an important commercial feature, since it affects the decisions of consumers purchasing meat and is closely associated with meat freshness and quality (Salakova et al., 2009). In the case of poultry meat, it is assumed that the natural color is from greyish white to matte red. In ovo injection of synbiotics significantly influenced the lightness of chicken meat at 45 min *post-mortem*. This parameter was significantly higher in the SB group compared to SI group ($P < 0.05$); no differences ($P > 0.05$) were detected for L* measured at 24 h among the experimental groups. Lightness (L*) measured 24 h *post-mortem* was similar to that reported by Jakubowska et al. (2014) and Park and Kim (2014). Redness (a*) and yellowness (b*) of meat measured 45 min and 24 h *post mortem* were found to be similar ($P > 0.05$) among groups. The color measurements at 24 h, when the color is stabilized, are within the acceptable range for commercial meats.

Meat color is correlated with WHC, low pH of meat is associated with low WHC. Present study revealed the most beneficial value of WHC in C group as compared to PB, SI, and SB groups ($P < 0.01$). Intermediate value was found in PI group ($P > 0.05$). Some studies reported that dietary probiotic supplements to chicken could improve meat quality attributes; it was found that dietary administration of probiotics to broiler chickens increased WHC value of breast meat compared to control birds (Ali, 2010). Hascik et al. (2009) found higher protein content in breast muscle of chickens fed supplemented with probiotics; they suggested that the increasing of protein content in breast muscle is directly related to the increase in water molecules binding to proteins, and thus subsequently might improve WHC.

Table 4. Effect of in ovo prebiotic and synbiotics administration on meat quality traits of broiler chickens at 35 d of age.

	Group[1]						
	C	PI	PB	SI	SB	SEM	*P*-value
pH 45 min	6.62	6.57	6.61	6.59	6.58	0.020	0.935
pH 24h	5.85^{a,b}	5.91^{a}	5.79^{b}	5.82^{a,b}	5.86^{a,b}	0.014	$P<0.05$
Color 45 min							
L*	47.10^{a,b}	47.62^{a,b}	47.10^{a,b}	45.81^{b}	48.54^{a}	0.323	$P<0.05$
a*	5.06	5.06	4.87	5.11	4.95	0.162	0.991
b*	3.32	2.77	2.75	3.28	2.90	0.142	0.559
Color 24 h							
L*	53.16	52.95	54.24	53.90	52.78	0.274	0.384
a*	6.45	6.82	6.69	6.17	6.06	0.208	0.757
b*	3.78	3.67	3.88	3.88	4.21	0.180	0.915
WHC (%)	19.94^{B}	21.06^{A,B}	23.28^{A}	22.56^{A}	22.85^{A}	0.328	$P<0.01$

^{A,B}Means within a row with different letters are significantly different ($P < 0.01$).
^{a,b}Means within a row with different letters are significantly different ($P < 0.05$).
[1]Group: C = Control, in ovo injection of physiological saline; PI = Prebiotic 1 (inulin); PB = Prebiotic 2 (Bi^{2}tos);
SI = Synbiotic 1 (inulin + *Lactococcus lactis* ssp. *lactis*); SB = Synbiotic 2 (Bi^{2}tos + *Lactococcus lactis* ssp. *cremoris*).
L*a*b* - color parameters: L*- lightness, a* - redness, b* - yellowness.
WHC- Water Holding Capacity.

In a recent study, Mahmoud et al. (2017) found that adding *B. subtilis* had no effect on the meat quality measurements, even if dietary levels of crude protein in the diet reduced meat WHC. Taking into account prebiotics, Park and Park (2011) observed a significantly higher WHC in breast muscle of chickens supplied with inulin compared with control birds. Some other studies noted that there was no synergistic effect of probiotics and prebiotics on chicken meat quality (Zhang et al., 2006).

Microstructural Features of Pectoral Muscles

Molecular and histological analysis of myogenesis allows for the development of effective methods for improving meat yield and quality. The structure and function of muscles are determined during incubation. The number of muscle fibres depends on many genetic and environmental factors. Rehfeldt et al. (1999) concluded that the number of muscle fibres in mammals and birds remains unchanged. However, studies of Knight and Kothary (2011) revealed that during embryonic development the number of fibres in skeletal muscles continues to increase. After hatching, red fibres transform into white fibres, and in the later growth phase, into intermediate fibres (Seideman et al., 1984). Considering this fact, we decided to evaluate the effect of bioactive compounds on the two types of muscle fibers: oxidative – βR and glycolytic – αW (Figure 2). According to Smith et al. (1993), glycolytic fibers (white) account for 96% of the pectoral muscle in broiler chickens. In our study their share was in the range of 79.95 to 86.86%. Breast muscle from group SI showed higher percentage of glycolytic fibers (+6.9%) compared with control ($P < 0.05$) and intermediate values ($P > 0.05$) were detected for PI, PB, and SB groups (Table 5). Bioactive compounds also had a significant effect on oxidative muscle

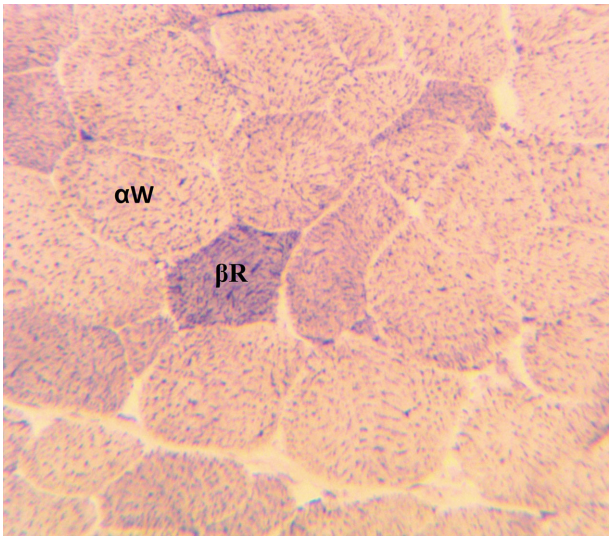

Figure 2. Muscle fibre types: αW (glycolytic) and βR (oxidative), NADH-TR tetrazolium reductase activity stain, magnification × 200.

fibres. A significantly lower share of oxidative fibres was found in group SI as compared to the control group ($P < 0.05$), while intermediate values were observed for the other groups ($P > 0.05$). Despite the fact that the chicken *musculus pectoralis superficialis* has a homogeneous structure, has been found a place in the chicken breast muscle in which we can found the population of the glycolytic and oxidative fibers. This area lies close to the wishbone and is characterized by a higher average level of staining for oxidative enzymes (Edman et. al, 1988). Intensive poultry production affected the growth of muscular tissue of birds, mainly of breast muscles in chicken broiler. The development of muscle tissue is not accompanied by angiogenesis, as a result of which the muscles are not well supplied with blood. As a result of a little myoglobin amount and low capillary density is a conversion of oxidative muscle fibers to glycolytic type (Scott et. al, 2001). In addition, as a result of intensive

Table 5. Effect of in ovo prebiotic and synbiotic administration on microstructural features of pectoral muscle of broiler chickens at 35 d of age.

	Group[1]						
	C	PI	PB	SI	SB	SEM	*P -value*
Oxidative fibres (%)	20.05[a]	14.18[a,b]	17.21[a,b]	13.14[b]	14.69[a,b]	0.950	$P < 0.05$
Glycolitic fibres (%)	79.95[b]	85.82[a,b]	82.79[a,b]	86.86[a]	85.31[a,b]	4.091	$P < 0.05$
Oxidative fibres diameter (μm)	32.21	34.39	36.02	30.34	32.40	0.830	0.281
Glycolitic fibres diameter (μm)	49.09	48.67	46.67	44.94	47.67	0.694	0.336
Fibre diameter (μm)	41.60	40.99	39.43	37.93	38.02	0.610	0.198
Fibre density (n./mm^2)	147.90	172.13	178.90	188.33	184.75	6.194	0.106

[a,b]Means within a row with different letters are significantly different ($P < 0.05$).
[1]Group: C = Control, in ovo injection of physiological saline; PI = Prebiotic 1 (inulin); PB = Prebiotic 2 (Bi^{2}tos); SI = Synbiotic 1 (inulin + *Lactococcus lactis* ssp. *lactis*); SB = Synbiotic 2 (Bi^{2}tos + *Lactococcus lactis* ssp. *cremoris*).

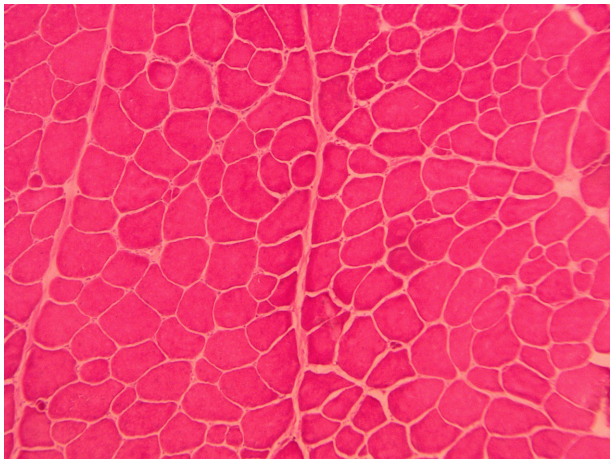

Figure 3. Cross-section of the *pectoralis superficialis* muscle of 35-day-old broiler chickens Ross 308, HE stain, (magnification × 100).

genetic selection of chickens in pectoral muscles we can observe a large number of pathological changes such as gigant fibers or atrophy (Dransfield and Sośnicki, 1999; Remignon et al. 2000).

Muscle weight is determined by the number and diameter of muscle fibers (Rehfeld et al. 1999). Scheuermann et al. (2004) reported greater density of fibers in muscles from male broilers compared to those from females. On the other hand, Chiang et al. (1995) found no correlation between bird sex and muscle density. In our study, bioactive compounds had no significant effect ($P < 0.05$) on the diameters of oxidative and glycoltic fibers, and the density of muscle fibers. Mean muscle fiber diameter range from 37.93 μm (SB) to 41.60 μm (C), what is reflected in the fiber density (Figure 3). The number of fibres per mm^2 was slightly highest ($P > 0.05$) in groups treated with synbiotics (SI and SB; 188.33 and 184.75, respectively). The lowest number of fibers per mm^2 was found in the control group ($P > 0.05$).

CONCLUSIONS

The effect of bioactive compounds injected in ovo on muscle microstructure is rarely investigated. Despite the important role that probiotics, prebiotics, and synbiotics have begun to play in animal nutrition, very few reports on this subject are available (Maiorano et al., 2012; Tavaniello et al., 2018). Findings from our study did not provide a conclusive explanation as to the effect of prebiotics and synbiotics injected in ovo on the quality parameters and microstructural features of pectoral muscles in broiler chickens, therefore this issue should be further investigated.

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REFERENCES

Ali, F. H. M. 2010. Probiotics feed supplement to improve quality of broiler chicken carcasses. WJDFS. 5: 93–99.
Apata, D. F., 2009. Antibiotic resistance in poultry. Int. J. Poult. Sci. 8:404–408.
Apata, D. F., 2012. The emergence of antibiotics resistance and utilization of probiotics for poultry production. Sci. J. Microbiol. 2:8–13.
Awad, W. A., K. Ghareeb, and J. Böhm. 2008. Intestinal structure and function of broiler chickens on diets supplemented with a synbiotic containing *Enterococcus faecium* and oligosaccharides. IJMS 9:2205–2216.
Awad, W. A., K. Ghareeb, S. Abdel – Raheem, and J. Böhm. 2009. Effects of dietary inclusion of probiotic and synbiotic on growth performance, organ weights, and intestinal histomorphology of broiler chickens. Poult. Sci. 88: 49–56.

Aziz Mousavi, S. M. A., A. Seidavi, M. Dadashbeiki, A. Kilonzo – Nthenge, S. N. Nahashon, V. Laudadio, and V. Tufarelli. 2015. Effect of a synbiotic (Biomin®IMBO) on growth performance traits of broiler chickens. Europ. Poult. Sci. 79:1–15.

Bailey, J. S., L. C. Blankenship and, and N. A. Cox. 1991. Effect of fructooligosaccharide on Salmonella colonization of the chicken intestine. Poult. Sci. 70:2433–2438.

Bednarczyk, M., M. Urbanowski, P. Gulewicz, K. Kasperczyk, G. Maiorano, and T. Szwaczkowski. 2011. Field and *in vitro* study on prebiotic effect of raffinose family oligosaccharides in chickens. Bull. Vet. Inst. Pulawy. 55:465–469.

Bednarczyk, M, P. Lakota, J. Zylinska, M. Chmielewska, J. Bardowski, A. Dankowiakowska, and G. Maiorano. 2013. *In vitro* and *in vivo* selection of bioactives enabling the stimulation of chicken microbiome. Ital. J. Anim. Sci. 12:132.

Bogucka, J., A. Dankowiakowska, G. Elminowska – Wedna, A. Sobolewska, J. Jankowski, M. Szpinda, and M. Bednarczyk. 2017. Performance and small intestine morphology and ultrastructure of male broilers injected *in ovo* with bioactive substances. Ann. Anim. Sci. 17:179–195.

Chaveerach, P., D. A. Keuzenkamp, L. J. A. Lipman, and F. Van Knapen. 2004. Effect of organic acids in drinking water for young broilers, on *Campylobacter* infection, volatile fatty acid production, gut microflora and histological cell changes. Poult. Sci. 83: 330–334.

Cheng, Y., Y. Chen, X. Li, W. Yang, C. Wen, Y. Kang, A. Wang, and Y. Zhou. 2017. Effects of synbiotic supplementation on growth performance, carcass characteristics, meat quality and muscular antioxidant capacity and mineral contents in broilers. J. Sci. Food Agric 97:3699–3705.

Chiang, W., M. B. Solomon, and K. L. Kotula. 1995. Muscle fiber type of selected muscles from broiler chickens in relation to age and sex. J Muscle Foods 6: 197–210.

Dankowiakowska, A., I. Kozłowska, and M. Bednarczyk. 2013. Probiotics, prebiotics and snybiotics in poultry – mode of action, limitation, and achievements. JCEA. 14: 467–478.

De Oliveira, J. E., E. Van der Hoeven – Hangoor, I. B. Van de Linde, R. C. Montijn, and J. M. B. M. Van der Vossen 2014. *In ovo* inoculation of chicken embryos with probiotic bacteria and its effect on posthatch Salmonella susceptibility. Poult. Sci. 93: 818–829.

Dransfield, E., and A. A Sośnicki. 1999. Relationship between muscle growth and poultry meat quality. Poult. Sci. 78:743–746.

Dunislawska, A., A. Slawinska, K. Stadnicka, M. Bednarczyk, P. Gulewicz, D. Jozefiak, and M. Siwek. 2017. Synbiotics for broiler chickens — in vitro design and evaluation of the influence on host and selected microbiota populations following in ovo delivery. PLoS One, 12, e0168587.

Edman, A. Ch., I. J. Lexell, M. Sjfistri, and J. M. Squire. 1988. Structural diversity in muscle fibres of chicken breast. Cell Tissue Res. 251:281–289.

Fallah, R., Kiani A., and A. Azarfar. 2013. A review of the role of five kinds of alternatives to in – feed antibiotics in broiler production. J. Vet. Med. Anim. Health. 5:317–321.

Gantois, I., R. Ducatelle, F. Pasmans, F. Haesebrouck, R. Gast, T. J. Humphrey, and F. Van Immerseel. 2009. Mechanisms of egg contamination by Salmonella Enteritidis. FEMS Microbiol. Rev.33:718–738.

Gibson, G. R., and M. B. Roberfroid. 1995. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. J. Nutr. 125: 1401–1412.

Guaadaoui, A., S. Benaicha, N. Elmajdoub, M. Bellaoui, and A. Hamal. 2014. What is a bioactive compound? A combined definition for a preliminary consensus. Int. J. Nutr. Food Sci. 3:174–179.

Grau, R., and R. Hamm. 1952. Eine einfache Methode zur Bestimmung der Wasserbindung im Fleisch. Fleischwirtschaft. 4:295–296.

Hajati, H., and M. Rezaei. 2010. The application of prebiotics in poultry production. Int. J. Poult. Sci. 9: 298–304.

Hascik, P., M. Kacaniova, I. Novakova, M. Fikselova, V. Kulisek, K. Vavrisinova, and H. Arpasova. 2009. Effect of probiotics on protein production in fattening chicken meat. Slovak J. Anim. Sci. 42: 22–26.

Jakubowska, M., J. Gardzielewska, T. Karamucki, A. Rybarczyk, D. Czernomysy – Furowicz, and P. Matusevicius. 2014. The effect of the lactic acid addition to drinking water on the hygiene and quality of chicken broiler meat. Acta Sci. Pol. Technol. Aliment. 13: 45–54.

Khan, R. U., and S. Naz. 2013. The applications of probiotics in poultry production. Worlds Poult. Sci. J. 69:621–632.

Khare, A., G. Thorat, A. Bhimte, and V. Yadav 2018. Mechanism of action of prebiotic and probiotic. J. Entomol. Zool. Stud. 6:51–53.

Knight, J. D. R., and R. Kothary. 2011. The myogenic kinome: protein kinases critical to mammalian skeletal myogenesis. Skelet. Muscle. 1:1–18.

Kizerwetter-Swida, M., and M. Binek. 2008. Bacterial microflora of the chicken embryos and newly hatched chicken. J. Anim. Feed Sci., 17:224–232.

Krawczyk, J., M. Puchała, and J. Obrzut. 2012. Population status of laying hens enrolled in the animal genetic resources conservation programme in Poland. Wiad. Zoot. R. L, 4:41–46.

Madej, J., and M. Bednarczyk. 2016. Effect of *in ovo*-delivered prebiotics and synbiotics on the morphology and specific immune cells composition in the GALT. Poult. Sci. 95: 19–29.

Mahmoud, K. Z., B. S. Obeidat, M. Z. Al-Sadi, and ShR. Hatahet. 2017. Effect of *Bacillus subtilis* supplementation and dietary crude protein level on growth performance and intestinal morphological changes of meat type chicken. Livest. Sci. 195: 99–104.

Maiorano, G., A. Sobolewska, D. Cianciullo, K. Walasik, G. Elminowska – Wenda, A. Sławińska, S. Tavaniello, J. Żylińska, J. Bardowski, and M. Bednarczyk. 2012. Influence of in ovo prebiotic and synbiotic administration on meat quality of broiler chickens. Poult. Sci. 91:2963–2969.

Maiorano, G., K. Stadnicka, S. Tavaniello, C. Abiuso, J. Bogucka, and M. Bednarczyk. 2017. *In ovo* validation model to assess the efficacy of commercial prebiotics on broiler performance and oxidative stability of meat. Poult. Sci. 96: 511–518.

Majd, E. N., M. Mayahi, and A. Sadeghi Moghadam. 2013. The effect of alphamune and biomin on histomorpholoigical structure of small intestine and caecal tonsil lymphoid tissue in broiler chicken. IJVR. 15 30–35.

Mista, D., B. Kroliczewska, A. Pecka – Kielb, V. Kapusniak, W. Zawadzki, S. Graczyk, A. Kowalczyk, E. Lukaszewicz, and M. Bednarczyk. 2017. Effect of *in ovo* injected prebiotics and synbiotics on the caecal fermentation and intestinal morphology of broiler chickens. Anim. Prod. Sci. 57:1884–1892.

Nowak, A., K. Śliżewska, and Z. Libudzisz. 2010. Probiotics – history and mechanisms of their effect. Food Sci. Technol. Quality. 4:5–19.

Park, J. H., and I. H. Kim. 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.

Park, S. O., and S. B. Park. 2011. Effect of dietary microencapsulated-inulin on carcass characteristics and growth performance in broiler chickens. J. Anim. Vet. Adv. 10:1342–1349.

Pedroso, A. A., A. B Batal, and M. D. Lee. 2016. Effect of *in ovo* administration of an adult-derived microbiota on establishment of the intestinal microbiome in chickens. Am. J. Vet. Res.. 77:514–526.

Pruszynska – Oszmalek, E., P. A. Kolodziejski, K. Stadnicka, M. Sassek, D. Chałupka, B. Kuston, L. Nogowski, P. Mackowiak, G. Maiorano, J. Jankowski, and M Bednarczyk. 2015. *In ovo* injection of prebiotics and synbiotics affects the digestive potency of the pancreas in growing chickens. Poult. Sci. 94:1909–1916.

Rehfeldt, C., N. C Stickland, I. Fiedler, and J. Wegner. 1999. Environmental and genetic factors as sources of variation on skeletal muscle fiber number. Basic Appl. Myol. 9, 5: 235–253.

Remignon, H., J. Zanusso, A. Gaëlle., and R. Babilé. 2000. Occurrence of giant myofibres according to muscle type, pre- or post-rigor state and genetic background in turkeys. Meat Sci. 56:337–343.

Salakova, A., E. Strakova, V. Valkova, H. Buchtova, and I. Steinhauserova. 2009. Quality indicators of chicken broiler raw and cooked meat depending on their sex. Acta Vet. Brno 78:497–504.

Scheuermann, G. N., S. F Bilgili, S. Tuzun, and D. R. Mulvaney. 2004. Comparison of chicken genotypes: myofiber number in pectoralis muscle and myostatin ontogeny. Poult. Sci. 83: 1404–1412.

Scott, W., J. Stevens, and S. A. Binder-Macleod. 2001. Human skeletal muscle fiber type classifications. Phys. Ther. 81:1810–1816.

Seideman, S. C., S. H. Cohen, and J. V. Schollmeyer. 1984. Some factors influencing ante – mortem changes in muscle: a brief review. Food Microstruc. 3:127–132.

Seifert, S., and B. Watzl. 2007. Inulin and oligofructose: review of experimental data on immune modulation. J. Nutr. 137:2563S–2567S.

Siwek, M., A. Slawinska, K. Stadnicka, J. Bogucka, A. Dunislawska, and M. Bednarczyk 2018. Prebiotics and synbiotics – *in ovo* delivery for improved lifespan condition in chicken. BMC Vet. Res. 14:402.

Slawinska, A., A. Plowiec, M. Siwek, M. Jaroszewski, and M. Bednarczyk. 2016. Long-term transcriptomic effects of prebiotics and synbiotics delivered in ovo in broiler chickens. PLoS One. 11:e0168899.

Slawinska, A., M. Siwek, J. Zylinska, J. Bardowski, J. Brzezinska, K. A. Gulewicz, A. Nowak, M. Urbanowski, A. Płowiec, and M. Bednarczyk. 2014. Influence of synbiotics delivered *in ovo* on immune organs' development and structure. Folia Biol. Krakow. 62: 277–285.

Sobolewska, A., J. Bogucka, A. Dankowiakowska, G. Elminowska – Wenda, K. Stadnicka, and M. Bednarczyk. 2017. The impact of synbiotic administration through *in ovo* technology on the microstructure of a broiler chicken small intestine tissue on the 1st and 42nd day of rearing. J Anim. Sci. Biotechnol. 8:1–8.

Smith, D. P., D. L. Fletcher, R. J. Buhr, and R. S. Beyer. 1993. Pekin duckling and broiler chicken pectoralis muscle structure and composition. Poult. Sci. 72: 202–208.

Swamy, M. N., and H. A. Upendra. 2013. Growth performance, crude protein, ether extract and total ash in the breast muscle of broiler chickens supplemented with probiotics. Int. J. Sci. Envir. Tech. 2:1000–1007.

Tavaniello, S., G. Maiorano, K. Stadnicka, R. Mucci, J. Bogucka, and M. Bednarczyk. 2018. Prebiotics offered to broiler chicken exert positive effect on meat quality traits irrespective of delivery route. Poult. Sci. 8: 2979–2987.

Tavaniello, S., R. Mucci, K. Stadnicka, A. Ongwech, M. Bednarczyk, and G. Maiorano. 2019. Effect of in ovo administration of different synbiotics on carcass and meat quality traits in broiler chickens. Poult. Sci. 0: 1–9 http://dx.doi.org/10.3382/ps/pey330.

Zhang, G, L. Ma, and M. P. Doyle. 2006. Efficiency of probiotics, prebiotics and synbiotics on weight increase of chickens. (*Gallus Domesticus*). http: //www. ugacfs.org/ research/pdfs/ Poultry 2006.pdf.