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Effect of Synbiotic Dietary Supplementation on Histological and Histopathological Parameters of *Pectoralis Major* Muscle of Broiler Chickens

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ABSTRACT

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Bioactive substances may constitute an alternative to antibiotics. Probiotics are mixtures of microorganisms that enhance the effectiveness and yield of nutrient absorption by competing for the substrate against pathogens that may cause intestinal infections. Prebiotics are organic substances which include complex carbohydrates and have an influence on the growth and activity of desirable intestinal microflora. Synbiotics are a combination of both of them. The aim of this study was to analyze the impact of synbiotics added to commercial feed on *Pectoralis major* muscle microstructure in broiler chickens. Research material consisted of 240 one-day-old Ross 308 female chicks. Birds were divided into 2 groups, 12 broilers each (replicated 10 times). The control group (C) was fed a commercial diet, and the SYN group was fed the same diet with added synbiotic: 0.8% of prebiotic RFO (raffinose family oligosaccharides) extracted from lupin seeds and 1% of probiotic Lavipan®. Birds were slaughtered at day 42. Immediately after slaughter, samples of the *Pectoralis major* muscle for histological analysis were taken and frozen in liquid nitrogen. The staining procedures performed were: hematoxylin and eosin staining to evaluate fibre diameter and histopathological changes, oil red staining to determine intramuscular fat content, NADH-TR (tetrazolium reductase) activity to distinguish muscle fibre types and alkaline phosphatase reaction for counting the number of capillaries. The tendency to reduced muscle fibre diameter in chickens supplemented with synbiotics indicates an increase in muscle fibre density, which gives meat a more delicate structure. When assessing the occurrence of histopathological changes, significantly more fibre splitting was observed in the control group. Moreover, the positive effect of feed supplementation with synbiotics on muscle microvascularization could have an important practical meaning, because low physical activity of chickens during rearing may lead to ischemic muscles, increased changes in the structure of muscle fibres, and reduction of meat quality.

Keywords: poultry; bioactive substances; muscle fibre; histopathological traits

Chicken meat is gaining an increasing importance in modern society, not only because it is cheap, but also because it has a desirable fatty acid profile, a relatively low cholesterol content (skin excluded), it is easy to cook, and it is not subjected to ethical or religious prejudices (Scanes 2007). Since the

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breast portion is the most valuable part of the broiler chicken carcass, the broiler industry is always interested in gathering information concerning progress in matters of performance, both qualitative and quantitative (fibre characteristics and muscle weight, respectively).

Intramuscular fat (IMF) content is a relevant information to evaluate the quality of meat because it influences flavour and the visual aspect (marbling, for example). In white meat, such as broiler meat, lipids accumulate in the connective tissue surrounding the fibres. Intramuscular fat content in breast meat of chicken is especially low and amounts to 1% (Hocquette et al. 2010).

By analyzing fibre diameter and number, it is possible to assess genetic variations in the yield of pectoral muscles. Generally, muscle fibre diameter varies from 10 to 100 μm, and its length goes up to 30 cm (depending on factors such as sex or feed intake). Large diameters determine dark and tough meat, with higher pH. An average diameter is ideal because it enhances the quality and quantity of meat, as has been reported by Choi and Kim (2009). In avian species, muscle weight increases due to the enlargement of fibres. This was due to the long selection of breeds that brought some setbacks like fibre necrosis triggered by a lower capillary density, which also increases oxidative metabolism among fibres. These factors can also lead to Deep Pectoral Myopathy (DPM), which causes necrotic lesions in one side or both sides of the breast muscle. Histopathological changes are a constant concern in the broiler industry, mainly due to genetic selection, contact with pathogens, type of feed provided, and ingestion of toxins. The following changes may occur in the muscle's microstructure: fibre atrophy, giant fibres, fibre splitting, necrosis, and connective tissue hypertrophy (Walasik et al. 2017). These changes may cause major alterations, such as white striping, which is characterized by the appearance of white lines parallel to fibre orientation on the surface of the breast muscle, ultimately co-related with the number of atrophic fibres (Petracci and Cavani 2012; Kuttappan et al. 2013). Also wooden breast (WB) is a myopathy which imparts tough consistency to raw breast fillets. Histologically, WB is characterized by polyphasic myodegeneration, which led to a variable amount of interstitial connective tissue contents and fibrosis, necrosis, lipidosis, and regenerative changes (Sihvo et al. 2014; Cruz et al. 2017). This genetic selection for fast-growing properties created muscles that surpass the capacity of life supporting systems such as the cardio-vascular system, and immaturity of connective tissue at slaughter age, causing poor cohesiveness of cooked meat (Petracci and Cavani 2012).

To improve the efficiency of the digestive process of broiler chickens, producers started using antibiotics as feed supplements. They were used mainly to prevent infections, treat sick animals, and to act as growth promoters (Hume 2011). Unfortunately, the usage of these substances was extremely exaggerated and bacteria started to develop resistance against them; some of these antibiotics were also used in human medicine, and later caused an increase in multi-drug resistant bacteria causing deadly infections (Threlfall et al. 2000). These occurrences demanded alternatives to growth promoters, antibiotics have been banned from use. Increasing attention has recently been paid to a search for new nutritional solutions, namely probiotics, prebiotics, and synbiotics.

Probiotics are a preparation or a product containing viable, defined microorganisms in sufficient numbers, which alter the microflora (by implantation or colonization) in a compartment of the host and by that exert beneficial health effects in this host (Havenaar and Huis in't Veld 1992). These microorganisms act in the gastrointestinal (GI) tract by competing with ingested pathogens, producing volatile fatty acids and stimulating immune response to foreign microorganisms (Chambers 2003).

Prebiotics are non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth/activity of one or a limited number of types of bacteria in the colon (Gibson and Roberfroid 1995). These substances do not change the composition of the native GI microflora. Instead they apply a selective stimulus to predetermined bacteria that are positive to the host (Chambers 2003). Prebiotics also lower the pH of the GI tract, increasing the absorption of minerals (calcium, for example) and, consecutively, bone mineralization (de Vrese and Schrezenmeir 2008).

Synbiotics are products that contain pro- and prebiotics. Prebiotics act on the homologous microorganisms by enhancing their desirable characteristics, like fatty acid production, or by helping in the colonization process. To our knowledge,

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there is no available information regarding the influence of dietary supplementation with synbiotics on the microstructure of chicken muscles. The aim of this study was to explore the potential of synbiotics (0.8% RFO – raffinose family oligosaccharides and 1% Lavipan®) added to a commercial diet, and to determine their influence on body weight, breast muscle weight, some histological parameters (fibre diameter, muscle fibre density, intramuscular fat, and microvascularization) and histopathological changes in the *Pectoralis major* muscle of broiler chickens.

MATERIAL AND METHODS

Birds. The experiment was conducted in commercial hatchery conditions (PIAST PASZE Ltd., Olszowa, Poland). The present experiment involved a total of 240 one-day-old female chicks (Ross 308), which were randomly selected from 20 000 birds. The chicks were randomised with different dietary treatments. Each treatment was replicated 10 times, and 12 female broilers housed together formed the experimental unit. Chicken broilers were reared in pens (1 × 1 m) on litter, as recommended by Aviagen. Group 1 (control) was fed with a commercial diet, and group 2 (SYN) was fed a commercial diet with added synbiotics: 0.8% RFO (prebiotic: raffinose family oligosaccharides extracted from lupin seeds) and 1% Lavipan® (*Lactococcus lactis* B/00039, *Carnobacterium divergens* KKP 2012p, *Lactobacillus casei* B/00080, *Lactobacillus plantarum* B/00081, and *Saccharomyces cerevisiae* KKP 2059p). The additives to the feed were used in the first 7 days of rearing. Food and water were administered *ad libitum*. The feeding process was split into three stages: Starter (1–10-day old), Grower (11–21-day old), and Finisher (22–42-day old) (Table 1). The chickens were electrically stunned and slaughtered on day 42 of life in a commercial poultry slaughterhouse near the farm.

Sample preparation. Directly after slaughter, samples of the *Pectoralis major* muscle were taken from the left side of the breast, at 2/3 the length of the keel around the bone crest (following the orientation of the muscle fibres). For histological investigations, 12 chickens per group, with a body weight similar to the mean for the group, were selected. After collection, the samples were frozen in liquid nitrogen at roughly –196°C until processed in a cryostat (Thermo Shandon/Thermo Fisher Scientific, UK) where 10 µm sections were cut to enable future processing.

Staining procedures. The staining procedures performed were: NADH-TR (tetrazolium reductase) activity to distinguish muscle fibre types

Table 1. Ingredients and chemical composition of the Starter, Grower, and Finisher diets for chickens

	Starter	Grower	Finisher
Raw materials (%)			
Wheat	45.70	47.80	50.20
Maize	25.20	21.90	19.30
Soybean meal	10.00	10.00	10.00
Rapeseed meal	10.00	10.00	10.00
Soybean oil	5.20	7.30	7.70
Calcium phosphate	2.25	1.50	1.00
Alimet liquid[1]	0.33	0.30	0.28
Vitamin-mineral premix 0.3% HyD without enzymes[2]	0.30	0.95	0.85
L-Lysine	0.29	0.94	0.63
Chalk	0.28	0.18	0.13
Salt	0.18	0.21	0.19
Sodium carbonate	0.17	0.10	0.12
L-Threonine	0.15	0.14	0.18
Vitamin-mineral premix[3]	0.30	0.30	0.30
Nutritional value			
ME (kcal/kg)	3073	3269	3329
Total protein (%)	21.50	20.10	19.20
Lysine (%)	1.30	1.10	1.10
Methionine (%)	0.60	0.50	0.50
Methionine + cysteine (%)	1.00	0.90	0.80
Calcium (%)	0.90	0.70	0.70
Phosphorus (%)	0.40	0.35	0.28
Sodium (%)	0.14	0.14	0.13

[1]methionine hydroxy analogue (88% methionine activity)
[2]vitamin-mineral premix for broilers for the 2nd rearing period with the addition of HyD (30% of vitamin D3) without enzymes
[3]contents per 1 kg premix: Vitamin A 5 000 000 IU, Vitamin D_3 1 400 000 IU, Vitamin E 18 200 mg, Vitamin K_3 1200 mg, Vitamin B_1 600 mg, Vitamin B_2 2000 mg, Vitamin B_6 1200 mg, Vitamin B_{12} 8000 mg, biotin (H) 80 000 mg, Fe 20 000 mg, Mn 40 000 mg, Zn 36 000 mg, Cu 6000 mg, I 400 mg, Se 140 mg, calcium pantothenate 4800 mg, nicotinic acid 20 000 mg, folic acid 400 mg, choline chloride 380 mg, phytase – 500 phytase units (FTU)

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differing in enzymatic activity (Figure 1A), oil red staining to determine intramuscular fat content (Figure 1B), alkaline phosphatase reaction for counting the number of blood vessels (Figure 1C), and HE staining (hematoxylin and eosin) to evaluate fibre diameter, histopathological changes and count of normal fibres, respectively (Figure 1D–F). All methods were performed following the same procedure, with the slides containing the samples being immersed in various chemical substances in specific time periods. After mounting the preparations (last step of the process, after chemical sequence), they were ready for the analysis process.

Sample analysis. An MN-800 F microscope (OPTA-TECH, Poland) equipped with an Opta-View camera was used to record microscopic images with muscle tissue on a computer disk. The MultiScan v.18.03 microscope imaging software (Computer Scanning Systems II Ltd, Poland) was used for measurements of fibre diameter, muscle fibre density, counting the percentage of oxidative and glycolytic fibres, total number of capillaries and number of capillaries per muscle fibre, histopathological changes, and percentage of normal fibres per 1.8 mm^2. The MultiScan program was also used for the contrast feature, which calculates the percentage of red colour (lipids – intramuscu-

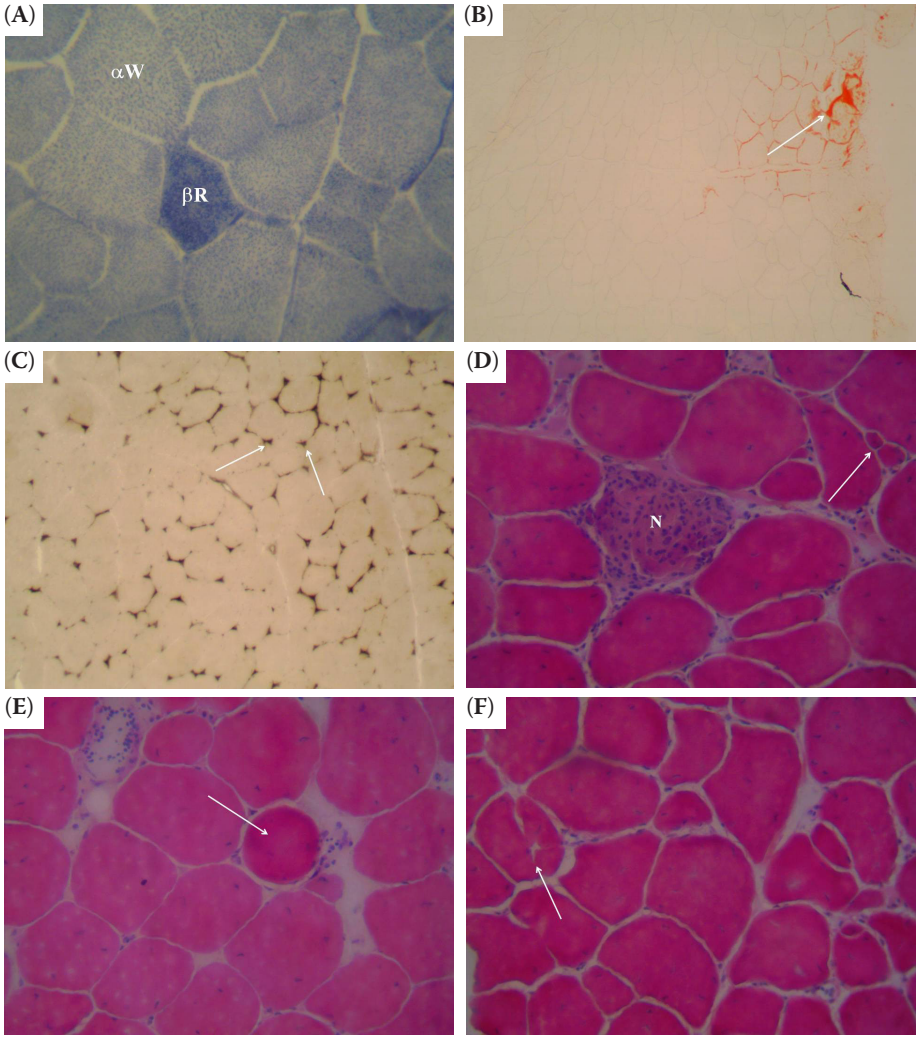

Figure 1. (**A**) Muscle fibre types: αW (glycolytic) and βR (oxidative), NADH-TR tetrazolium reductase activity stain, magnification ×200; (**B**) intramuscular fat (arrow), Red Oil stain, magnification ×100; (**C**) capillaries (arrows), alkaline phosphatase stain, magnification ×100; (**D**) necrotic (N) and atrophic fibre (arrow), hematoxylin and eosin (HE) stain, magnification ×200; (**E**) giant fibre (arrow), HE stain, magnification ×200; (**F**) fibre splitting (arrow), HE stain, magnification ×200

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lar fat). The percentage of intramuscular fat was determined per 3.6 mm^2 area.

Statistical analysis. The results were subjected to one-way analysis of variance (ANOVA) using STATISTICA AXAP v. 10.0 MR1. Arithmetic mean (x) and standard error of the mean (SEM) were calculated. Significant differences (at $P < 0.05$, $P < 0.01$) between the groups were analysed with Tukey's test. The relationships between investigated traits were examined by simple correlation coefficients.

RESULTS AND DISCUSSION

Table 2 summarizes body weight, breast muscle weight, and histological parameters in the *Pectoralis major* muscle of chickens at 42 days of age. The final body weight of chickens ranged from 2620 g in SYN group to 2643 g in control group, and breast muscle weight ranged from 526.6 g in control group to 529.4 g in SYN group. No statistically significant differences between the groups in terms of body weight and breast muscle weight of chickens were found. Taherpour et al. (2009) studied the effect of a dietary probiotic (PrimaLac), a prebiotic (Fermacto®), and butyric acid glycerides (Baby C4) on the performance of Ross 308 broiler chickens. Their results showed that all the treatments, except the treatment containing only the prebiotic, produced better final body weight in comparison with the control treatment. Conversely, Kral et al. (2012) obtained weight decrease of Cobb 500 broiler chickens at 42 days of age, which were fed a mixture with the addition of probiotics. Maiorano et al. (2012) found no effect of prebiotics (RFO – Raffinose Family Oligosaccharides) and synbiotics (RFO + bacteria: *Lactococcus lactis* spp. *lactis* and *Lactococcus lactis* spp. *Cremoris* as well as commercial synbiotic Duolac™) administered *in ovo* on the final body weight of chickens. According to the study performed by Maiorano et al. (2017), the administration of two different prebiotics was associated with small increases in body weight, which has a significant impact in big rearing systems. The supplemented broilers proved to be heavier and with greater carcass yield, as well as breast muscle weight, which confirms the results obtained in the present study with synbiotic supplementation. Bioactive substances do not act in the same way in every breed, as it was shown by Hanning et al. (2012). Using three different prebiotics (galactooligosaccharides, fructooligosaccharides, and plum fibres) on two different breeds of broilers (Cornish White Rock and Naked Neck), different effects on body weight were found: fructooligosaccharides enhanced the body weight of Cornish White Rock chickens, and the Naked Neck breed were heavier when supplemented with plum fibres.

The biggest fibre diameter was found in the control group (55.81 μm) and the smallest in the SYN group (52.79 μm). As for muscle fibre density (per 1.8 mm^2 of section area), it was the other way around, with 301.33 fibres/1.8mm^2 and 320.92 fibres/1.8mm^2 for the control and SYN groups, respectively. No statistically significant differences between the groups in terms of fibre diameter and muscle fibre density were observed. However, a tendency to reduce the thickness of the muscle fibres with a simultaneous increasing of the muscle fibre density in the SYN group was observed. As noted by Choi and Kim (2009) and Maiorano et al. (2017), smaller diameter of the fibres favourably affects meat quality, and might be considered an indicator of a more delicate structure of meat. As expected, the chicken breast muscle is characterised by a prevalent glycolytic metabolism, as white fibres represent over 95% of all fibres.

Fat tissue content determined by histochemical methods also showed no significant differences between mean values (1.48% in the control group

Table 2. Body weight, breast muscle weight, and histological parameters in *Pectoralis major* muscle of chickens (values are means ± SEM)

		Control	SYN
Body weight (g)		2643 ± 57.5	2620 ± 45.1
Breast muscle weight (g)		526.6 ± 18.5	529.4 ± 13.7
Fibre diameter (μm)		55.81 ± 1.85	52.79 ± 1.28
Muscle fibre density (fibres *n*/1.8 mm^2)		301.33 ± 24.06	320.92 ± 13.63
Muscle fibre type (%)	βR	4.21 ± 0.88	3.65 ± 0.68
	αW	95.79 ± 0.88	96.35 ± 0.68
Intramuscular fat content (%)		1.48 ± 0.42	1.91 ± 0.36
Capillaries *n* per 1.8 mm^2		442^b ± 35.54	567^a ± 40.16
Capillaries *n* per muscle fibre		1.21^b ± 0.06	1.50^a ± 0.11

SYN = prebiotic RFO (raffinose family oligosaccharides) (0.8%) + probiotic Lavipan® (1%)

^{a,b}values significantly differ at $P < 0.05$ level

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vs 1.91% in the SYN group, respectively). Similarly, Maiorano et al. (2012) did not find any significant effect of prebiotic RFO administered *in ovo* on the 12th day of incubation on abdominal fat in Ross broiler chickens. Prebiotic substances like betaine have been proven to reduce abdominal fat deposition in broiler chickens and abdominal fat yield percentage in ducks (Fouad and El-Senousey 2014). Regarding intramuscular fat content, studies have shown that prebiotics and synbiotics delivered *in ovo* at day 12 of embryonic development significantly increase this percentage in comparison to the control group (Dankowiakowska et al. 2014). Santoso et al. (1995) proved a significant result on the decrease of abdominal fat when supplementing the diet of broiler chickens with *Bacillus subtilis*. These data suggest that the supplementation of broiler feed with synbiotics is a good way to improve the quality of broiler carcasses without using antibiotics, diminishing the amount of abdominal fat (the biggest source of saturated fatty acids) and increasing intramuscular fatty tissue content (because the tendency of increased intramuscular fat in the SYN group was observed), resulting in healthier and more valuable meat. According to Maiorano et al. (2017), intramuscular fatty tissue content is slightly higher in broilers supplemented with prebiotics, which may be an aggravating factor in lipid oxidation that was increased with *in ovo* injection of prebiotics. According to Dinh et al. (2011), broiler breast muscle with 100% white fibres has by 50% less cholesterol content than the homologous meat cut of ducks. This represents important information about meat quality and the healthiness of the meat, which is becoming the prime concern for consumers. Muscle fibre type was also analyzed, and the results obtained were according to what would be expected, since breast muscle (white meat) is supposed to have > 95% white fibres (αW).

Concerning the percentage of normal fibres and histopathological changes in the *Pectoralis major* muscle, presented in Table 3, the SYN group had a bigger percentage of normal fibres and fewer atrophic fibres, but no statistically significant differences between the groups were observed. Fibre atrophy is one of the main causes of white striping (Petracci and Cavani 2012). According to Kuttappan et al. (2013), white striping is a condition characterized by the occurrence of white striations parallel to the muscle fibres direction on the muscle surface, and it causes depletion of fat and protein content in meat. The smaller percentage of normal fibres in the control group indicates a bigger percentage of pathological fibres (i.e. fibre atrophy, fibre necrosis with phagocytosis, fibre splitting). Necrotic fibres cause death of the muscle and hence meat spoilage. This can be caused by an excess of intracellular calcium in fibres (Sandercock et al. 2009) or by hypertrophy of connective tissue, which causes arterial blockage and low capillary proliferation, causing hypoxia of the muscle (Dransfield and Sosnicki 1999).

Fibre splitting mean values were shown to occur at a higher percentage in the control group ($P < 0.05$) when compared to the SYN group (1.42% vs 0.86%, respectively), which may indicate that supplementing chickens with bioactive substances reduces the occurrence of splitting. The reason for splitting is the overload of cells, probably caused by too little blood supply to the fibres. This is confirmed by the results shown in Table 2. In the control group a lower number of capillaries on the surface of 1.8 mm^2 compared to the experimental group was observed (442 vs 567, respectively; $P < 0.05$). Moreover, the control group had significantly fewer capillaries per muscle fibre compared to the SYN group (1.21 vs 1.50, respectively; $P < 0.05$), which was characterized by the lowest percentage of atrophy fibres. Borisov et al. (2001) are of the opinion that both denervation and vascular tissue degeneration affect the occurrence of atrophy fibres. The number of capillaries supplying blood to single muscle fibres increases in a linear way according to the cross section area

Table 3. Normal fibres and histopathological changes in *Pectoralis major* muscle of chickens (values are means ± SEM)

	Control	SYN
Normal fibres (%)	94.88 ± 0.79	96.35 ± 0.23
Fibre atrophy (%)	3.38 ± 0.62	2.58 ± 0.24
Giant fibres (%)	0.04 ± 0.03	0.05 ± 0.04
Fibre necrosis with phagocytosis (%)	0.28 ± 0.09	0.16 ± 0.05
Fibre splitting (%)	1.42^a ± 0.24	0.86^b ± 0.13

SYN = prebiotic RFO (raffinose family oligosaccharides) (0.8%) + probiotic Lavipan® (1%)

^{a,b}values significantly differ at $P < 0.05$ level

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of muscle fibre (Hudlicka 1985). In fast growing birds the number of capillaries decreases and this is the reason why ischemic and degenerative muscle changes occur (Sosnicki et al. 1991). Furthermore, in white muscles with the dominance of glycolytic metabolism a more weakly developed network of capillaries exists in comparison with red muscles with the dominance of oxidative metabolism (Elminowska-Wenda et al. 2005).

The administration of synbiotics proved to be more effective against histopathological changes. This results in better meat quality and may be due to the synergy mechanism that combines desirable traits of probiotics and prebiotics, ensuring that the administered microbiota reach the gut thanks to prebiotic action. Fast-growing birds and those with high meatiness have the most extensive histopathological changes, most of the time caused by insufficient blood supply, which also inflicts myopathy (Maiorano et al. 2012).

This is caused also by fibres with big diameters, which is in accordance with the SYN group, i.e. the group with the smallest mean value for fibre diameter and the one with fewer histopathological changes and better microvascularization. Bioactive substances may have an attenuating effect in this disease, which has a high incidence in Ross 308 broilers (Bianchi et al. 2006), and is confirmed by the results obtained in the present study, where both substances administered increased the number of capillaries in muscle sections. Bioactive substances may have an important role against Deep Pectoral Myopathy by inducing smaller fibre diameter and a bigger number of blood vessels in the breast muscle of broiler chickens. Ross 308 broilers are selected for greater weight of breast muscle. In chickens, muscles become heavier not by hyperplasia (increase of the number of fibres), but by postnatal hypertrophy, which, according to MacRae et al. (2007), is linked to an increase in histopathological changes. It is these authors' belief that embryonic hyperplasia should be the trait to select in order to improve broiler growth potential and may also alleviate muscle damage. According to Cianciullo (2012), by selecting broilers with heavier muscles, the resources for mature visceral organs are redirected to muscles. This causes ascites, which is directly linked to the limited cardiopulmonary capacity of the animal to attend to body oxygen demands. Fibre atrophy is the result of insufficient nutrient supply and the predominance of catabolism processes as a result of a general reduction of metabolic rate. So, abrupt increases in fibre size can cut oxygen supply, which leads to necrosis and fibre splitting, triggered by hypoplasia. Connective tissue hypertrophy also aids in this process by applying pressure in surrounding blood vessels, leading to oxygen deficiency and degenerative changes. In this particular study, synbiotic administration induced smaller fibre diameters and more fibres per 1.8 mm^2. Radial fibre growth may outrun support systems, causing fibre atrophy due to metabolic stress. Accelerated muscle growth is connected with reduced oxidative capacity, which is often the case in the pectoral muscle of chickens, where glycolytic (white) fibres are predominant. Cianciullo (2012) observed more histopathological changes in broilers with feed supplementation of synbiotics, but fibre splitting was predominant in prebiotic supplementation due to bigger oxygen diffusion distances. Microscopic images in Figure 1 show the characteristic pattern of histochemically different muscle fibres, the distribution of intramuscular fat, capillaries content, and different histopathological changes in the *Pectoralis major* muscle.

Table 4. Correlation coefficients (r) between traits of *Pectoralis major* microstructure and body weight and breast muscle weight of chickens

Trait	Body weight	Breast muscle weight
	(g)	
Fibre diameter (μm)	0.40	0.46*
Muscle fibre density (fibres n/1.8 mm^2)	–0.49*	–0.56**
Intramuscular fatty tissue content (%)	0.21	0.44*
Normal fibres (%)	–0.27	–0.27
Fibre atrophy (%)	0.24	0.18
Giant fibres (%)	0.15	0.05
Fibre necrosis with phagocytosis (%)	0.26	0.37
Fibre splitting (%)	0.12	0.27

correlation coefficients significant at *$P < 0.05$, **$P < 0.01$

The relationships between the microstructure traits of the muscle tissue of broiler chickens and body weight and weight of the breast muscle expressed by the size of the correlation coefficients are shown in Table 4. It has been shown that the

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greater pectoral muscle mass of broiler chickens was positively correlated with the diameter of muscle fibres (r = 0.46*). Therefore, the greater the body weight and pectoral muscle weight, the lower the muscle fibre density per unit area of examined muscle (correlation coefficients between these traits were negative, and amounted to r = –0.49* and r = –0.56**). Moreover, a positive correlation between the muscle mass and the presence of intramuscular fat (r = 0.44*) was also observed. Other correlations between microstructure characteristics and body weight, as well as the weight of breast muscle, turned out to be not statistically significant. In sum, the differentiation of meatiness in broiler chickens was caused by the occurrence of muscle fibre hypertrophy. This was confirmed by the studies of Le Bihan-Duval et al. (2008) and Koomkrong et al. (2015). Le Bihan-Duval et al. (2008) found a high heritability in breast meat quality traits like cooking loss and drip loss. Breast muscle weight was genetically related to fibre size, and other results indicated that the selection for increased breast muscle is expected to lead to greater fibre hypertrophy (MacRae et al. 2007). Koomkrong et al. (2015) related tender meat to large fibre diameter in muscle, which leads to less connective tissue, which confirms the results obtained in the present study, where the group with bigger fibre diameter (control) had the smallest percentage of connective tissue hypertrophy.

CONCLUSION

The results of the study show the influence of given synbiotics on the microstructure of the *Pectoralis major* muscle. The tendency to reduced muscle fibre diameter in chickens supplemented with synbiotics indicates an increase in muscle fibre density, which gives meat a more delicate structure. Also, intramuscular fat mean values point to a tendency of improvement in the supplemented group, which may have a positive impact on meat juiciness and flavour. Moreover, the positive effect of the feed supplementation with synbiotics on muscle microvascularization could have an important practical meaning, because low physical activity of chickens during rearing may lead to ischemic muscles, increased changes in the structure of muscle fibres, and reduction of meat quality.

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