Influence of in ovo prebiotic and synbiotic administration on meat quality of broiler chickens

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ABSTRACT A trial was conducted to evaluate the effect of in ovo injection of prebiotic and synbiotics on growth performance, meat quality traits (cholesterol content, intramuscular collagen properties, fiber measurements), and the presence of histopathological changes in the pectoral muscle (PS) of broiler chickens. On d 12 of incubation, 480 eggs were randomly divided into 5 experimental groups treated with different bioactives, in ovo injected: C, control with physiological saline; T1 with 1.9 mg of raffinose family oligosaccharides; T2 and T3 with 1.9 mg of raffinose family oligosaccharides enriched with different probiotic bacteria, specifically 1,000 cfu of *Lactococcus lactis* ssp. *lactis* SL1 and *Lactococcus lactis* ssp. *cremoris* IBB SC1, respectively; T4 with commercially available synbiotic Duolac, containing 500 cfu of both *Lactobacillus acidophilus* and *Streptococcus faecium* with the addition of lactose (0.001 mg/embryo). Among the hatched chickens, 60 males were randomly chosen (12 birds for each group) and were grown to 42 d in collective cages (n = 3 birds in each 4 cages: replications for experimental groups). Broilers were fed ad libitum commercial diets according to age. In ovo prebiotic and synbiotic administration had a low effect on investigated traits, but depend on the kind of bioactives administered. Commercial synbiotic treatment (T4) reduced carcass yield percentage, and the feed conversion ratio was higher in T3 and T4 groups compared with other groups. The abdominal fat, the ultimate pH, and cholesterol of the PS were not affected by treatment. Broiler chickens of the treated groups with both slightly greater PS and fiber diameter had a significantly lower amount of collagen. The greater thickness of muscle fibers (not significant) and the lower fiber density (statistically significant), observed in treated birds in comparison with those of the C group, are not associated with histopathological changes in the PS of broilers. The incidence of histopathological changes in broiler chickens from examined groups was low, which did not affect the deterioration of meat quality obtained from these birds.

Key words: broiler chicken, prebiotic and synbiotic, in ovo injection, meat quality, histology

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INTRODUCTION

In the last decade, the need in European Union countries for improving the quality of the raw meat has been emphasized. Meat is a complex, composite substance. It consists of myofibers, connective tissue, and lipids. It has been established that collagen, the major component of the intramuscular connective tissue (Light et al., 1985), plays a key role in determining meat toughness (McCormick, 1999; Purslow, 2005) of different domestic animals, including birds (Baéza et al., 1998; Maiorano et al., 2011). Raw chicken meat is generally very soft and, when cooked, it can even be cohesive. However, a newer emerging quality issue in poultry is the poor cohesiveness of meat related to immaturity of intramuscular connective tissue; it is believed that genetic progress has put more stress on the growing bird and has resulted in histological and biochemical modifications of the muscle tissue by impairing some meat quality traits (Petracci and Cavani, 2012). The quality and composition of poultry meat are influenced by numerous factors such as genotype, sex, age, diet, rearing conditions, and preslaughter treatment of birds (reviewed in Fletcher, 2002), and probiotics supplied in feed (Salma et al., 2007; Zhou et al., 2010; Khan et al., 2011). The effect of bioactives on meat quality is not homogeneous. Many promising studies demonstrate that well-established probiotics or prebiotics (or both)

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possess, for example, hypocholesterolemic effects. However, other studies have also shown that probiotics and prebiotics had insignificant effects on lipid profiles, disputing the hypocholesterolemic claim (reviewed in Ooi and Liong, 2010). A major problem with their use is efficient administration under fully controlled conditions. To be effective, bioactives have to be administered to the animal as early as possible (reviewed in Schneitz, 2005). To eliminate some of the factors that could negatively influence the responses of birds to pre- and probiotics applied, in ovo technology has been used, that enables administration of the given substance in a solution, directly inside the eggs during their incubation. It has been proven that a single in ovo injection of the raffinose family oligosaccharides (**RFO**) leads to the long-term maintenance of a high level of intestinal *Bifidobacteria* (Villaluenga et al., 2004; Pilarski et al., 2005). The effects of RFO in ovo application in the field conditions (Bednarczyk et al., 2011) proved that this substance delivered in very low doses (1.9 mg/embryo) can replace the traditional method of administrating prebiotic in the diet. Additionally, our last unpublished data suggest a higher effectiveness of the combinations of RFO and well-characterized strains of lactic acid bacteria (synbiotics) in relation to prebiotic (A. Sławińska, unpublished data). However, according to the available information, no research has yet been conducted to study the effect of prebiotic and synbiotics in ovo administered on chicken meat quality. Only limited information is available in the literature (Pilarski et al., 2005) regarding the effect of prebiotic on muscle cholesterol content.

The objective of the present experiment was to evaluate the effects of in ovo prebiotic used alone or in combination with strictly selected and characterized probiotic bacteria (synbiotics; Bardowski and Kozak, 1981; Boguslawska et al., 2009) administration on growth, meat quality, and on the presence of histopathological changes in the pectoral muscle of broiler chickens.

MATERIALS AND METHODS

Birds

Broiler chicken (Ross 308) hatching eggs (480) were incubated in a commercial hatchery Drobex (Solec Kujawski, Poland), a Petersime incubator (vision version, Petersime NV, Zulte, Belgium). On d 12 of incubation, the eggs were randomly divided into 5 experimental groups treated with different bioactives, administered in ovo. Prior to the injection, the eggs were candled and those unfertilized or with dead embryos were discarded. An aqueous solution at the equal volume of 0.2 mL was injected manually into the air chamber by using self-refilling syringes (Socorex, Ecublens, Switzerland). The **C** group (control) was injected with physiological saline. The **T1** group was injected with a solution containing 1.9 mg of raffinose family oligosaccharides (**RFO**). For the **T2** and **T3** groups, the injection solutions consisted of homemade synbiotics: 1.9 mg of RFO enriched with different probiotic bacteria, specifically, 1,000 cfu of *Lactococcus lactis* ssp. *lactis* SL1 (group T2) and 1,000 cfu of *Lactococcus lactis* ssp. *cremoris* IBB SC1 (group T3). The **T4** group was injected with a commercially available synbiotic Duolac (Biofaktor, Skierniewice, Poland) that contained combined 500 cfu of *Lactobacillus acidophilus* and 500 cfu of *Streptococcus faecium* with addition of lactose (0.001 mg/embryo). After injection, each hole was sealed with adhesive tape and the egg incubation was continued until hatching.

Among the hatched chickens, 60 males were randomly chosen (12 birds for each group) and reared according to the animal welfare recommendations of European Union directive 86/609/EEC in an experimental poultry house that provided good husbandry conditions (e.g., stocking density, litter, ventilation). The birds were grown to 42 d of age in collective cages (n = 3 birds in each 4 cages: replications for experimental groups). The cages (30 × 40 × 35 cm; length × width × height) had wire floors and solid metal walls, a feeder space of 12.2 cm/bird, and 1 water nipple. The lighting program was 23L:1D. The conditions of management of broiler chickens were the same in all groups. The broilers were fed ad libitum commercial diets (Table 1) according to their age, and water was provided ad libitum. Amounts of feed offered to each cage were recorded, and uneaten feed in each cage was weighed daily (from 1 to 42 d). Cumulative feed intake and feed conversion ratio (**FCR**) were calculated on a cage basis.

Slaughter Surveys and Analyses

At 42 d of age, broilers (12 for each group), identified by numbered permanent wing bands, were weighed individually (after a fasting period of 12 h) and transported within 1.0 h (including careful catching and loading) to a commercial poultry slaughterhouse. After careful unloading and hanging in randomized order, the birds were electrically stunned and slaughtered (according to the local Animal Research Ethics Committee at University of Technology and Life Sciences in Bydgoszcz), hot carcass weight was recorded, and carcass yield percentage was calculated.

At slaughter, samples of the right pectoral muscle of 40 animals, 8 birds from each experimental group, were taken and frozen in liquid nitrogen (−196°C) for histological and histopathological analyses. Pectoral muscle pH was recorded at 45 min ($\mathbf{pH_{45}}$), 12 h ($\mathbf{pH_{12}}$), and 24 h ($\mathbf{pH_{24}}$) postmortem using a portable HI 9625 pH meter (Hanna Instruments, Padova, Italy).

The left pectoral muscle was removed from all carcasses (n = 60) and its percentage was calculated based on hot carcass weight. Afterward, it was vacuum packaged and stored frozen (−40°C) until intramuscular collagen (**IMC**) and cholesterol analyses. In addition, backfat was measured and its percentage was calculated based on hot carcass weight.

Table 1. Composition and nutritive value of the diets

Item (% unless noted)	Period 1 to 14 d	Period 15 to 42 d
Component		
Wheat	35.0	40.0
Maize	17.05	22.85
Soybean meal 45%	37.0	27.8
Soybean oil	5.7	4.6
Calcium monophosphate	1.3	1.3
Limestone	0.6	0.5
$NaHCO_3$	0.1	—
Lysine 20%	0.9	0.8
Methionine 20%	1.0	0.8
NaCl	0.35	0.35
Vitamin-mineral premix[1]	1.0	1.0
Calculated nutritional value		
ME (kcal/kg)	3,150	3,100
N × 6.25	23.0	20.0
Ca	1.1	1.2
P available	0.47	0.60
Na	0.23	0.23
NaCl	0.50	1.0

[1]Provided the following per kilogram of diet: vitamin A (retinyl acetate), 13,000 IU; vitamin D_3 (cholecalciferol), 4,000 IU; vitamin E (DL-α-tocopheryl acetate), 80 IU; vitamin K (menadione sodium bisulfite), 3 mg; riboflavin, 6.0 mg; pantothenic acid, 6.0 mg; niacin, 20 mg; pyridoxine, 2 mg; folic acid, 0.5 mg; biotin, 0.10 mg; thiamine, 2.5 mg; vitamin B_{12}, 20 μg; Mn, 120 mg; Zn, 90 mg; Fe, 30 mg; Cu, 10 mg; I, 1.5 mg; Se, 0.2 mg; and ethoxyquin, 100 mg.

Histological and Histopathological Analyses

The muscle samples were cut into 10-μm cross-sections in a Leica cryostat. The samples were stained with hematoxylin and eosin according to the method of Dubowitz and Brooke (1973) to measure the diameters and the number of muscle fibers and to determine the extent of histopathological changes in the pectoral muscle. Using the InterVideo WINDVR program (Kworld Computer Co. Ltd., New Taipei, Taiwan), 12 images of microscopic pictures were taken per sample, using magnification 12.5 × 10. Afterward, all muscle fibers were counted and their diameters were measured at the surface of a 1-mm^2 cross-section of the pectoral muscle of the chicken using the computerized image analysis system MultiScanBase v. 14.02 (Computer Scanning System Ltd., Warszawa, Poland).

Percentage of histopathological changes in muscle structure was evaluated in an area of 2 mm^2 cross-section. Changes in fiber size and shape, and degenerative changes of fibers were analyzed according to Dubowitz and Brooke (1973).

Measurement of Muscle Cholesterol

Cholesterol was extracted using the method of Maraschiello et al. (1996) and then quantified by HPLC. A Kontron HPLC (Kontron Instruments, Milan, Italy) model 535, equipped with a C18 reverse-phase column (250 × 4.6 mm × 5 μm; Phenomenex, Torrance, CA), was used. The mobile phase was acetonitrile/2-propanol (55:45 vol/vol) at a flow rate of 1.2 mL/min. The detection wavelength was 210 nm, and retention time was 13.89 min.

Collagen and Statistical Analyses

Approximately 100 g of muscle (wet weight) was thawed at room temperature, trimmed of fat and epimysium, lyophilized for 48 h, weighed, and hydrolyzed in Duran tubes (Schott AG, Mainz, Germany) in 5 mL of 6 *N* HCl at 110°C for 18 to 20 h (Etherington and Sims, 1981) for determination of hydroxyproline (Woessner, 1961) and crosslinking. All analyses were carried out in duplicate. The IMC concentration was calculated, assuming that collagen weighed 7.25 times the measured hydroxyproline weight (Eastoe and Leach, 1958) and expressed as micrograms of hydroxyproline per milligram of lyophilized tissue. Hydroxylysylpyridinoline (**HLP**) concentration, the principal nonreducible crosslink of muscle collagen (McCormick, 1999), was concentrated and separated from the bulk of the other amino acids by selective elution from a CF 1 cellulose column (Skinner, 1982), using the HPLC procedure developed by Eyre et al. (1984). The HPLC was equipped with a Kontron 450 MT2 (Kontron Instruments, Milan, Italy) data system and with an Altex (Beckman Instruments, Fullerton, CA) Ultrasphere-ODS (C-18; small pore; 4.6 × 250 mm) column. Pyridoxamine.2HCl (Sigma Chemical, St. Louis, MO) was added as an internal standard to the eluent containing HLP and analyzed by HPLC. Identification of HLP in tissue hydrolyzates was made by comparison with a purified HLP standard and the known relationship between the elution time of HLP and pyridoxamine (Eyre et al., 1984). Purified HLP standard was routinely prepared from bovine cartilage hydrolyzates using the technique described by Eyre et al. (1984). The concentration of HLP residues in samples was calculated based on the concentration of collagen in each hydrolyzate and assuming that the molecular weight of collagen was 300,000 and the molar fluorescence yield of pyridoxamine was 3.1 times that of HLP (Eyre et al., 1984). The HLP was expressed as moles of HLP per mole of collagen and also as micrograms of HLP per milligram of lyophilized tissue.

To verify significant differences in relation to the treatments, the data were evaluated by using 1-way ANOVA and means were separated by Scheffe's battery of pairwise tests (SPSS Inc., 2010).

RESULTS AND DISCUSSION

Slaughter traits, FCR, and pH of broiler chickens are presented in Table 2. Compared with the C group, the treatment groups had slightly higher final BW, more evident in the T1 group (+4.54%), but these differences were not statistically significant. Chickens from T1 group showed also a slightly higher carcass weight compared with other groups ($P > 0.05$). Carcass yield percentage was higher (+2.9%) in C group than in T4

Table 2. Effect in ovo prebiotic and synbiotics administration on slaughter traits, FCR,[1] and pH of broiler chickens

	Group[2]						
Item	C	T1	T2	T3	T4	SEM	*P*-value
n	12	12	12	12	12		
Final BW (g)	2,708.4	2,837.1	2,717.7	2,799.3	2,784.5	26.34	0.491
Carcass weight (g)	1,993.0	2,048.2	1,977.1	2,010.1	1,965.9	19.79	0.726
Carcass yield (%)	73.6^{a}	72.2^{ab}	72.7^{ab}	71.8^{ab}	70.7^{b}	0.31	0.043
FCR	1.54^{b}	1.55^{b}	1.53^{b}	1.64^{a}	1.67^{a}	0.01	0.001
Pectoral muscle (%)	24.6	28.3	24.7	26.2	27.0	0.48	0.070
pH_{45min}	6.55^{ab}	6.41^{c}	6.45^{bc}	6.61^{a}	6.50^{ac}	0.02	0.003
pH_{12h}	5.97	5.95	5.92	5.91	5.95	0.01	0.720
pH_{24h}	5.87	5.87	5.82	5.82	5.84	0.01	0.632

$^{a–c}$Means within a row lacking a common superscript differ ($P < 0.05$).

[1]FCR = feed conversion ratio (cumulative feed intake/weight gain); cage was used as the experimental unit.

[2]C = control group; T1 = prebiotic group; T2 = synbiotic group (prebiotic + 1,000 cfu of *Lactococcus lactis* ssp. *lactis* SL1); T3 = synbiotic group (prebiotic + 1,000 cfu of *Lactococcus lactis* ssp. *cremoris* IBB SC1); T4 = commercial symbiotic group (*Lactococcus acidophilus* + *Streptococcus faecium*).

group ($P < 0.05$); the proportion of carcass yield of T1, T2, and T3 broilers was intermediate between C and T4 ($P > 0.05$).

The FCR, ranging from 1.53 to 1.67, was found to be similar among the groups C, T1, and T2 ($P > 0.05$); however, FCR was higher in T3 and T4 groups compared with other groups ($P < 0.05$). Poultry feed efficiency has been traditionally measured as FCR, the ratio of feed intake to weight gain. In the present study, FCR depends on the combination of prebiotic and probiotic in ovo injected, similar to the effect of bioactives administered in chickens feed (Falaki et al., 2011).

The abdominal fat percentage, ranging from 1.2 to 2.4% (SEM = 0.20), was similar ($P > 0.05$) among the groups. The pectoral muscle percentage was slightly influenced by treatment. In fact, compared with C and T2 broilers, those of T1, T3, and T4 groups had slightly higher ($P = 0.070$) pectoral muscle percentage. Similarly, Pilarski et al. (2005) found a slight but not significant influence of different doses of fructooligosaccharides or α-galactoside (**RFO**) injection on the final body, carcass, breast muscle, and leg weight, and on abdominal fat ratio of Hubbard broilers.

Conversely, in the study conducted under commercial conditions on 1,381,212 broilers (M. Bednarczyk, unpublished), a prebiotic (RFO) administered in ovo improved performance. The registered broilers survivability, final BW, and FCR were 4.7%, 2,304 g, 1.87, and 4.2%, 2,325 g, and 1.86, in controlled and injected groups (RFO), respectively. In another study, Bednarczyk et al. (2011) observed under field conditions that the prebiotic effect of RFO (administered by single in ovo injection) and growth promotant antibiotic (in water) had a similar effect on broiler performance (survivability, growing period, and European production efficiency factor); instead, BW and FCR were significantly higher in injected groups in comparison with the control. The lack of consistency between broiler performance registered in an experimental farm or in field conditions can be explained by the study of Timmerman et al. (2006). Based on their 4 studies in combination with 9 other studies published earlier, the authors suggested that the effect of probiotics becomes smaller when productivity rates of broilers is higher. Additionally, Patterson and Burkholder (2003) suggested that environmental and stress status influence efficacy of prebiotics and probiotics, and are more effective when the animal is producing well below its genetic potential.

In general, the pH_{45} differed significantly ($P = 0.003$) among groups. On the contrary, the parameters pH_{12} and pH_{24} were found to be similar ($P > 0.05$) among groups. The ultimate pH values observed in our study (ranging from 5.82 to 5.87) varied within the pH range accepted for commercial meats. It is well known that the ultimate pH of the muscle is an important contributing factor to meat quality expressed as tenderness, color, and storage life (Van Laack et al., 2000).

Fiber histological characteristics and collagen properties in the pectoral muscle are presented in Table 3. A greater thickness (diameter) of muscle fibers was found in the experimental groups of chickens compared with the control group; however, the differences were not statistically significant, possibly due to significant variation of BW within the experimental groups. In contrast, differences ($P = 0.045$) in the total number of muscle fibers in the 1 mm^2 of the cross-section of pectoral muscle were more evident between C and T3 groups ($P < 0.05$). The number of muscle fibers did not differ significantly among the T1, T2, and T4 groups. However, the C and T2 groups with the highest number of the muscle fibers (285.1 and 255.0 number/mm^2, respectively) showed the lowest pectoral muscle percentage (24.4 and 24.7%, respectively).

On one hand, it can be assumed that the application of prebiotics or synbiotics has a positive impact on muscle weight. On the other hand, it probably leads to even greater congestion of already hypertrophic fibers. In studies that Lisowski et al. (2003) carried out on meat type chickens, a beneficial effect of the RFO on breast muscle weight and on carcass percentage was observed. According to Elminowska-Wenda et al. (2004), accelerated growth of skeletal muscle in different spe-

Table 3. Effect in ovo prebiotic and synbiotic administration on pectoral muscle qualitative traits of broiler chickens

	Group[2]						
Item[1]	C	T1	T2	T3	T4	SEM	*P*-value
n	12	12	12	12	12		
Fiber diameter (μm)	45.9	50.5	51.2	51.6	50.8	0.85	0.200
Fiber density (no./mm^2)	285.1^{a}	239.6^{ab}	255.0^{ab}	225.6^{b}	249.2^{ab}	6.91	0.045
IMC (μg/mg of lyophilized muscular tissue)	25.27^{a}	19.39^{bc}	20.49^{bc}	21.06^{b}	18.87^{c}	2.67	0.000
HLP (mol/mol of collagen)	0.065	0.079	0.076	0.075	0.085	0.003	0.400
HLP (μg/mg of lyophilized muscular tissue)	2.33	2.20	2.23	2.21	2.35	0.10	0.980

$^{a–c}$Means within a row lacking a common superscript differ ($P < 0.05$).

[1]Fiber density and fiber diameter = 8 animals for each group; fiber diameter = 200 fibers; IMC = intramuscular collagen; HLP = hydroxylysylpyridinoline.

[2]C = control group; T1 = prebiotic group; T2 = synbiotic group (prebiotic + 1,000 cfu of *Lactococcus lactis* ssp. *lactis* SL1); T3 = synbiotic group (prebiotic + 1,000 cfu of *Lactococcus lactis* ssp. *cremoris* IBB SC1); T4 = commercial symbiotic group (*Lactococcus acidophilus* + *Streptococcus faecium*).

cies of birds is associated with reduced oxidative capacity. This is most often the case in the pectoral muscle of broiler chickens, and it confirms the high proportion of glycolytic fibers (white) that function through the anaerobic metabolism of glycogen.

The effect of prebiotic and synbiotics administration had a partial influence on IMC properties (Table 3). In general, the IMC concentration was notably reduced by the treatments ($P = 0.001$). In particular, the IMC concentration was higher ($P < 0.05$) in meat of control chickens than for treated groups, and a lower value was found in the meat of the T4 group. The IMC content found in the T4 group differed ($P < 0.05$) from that of chickens of the T3 group. Muscle HLP concentration (μg/mg) and collagen maturation (mol of HLP/mol of collagen) did not differ ($P > 0.05$) among experimental groups. Velleman et al. (1996) found a similar collagen content (25%) in the pectoral muscle of 6-wk-old White Leghorn chickens, but more crosslinked (almost 0.5 mol of HLP/mol of collagen). This marked difference in collagen maturity could be due mainly to the modern chicken strains (Petracci and Cavani, 2012). In fact, in fast-growing birds, collagen is immature, resulting in low heat stability. Consequently, poultry meat is tender, but may turn fragile, even mushy (Puolanne and Voutila, 2009) and cooked chicken breast meat is generally fragmented (soft; Voutila et al., 2009).

The amount of collagen is related both to muscle growth and fiber diameter (Das et al., 2010). In agreement with Das et al. (2010), the broiler chickens of the present study with a slightly greater pectoral muscle, in which the fiber diameter was slightly higher, had a lower amount of collagen. McCormick (1999) suggested that mature crosslinks and collagen concentration have an additive effect on the toughening of meat. In other words, the role of collagen on meat tenderness depends not only on the crosslinks but also on the amount of collagen. Maiorano et al. (2001) give a tenderness index, which is the amount in HLP crosslinks per gram of lyophilized muscular tissue in different muscles in goat meat. In agreement with the suggestions of McCormick (1999) and Maiorano et al. (2001), the results of HLP crosslinks concentration (μg/mg) indicate that meat produced from all birds could be similar in background toughness.

The cholesterol content determined in this study (ranging from 70.45 to 78.12 mg/100 g, SEM = 1.30) was found to be similar among experimental groups ($P > 0.05$). Our results contrast with the findings of Pilarski et al. (2005) in Hybro G broiler breeder eggs, who reported that the fructooligosaccharides caused a decrease of breast muscle cholesterol in comparison with the control group. The cholesterol values found in the present study are higher than those reported by Pilarski et al. (2005) in breast muscle of 42-d-old broiler chickens (ranging from 49.3 to 54.7 mg/100 g). In contrast, Salma et al. (2007) observed higher cholesterol values (93.6 mg/100 g of meat) in pectoralis major of 56-d-old male Chunky broilers. Cholesterol content in chicken meat can be altered by varying the composition of diet, age, and sex (Wang et al., 2006), as well as the use of different methodologies for cholesterol quantification or for sampling (Bragagnolo and Rodriguez-Amaya, 2002).

There were no statistically significant differences ($P > 0.05$) between groups for any of the pathologies analyzed. The most histopathological changes were observed in the T2 group (homemade synbiotic) and the least in the T4 group (commercial synbiotic; overall means: C = 4.12%, T1 = 6.37%, T2 = 6.80%, T3 = 5.22%, T4 = 3.59%; SEM = 0.44). Most of the incorrect size fibers were classified as small-diameter fibers. They ranged from 2.71 to 4.80% (SEM = 0.31) of the total number of fibers. The splitting of fibers was the second most common pathological change, that occurred most frequently in the T1 group, and the least in the C group (overall means: C = 0.56%, T1 = 1.55%, T2 = 1.40%, T3 = 1.04%, T4 = 0.78%; SEM = 0.13). Changes in the shape of fibers were less evident than the histopathological changes in all groups (ranging from 0.10 to 0.65%; SEM = 0.08). The necrotic lesions were observed only in individual cases and affecting individual muscle fibers. Their incidence ranged from 0.04% (T4 group) to 0.19% (T1 group; SEM = 0.04). There was no lymphocyte infiltration, which leads to inflammation in the developing chicken pectoral muscle cells.

The study of Elminowska-Wenda et al. (2004) indicated that histopathological changes are most extensive in birds with a fast growth rate and high meatiness. Hoving-Bolink et al. (2000) showed that many cases of myopathy in modern lines of chickens and turkeys with fast growth are caused by inadequate blood supply to the pectoral muscle. The increase in the diameter of muscle fibers, to which transportation of oxygen by capillaries is limited, may cause muscle cell hypoxia and necrosis.

In light of this result, the greater thickness of muscle fibers (not significant) and the lower fiber density (statistically significant; Table 3), observed in treated birds in comparison with those of the C group, are not associated with histopathological changes in the pectoral muscle of broilers.

In conclusion, in ovo prebiotic and synbiotic administration had little effect on most of the investigated traits. The commercial synbiotic treatment (T4) reduced carcass yield percentage, and the FCR was higher in the T3 and T4 synbiotic groups compared with other groups. The abdominal fat, ultimate pH, and cholesterol of the pectoral muscle were not affected by in ovo administration. Broiler chickens of treated groups with both slightly greater pectoral muscle and fiber diameter had lower amounts of collagen. The effect of in ovo prebiotic and synbiotics administration on histopathological changes in the pectoral muscle was low, which did not affect the deterioration of meat quality obtained from these birds. The findings of the histopathological evaluation of the pectoral muscle were not associated with fiber diameter and fiber density. In light of the present results, we believe in ovo prebiotic and synbiotic administration has a low impact under experimental conditions. However, further research is needed to increase knowledge regarding the effect of in ovo prebiotic and synbiotic administration on the growth and meat quality of broiler chickens in both experimental and field conditions.

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