DOI: 10.34101/ACTAAGRAR/1/8455

The effect of diet composition, a probiotic and a symbiotic treatment on the ileal microbiota composition of one-week-old broiler chickens

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SUMMARY

A healthy microbiota present in the small intestine contributes significantly to small intestinal function, including digestion, nutrient absorption and health. The current study investigated the effects of a prebiotic, a probiotic and a symbiotic supplementation on ileal microbiota composition of broilers at 7 days of age. In a total of 574 male Ross 308 day-old chickens were divided into four groups using six replicate pens and 24 chickens per pen. A maize-soybean based control diet (C), a control diet supplemented with probiotics (Broilact; Br), a control diet supplemented with symbiotic (inulin, yeast, Bacillus subtilis*; Sy) and a wheat based diet supplemented with wheat bran (W) were formulated. On day 7 of life, two chickens per pen were slaughtered and ileal chymus samples were collected. For microbiota analysis 16S rRNA (V3–V4 region) gene targeted Illumina MiSeq sequencing was used. Feeding all diets supplemented increased the diversity to varying degrees compared to the control (C) diet (p=0.006). As a conclusion, all supplementation substantially influenced ileal microbiota of broiler chickens at an early age. All these results could offer some information for the future study on the relationship between early intestinal microbiota and the compounds of the feed.*

Keywords: *broilers; ileal microbiota; prebiotic; probiotic; 16s rRNA sequencing*

INTRODUCTION

The intestinal flora of broilers plays an important role for growth performance and health. However, knowledge about the composition of the gut microflora and microbial ecology of the gastrointestinal tract is restricted (Bjerrum et al., 2006). New molecular studies using 16S ribosomal DNA for phylogenetic analysis have resulted more detailed insight into the composition of this ecosystem (Bjerrum et al., 2006; Amit-Romach et al., 2004). Several studies demonstrated that different intestinal regions have different communities of bacteria and the diversity of the microbiota increases with the age of chickens (Gong, 2008). A healthy microbiota what present in the small intestine contributes significantly to small intestinal function, including digestion and nutrient absorption, which is the most limiting factors of growth rate (Moran, 1982). However, in commercial poultry production, the development of intestinal microbiota in the chickens is often impeded by modern practices. This includes facility hygiene, routine medication, and artificial egg incubation, hatching and chicken rearing. Because of this chickens are susceptible enough to colonization by bacterial pathogens (Barrow, 1992). The use of antimicrobial growth promoters is under debate in many regions mondial. Several countries, for example in the EU have already banned these compounds in animal diets. The use of therapeutic is still in the broiler industry worldwide and although they are fundamental to maintain animal welfare and to treat chickens from diseases, these compounds are also often used in a prophylactic manner (Van Immerseel, 2017). In order to improve the immunity and feed utilisation efficiency of livestock and poultry, research into environmentally friendly feed additives has become one of the main challenges of animal production in the 21st century (Wang, 2017). It is well known that the composition and structure of the feed as well as the housing conditions influence the composition of the intestinal microbial community of chickens (Apajalathi et al., 2004; Engberg et al., 2004). Different studies trying to find alternatives to growth-promoting antibiotics suggest that the antibiotics can be replaced by probiotics, prebiotics, organic acids, or herbs. Of these animal and poultry feed additives, probiotics and prebiotics have drawn high attention (Zarei et al., 2017). It is generally accepted that shifts in the intestinal microbiota composition may be the result of dietary changes, such as the addition of cereal fibers which exert prebiotic-like effects (Torok, 2011). Non-starch polysaccharides (NSPs) of wheat are present in the bran and endosperm of the seed and are composed of arabinoxylans (AX), β-glucans, cellulose and arabinogalactan-peptides (Parsaie et al., 2007). Soluble arabinoxylans increase viscosity in the digestive tract and impairs the digestibility of nutrients and increase the water content of excreta. Supplementation of broiler diets with exogenous enzymes, like xylanase decrease the negative effects of soluble AX. The degradation of AX also results in xylan oligosaccharides (XOS) that can exert prebiotic effect. Thereby feeding NSP-ases can influences the microbiome of both the ileum and caecum (Bedford, 2018). The most commonly used probiotics in the poultry industry are Lactobacilli, Bifidobacteria, Bacillus and Saccharomycetes. The host can benefit from each ones based on different mechanisms (Liu et al., 2012). Accordingly, the use of multistrain probiotics is better than using monostrain probiotics, because those different strains of the genera show symbiosis, additive relationships towards each other which enhances growth and metabolic activity of host animal (Kazemi et al., 2019). Nurmi and Rantala (1973) demonstrated that oral administration of caecal

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bacterial flora from adult birds into newly hatched chickens increased the chickens resistance to salmonella infection. Their results were later confirmed in different laboratories. Great interest was paid to the phenomenon, now often referred to as competitive exclusion or the Nurmi concept (Pivnick and Nurmi, 1982). Synbiotics consist of a combination of synergistically interacting probiotics and prebiotics. These can be aimed at improving the resistance and stability of health-promoting organisms in the gut of birds by providing a substrate for fermentation (Sobolevska et al., 2017).

To grant the greatest protection of the immune system for newly hatched chickens, external supplementation with bioactive substances should occur as early as possible. Therefore, the aim of this study was to investigate the effects feeding maize or wheat based dies and the supplementation of maize based diets with a multistrain probiotic and a symbiotic on the composition of the ileal microbial community of broiler chickens at 7 days of age using next generation sequencing technology.

MATERIALS AND METHODS

Study designed

A total of 576 ROSS 308 one-day-old male broiler chickens were randomly devided into four groups of 24 chickens each in 4 replicate floor pens with chopped wheat straw bedding. Feed and water were available ad libitum. In the experiment, four dietary treatments were used as followes. A maize soybean based diet was used as a control (C). Beside the control diet, three different diets were fed: control diet with Broilact supplementation (Br), control diet supplemented with symbiotic feed additive (Sy) and a wheat-based diet containing 3% of wheat bran (W). The product Broilact® (Broilact, Europharmavet Kft. 1077 Budapest, Rózsa str. 10–12. Hungary) is an undefined but limited mixture of normal intestinal bacteria derived from a healthy adult hen and screened to ensure the absence of specific pathogens (Nurmi et al., 1987). This product was given the first two days to chickens via crop inoculation, two times at $1.25x10^7$ CFU/0.5 ml dosage. The symbiotic treatment was contained three product: *Bacillus subtilis*, DSM17299 bacterial strain (0.4 g kg^{-1}, $1.6x10^6$ CFU/g; Gallipro, Biochem GmbH, Küstermeyerstrasse 16. 49393 Lohne, Germany), inulin (5 g kg^{-1}, Oratfi HSI, Beneo GmbH, Aandorenstraat 1, B. 3300 Tienen Belgium) and yeast (Saccharomyces cerevisiae boulardii, 1x109 CFU/kg Levucell SB 20, Lallemand GmbH. Ottakringer Straße 89, A-1160 Wien, Austria). Diets were formulated to be isoenergetic and isonitrogenous, fitting to the requirements of Ross 308 broiler chickens (Aviagen, 2019). Each diet contained the NSP-degrading enzyme supplementation, Econase XT (AB Vista Ltd., Marlborough, Wiltshire, SN8 4AN). The composition and nutrient content of the experimental diets are shown in *Table 1–2*. Computer controlled housing and climatic conditions were maintained according to the breeder suggestions (Aviagen, 2019).

On day 7 of life, 2 chickens per pen were slaughtered and digesta samples were taken from ileum (about 3 cm proximal to Meckel's diverticulum). All samples were immediately snap-freezed and stored at -80 °C until further processing.

Table 1. **Composition of the diet (g kg^{-1} as fed)**

Ingredient (g kg^{-1})	C	Br	Sy	W
Maize	431	431	426	140
Wheat	0	0	0	300
Wheatbran	0	0	0	30
Extr. soybean meal	464	464	464	410
Sunflower oil	56	56	56	68
Limestone	18	18	18	18
MCP	16	16	16	16
NaCl	3	3	3	3
$NaHCO_3$	1	1	1	1
L-Lyzine (54%)	2	2	2	3
DL-Methionine	4	4	4	4
L-Threonine	0	0	0	1
L-Valine	0	0	0	1
Premix[1]	5	5	5	5
Fitase (Quantum Blue)	0.1	0.1	0.1	0.1
Xylanase (Econase XT)	0.1	0.1	0.1	0.1
Inulin	0	0	5.0	0
Yeast	0	0	0.05	0
B. subtilis	0	0	0.4	0
Total	**1000**	**1000**	**1000**	**1000**

[†]Premix was supplied by UBM Ltd. (Pilisvörösvár, Hungary). The active ingredients contained in the premix were as follows (per kg of diet): retinyl acetate – 5.0 mg, cholecalciferol – 130 µg, dl-alpha-tocopherol-acetate – 91 mg, menadione – 2.2 mg, thiamin – 4.5 mg, riboflavin – 10.5 mg, pyridoxin HCL – 7.5 mg, cyanocobalamin – 80 µg, niacin – 41.5 mg, pantothenic acid – 15 mg, folic acid – 1.3 mg, biotin – 150 µg, betaine – 670 mg, Ronozyme® NP – 150 mg, monensin-Na – 110 mg (only grower), narasin – 50 mg (only starter), nicarbazin – 50 mg (only starter), antioxidant – 25 mg, Zn (as $ZnSO_4 \cdot H_2O$) – 125 mg, Cu (as $CuSO_4 \cdot 5H_2O$) – 20 mg, Fe (as $FeSO_4 \cdot H_2O$) – 75 mg, Mn (as MnO) – 125 mg, I (as KI) – 1.35 mg, Se (as Na_2SeO_3) – 270 µg;

Table 2. **Analysed nutrient composition of experimental diets (%)**

Ingredient (%)	C	Br	Sy	W
crude protein	24.3	24.3	24.2	23.9
crude fat	7.2	7.2	7.5	8.3
crude fiber	3.8	3.8	3.9	4.2
ash	7.0	7.0	6.9	7.0
Ca	1.0	1.0	1.04	1.1
P	0.7	0.7	0.7	0.8
starch	31.4	31.4	31.3	30.8
ME (MJ kg^{-1})	12.1	12.1	12.1	12.3

Abbreviations: C – maize based diet; Br – Control group supplemented with Broilact ; Sy – Control group supplemented with *Bacillus subtilis*, inulin and yeast; W – wheat-based diet supplemented with 3% wheat bran; ME – metabolisable energy

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16s rRNA analysis

Bacteria were identified by the analysis of the V3–V4 region of the 16S rRNA gene using Illumina Miseq platform. Sequences were analyzed using Quantitative Insights Into Microbial Ecology 2 (QIIME2) version 2020.2 software package (Bolyen et al., 2019). Sequences were filtered based on quality scores and the presence of ambiguous base calls using the quality-filter q-score options. Representative sequences were found using a 16S reference as positive filter as implemented in the deblur denoise-16S method. Finally, sequences were classified by taxon in Operational Taxonomic Units (OTUs) using a fitted classifier base on SILVA 132 database (Quast et al., 2013).

Alpha diversity (e.g., Observed, Shannon and Simpson indices) for individual samples was estimated using MicrobiomeAnalyst (Chong et al., 2020). Samples analyzed with MicrobiomAnalyst were filtered for low abundance based on prevalence of OTUs, and for low variability using the inter-quantile range assessment. Alpha diversity analysis is used to study diversity within a specific environment or within a single sample. Bacterial alpha diversity in the ileal microbiota of broiler chickens was estimated by calculating the Identified OTU numbers, Chao's, Shannon's, and Simpson's indices of richness and diversity. The Chao1 was used to estimate species richness; Shannon's and Simpson's index were used to indicate species diversity. The Shannon index is more sensitive to rare species and the Simpson index to dominant species.

Beta diversity analysis is used to compare the differences of samples groups in terms of species diversity. It revealed that the microbial composition of the ileum in the control and treated group was different.

Statistical analysis

The significance of differences in alpha diversity among sample groups was tested using analysis of variance (ANOVA) with a Benjamini-Hochberg post hoc test and correction of p-values for multiple comparisons. Both platforms were also used to generate beta diversity indices and to visualize community (dis)similarities using Jensen-Shannon divergence with tests of significance using permuted analysis of variance (PERMANOVA). The mean relative abundances of microbial communities, richness, and evenness were analyzed by ANOVA and Benjamin-Hochberg FDR correction (q) using $q<0.05$ value.

RESULTS AND DISCUSSION

A total 478.619 quality controlled sequences were generated from each of the 24 samples. The read number were with an average of 20.608 reads per control (C), 22.152 reads per Br, of 20.555 reads per Sy and 16.452 reads per W treatment samples. The sequences were clustered into 1.558 operational taxonomic units (OTU) using 97% similarity. Results were assigned into 5 taxonomic levels (phylum, class, order, family and genus). A total of 9 phyla, 15 classes, 30 orders, 47 families, and 87 genera were found in the examined sample groups.

Alpha-diversity

The Shannon's and Simpson's indices apparently indicated that the three treatment contained more diverse bacterial communities compared to control group. The Chao's and Observes indices were higher at tendency level in the Br group than in the C group (*Table 3*).

Table 3. **Bacterial alpha diversity based on the main effects of diet and ileal site of broiler chickens at 7 days of age**

Treatment	Chao1 (average ± SD)	Shannon (average ± SD)	Simpson (average ± SD)	Identified OTU-s[5] (average ± SD)
C[1]	117.92±0.78[b]	1.94±0.50[a]	0.69±0.16[a]	113.67±32.37[a]
Br[2]	175.87±1.17[a]	2.96±0.25[b]	0.89±0.03[b]	174.83±32.04[b]
Sy[3]	122.48±0.80[b]	2.37±0.59[b]	0.80±0.14[b]	122.00±46.57[a]
W[4]	124.32±1.08[b]	2.70±0.43[b]	0.87±0.05[b]	123.50±48.75[a]
p-value	0.063	0.006	0.011	0.065

Abbreviations: [1]C – maize based diet; [2]Br – Control group supplemented with; [3]Sy – Control group supplemented with *Bacillus subtilis*, inulin and yeast; [4]W – wheat-based diet supplemented with 3% wheat bran; [5]OTU - opetational taxonomic units, Means in each row with no common superscript letter are significantly different ($p < 0.05$).

Beta diversity

The Principal coordinate analysis (PCoA) plots analyzed using Permanova for Jensen-Shannon divergence ß-diversity demonstrated significance difference (F-value: 3.4206, R-squared: 0.3391, p=0.001) in ileal samples among the four treatment. The diversity in the chickens of control group was lower than that in the the three other treatments (*Figure 1*).

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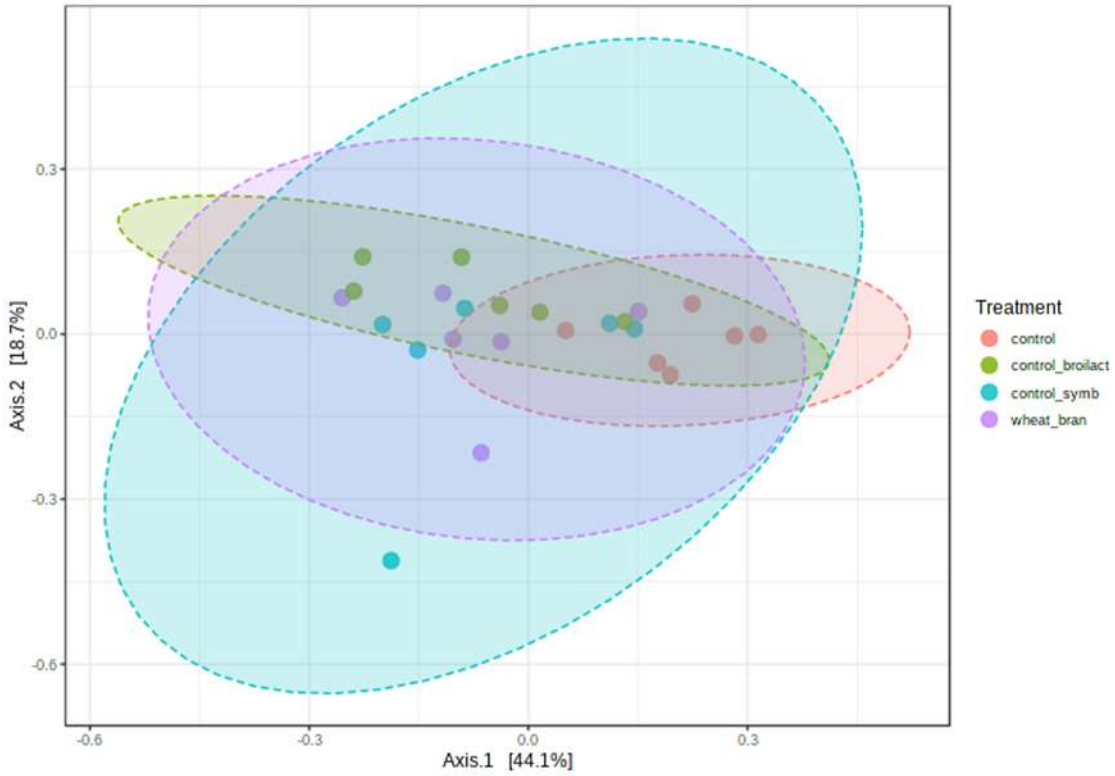
Figure 1. **Principal coordinates analysis (PCoA) of Jensen-Shannon divergence (P = 0.001) distance of ileal digesta bacterial community between the chickens that fed M, Br, Sy and W diets**



Abbreviations: control – maize based diet; control_broilact – Control group supplemented with Broilact (Europharmavet Kft. 1077 Budapest, Rózsa str. 10–12. Hungary); control_symb – Control group supplemented with *Bacillus subtilis*, inulin and yeast; wheat_bran – wheat-based diet supplemented with 3% wheat bran.

Several previous studies examined the composition of ileal microflora of broiler chickens. Xiao et al. (2017) reported that the microbiota of ileum showed the lowest diversity among the different gastrointestinal segments. This finding was in accordance with the other studies showing that the microbiota in caecum were more diverse than in ileum (Bjerrum et al., 2006; Dumoneeaux et al., 2006; Mohd Shaufi et al., 2015). Rodrigues (2020) found that the alpha-diversity measurements decreased in the ileum with increasing age. The microbiome was less diverse on the tenth day compared to the third day of life. To their knowledge, the drop in ileal diversity was potentially accounted for by the relative overabundance of few dominating species as as the age of the birds increased. In face of declining biodiversity, the host-microbiota may lose redundant species (Foster et al., 2017). This means that members of the community which are phylogenetically unrelated, but have similar functional niches can be substituted for one another with little impact on the ecosystem (Lozupone et al., 2012; Tian et al., 2017).

In our study throughout the four dietary groups, 9 bacterial phyla were identified with *Firmicutes*, *Proteobacteria* and *Cyanobacteria* being the most abundant ones. The listed three phyla represented more than 98% of the examined bacterial population. Bacterial composition at the phylum level did not differed ($q > 0.05$) among dietary treatments (*Figure 2*). Relative abundance of the *Planctomycetes* ($p=0.02$; $q=0.15$) and *Bacteroidetes* ($p=0.03$; $q=0.15$) phylum tended to be higher in the Sy and W group compared to the C group.

The phylum *Planctomycetes* is a group of budding, peptidoglycan-less bacteria of increasing significance for microbial evolution, ecology, cell biology and genomics. This phylum which represents a deep-branching group within the Bacteria on the basis of 16S rRNA sequence phylogenetics (Fuerst, 2004). These bacteria have been identified in diverse freshwater, marine and soil habitats and even invertebrate animals (Fuerst, 1995; Wang et al., 2002). The experimental data, however, remain scarce due to the low number of characterized representatives of this phylum, but some *Planctomycetes* may be involved in degrading polymeric organic matter. (Ivanova et al., 2017). The rate of the *Planctomycetes* phylum was higher in the Sy and W groups than the control group. These groups received prebiotic components to the feed. This could correlate to the polymeric degrading attitude of *Plactomycetes*.

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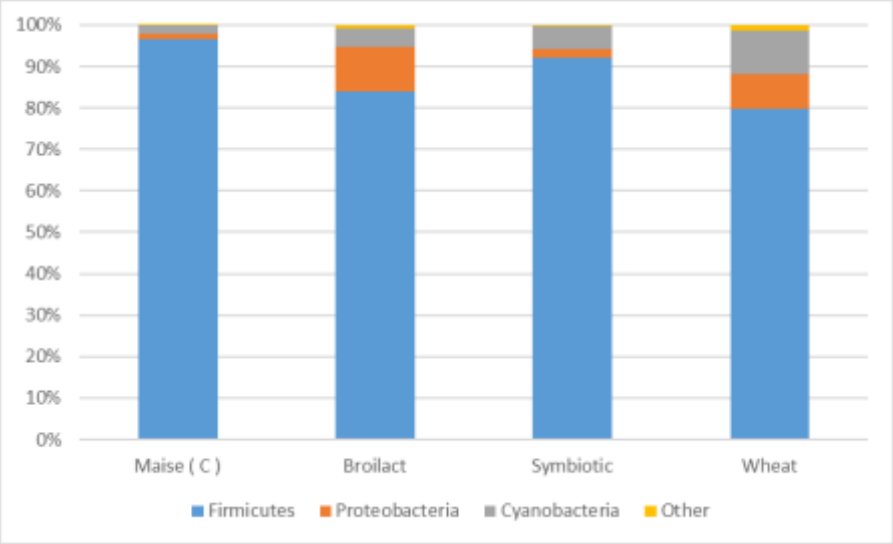
Figure 2. **Relative proportions (%) of the most abundant phyla in the ileum of broiler chickens (day 7 of life) fed different diets**



Abbreviations: Maize based diet; Control group supplemented with Broilact (Europharmavet Kft. 1077 Budapest, Rózsa str. 10–12. Hungary); Control group supplemented with *Bacillus subtilis*, inulin and yeast; Wheat-based diet supplemented with 3% wheat bran;

At the class level, the *Alphaproteobacteria* was higher (q=0.007) in W compared the C group and at the order level. Relative abundance of the *Rickettsiales* was significant higher (q=0.008) in W compared than that in the C group.

At the family level the *Lactobacillaceae* was the dominant in all treatment, but the relative abundance tended to decrease in the chickens fed the by the Br diet. (p=0.239 q=0.492). The *Mitochondria* (q=0.01) was significant higher in the W group, and the *Bacillaceae* was higher in the Sy group than in the control group (p=0.001; q=0.04). *Enterobacteriaceae* had a trend to be lower (p=0.05; q=0.48) in control comparising to the Br group (*Table 4*).

Table 4. **The relative abundance (>1%) of the 12 most abundant bacterial family in ileal content of broiler chickens at day 7 of life**

Treatment	C[1]	Br[2]	Sy[3]	W[4]	p-values	q-values
Lactobacillaceae	79.4%	49.8%	75.2%	67.6%	0.239	0.492
Lachnospiraceae	6.9%	7.5%	0.5%	1.3%	0.3812	0.551
Ruminococcaceae	4.8%	3.5%	1.3%	0.4%	0.616	0.628
Not_Assigned	2.0%	4.4%	5.5%	10.5%	0.064	0.480
Enterococcaceae	1.7%	18.2%	13.8%	7.3%	0.096	0.480
Streptococcaceae	1.7%	1.3%	0.2%	0.8%	0.378	0.551
Peptostreptococcaceae	1.1%	1.1%	0.0%	0.2%	0.411	0.551
Enterobacteriaceae	0.8%	9.5%	0.1%	1.2%	0.056	0.480
Clostridiaceae_1	0.6%	0.8%	0.7%	1.6%	0.121	0.480
Caulobacteraceae	0.3%	0.5%	0.9%	1.5%	0.154	0.480
Mitochondria	*0.1% b*	*0.3% ab*	*0.5% ab*	*4.5% a*	*0.0002*	*0.014*
Bacillaceae	*0.0% b*	*0.0% b*	*0.1% a*	*0.0% b*	*0.001*	*0.045*

Abbreviations: [1]C – maize based diet; [2]Br – Control group supplemented with Broilact; [3]Sy – Control group supplemented with *Bacillus subtilis*, inulin and yeast; [4]W – wheat-based diet supplemented with 3% wheat bran; [5]Benjamin-Hochberg FDR correction; Means in each row with no common superscript letter are significantly different (q < 0.05).

The *Lactobacillaceae* is a highly prevalent member of ileal microbiota during the poultry lifetime, producing short-chain fatty acids and/or lactic acid (Rivière et al., 2016). This contribute to the inhibition of many acid-sensitive bacteria, such as *Enterobacteriaceae*, by lowering the pH of the intestinal contents (Cisek and Binek, 2014). During the growth of the chicken takes about 2 week for *Lactobacilli* to become the predominant bacteria (Barnes et al., 1973). The predominance and endurance of most species of *Enterobacteriaceae* within the intestine is a standard marker of dysbiosis (Rivera-Chávez et al., 2017), even though it is well-known that this family is a pioneer colonizer in the gut (Donaldson

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et al., 2017). In addition, members of *Enterobacteriaceae* may also delay or block the growth of beneficial microbiota due to their utilization of important early ecological niches (Pedroso et al., 2016). In agreement of this study, Juricova et al. (2013) demonstrated that chicken infected with *Salmonella Enteritidis* at one and 4 days of age delayed the microbiota development. The reason is probably that the number of *Enterobacteriaceae* population increased and at the same time the *Clostridiales* and *Lactobacillales* relative abundances decreased. In previous studies the Broilact were able to inhibit *Campylobacter spp*. binding to chicken intestinal mucus in the exclusion assay (Ganan et al., 2012), and became established in the gut of the newly hatched chickens while the colonization of *Salmonella* was inhibited (Schneitz et al., 2016). In our experiment the members of *Enterobacteriaceae* may also delay the growth of beneficial microbiota in the Br group, because here was the highest rate of this family and the lowes rate of the family *Lactobacillaceae*.

The family *Mitochondria* is the member of the *Rickettsiales* order and the *Alphaproteobacteria* class. *Rickettsiae* are obligate intracellular endosymbionts. These are parasites of eukaryotic cells. (Emeyanov, 2001). The Mitochondria are important organelles in eukaroytic cells, not a bacterial family. The alphaproteobacterial origin of mitochondria is generally indisputable, but attempts to resolve the phylogenetic position of mitochondria in the alphaproteobacterial species tree have failed to reach consensus. Most studies support the idea that mitochondria evolved from an ancestor related to *Rickettsiales* (Fitzpatrick et al., 2006; Ferla et al., 2013). *Alphaproteobacterial* and mitochondrial sequences display a high degree of compositional heterogeneity, they are sensitive to the compositional bias artefact in which unrelated lineages with similar sequence compositions falsely group together (Martjin et al., 2018). The sequences of *Mitochondria* were analysed by the SILVA Sequence Database. According to the results, the sequences are same to plant sequences and the assigned *Rickettsiales* possibly represent mitochondria of wheat not bacterial sequences. This may skew the results.

At the genus level the most abundant bacteria was the *Lactobacillus* in all groups. This is consistent with the previous results showing that the Ileal digesta of broilers contains between 10^8 and 10^9 bacteria per gram (Gong et al., 2002; Apajalahti et al., 2004) with *Lactobacillus spp*. (70%), *Clostridiaceae* (11%), *Streptococcus spp*. (6.5%), and *Enterococcus spp.* (6.5%) being most abundant (Lu et al., 2003).

There was no significant differences between the four treatment but several trend differences were found, especially for the Br treatment. The *Sellimonas* (p=0.001; q=0.1), *ASF356* (p=0.009; q=0.3), *Ruminococcus_torques_group* (p=0.01; q=0.4), *Merdibacter* (p=0.04; q=0.5), *Ruminococcaceae_UCG_013* (p=0.04; q=0.5), *Romboutia* (p=0.04; q=0.5), and *Rothia* (p=0.04; q=0.5) genera were present with a relatively higher frequency in the Br than in the C group. The abundance of *Tyzzerella* was (p=0.02; q=0.5) higher in the C than in the other three group. The *Bacillus* genus (p=0.001; q=0.1) was higher in the Sy than the control group.

In a study, Farkas et al. (2020) identified the composition of the Broilact product also with 16s rRNA analysis. According to that results, the dominant phyla were the *Firmicutes, Proteobacteria* and *the Bacteroidetes* (43.5%, 43.7% 12.6%, respectively). Though most genera showing changes belonged to the *Clostridia* phyla.

Some previous study reported that the B. subtilis modulates the intestinal microflora (La Ragione and Woodward, 2003) and selectively supports the growth of lactic acid producing bacteria (Knarreborg et al., 2008). The symbiotic treatment in this study contained *Bacillus subtilis, DSM17299* bacterial strain. This can be explanation that the *Bacillus* genus abundance was relative higher in this treatment group than in the other three groups.

CONCLUSIONS

This study examined the impact of early intestinal colonisation by a prebiotic, a probiotic and symbiotic treatment on the development of ileal microbiota. An increase in the rate of *Bacillus genus* in the small intestine has been observed with Symbiotic treatment. Broilact tended to decrease lactobacilli and increased the proportion of the *Enterobacteriaceae family*. *Enterobacteriaceae* strains, as pioneer colonizers, delayed the microbial consortium establishment in the ileum of young broilers. The treatment in W and Sy groups resulted higher rate of the *Planctomycetes* phylum. Finally, an essential contribution of this study is that all of the three treatments increased the diversity compared to the control treatment, but the Broilact was resulted the highest difference. In future researches, it would be worth to study whether how this significant diversity changes with the age of broilers. All these results could offer some information for the future study on the relationship between early intestinal microbiota and the compounds of the feed.

ACKNOWLEDGEMENTS

This work was supported by the Hungarian Government and the European Union, with the co-funding of the European Regional Development Fund in the frame of Széchenyi 2020 Programme GINOP-2.3.2-15-2016-00054 project and by the EFOP-3.6.3-VEKOP-16- 2017-00008 project. The project is co-financed by the European Union and the European Social Fund.

DOI: 10.34101/ACTAAGRAR/1/8455

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