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ORIGINAL PAPER

EFFECT OF BIOPLUS YC PROBIOTIC ON THE FATTY ACID PROFILE AND LIPID INDICES IN PORK*

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Abstract

The objective of this study was to assess how probiotic bacteria added to diets of 60 hybrid barrows (Landrace–Yorkshire x Duroc) affected the basic chemical composition of meat, the fatty acid profile of intramuscular fat (IMF), m. *longissimus lumborum* (LL) and lipid quality indices, because IMF cannot be removed before consumption and this inevitably has an impact on human health. After 78 days, the pigs were divided into the control group (CT, n=30) and the experimental group (BP, n=30), which until 97 days of rearing were supplied with BioPlus YC probiotic (*Bacillus licheniformis* DSM 5749 and *Bacillus subtilis* DSM 5750). After slaughter, 12 carcasses of similar weight (90 ± 5 kg) from each group were selected for testing. Supplementation with probiotic *Bacillus* did not affect the basic chemical composition of meat. It was established that BioPlus YC had a significant impact on capric – C10:0 (0.08 vs. 0.13), eicosatrienoic – C20:3 n-3 (1.76 vs. 2.14) and ΣPUFA n- 3 acids (1.88 vs. 2.41), causing a reduction in their percentage, which was thereby different than in the CT group. Among lipid quality indices, IMF *LL* of pigs from the BP group, the thrombogenicity index (TI) was characterized by a significantly (P≤0.01) higher value than in the CT group (1.08 vs. 0.99). These results suggest that further research is needed to study differences in compounds affecting the fatty acid profile of pork, and to confirm the association of the lipid profile with the use of BioPlus YC probiotic in pig fattening.

Keywords: pig, meat, probiotic, fatty acid, lipid quality indices.

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INTRODUCTION

Pork is an important component of human diet in many countries in the world. China is the largest producer and consumer of pork globally, while the European Union comes second in this respect (Szűcs, Vida 2017). Pork is consumed because of deep-rooted habits and culinary traditions as well as its affordability. In recent years, there has been growing awareness of how human health is negatively affected by the consumption of saturated fatty acids (SFA) while being positively affected by the consumption of monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids. According to the WHO (2003) and European Food Safety Authority (EFSA 2010), one's total consumption of fat should not exceed 30% of total energy intake so as to avoid unhealthy weight gain. The intake of saturated fats should be less than 10% of total energy intake, or as low as possible. Moreover, consumers are also paying attention to the overall nutrition value of meat and its products, including its fat content and fatty acid profile, as these components of human nutrition may also be crucial in the prevention of cardiovascular diseases and cancers. Consumers are becoming increasingly interested in safe, tasty, and healthy meat products (Nuernberg et al. 2015). Furthermore, there has been a growing concern about antibiotic residues in meat, and it is assumed that the continuous use of antibiotics may increase bacterial resistance, which can threaten both animal and human health (Van der Fels-Klerx et al. 2011).

For many years, rearing has led to a significant reduction of fatness in pig carcasses, and thus to the improvement of nutritive and health value of this meat. It is generally known that the fat content in pork and its fatty acids profile are influenced by the following factors: sex, age, breed, body ratio, protein ratio; also, energy in feed, feeding method, energy consumption and type of fat, as well as the efficiency of the metabolism of fatty acids in the body, with intraindividual variations due to genetic disposition, play a role (Nevrkla et al. 2017). The fatty acid profile of meat can be easily modified through feeding, thereby improving the quality of pork for the consumer and meeting nutritionists' recommendation. Pig feeding solutions have been sought that would include supplementary formulas to improve pigs' welfare, resistance to diseases, but also to raise the safety of raw meat harvested from those animals. Probiotics are an example of such formulas. Following the ban on antibiotics, probiotics have been suggested as the most desirable alternative for livestock rearing owing to their beneficial effects. Among several bacterial species used as probiotics, *Bacillus spp*. has been considered as the most appropriate probiotic because its spores can resist harsh environments, thus allowing extensive storage at ambient temperature (Ross et al. 2012, Markowiak, Śliżewska 2018).

The usage of *Bacillus spp*. in animal nutrition is regulated by the EFSA. The species *B. subtilis* and *B. licheniformis* have been given a Quali-

fied Presumed Safety (QPS) status, provided they prove to be non-toxigenic (EFSA 2017). Previous studies on dietary supplementation with *Bacillus spp*. have reported their positive impact on the health status and productivity of pigs during weaning, growing and finishing stages of growth, and on the quality of meat-carcass (Balamuralikrishnan et al. 2016, Liu et al. 2018). Moreover, studies demonstrated an impact of probiotic administration on the reduction of serum fatty levels in animals (Joysowal et al. 2018). However, there is not enough information about the meat fatty acid profile after supplementation with beneficial bacteria.

The objective of this research was to assess the impact of feed supplementation with BioPlus YC probiotic on the chemical composition and profile of fatty acids in pig muscles and on the lipid quality indices calculated on their basis, because intramuscular fat cannot be removed before consumption and this inevitably has an impact on human health.

MATERIAL AND METHODS

Animals, diets, slaughtering and meat sampling

An animal care and use committee approval was not obtained for this study because pork samples were collected after slaughter from a commercial pork packing plant.

The study has been conducted on 60 hybrid barrows (Landrace – Yorkshire x Duroc) in the spring season. The animals were maintained at a commercial pig production farm in the Pomeranian Voivodeship (Poland). During the fattening, the pigs were kept in the same environmental conditions, and were fed *ad libitum* with two balanced dry loose feed mixes from the farm's mixing plant (Rosta from 20 till 50 kg body weight and Finisher from 45 till 100 kg body weight). The specification of components and chemical compositions can be found in Table 1. The fatty acid profile of the feeds is in Table 2. On the day the fattening was commenced (78th day), the pigs were divided into two groups: the control (CT, n=30) and the experimental (BP, n=30). Pigs from the BP group were supplemented with BioPlus YC probiotic (by Chr. Hansen) at an amount of 400 g t^{-1}. The supplement contains a complex of probiotic bacteria, i.e. *Bacillus licheniformis* DSM 5749 (1.6 × 109 CFU g^{-1}) and *Bacillus subtilis* DSM 5750 (1.6 × 109 CFU g^{-1}) spores in a 1:1 ratio.

After reaching the weight of approximately 110 kg (on day 97), the pigs were transported from the farm to the Meat Plant, 79 km away (1.5 h drive), with the average temp. on the hold during transport of 22.6°C. After unloading the truck, which took 30 min, the pigs spent the next 16 h in a lairage, where the average temp. was 15°C. The duration of fasting time before slaughter was 24 h. The average results of fattening and slaughter provided

Table 1

Composition of the experimental diets

Items	Rosta 20-50 kg body weight	Finisher 45-100 kg body weight
Ingredient (g kg^{-1} on a DM basis)		
Wheat grain	11.60	11.60
Barley grain	10.60	10.60
Triticale grain	10.60	10.60
Wheat bran	-	15.60
NaCl	0.70	0.61
Complementary feed	1.25	1.00
Other[#]	62.25	49.99
Chemical composition		
Metabolizable energy (MJ kg^{-1})	11.80	11.60
Net energy (MJ kg^{-1})	9.74	9.51
Crude protein (%)	17.42	15.88
Total fibre (%)	3.80	4.66
Crude fat (%)	3.38	3.03
Calcium (%)	0.77	0.58
Total phosphorous (%)	0.60	0.45
Lysine (%)	1.26	0.98
Methionine (%)	0.39	0.28
Methionine+cysteine (%)	0.75	0.66
Threonine (%)	0.80	0.64
Tryptophan (%)	0.24	0.19
Isoleucine (%)	0.66	0.58
Valine (%)	0.79	0.72

[#] Other: post-extraction soy meal, toasted, post-extraction rapeseed meal, rapeseed rape EP-100, narrow-leaved lupine, animal fat, fine grained chalk ($CaCO_3$ min. 94%, Ca – 37.6%), phosphate 1-CA[2] (additive contains min. 22% P and 15% Ca) – protected feed formulation.

by the farm are shown in Table 3. Table 4 presents the daily consumption of fatty acids by pigs in both groups, calculated on the basis of the average daily consumption of feed and determined fatty acid profile in the pig diet.

On the slaughter line, after the pigs were stunned (Butina CO_2 gas stunning system, Denmark), the lean meat percentage in the carcass was non-invasively ultrasonically measured (AutoFom, SFK Technology, Denmark). Before cooling, the hot carcass weight was determined at an accuracy of 100 g. Next, the carcasses were progressively chilled for 24 hrs. At first, they were cooled at a temp. of +1°C for 7-8 h, and subsequently, the carcasses were cooled at a temp. between –3°C and –4°C for 6-7 h, and then

Table 2

Fatty acid profile (% of total fatty acids) of the experimental diets

Items	Rosta	Finisher
SFA		
Lauric (C12:0)	0.57	0.53
Myristic (C14:0)	1.03	1.12
Palmitic (C16:0)	22.00	20.90
Margaric (C17:0)	0.16	0.16
Stearic (C18:0)	3.65	3.42
Arachidic (C20:0)	0.12	0.15
MUFA		
Myristoleic (C14:1 *n*-5)	0.15	0.14
Oleopalmitic (C16:1 *n*-7)	2.50	2.16
Oleic (C18:1 *n*-9)	31.15	34.20
Eicosanoic (C20:1 *n*-9)	0.41	0.49
PUFA		
α-Linolenic (C18:3 *n*-3)	3.94	4.14
Total *n*-3	3.94	4.14
Linoleic (C18:2 *n*-6)	33.32	31.68
γ-linolenic (C18:3 *n*-6)	0.05	0.04
Eicosadienoic (C20:2 *n*-6)	0.06	0.06
Dihomo-γ-linolenic (C20:3 *n*-6)	0.02	0.04
Total *n*-6	33.45	31.82
Unidentified fatty acids	0.89	0.80
Σ SFA	27.53	26.28
Σ MUFA	34.21	36.99
Σ PUFA	37.39	35.96
Σ *n*-6/Σ *n*-3 PUFA	8.49	7.69

SFA – saturated fatty acids, MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acid

at 4–6°C for the remaining time (about 10 h). Based on the defined hot carcass weight, 24 carcasses of similar weight (90 ± 5 kg) were selected (twelve from each group) to determine chemical properties of *longissimus lumborum* (*LL*) muscle samples (1^{st}-4^{th} lumbar vertebrae). After cooling, the samples were transported in thermoses to the laboratory. Next, they were minced and vacuum-packed in polyethylene bags, and frozen at -19°C to analyse their basic chemical composition. For the fatty acid analysis, the samples were frozen and kept at -80°C for 12 wk. The samples were thawed at 4°C for 12 h before analysis.

Table 3

Average results of fattening and slaughter (from the farm)

Items	CT	BP
Initial body live weight – BLW (kg)	28.20	27.90
Final BLW (kg)	110.30	111.20
Body weight gain (kg)	82.10	83.30
Daily weight gain (kg d^{-1})	0.89	1.00
Daily feed intake (kg)	2.44	2.54
Feed efficiency (kg feed/ kg live weight gain)	2.75	2.53
Feed consumption for the production of pigs (kg)	226.10	205.50
Energy utilization coefficient (MJ)	2.82	2.59
Feed consumption for the production of pigs (MJ)	231.30	210.30
Gain:feed ratio	0.36	0.39
Hot carcass weight (kg)	88.82	88.77
Lean meat in carcass (%)	56.77	56.81

CT – control treatment, BP – BioPlus YC treatment

Chemical composition of meat

The basic chemical composition of *LL* muscle was determined in accordance with the official analytical methods of the AOAC (Latimer 2016): moisture content by the oven-drying of 2 g samples at 102°C for 12 h to a constant weight in a SUP-4M laboratory dryer, Wawa-Med, Warsaw Poland (950.46B, p. 39.1.02); total nitrogen with the Kjeldahl method, converted into an amount of protein on a Kjeltec 2100 Foss Tecator distiller, Hillerød, Denmark (992.15, p. 39.1.16), and crude fat content by petroleum ether extraction using a Soxtec HT6 apparatus by Foss Tecator, Hillerød, Denmark (960.39 (a), p. 39.1.05). The ash (total mineral content) was determined by incineration at 550°C for 10 h in an FCE 7SHM muffle furnace Czylok, Jastrzębie Zdrój, Poland (920.153, p.39.1.09).

Fatty acid analysis

The composition of fatty acids in pig diet and *LL* muscles was determined using the gas chromatography technique on an AGILENT Tech. 7890A Chromatograph, equipped with a flame-ionization detector (FID). Crude ground samples of feed and meat were homogenized in a T 25 Ultra Turrax (IKA-Werke GmbH & Co. KG, Staufen, Germany). The fat from pig diet and meat was extracted with the procedure described by Folch et al. (1957). According to the method, samples were homogenized by the use of a chloroform: methanol (2:1; v/v) solution. The extraction mixture contained 0.001% (w/v) of butylated hydroxytoluen as an antioxidant. The organic solvent was evaporated under the nitrogen stream. The crude lipid extracts were then saponified with KOH in methanol. Afterwards,

Table 4

Calculated average daily intake of fatty acids by pigs (g kg^{-1} feed)

Items	CT	BP
SFA		
Lauric (C12:0)	1.29	1.35
Myristic (C14:0)	2.73	2.84
Palmitic (C16:0)	50.90	53.00
Margaric (C17:0)	0.39	0.41
Stearic (C18:0)	8.34	8.69
Arachidic (C20:0)	0.37	0.38
MUFA		
Myristoleic (C14:1 *n*-5)	0.34	0.36
Oleopalmitic (C16:1 *n*-7)	5.27	5.49
Oleic (C18:1 *n*-9)	84.40	86.90
Eicosanoic (C20:1 *n*-9)	1.20	1.24
PUFA		
α-Linolenic (C18:3 *n*-3)	10.10	10.50
Total *n*-3	10.10	10.50
Linoleic (C18:2 *n*-6)	77.30	80.50
γ-linolenic (C18:3 *n*-6)	0.10	0.10
Eicosadienoic (C20:2 *n*-6)	0.15	0.15
Dihomo-γ-linolenic (C20:3 *n*-6)	0.10	0.10
Total *n*-6	77.70	80.90
Unidentified fatty acids	1.95	2.03
Σ SFA	64.00	66.70
Σ MUFA	91.30	94.00
Σ PUFA	87.80	91.40
Σ *n*-6/Σ *n*-3 PUFA	7.69	7.70

CT – control treatment, BP – BioPlus YC treatment, SFA – saturated fatty acids, MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acids

the methyl esters of fatty acids (FAMEs) were prepared by transesterification with boron trifluoride in methanol (BF3) according AOCS official method Ce 2-66 (AOCS 1997). The resulting FAMEs were analyzed on a fused silica CP-Sil 88 (Chrompack, Netherlands) capillary column J&W Scientific HP-88 series (100 m x 0.25 mm x 0,20 µm). The analysis was carried out at a detector temp. of 280°C, and injector temp. of 250°C. The injection was made automatically at 1.0 mL volume with the 1:50 split ratio. Helium was employed as the carrier gas, at a flow rate 2 mL min^{-1}. The separation was conducted at the programmed temperature. The initial column temp. was 120°C, which was maintained for 1 min, then raised to 230°C

by an increased rate at $5^{o}C^{-1}$ min^{-1} and finally held for 20 min. FAMEs were identified by comparison of the retention times with those of a mixture of external standard methyl esters from Supelco (Supelco 37 FAME Mix C4-C24 Component, Sigma-Aldrich, St. Louis, Missouri, USA). The fatty acids were calculated as a percentage (w/w) of total fatty acids with the Agilent ChemStation programme.

Lipid quality indices

Fatty acids were grouped as follows: saturated fatty acids (Σ SFA) = = C4:0+C6:0+C8:0+C10:0+C11:0+C12:0+C14:0+C16:0+C18:0; monounsaturated (ΣMUFA) = C16:1*n*-7+C18:1*n*-9 cis+C18:1*n*-9 trans; polyunsaturated fatty acids (ΣPUFA) = Σ*n*-3 PUFA (C18:3*n*-3+C20:3*n*-3+C22:6*n*-3) and Σ*n*-6PUFA (C18:2*n*-6); unsaturated (ΣUFA) = ΣMUFA+Σ PUFA.

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

(1) Desirable Fatty Acids (DFA) hypocholesterolemic acids: Σ UFA+C18:0 *n*-6 (Díaz et al. 2002):

(2) OFA hypercholesterolemic fatty acids: Σ SFA – C18:0 *n*-6 (Diaz et al. 2002);

(3) Polyunsaturated/saturated fatty acids ratio (P/S): [(C18:2*n*-6+C18:3*n*-3)/(C12:0+C14:0+C16:0)];

(4) Atherogenic index (AI): [C12:0+(4×C14:0)+C16:0+C18:0]/[Σ MUFA+ +Σ PUFA*n*-6+Σ PUFA*n*-3] (Ulbricht, Southgate 1991);

(5) Thrombogenicity index (TI): (C14:0+C16:0+C18:0)/ /[(0.5×ΣMUFA)+(0.5×ΣPUFA *n*-6)+(3×ΣPUFA*n*-3)+(ΣPUFA*n*-3/Σ PUFA*n*-6)] (Ulbricht, Southgate 1991);

(6) Staturation Index (SI): (C14:0+C16:0+C18:0)/(ΣMUFA cis+ΣPUFA);

(7) h/H – hypocholesterolemic fatty acids/hypercholesterolemic fatty acids ratio: [C18:1 cis *n*-9+C18:2*n*-6+C18:3*n*-6+C18:3*n*-3+C20: :3*n*-6+C20:4*n*-6+C20:5*n*-3+C22:4*n*-+C22:5*n*-3+C22:6*n*-3)]/ /(C14:0+C16:0);

(8) Peroxidisability index (PI): (%monoenoic acid×0.025)+(% dienoic acid×1)+(% trienoic acid×2)+(% tetraenoic acid×4)+(% pentaenoic acid×6)+(% hexaenoic acid×8) (Erickson 1992);

(9) Nutritive value index (NVI): (C18:0+C18:1*n*-9)/C16:0 (Sari et al. 2015);

(10) D^{9} desaturase activity index for 16:0 was estimated using the following ratio DI (16): Δ^{9}-desaturase index = 100 [C16:1*n*-9/ /(C16:1*n*-9+C16:0)] (Malau-Aduli et al. 1998);

(11) D^{9} desaturase activity index for 18:0 was estimated using the following ratio: DI (18): Δ^{9}-desaturase index = 100 [C18:1*n*-9/ /(C18:1*n*-9+C18:0)] (Malau-Aduli et al. 1998);

(12) Total desaturation index: TDI = MUFA (C16:1*n*-7+C18:1*n*-7+C18: :1*n*-9)/SFA (C14:0+C16:0+C18:0) (Green et al. 2010);

(13) Elongation index (EI) =100[(C18:0+C18:1*n*-9)/
/(C16:0+C16:1+C18:0+C18:1*n*-9)] (Green, Olson 2011).

Statistical analysis

All samples were analyzed in triplicate. The findings were log-transformed to attain or approach normal distribution, and subsequently one-way analysis of variance (ANOVA) was used in the orthogonal system. Statistical significance of differences between the averages of the groups was calculated using the Tukey's multiple comparison test, at the levels of significance $P \leq 0.05$ and $P \leq 0.01$, with the use of Statistica®13.1 software. The tables show average values and their standard deviations.

RESULTS AND DISCUSSION

Chemical composition of the meat

The differences in the basic chemical composition of pork may depend, for instance, on the content of nutrients in the diet and the amount of consumed feed, as well as the type of used supplements. In pigs, there is a significant correlation between the fat profile of the dietary fat source and adipose tissue in the carcass (Nguyen et al. 2003).

In this study, it has been established that the concentrations of dry mass, total protein, raw fat and ash in the *L.L* muscles were not significantly different (Table 5). The absence of differences in the basic chemical composition of the meat may be explained by similarities in the body weight of the animals (28.2 vs. 27.9 kg), daily feed intake (2.44 vs. 2.54 kg d^{-1}) and its use (2.75 vs. 2.53 kg feed kg^{-1} live weight gain) by both groups of animals (Table 3). These facts resulted in similarities in daily growth (0.89 vs. 1.0 kg d^{-1}) and the final body mass (110.3 vs. 111.2 kg) of pigs in both groups.

The influence of probiotics on the regulation of feed intake (by affecting hormones that influence metabolic function and areas in the brain associated with eating behaviour, so-called "gut microbiota-brain axis" represents a bidirectional signalling axis that regulates body weight by balancing appetite, storage, and energy expenditure), on the regulation of intestinal transit and on the retrieval and use of energy are generally known (Tolhurst et al. 2012, De Clercq et al. 2016). Similarly, Parra et al. (2010) did not establish a significant impact of feed supplementation with Bioplus 2B (*Bacillus licheniformis* and *B. subtilis* mixture) in Iberian pigs on the protein and fat content in *serratus ventralis* (*SV*) muscles.

Tufarelli et al. (2017) stated a significantly higher ($P \leq 0.05$) content of protein in the meat of pigs [(Landrace × Yorkshire) × Talent] supplemented with SLAB51 probiotic (Mendes SA, Lugano, Switzerland) that included

Table 5

Basic chemical composition (%) of *longissimus lumborum* (*LL*) muscle and main fatty acids (% of total fatty acid) of intramuscular fat

Item	CT	BP	*P* value
Basic chemical composition *LL* muscle			
Dry matter	25.52±0.92	25.34±0.66	0.585
Total protein	20.91±0.62	20.73±0.52	0.447
Crude fat	2.59±0.63	2.58±0.49	0.958
Ash	1.20±0.04	1.19±0.09	0.667
Fatty acids intramuscular fat *LL*			
SFA			
Butyric (C4:0)	10.21±2.1	8.96±7.2	0.152
Caproic (C6:0)	0.60±0.37	0.86±0.45	0.191
Caprylic (C8:0)	0.09±0.06	0.09±0.06	0.865
Capric (C10:0)	0.13±0.04^{a}	0.08±0.07^{b}	0.036
Undecanoic (C11:0)	0.05±0.07	0.06±0.07	0.784
Lauric (C12:0)	0.04±0.07	0.02±0.05	0.659
Myristic (C14:0)	1.37±0.21	1.30±0.23	0.589
Palmitic (C16:0)	22.80±0.7	23.60±2.1	0.329
Stearic (C18:0)	9.28±0.77	9.96±1.03	0.223
MUFA			
Palmitoleic (C16:1 *n*-7)	3.52±0.38	3.52±0.46	0.666
Oleic (C18:1 *n*-9 cis)	35.84±1.43	37.63±2.85	0.065
Elaidic (C18:1 *n-9 trans*)	3.63±0.25	3.64±0.44	0.175
PUFA			
α-Linolenic (C18:3 *n*-3)	0.22±0.33	0.12±0.25	0.406
Eicosatrienoic (C20:3 *n*-3)	2.14±0.34^{A}	1.76±0.41^{B}	0.015
Docosahexaenoic (C22:6 *n*-3)	0.05±0.02	0.002±0.01	0.349
Linoleic (C18:2 *n-6*)	8.68±2.62	8.69±1.23	0.949

a, *A*, *b*, *B* – means with different letters in the same row are significantly different at: small letters – $p<0.05$, capitals – $p<0.01$, CT – control treatment, BP – BioPlus YC treatment, SFA – saturated fatty acids, MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acids

a mixture of bacteria (*Streptococcus thermophilus* DSM 32245, *Bifidobacterium animalis ssp. lactis* DSM 32246 and DSM 32247, *Lactobacillus acidophilus* DSM 32241, *Lactobacillus helveticus* DSM 32242, *Lactobacillus paracasei* DSM 32243, *Lactobacillus plantarum* DSM 32244, *Lactobacillus brevis* DSM 27961) in comparison to the control group. Alike, (Joysowal et al. 2018) demonstrated that pork sourced from pigs (crossbred HD K-75 Landrace x x local pigs) supplemented with *Lactobacillus acidophilus* NCDC-15 and

Pediococcus acidilactici FT28 probiotics was characterized by an increased ($P \leq 0.05$) content of total protein and ash in comparison to the meat of pigs with the same genotype but not supplemented by probiotics. According to the authors, this resulted from an increased intake of feed by pigs from supplemented groups, its better use (Feed Conversion Ratio), better digestibility of nutrients, larger nitrogen retention and larger body mass increase, in comparison to the control group.

Fatty acid profile

The commensal bacteria in the gastrointestinal tract (GIT) in interaction with probiotic bacteria ferment carbohydrates, principally non-digestible carbohydrates (that are not used by the host), into CO_2, H_2 and CH_4 and short-chain fatty acids (SCFA), primarily acetic, propionic and butanoic (Patterson et al. 2014). Short-chain fatty acids have been pointed out as a link between the diet, gut microbiota, and host energy metabolism. Acetate enters the peripheral circulation to be metabolized by muscles and other tissues, while propionate is taken up by the liver. Propionate in the liver partakes in *gluconeogenesis*, and glucose thus created undergoes glycolysis into pyruvic acid, which after decarboxylation is transformed into Acetyl-CoA. This compound is included in the Krebs cycle (to create ATP), but can be also used in cholesterol biosynthesis, ketogenesis and in *de novo* synthesis of fatty acids (lipogenesis), which may undergo esterification into triacylglycerols, creating a pool of lipids in muscles or liver (LeBlanc et al. 2017).

Saturated fatty acids

It is well known that an increase in the consumption of SFA by humans is correlated to the risk of cardiovascular disease (CVD). The analysis of the fatty acids of intramuscular fat in the *LL* muscle (Table 5) showed that palmitic acid (C16:0) and stearic acid (C18:0) were the main triacylglycerols of SFA, and they did not show significant differences between the treatments. However, IMF of the pigs supplemented with Bio Plus YC, in comparison to the control group, was characterized by a significantly lower ($P \leq 0.05$) proportion of the C10:0. The higher content of capric acid in the *LL* muscle in the CT group may be beneficial for consumers, according to the Nurse's Health Study (NHS), and no significant increase in the coronary heart disease (CHD) risk was associated with the consumption of short- to medium-chain SFA (4:0 to 10:0) in a mixed diet (Hu et al. 1999). Yet, the lowered C10:0 in IMF of the *LL* muscle did not significantly affect changes in the total percentage of SFA (Table 6). Alike, Tufarelli et al. (2017) did not observe a significant difference in the SFA proportion in IMF of the *longissimus dorsi (LD)* of pigs [(Landrace × Yorkshire) × Talent] that were supplemented with SLAB51 probiotic. Likewise, Parra et al. (2010) did not establish a significant impact of supplementation Iberian pigs with Bioplus

Table 6

Lipid quality indices intramuscular fat *longissimus lumborum* (*LL*) muscle

Items	CT	BP	*P* value
Σ SFA	44.50±1.63	44.92±4.84	0.879
Σ MUFA	43.00±1.76	44.78±3.66	0.184
Σ PUFA *n*-3	2.41±0.35^{A}	1.88±0.38^{B}	0.001
Σ PUFA *n*-6	8.68±2.62	8.09±1.23	0.882
Σ PUFA	11.09±2.62	9.97±1.53	0.503
Σ UFA	54.09±3.06	54.76±4.04	0.704
Σ DFA (Σ UFA + C 18:0)	63.37±3.10	64.72±4.61	0.465
Σ OFA (Σ SFA – C 18:0)	35.25±1.82	34.96±5.48	0.702
Σ DFA/Σ OFA	1.80±0.15	1.90±0.37	0.500
Σ UFA/Σ SFA	1.22±0.09	1.24±0.19	0.816
Σ PUFA/Σ SFA	0.25±0.06	0.23±0.05	0.334
Σ MUFA /Σ SFA	0.97±0.07	1.00±0.16	0.457
P/S ratio	0.37±0.11	0.33±0.04	0.373
PUFA Σ *n*-6/Σ *n*-3 ratio	3.69±1.22	4.40±0.65	0.155
PUFA Σ *n*-3/Σ *n*-6 ratio	0.60±1.28	0.25±0.06	0.281
AI	0.70±0.04	0.70±0.01	0.387
TI	0.99±0.03^{B}	1.08±0.04^{A}	<0.001
SI	0.71±0.05	0.73±0.05	0.254
h/H ratio	0.52±0.11	0.48±0.04	0.294
PI index	12.50±2.93	11.10±1.54	0.378
NVI	2.14±0.08	2.18±0.10	0.380
DI (16)	13.4±1.0	13.0±0.8	0.287
DI (18)	80.93±1.44	80.54±1.66	0.545
TDI	1.28±0.04	1.28±0.05	0.957
EI	64.97±0.97	65.42±1.11	0.308

A, *B* – means with different letters in the same row are significantly different at $P<0.01$;
CT – Control treatment, BP – BioPlus YC treatment, SFA – saturated fatty acids, MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acid;
DFA – hypocholesterolemic fatty acids = (Σ UFA+C 18:0 *n*-6);
OFA – hypercholesterolemic fatty acids = (Σ SFA – C 18:0 *n*-6);
P/S – polyunsaturated/saturated fatty acids ratio = [(C 18:2 *n*-6 + C 18:3 *n*-3)/(C 12:0 + C 14:0 + C 16:0)];
AI – atherogenicity index = [12:0 + (4×C14:0)+C16:0+C18:0]/[Σ MUFA+Σ PUFA *n*-6+Σ PUFA *n*-3];
TI – thrombogenicity index = (C 14:0+C 16:0+C 18:0)/ (0.5×ΣMUFA) + (0.5×ΣPUFA *n*-6) + (3×ΣPUFA *n*-3) + (ΣPUFA *n*-3/ ΣPUFA *n*-6);
SI – staturation index = [C14:0 + C16:0 + C18:0]/[ΣMUFA cis + ΣPUFA];
h/H – hypocholesterolemic fatty acids/hypercholesterolemic fatty acids = [(C 18:1 cis *n*-9 + C 18:2 *n*-6 + C 18:3 *n*-6 + C 18:3 *n*-3 + C 20:3 *n*-6 + C 20:4 *n*-6 + C 20:5 *n*-3 + C 22:4 *n*-6 + C 22:5 *n*-3 + C 22:6 *n*-3)/ /(C14:0+C16:0)];
PI – peroxidisability index = (% monoenoic acid×0.025)+(% dienoic acid×1) + (% trienoic acid×2) + + (% tetraenoic acid×4) + (% pentaenoic acid×6) + (% hexaenoic acid×8);
NVI – nutritive value index = [(C 18:0+C 18:1 *n*-9)/ C 16:0];
DI(18): Δ^{9} – desaturase index = 100 [C18:1 *n*-9/(C18:1*n*-9 + C18:0)];
DI(16): Δ^{9} – desaturase index = 100 [C16:1 *n*-7/(C16:1 *n*-7+C16:0)];
TDI – total desaturation index = MUFA (C16:1,*n-7*+ C18:1,*n-7*+C18:1,*n-9*)/SFA (C14:0+C16:0+C18:0);
EI – elongation index = 100 [(C18:0+C18:*1n-9*)/(C16:0+C16:1+C18:0+C18:1*n-9*)].

2B probiotic (*Bacillus licheniformis* and *B. subtilis* mixture) on changes in the percentage of SFA in IMF of the *serratus ventralis (SV).* On the other hand, Ross et al. (2012) stated that the proportion of SFA in the *LD* muscle was significantly lower ($P \leq 0.05$) as a result of supplementation with *L. amylovorus* and *Enterococcus faecium* mixed culture (10^8 CFU mL^{-1}) probiotics in fattening pigs.

Monounsaturated fatty acids

In preventing human CVD, it is beneficial to consume food containing monounsaturated fatty acids (MUFA), which have a favourable effect on the blood lipid profile (Kien et al. 2014).

The main role of Δ^9desaturase (1 stearoylo-CoA, SCD1) in animal bodies is to limit the availability of palmitic acid (C16:0) by its conversion to oleopalmitic acid (C16:1 *n*-7), and thus to provide fluidity and permeability to the biological membrane. Another method to limit the presence of palmitic acid is to accelerate its elongation into stearic acid (C18:0) and its desaturation into oleic acid (C18:1 *n*-9). The C18:1 *n*-9, as the basic product of Δ^9desaturase, is also the main fatty acid in triacylglycerols of mammals, which is used for the synthesis of phospholipids and cholesterol esters (Costa et al. 2013).

In the present study, no significant impact of using BioPlus YC probiotic on the MUFA profile in IMF of the *LL* muscle was detected. This was a consequence of the similarity in the average amount of SFA and MUFA consumed in the feed by pigs in both groups (Table 5), and of the similar indicators of Δ^9desaturase DI (16) and DI (18) in the muscles of BP (Table 6) in comparison to the control group CT (13.4 vs. 13.0 and 80.9 vs. 80.5). Statistically, no significant differences have been found in the value of the total desaturation index (TDI) (Table 6), which is a product-to-precursor ratio used to indirectly calculate the activity of enzymes responsible for desaturation of SFA to MUFA.

Tufarelli et al. (2017) did not find significant differences in the MUFA proportion in IMF *LD* of pigs [(Landrace × Yorkshire) ×Talent] that were supplemented with SLAB51 probiotic, in comparison to the control group. On the other hand, Ross et al. (2012) indicated a significant ($P \leq 0.05$) increase of MUFA in IMF *LD* of pigs supplemented with *L. amylovorus* and *Enterococcus faecium* mixed culture (108 CFU mL^{-1}) in comparison to the control group. While, Chang et al. (2018) found a significant ($P \leq 0.05$) decline of MUFA in IMF*LL* of pigs (Landrace × Yorkshire × Duroc) that were supplemented with a probiotic containing *Lactobacillus plantarum* (2.2×108 CFU mL^{-1}).

Polyunsaturated fatty acids

The use of probiotics in pig significantly influenced ($P \leq 0.01$) the percentage of eicosatrienoic acid in IMF *LL* muscle. The α-linolenic acid (C18:3 *n*-3 ALA), present in small amounts in IMF *LL* of these animals (Table 5),

changed into eicosatrienoic acid under the influence of Δ^6-desaturase. This caused a significant ($P \leq 0.01$) decrease its proportion in the fatty acid profile compared to the control group CT (1.80 vs. 2.10%).The desaturation and elongation of C18:3 *n*-3 to long-chain polyunsaturated *n*-3 fatty acids such as eicosapentaenoic acid (C20:5 *n*-3 EPA) and docosahexaenoic acid (C22:6 *n*-3 DHA) may be severely inhibited by the presence of a high proportion of linoleic acid (C18:2 *n*-6 LA) in the diet. The C18:2 *n*-6 acid provided with the feed is necessary for the synthesis of arachidonic acid (C20:4 *n*-6 AA) in animal cells in the processes of desaturation and elongation by γ-linolenic acid (C18:3 *n*-6 GLA) and dihomo-γ-linolenic acid (C20:3 *n*-6 DGLA). In the BP group, the average daily consumption of feed (Table 3) was higher than in the CT group (2.54 vs. 2.44 kg d^{-1}), which caused the higher consumption of C18:2 *n*-6 (Table 4) (80.5 vs. 77.3 kg d^{-1}). Although the pigs from BP consumed more LA, no significantly increased synthesis of this acid was found in IMF *LL* in comparison to the CT group (0.12.vs. 0.22%). Moreover, no C20:4 *n*-6was found in IMF of the *LL* muscle of either group, which indicates low activity of Δ^6-desaturase and Δ^5-desaturase, which are responsible for the conversion of C18:2 *n*-6 by C18:3 *n*-6 into C20:4 *n*-6. This is beneficial, as C20:4 *n*-6 is a precursor of eicosanoids (with the use of cyclooxygenases and lipoxygenases), which has pro-inflammatory characteristics. A lack of significant differences in the values of the elongation index EI (Table 6) between the groups of the studied animals also confirms an insignificant difference in the proportion of PUFA in IMF *LL*. In nonruminants, the fatty acid elongases are rate-limiting enzymes controlling the synthesis of long-chain fatty acids (LCFA); e.g., 16-18 carbons (Green, Olson 2011), some of which are substrates for the biosynthesis of PUFA. Kazala et al. (1999) suggested that fatty acid elongation was unable to keep pace with the *de novo* production of C16:0 in animals that deposited greater amounts of intramuscular fat. Similarly, Parra et al. (2010) found no influence of feed supplementation with Bioplus 2B probiotic on the content of PUFA in IMF *SV* of Iberian pigs. However, Ross et al. (2012) confirmed ($P \leq 0.05$) an increase in C18:2 *n-6* and C18:3 *n-3* acid content in IMF *LD* of pigs supplemented with *L. amylovorus* and *Enterococcus faecium* mixed culture, in comparison with the control group. According to Tufarelli et al. (2017), an increased ($P \leq 0.05$) content of PUFA was found in IMF *LD* of pigs [(Landrace × Yorkshire) ×Talent] supplemented with SLAB51 probiotic in comparison with the control group. Also, Chang et al. (2018) indicated a higher ($P \leq 0.05$) content of PUFA in IMF *LD* of pigs (Landrace × Yorkshire × Duroc) supplemented with a probiotic that contained *Lactobacillus plantarum* (2.2×108 CFU mL^{-1}).

Lipid quality ratios

Considering possible influence of fatty acids consumed in human diet on the risk of cardiovascular and metabolic diseases and cancers, lipid quality indices were used to assess it.

The study did not show any significant impact of the BioPlus YC probiotic on ΣSFA, ΣMUFA, ΣPUFA, ΣUFA, ΣDFA, ΣOFA, ΣDFA/ΣOFA, ΣUFA/ΣSFA, ΣPUFA/ΣSFA and ΣMUFA/ΣSFA in IMF *LL* of pigs (Table 6). Similarly, Chang et al. (2018) did not indicate significant differences in the SFA and UFA proportion in IMF *LD* of pigs (Landrace × Yorkshire × Duroc) supplemented with a probiotic containing *Lactobacillus plantarum*. Conversely, Ross et al. (2012) demonstrated significantly ($P \leq 0.05$) lower content of SFA and higher of MUFA and PUFA in IMF *LD* of pigs supplemented with *L. amylovorus* and *Enterococcus faecium* probiotic mixed culture. They also found a significantly lower value of MUFA:PUFA indicators and higher values of MUFA:SFA.

Currently, PUFA acids from the groups *n*-6 and *n*-3 in animal diet compete for desaturation enzymes, which participate in the synthesis of their metabolites, although Δ^4 and Δ^6 desaturase use PUFA *n*-3 acids more often than PUFA *n*-6. In this study, the probiotic BioPlus YC did not significantly influence the PUFA *n-6* profile in IMF *LL* (Table 6), but it caused a significant ($P \leq 0.01$) decrease (at 0.5%) in the synthesis of acids from the family of PUFA *n*-3, in comparison to the CT group.

The ΣPUFA *n*-6/Σ PUFA*n*-3 ratio is the factor that shows whether fatty acids control the hypocholesterolemic index. The *n*-3 acids play a major role in regulating the thrombogenic index, while *n*-6 acids are dominant in the atherogenic ones. A healthy animal product can be characterized by low atherogenic and thrombogenic indices. Furthermore, animal products with low thrombogenicity decrease the threat of atrial fibrillation (Attia et al. 2017). The ΣPUFA *n-6*/Σ PUFA *n*-3 ratio in IMF *LL* for probiotic group was slightly higher than that recommended for human health (<4.0), although it did not significantly differ between the groups of the studied animals. According to Fernandes et al. (2014), the ΣPUFA *n*-6/ΣPUFA *n*-3 ratio ranged from 5.0 to 6.0 and was close to recommended, suggesting that these species could be categorized as beneficial to human health consumption. Similarly, Parra et al. (2010) did not indicate any impact that supplementation with Bioplus 2B probiotic would have on changes in Σ PUFA *n*-6/Σ PUFA *n*-3 ratio in IMF *SV* Iberian pigs.

In the diet used for ischemic heart disease (IHD) prevention, it is recommended that the indicator of ΣPUFA/Σ SFA is higher than 0.45 (Hoenselaar 2012). Therefore, foods with a lower value than that which is recommended have been considered undesirable in human diet because of their potential to increase cholesterol in blood. In our study, we have not found significant differences in the value of this indicator between the groups of pigs, although these values were lower than recommended. Also, Parra et al. (2010) did not find any impact of the supplementation with Bioplus 2B probiotic on changes in the values of Σ PUFA/Σ SFA in IMF *SV* of Iberian pigs. However, Ross et al. (2012) noted a significant increase in the value of this indicator in IMF *LD* of pigs supplemented with a *L. amylovorus* and *Enterococcus faecium* probiotic mixed culture.

There have been several reports on the effect of various P:S ratios of dietary fatty acids on lipid metabolism, which is associated with the serum HDL-C concentration, and its recommended value is >0.45. In both studied groups of pigs, the value of this indicator was lower than recommended and did not significantly differ between the groups of studies animals.

In terms of human health, Ulbricht and Southgate (1991) recommended that the atherogenic index (AI) lower than 0.5 represents the relationship between hypercholesterolemic (favouring the adhesion of lipids to cells of the immunological and circulatory system) and protective fatty acids (inhibiting the aggregation of plaque and diminishing the levels of esterified fatty acid, cholesterol and phospholipids, thereby preventing the appearance of micro- and macro- coronary diseases). In our research, the value of this index did not significantly differ between studied groups of pigs, but it was higher than recommended. Ross et al. (2012) indicated ($P \leq 0.05$) a lower value of AI in IMF *LD* of pigs as a result of supplementation with *L. amylovorus* and *Enterococcus faecium* mixed culture, in comparison to the control group (1.32 vs. 2.08), which they explained as a result of lowered SFA.

The thrombogenicity index was defined as the relationship between the pro-thrombogenic (saturated) and the anti-thrombogenic fatty acids (Ulbricht, Southgate 1991). The TI shows a tendency to forming clots in blood vessels. In terms of human health, it is recommended that the TI should be less than 1.0 (Fernandes et al. 2014). In our research, we discovered that lipids in muscles from the CT group pigs were characterized by a significantly ($P \leq 0.01$) lower value of the TI that those from the BP group (0.99 vs. 1.08), which is more beneficial for consumers.

The saturation index (SI) is a commonly used criterion to describe the dietetic value of fat. In our research, the value of the SI in IMF *LL* of the analysed animals was similar in both groups. Nutritionally, higher hypocholesterolemic fatty acids/hypercholesterolemic fatty (h/H) values are considered more beneficial for human health. However, in our study, the values of this indicator in IMF *LL* in both animal groups were similar.

Increasing PUFA in pig diets can shift the fat profile towards more favourable composition; the increased level of unsaturation, regardless of a dietary source, causes decreased oxidative stability in the pork products (Kouba et al. 2003). The peroxidisability index (PI) is used to assess the stability of PUFA included in food products, and to protect them from possible oxidation processes, but the higher PI value, the greater protective potential for coronary artery disease (Kang et al. 2005). However, in this study, supplementation of pig feeds with probiotics did not significantly influenced the value of the PI indicator in IMF *LL* of the analyzed animals.

In terms of the nutritive value of lipids of pig meat, the nutritive value index (NVI) was found to be similar in both groups. Thus, a significant impact of the probiotic supplementation used in pig fattening was not confirmed to have significantly improved the nutritive value of their meat.

CONCLUSION

1. This study shows that using BioPlus YC probiotic in feed supplementation of pigs did not cause significant changes in the basic chemical composition of muscles. However, the fatty acid profile of their *longissimus lumborum* muscle was significantly affected. Moreover, pigs which were fed diets containing the probiotic vs. the control group were characterized by a significantly lower value of: Σ PUFA *n*-3 and higher TI; thus, potentially it is not favourable for obtaining a balanced human nutrition.

2. These results suggest further research is needed to study differences in compounds affecting the lipid profile of pig meat. The varied results of studies into the effect of probiotics may result from such aspects as bacterial strains, level of supplementation, composition of diet, feeding management, feed form, or interaction with other dietary additives. To confirm these findings, more research should be conducted on pigs of different breeds and based on more samples.

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