THESIS OF DOCTORAL
(PhD) DISSERTATION

NIKOLETTA AMANDA SUCH

GEORGIKON CAMPUS

KESZTHELY

2022

HUNGARIAN UNIVERSITY OF AGRICULTURAL AND LIFE SCIENCES

GEORGIKON CAMPUS

COMPLEX EVALUATION OF PRE- AND PROBIOTIC FEED ADDITIVES IN BROILER CHICKENS

DOI: 10.54598/002960

Nikoletta Amanda Such

Keszthely

2022

Name of the doctoral school: Festetics Doctoral School

Study field: Animal Husbandry

Head of the doctoral school: Dr. Anda Angéla DSc, University professor, doctor of the Hungarian Academy of Sciences
MATE, Georgikon Campus, Institute of Crop Sciences, Department of Agronomy

Doctoral supervisor: Dr. Dublecz Károly CSc, University professor
MATE, Georgikon Campus, Institute of Animal Physiology and Animal Nutrition, Department of Animal Nutrition and Nutritional Physiology

The candidate has fulfilled all the requirements of the Doctoral Regulations of the Hungarian University of Agricultural and Life Sciences and has taken into account the comments and suggestions made in the workshop discussion of the thesis when revising it, therefore the thesis may be submitted for the defence procedure.

…… ……

Approval by the Head of the Doctoral School

Approval of the Supervisor

1. Background and objectives

The gastrointestinal tract (GIT) of poultry is colonized by a diverse group of bacterial, fungal, and protozoan species, including more than 900 bacterial species in the GIT of broilers (Apajalathi, 2004). The host maintains a synbiotic relationship with its microbial inhabitants, in which the microbes play several beneficial roles. The gut microbiota provides protection against pathogenic bacteria involved in the digestion and utilization of nutrients and helps in the normal development of gut morphology (Thompson, 2013; Oakley, 2014). In order to support the establishment and maintenance of balanced gut microbiota, lots of feed additives, such as organic acids, probiotics, or prebiotics can be used (Popova, 2017; Al-Khalaifah, 2018). In the case of poultry, the support of the intestinal flora deserves special attention, since the chicks that hatch from the eggs in the hatchery have no contact with the laying hens, who could give them the appropriate intestinal flora (Kers, 2018). Therefore, it is important what kind of microflora the young birds come into contact with in the first days of their lives. Among the effects of animal production on the environment, one of the most important areas is ammonia emission. Relatively little information is available on whether the use of pre- and probiotics affects the urease activity of the faecal microflora, thereby the amount of N-containing substances that evaporate from the litter.

Based on the above, the objectives of the thesis can be summarised as follows:

How certain pre- and probiotics alone, in combination, and when fed in different ways influence the production parameters of broiler chickens, the

composition of the intestinal microbiota, the reactions of the chickens' immune response and the ammonia emission from the excreta.

Furthermore, our goal was to establish whether two probiotic bacteria species producing different metabolic products (*Lactobacillus farciminis, Clostridium butyricum*) alone or in combination with wheat bran supplementation. Beside the production parameters, the small intestine enzyme activity, the caecal short chain fatty acid concentration, the morphological parameters of the ileum and cecum, as well as the composition of the caecal microbiota were investigated. Furthermore, the dry matter content and the ratio of the different nitrogen forms of excreta, and the effect of the treatments on ammonia emission was also examined.

After that, we were interested in whether the maruration of bacterial flora in the intestinal tract of chickens can be influenced by different nutritional treatments. This time a multi-strain bacterial culture was given to one and two day old chickens. In addition, a symbiotic treatment containing *Bacillus subtilis, Saccharomices cerevisaee* and inulin was used through the whole fattening. Furthermore, beside a traditional corn-based diet, wheat and wheat bran containing diets were fed in order to investigate the effect of soluble arabinoxylans. During the experiment our goal was to study the production parameters and the change in microbiota composition in the intestinal content of the caecum, intestinal content of the ileum, and ileal epithelium with the age of the chickens. We also aimed to extend the examination of the treatments on the immune response against Gumboro disease vaccination. Similarly to the first experiment excreta analysis was performed also in this trial.

2. Materials and methods

2.1. First trial

In the first trial two single probiotics, *Lactobacillus farciminis, Clostridium butyricum* and the effects of feeding wheat bran were studied alone and in their combinations. In this experiment production traits, gut morphology, jejunal pancreatic enzyme activity, the microbiota composition of the caecum and some characteristics of the excreta were determined.

2.1.1. Animals and treatments

All husbandry and euthanasia procedures were performed in accordance with the Hungarian Government Decree 40/2013 and in full consideration of animal welfare ethics. The animal experiment was approved by the Institutional Ethics Committee (Animal Welfare Committee, Georgikon Campus, Hungarian University of Agriculture and Life Science) under the license number MÁB-5/2018. A total of 574 Ross 308 broiler hybrids were used in the experiment. Day-old cockerels were purchased from a commercial hatchery and sorted randomly into 6 dietary treatment groups. Chickens were arranged in 4 replicate pens with 24 chicken per pen. Dietary treatment groups included: control group (K), *Clostridium butyricum* (K+VB) supplemented group, *Lactobacillus farciminis* (K+TB) supplemented group, wheat bran supplemented group (BK), wheat bran + *Clostridium butyricum* (BK+VB) supplemented group and wheat bran + *Lactobacillus farciminis* supplemented group (BK+TB). The control diet was based on corn and soybean. The chickens received starter (day 1–10), grower (day 11–24), and finisher (day 25–40) diets. Feed and water were provided ad libitum. The experimental diets were formulated according to the recommendations for the Ross 308 hybrids. The probiotic

supplementation consisted of spores of a single strain butyric acid-producing bacteria, *C. butyricum* CBM 588 (Miya-Gold®, Huvepharma, Sofia, Bulgaria; $2{,}5x10^9$ CFU/kg) or lactic acid-producin bacteria, *L. farciminis* CNMA67-4R, (Biacton, $5x10^9$ CFU/kg). The wheat bran supplemented diets contained 3, 6 and 6% wheat bran in the starter, grower and finisher diets, respectively. Chickens were kept on chopped straw bedding in floor pens at a stocking density of 10 chickens/m^2, which was in accordance with the European Union Council Directive 2007/43/CE. The computer-controlled environmental conditions matched the breeder's recommendations.

2.1.2. Sampling

Growth rate and feed intake of birds was measured and feed conversion calculated on pen basis for each feeding phase and the whole fattening period. On day 40, 2 chickens were randomly selected from each pen (8 per dietary treatment) and euthanized by bleeding out the jugular vein under carbon dioxide anaesthesia. Immediately after the opening of the abdominal cavity, tissue and chymus samples were taken from the cecum. Fresh chymus samples were used for the determination of pH values. Chymus samples collected from the cecum were stored at -20 C° for bacterial cultivation and at -80 C° in a deep freezer until laboratory analyses of SCFA and next generation sequencing. Tissue samples for the histomorphology analyses were fixed and stored in 5% phosphate-buffered formalin. At day 40, eight chicks, with similar body weight, were transferred from each pen to individual balance cages, and representative excreta samples were collected, after 3 days adaptation period, on day 43. In this phase, birds were fed the same finisher diets as before, in pens. A

minimum of 200 g excreta was collected from each animal and stored in a refrigerator at -20 °C. Before the analyses, excreta were homogenized properly, then the pH, dry matter content, total N, ammonium-N (NH_4^+-N) and uric acid-N contents determined.

2.1.3. DNA extraction, PCR amplification of the 16S rRNA genes, and Illumina MiSeq sequencing

Bacterial DNA sequence analysis were completed only for 4 treatment groups (K, K+VB, BK, BK+VB). Bacterial DNA was amplified with tagged primers covering the V3–V4 region of the bacterial 16S rRNA gene. Polymerase chain reactions (PCRs) and DNA purifications were performed according to the Illumina Demonstrated Protocol. Equimolar concentrations of libraries were pooled and sequenced on an Illumina MiSeq platform using the MiSeq Reagent Kit v3 (600-cycle, Illumina Inc., San Diego, CA, USA) 300 bp read length paired-end protocol. Sequences were analyzed by Quantitative Insights Into Microbial Ecology (QIIME 2, version 2020.2.) software. Operational taxonomic units (OTUs) were clustered by an open-reference OTU picking strategy based on 97% similarity level. Greengenes Database (version 13.8) and UCLUST algorithm were applied for OTU clustering. Taxonomic identification was assigned by RDP naïve Bayesian classifier with a confidence threshold of 0.8.

2.1.4. Chemical analyses

Fresh caecal contents were diluted with distilled water (1:5) immediately after collection and shaken manually for 1 min. pH measurements were carried out with a SNEX electrode (pH200A Portable pH meter equipped with CS1068 SNEX pH Sensor (CLEAN Instruments, Shanghai, China).

Gas chromatography (TRACE 2000, Thermo Scientific, Waltham, MA, USA) method was applied for SCFA analysis. Standard mixtures of SCFAs (1, 4, 8 and 20 mM), consisting of acetate, propionate, n-butyrate and n-valerate as external standards, were used for calibration.

2.1.5. Histomorphological analysis

Tissue samples were taken from the left caecum close to the apex. Samples were fixed in 5% phosphate-buffered formalin. Processing consisted of serial dehydration, clearing and wax impregnation. Tissue sections were cut in 5 μm thicknesses (3 cross sections) from each of the 8 chickens per treatment. The sections were cut by a microtome and fixed on slides. A routine staining procedure was carried out with hematoxylin and eosin. The slides were examined under a Leica DMi8 Microscope (Leica Microsystems CMS GmbH,Wetzlar Germany) fitted with a digital video camera. Images were analyzed with ImageJ software (version 1.47) developed by the National Institutes of Health (Bethesda, MD, USA). A total of 10 intact, well-oriented villus-crypt units were selected in triplicate from each intestinal cross section.

2.1.6. Excreta analysis

The total N was determined according to the Kjeldahl method by a Foss-Kjeltec 8400 Analyzer Unit, the ammonium-N by the method of Peters (2003), and the uric acid-N as described by Marquardt (1983). The sum of ammonium-N and uric acid-N was considered as urinary N content. The in vitro ammonia emission measurement was carried out at two time points with Draeger equipment (model X-am 5600; Drägerwerk AG & Co. KGaA, Lübeck, Germany).

2.1.7. Feed Analyses

Experimental diets were analyzed for dry matter (ISO 6496), crude protein (ISO 5983-1:2005), crude fat (ISO 6492), crude fiber (ISO 6865:2001), total P (ISO 6491:2001) and Ca (ISO 6869:2001) content. A polarimetric method was used for starch content measurement in line with the European Directive 152/2009.

2.1.8. Statistical Analyses

Growth characteristics, SCFA, pH, histomorphology and extreta data were analysed with two-way ANOVA using SPSS 24.0 software. Differences were considered significant at a level of $p < 0.05$, and trends were observed for p 0.1 - 0.05. The results of the 16S rRNA analysis were evaluated and visualized with Microbiome Analyst, filtered for low abundance sequences (<4) based on the mean abundance of OTUs, and for low variability (<10%) using interquartile range assessment. After being filtered, OTU abundances were transformed by relative log expression. The false discovery rate (FDR) was calculated using the Benjamini and Hochberg method, and q-values less than 0.05 were considered statistically significant. Abundances of microbial taxa were expressed as percentages of total 16S rRNA gene sequences.

2.2. Second trial

In the second trial beside a corn-soybean based control diet the effects of a bacterial culture, isolated from the caeca of healthy laying hens (Broilact), a symbiotic treatment (*Bacillus subtilis, Saccharomyces cerevisiae*, inulin) and wheat based and wheat bran supplemented diets were used. Broilact

only at day 1 and 2, while the other treatments were carried out throughout the whole production cycle. In the second experiment beside the production traits, the development of the gut microflora with the age of chickens was evaluated from the samples of ileal digesta, ileal mucosa and caecal contents.

2.2.1. Animals and treatments

The animal experiment was approved by the Institutional Ethics Committee (Animal Welfare Committee, Georgikon Campus, Hungarian University of Agriculture and Life Science) under the license number MÁB-9/2019. A total of 574 Ross 308 broiler hybrids were purchased from a commercial hatchery (Gallus Ltd. Devecser, Hungary) and placed into 24 floor pens, 20 chickens per pen (10 chickens per m2). Each treatment was replicated 6 times. A maize–soybean-based basal diet was fed without feed additive in the control group (C). Birds of the second treatment (Br) were fed the control diet, and the solution of the product Broilact® was given to the birds via crop inoculation in two equal doses (1.25×10^7 CFU/0.5 mL) at day 0 and 1. All the chickens of the two other treatment groups were inoculated with drinking water. The product Broilact® (Europharmavet Ltd., H-1077 Budapest, Rózsa str. 10–12., Hungary) is a refined gut microbiota derived from healthy adult hens and was screened to ensure the absence of specific pathogens. The basal diet was supplemented with a synbiotic additive mixture in the third treatment group (Sy) and fed throughout the whole trial. The synbiotic additive mixture contained three products: GalliPro®200, at a dose of 0.4 g/kg diet (*Bacillus subtilis*, DSM17299 bacterial strain; 1.6×10^6 CFU/g, Biochem Ltd., Küstermeyerstrasse 16. 49393 Lohne, Germany); Orafti® HSI containing

inulin, at a dose of 5 g/kg diet (Beneo Ltd., Aandorenstraat 1, B. 3300 Tienen, Belgium); and a yeast-product, Levucell® SB20, at a dose of 0.05 g/kg diet, providing 1×10^9 CFU viable yeast cells per kg of diet (*Saccharomyces cerevisiae boulardii*, 2×10^{10} CFU/g, Lallemand Ltd., Ottakringer Str. 89, A-1160 Vienna, Austria). In the case of the fourth treatment a wheat-based diet was fed (W), which contained 30% wheat and 3%, 6% and 6% wheat bran in the starter, grower and finisher phases respectively. The keeping of the animals was the same as described in the previous experiment.

2.2.2. Sampling

The investigation of the performance parameters was the same as described in the previous experiment. On days 7, 14, 21, and 40, 2 chickens per pen, 12 birds per treatment, were selected randomly, slaughtered, and blood, mucosa and digesta samples collected. Blood samples were centrifuged at 5000x g for 10 min at 10 °C, and the serum was separated and stored at 5 °C until analysis. The collected serum samples were analysed for Gumboro antibody titres using an IBD specific ELISA kit (ID VET, ID Screen, IBD indirect test), which measures IgG and IgM antibodies. Ileal chymus (IC) and ileal mucosa (IM) samples were taken from a 10 cm long ileal segment, starting 3 cm after the Meckel's diverticulum. Caecum chymus (CC) samples were collected from the left sac. Ileal and caecal contents were pushed out gently without damaging the gut structure. The luminal contents were homogenized with sterile cell spreaders and about 2 g samples were taken into a sterile container. After the gut content collection, the ileal part was washed with sterile, ice-cold phosphate buffer solution (PBS) until the mucosa was completely cleaned from the digesta. Mucosa samples were

collected aseptically by scraping off the mucosa from the internal wall of the ileal part with a glass slide. The samples were homogenized as described earlier at the ileal sampling. All samples were immediately snap-frozen in liquid nitrogen and stored at -80 °C until analysis. Before DNA extraction, the samples of two birds of the same pen were pooled. Thus, the microbiota analysis of each gut segments was carried out in 6 replicates. The actual microbial composition of Broilact was also determined in three replicates. On day 40 about 200 g fresh excreta samples were collected from each pen on nylon foils. Samples were mixed thoroughly, frozen, and stored at -20 °C until further processing.

2.2.3. Analysis

The analysis of the microbiota composition and excreta parameters was the same as described in the first experiment.

2.2.4. Statistical and bioinformatical analysis

The production traits, Gumboro titers and excreta parameters were evaluated with two-way analysis of variance (ANOVA, $p \leq 0.05$). The analysis of the bioinformatic parameters were the same as described in the previous experiment. Sequences were clustered into Operational Taxonomic Units (OTUs) using vsearch algorithm open-reference clustering, based on a 97% similarity to the SILVA reference database. Alpha diversity metrics (Chao1, Shannon, and Simpson) and beta diversity metrics (Bray–Curtis dissimilarity) were estimated using QIIME2-diversity and Calypso (San Francisco, CA, USA) online software (Version 8.84). Statistical analysis was performed with SPSS statistical software version 23.0 (IBM Corp. Released 2015) and Calypso. Alpha diversity indices and microbial composition at different taxonomical levels and in

different intestinal samples (IC, IM and CC) were compared using two-way ANOVA test with Tukey's HSD multiple group comparison's post hoc test, using the dietary treatments (C, Br and Sy) and the age of birds (7, 14, 21, and 40 day) as main factors. Dietary treatment effects at each sampling time were also evaluated by one-way ANOVA. Benjamini–Hochberg false discovery rate (BH-FDR) correction (FDR p-value) was used to adjust for multiple testing.

3. Results and discussions

None of the treatments affected the production traits in the first experiment. On the other hand, wheat bran modified gut morphology, increased both in the ileum and caecum the crypt depths and the muscle layer thickness. None of the probiotic feed additives, wheat bran or their combination had significant effect on the trypsin, lipase, and amylase activity of the jejunal chyme. Similarly, treatments failed to modify the SCFA composition and pH of the caecal contents. The *Lactobacillus* counts of ileum and caecum and the coliform bacteria content of the caeca was not changed.

Table 1. The relative abundance of microbiota at genus level in the caecal chymus as affected by dietary treatments.

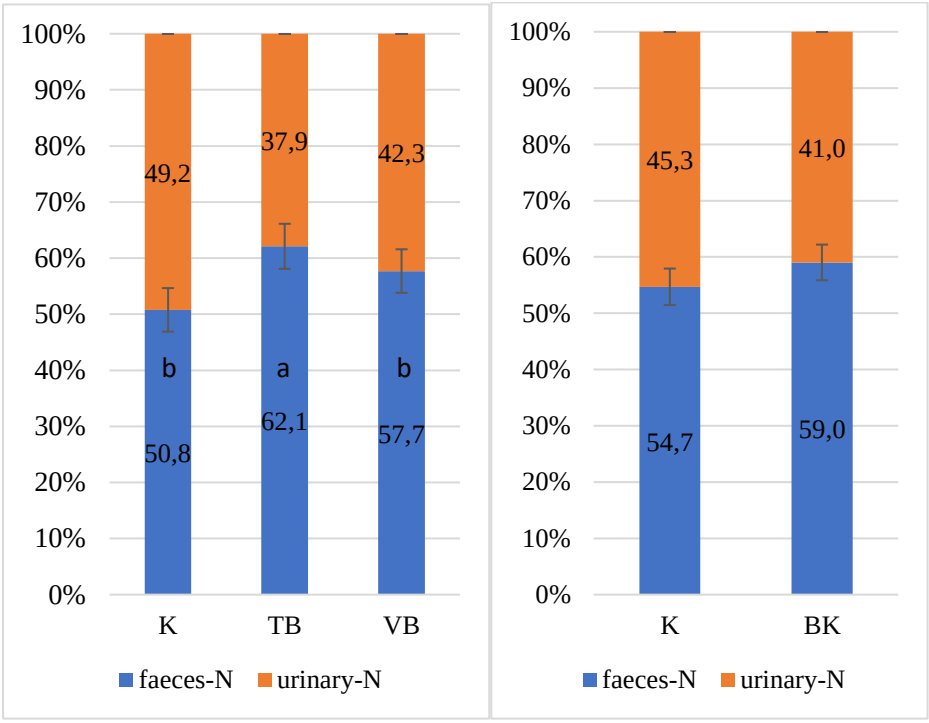
	K	**K+VB**	**BK**	**BK+VB**	**SEM**	**p-value**	**q-value**
Bacteroides	54,1	48,1	46,8	48,9	2,37	0,865	0,956
Oscillospira	2,57	1,91	1,43	2,12	0,201	0,521	0,956
Akkermansia	*2,17^{a,b}*	*0,02^{c}*	*7,77^{a}*	*1,17^{b}*	*1,071*	*<0,001*	***0,004***
Faecalibacterium	2,09	1,88	0,77	1,72	0,25	0,936	0,956
Ruminococcus	1,83	1,5	1,33	1,78	0,143	0,667	0,956
Streptococcus	1,24	1,37	0,9	0,91	0,181	0,813	0,956
Lactobacillus	0,55	0,89	0,94	0,42	0,142	0,469	0,956
Dehalobacterium	0,52	0,71	0,77	0,51	0,1	0,606	0,956
Anaeroplasma	0,11	0,6	0,24	0,31	0,086	0,016	0,106
Clostridium	0,2	0,17	0,31	0,32	0,04	0,895	0,956
Coprococcus	0,24	0,16	0,22	0,22	0,022	0,43	0,956
Butyricicoccus	0,31	0,16	0,18	0,19	0,027	0,133	0,663
Turicibacter	0,27	0,23	0,14	0,17	0,028	0,856	0,956
Anaerotruncus	0,41	0,06	0,2	0,09	0,065	0,007	0,072
Blautia	0,2	0,19	0,12	0,15	0,019	0,956	0,956

a, b: values within the mean (Age) rows with different lowercase letters were significantly different ($p < 0.05$). Control – commercial maize-based diet; K+VB – Control group supplemented with $2.5x10^9$ colony forming units/kg *Clostridium butyricum* spores; BK – Control group supplemented with 3,6 and 6% of wheat bran; BK+VB – Control group supplements with wheat bran and Clostridium butyricum spores; SEM – standard error of the mean. *q-value is the corrected p-values with the false detection ratio (FDR) with Benjamini-Hochberg method.*

On the other hand, using the new generation 16S rRNA sequencing technique we could detect probably first time that in the caeca of broiler chickens, wheat bran can increase the abundance of genera *Akkermansia*.

Both probiotic bacteria treatments increased the dry matter content and the ratio of faecal N of excreta, while wheat bran significantly decreased the amount of NH_4^+-N. Besides that, wheat bran supplementation of diets increased the number of ureolytic bacteria, and this was increased the speed of ammonia emission from the manure.

Figure 1. Treatment effects on the ratio of the faecal and urinary N.



a,b means with different superscripts are significantly different. K – commercial maize-based diet; TB –*Lactobacillus farciminis* spores supplementation VB – *Clostridium butyricum* spores supplementation; BK – Control group supplemented with 3,6 and 6% of wheat bran;

In the second experiment the wheat-based diet with wheat bran supplementation improved the production treats of the chickens compared

with the control, Broilact and symbiotic treatments. There was no difference in the feed consumption, but the results of the wheat-based diets were more favourable in terms of body weight gain and feed conversion ratio.

Table 2. Effect of dietary treatments on the body weight and daily weight gain of broiler chickens.

Treatment	Body weight				Daily weight gain			
	day 0	**day 7**	**day 21**	**day 40**	**Starter**	**Grower**	**Finisher**	**Total**
g/bird								
K	43	260[b]	1057[b]	2397[b]	217[b]	797	1340[b]	2354[b]
B	43	283[a]	1126[a]	2553[a]	239[a]	843	1427[a]	2509[a]
Br	43	256[b]	1065[b]	2398[b]	213[b]	808	1333[b]	2355[b]
Sz	43	260[b]	1073[b]	2441[b]	217[b]	812	1369[b]	2398[b]
SEM	0.07	2.78	9.58	18.57	2.76	7.83	12.69	18.57
p-values	0.711	***0.000***	***0.035***	***0.002***	***0.000***	0.196	***0.025***	***0.002***

(K) control treatment, (Br) Broilact, (B)-wheat based treatment, (Sz)-symbiotic treatment; a,b means with different superscripts are significantly different.

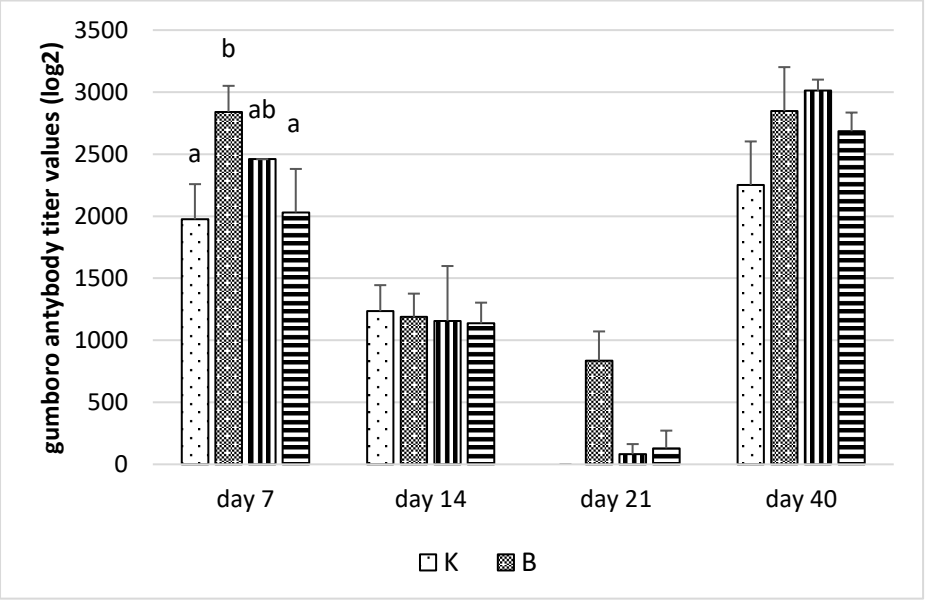
Table 3. Effect of dietary treatments on the feed intake and feed conversion ratio of broiler chickens.

Treatment	Feed intake				Feed conversion ratio			
	Starter	**Grower**	**Finisher**	**Total**	**Starter**	**Grower**	**Finisher**	**Total**
g/bird						kg/kg		
K	302	1420	2299	4020	1.30[a]	1.58	1.64[a]	1.59[a]
B	298	1452	2164	3913	1.18[b]	1.52	1.42[b]	1.43[b]
Br	304	1451	2284	4038	1.34[a]	1.59	1.61[a]	1.58[a]
Sz	290	1443	2293	4026	1.26[a]	1.57	1.59[a]	1.55[a]
SEM	2.30	10.41	38.25	42.51	0.01	0.009	0.03	0.10
p-values	0.16	0.702	0.574	0.734	***0.003***	0.090	***0.058***	***0.026***

(K) control treatment, (Br) Broilact, (B)-wheat based treatment, (Sz)-symbiotic treatment; a,b means with different superscripts are significantly different.

All pre- and probiotic treatments improved the seroconversion and the CV% of blood Gumboro titers compared with the control group. The maternal antibody titres of the wheat-based diet group at day 7 were significantly higher than those of the control group. The highest humoral antibody titres at day 40 were measured in the Broilact group, although these differences were not significant.

Figure 2. Infectious bursal disease (IBD) virus antibody titres in the different treatment groups (mean ± SD).

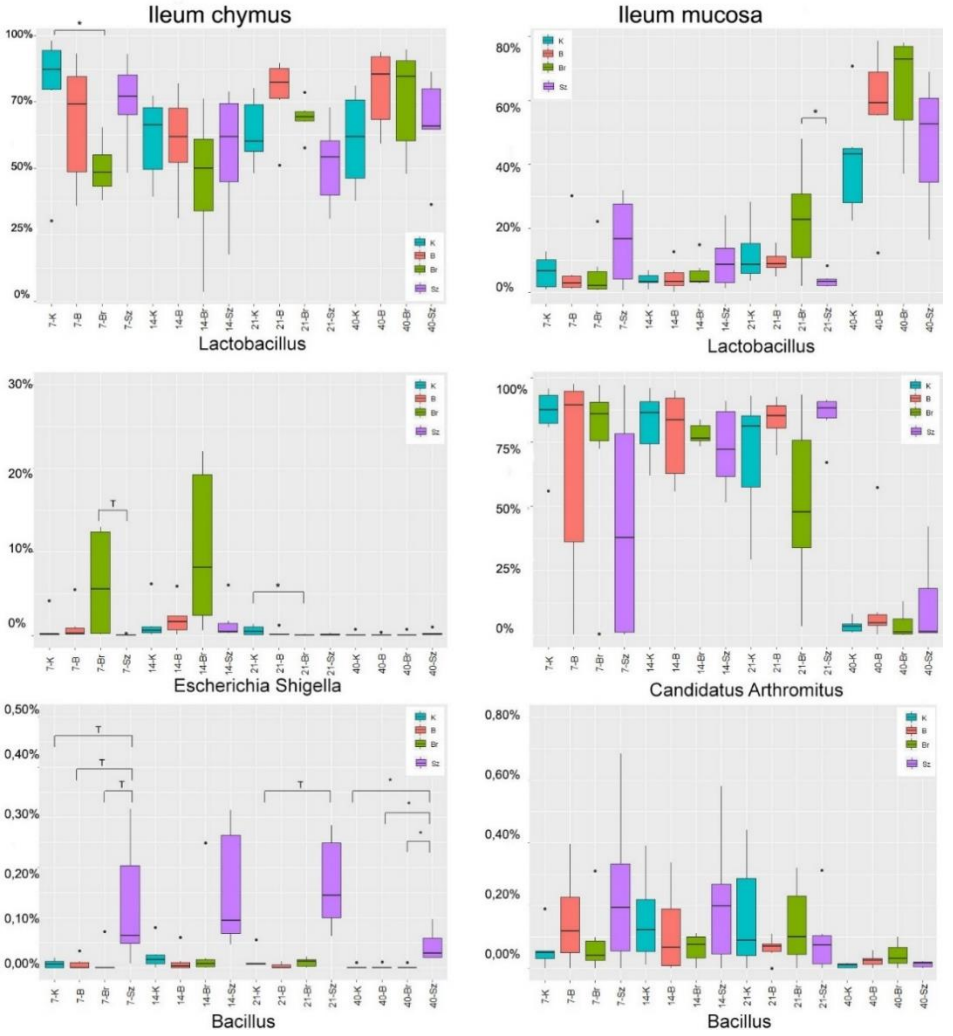


(K) control treatment, (Br) Broilact, (B)-wheat based treatment, (Sz)-symbiotic treatment; a,b means with different superscripts are significantly different.

In our second trial we could prove, that the bacteriota composition of the digestive tract is influenced mostly by the sampling places and by the age of chickens. Dietary effects were smaller and could have been detected mostly in the first week. The increase of bacterial diversity increased with the age of birds continuously except the ileal content where it reached the

plateau at the end of the third week. Compared to the other two sampling places, according to the Simpson index, variation of diversity was the highest in the ileum mucosa. Of the three intestinal sections, the microbial diversity of ileum mucosa was the lowest before day 40. In the caecum chymus, the diversity of microbiota also increased continuously with the age of chickens, and significant differences were found between the different age categories. The determinant phyla in all gut segments and age categories were Firmicutes. Its relative abundance was the highest in IM. No age-related trend was found in the different sampling places. Proteobacteria was one of the minor phyla in all three gut areas with a decreasing relative abundance over time. In ileum chymus and mucosa, its relative abundance was significantly higher at day 14 ($p < 0.05$) and dropped below 1% later on in IC. Beside the age effects only *Cyanobacteria* in ileal mucosa and *Lentisphareae* in caecal content was affected by dietary treatments. In the first case the symbiotic, in the second case the wheat treatment caused significantly higher abundance. At genus level, in the ileal gut content *Enterococcus* (Broilact and symbiotic) and *Bacillus* (symbiotic), in the caeca *Ruminiclostridium_5* (wheat) showed significant increase. Age had more pronounced effects on the bacteriota composition in all three sampling places.

Figure 3. Boxplots showing the abundances of the taxonomic groups at genus level in the ileal chymus (IC) and ileal mucosa (IM) as affected by the dietary treatments and age of birds.



Dots represent the outlier values. (K) control treatment, (Br) Broilact, (B)-wheat based treatment, (Sz)-symbiotic treatment;

In this trial excreta dry matter content was increased significantly when wheat-based diets were fed. In spite wheat treatment increased the urinary N ratio of excreta the quickest NH_3 emission was registered in the

symbiotic samples. The dynamics of the emission from the excreta of wheat treatment was slower. The reason for that could be different ratio of the ureolytic bacteria between the two treatments.

4. Conclusion

Comparing the results, we can conclude that the effect of feeding pre- and probiotic-supplemented diets is not primarily manifested in the improvement of production parameters. Wheat bran and probiotics had only a small effect on the intestinal properties of broiler chickens, without significant effects on production traits. The use of wheat bran as a prebiotic does not always mean increased fermentation short chain fatty acid production in the caeca. The main factors affecting the composition of the microbiota are the age of the birds and the sampling site. The *Akkermansia* genus is a member of the *Verrucomicrobia* phylum, which was able to multiply in the cecum as a result of wheat bran supplementation. Our results suggest that promotion the colonization of the genus Akkermansia by nutrition may be important not only in mammals but also in the hindgut sections of the chicken. The antibody titer results of Gumboro disease suggest that the development of adaptive immune competence of chickens is also influenced to a small extent by the composition of the feed.

From the results obtained during our first experiment, it can be seen that the addition of wheat bran to the diet increased the urease activity of the excreta and thus modify the dynamics of ammonia emission. The results of the second study confirm this. The results show that influencing the microbiome by nutrition can be a factor for reducing urinary N. In our study, the symbiotic treatment decreased the proportion of urine-N in the excreta. This results in lower ammonia emissions from the manure. However, in the short term, due to the greater microbial activity, the dynamics of the emission became more intense.

5. New scientific results

1. Adding 3, 6 and 6% wheat bran to the diet in the starter, grower and finisher phase results deeper crypts in the cecum, which indicates a more intensive regeneration of the caecal epithelium.

2. The abundance of genus *Akkermansia* increased in the caecum of chickens when 3, 6 and 6% wheat bran is fed in the starter, grower and finisher diets.

3. As a result of feeding *Lactobacillus farciminis (*$5x10^9$ CFU/kg) and *Clostridium butyricum* ($2,5x10^9$) supplements, the dry matter content of the faeces increases significantly and reduce the ratio of urinary N in excreta.

4. Feeding wheat-based diets (30%) supplemented with wheat bran (3, 6, 6% in the starter, grower and finisher phase) improves weight gain and feed conversion ratio of broilers and increase the ratio of urinary- N and ureolytic bacteria in the chicken's excreta compared to corn-based diets.

5. During the investigation of the effect on the development of the immune response against Gumboro disease, we found that at the 7th day the maternal antibody levels remain higher when wheat based and wheat bran supplemented diets are fed. Broilact (1.25×10^7 CFU/0.5 mL), the symbiotic feed additives (Bacillus subtilis, DSM17299 bacterial strain; 1.6×10^6 CFU/g, inulin, 5 g/kg; *Saccharomyces cerevisiae boulardii*, 2×10^{10} CFU/g) and wheat based diets all have positive effect on the humoral immune response of chickens and the uniformity of the immune response reactions.

Bibliography

1. Apajalahti, J.; *et al.* (2004) Characteristics of the gastrointestinal microbial communities, with special reference to the chicken. Worlds. Poult. Sci. J., 60, 223–232.

2. Thompson, C.L.; *et al.* (2013) Immune-modulating gut symbionts are not "candidatus Arthromitus". Mucos. Immunol., 6, 200–201.

3. Oakley, B.B.; *et al.* (2014) The chicken gastrointestinal microbiome. FEMS Microbiol. Lett., 360, 100–112.

4. Popova, T. Effect of probiotics in poultry for improving meat quality. Curr. Opin. Food Sci. 2017, 14, 72–77.

5. Al-Khalaifah, H.S. Benefits of probiotics and/or prebiotics for antibiotic-reduced poultry. Poult. Sci. 2018, 97, 3807–3815.

6. Kers, J. G. *et al.* (2018) 'Host and Environmental Factors Affecting the Intestinal Microbiota in Chickens.', Frontiers in microbiology, 9, p. 235.

7. Quast, C. *et al.* (2013) 'The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools', Nucleic Acids Research. 41(D1), pp. D590–D596.

8. Peters, B. J. *et al.* (2003) Recommended Methods for Manure Analysis, ASA-CSSA-SSSA ANNUAL MEETING ABSTRACTS. Madison, Wisconsin

9. Santoso, U. *et al.* (1999) 'Dried Bacillus subtilis Culture Reduced Ammonia Gas Release in Poultry House', Asian-Australasian Journal of Animal Sciences. 12(5), pp. 806–809

10. Zakrzewski, M. *et al.* (2017) 'Calypso: A user-friendly web-server for mining and visualizing microbiome-environment interactions', Bioinformatics. 33(5), pp. 782–783.

11. Bolyen, E. et al. (2019) 'Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2', Nature Biotechnology. 37(8), pp. 852–857.

6. Publications related to the topic of the dissertation

Publications in peer-reviewed journals in foreign languages:

Such N., F. Husvéth, A. Márton, I. A. Koltay, Z. Szokó, K. Dublecz (2019): The effect and accumulation of antibiotics and their residues in horticultural plants: a review. Journal of Central European Agriculture. 20(1), 431-446

Such N., A. Molnár, V. Farkas, L. Pál, F. Husvéth, I. A. Koltay, M. A. Rawash, Á. Mezőlaki, K. Dublecz (2020): Feeding two single strain probiotic bacteria and wheat bran failed to modify the production traits but altered some gut characteristics in broiler chickens. Journal of Central European Agriculture, 21(3), p.499-507

Molnár A.*, N. **Such*,** V. Farkas, L. Pál, L. Menyhárt, L. Wágner, F. Husvéth, K. Dublecz (2020): Effects of Wheat Bran and Clostridium butyricum Supplementation on Cecal Microbiota, Short-Chain Fatty Acid Concentration, pH and Histomorphometry in Broiler Chickens. Animals, 10, 2230.

Such N., V. Farkas, A. Molnár, G. Csitári, L. Pál, M. A. Rawash, I. A. Koltay, F. Husvéth, K. Dublecz (2021): The effect of diet composition, a probiotic and a symbiotic treatment on the ileal microbiota composition of one-week-old broiler chickens. Acta Agraria Debreceniensis. 1. 207-213. ISSN: 1587-1282

Such, N.; Csitári, G.; Stankovics, P.; Wágner, L.; Koltay, I.A.; Farkas, V.; Pál, L.; Strifler, P.; Dublecz, K. (2021): Effects of Probiotics and Wheat Bran Supplementation of Broiler Diets on the Ammonia Emission from Excreta. Animals, 11, 2703.

Such, N.; Farkas, V.; Csitári, G.; Pál, L.; Márton, A.; Menyhárt, L.; Dublecz, K. (2021): Relative Effects of Dietary Administration of a Competitive Exclusion Culture and a Synbiotic Product, Age and Sampling Site on Intestinal Microbiota Maturation in Broiler Chickens. Vet. Sci. 8, 187.

Publications in peer-reviewed journals in Hungarian:

Such N., Molnár A., Pál L., Farkas V., Menyhárt L., Husvéth F., Dublecz K. (2021): Pre- és probiotikum-kezelések kedvező hatása a Gumboro-betegség elleni vakcinázás egyes paramétereire brojlercsirkékben. Magyar Állatorvosok Lapja, 143. 119-127.

Such N., Farkas V., Pál L., Koltay I. A., Mezőlaki Á., M. A. Rawash, Husvéth F., Dublecz K., Molnár A. (2021): Pecsenyecsirke tápok búzakorpa kiegészítésének hatása a vakbél mikrobiom összetételére új generációs szekvenálási módszer alkalmazásával. Állattenyésztés és Takarmányozás, 70. 1. 25-34. ISSN: 0230-1814

Publications in full in a peer-reviewed conference volume:

Such N., V. Farkas, I. A. Koltay, M. A. Rawash, K. Dublecz, A. Molnár (2019): Wheat bran supplementation increases the abundance of Akkermansia muciniphila whereas single strain probiotic Clostridium butyricum fails to influence caecal microflora composition of broiler chickens. 18. BOKU-Symposium Animal Nutrion, Wien, 30. April, konF.iakötet 189-192

Such N., Koltay I. A., M. A. Rawash, Mezőlaki Á., Csitári G., Wágner L., Péterné Farkas E., Molnár A., Dublecz K. (2019): Búzakorpa etetésének

hatása a broiler csirke ürülékének ammónia kibocsájtására. XXV. Ifjúsági Tudományos Fórum, május 23. Keszthely

Such, N., I. Koltay, L. Pál, L. Wágner, M. A. Rawash, Á. Mezőlaki, A. Márton, A. Molnár, K. Dublecz (2019): Feeding two single strain probiotic bacteria and wheat bran failed to modify the production traits but altered some gut characteristics in broiler chickens. 22nd European Symposium in Poultry Nutrition, 10-13 June. ISBN: 978-83-942760-6-5

Such, N (2019): Wheat bran supplementation increase Akkermansia muciniphila in the chicken caecum. ConF.e of Agronomy Students, Cacak. Preceedings book. 11. 11.364-372. ISSN: 2334-99883

Such N., V. Farkas, I. A. Koltay, M. A. Rawash, Á. Mezőlaki, L. Pál, F. Husvéth, K. Dublecz, A. Molnár (2019): The effect of wheat bran supplementation on the microflora of broiler chickens, 61th. Georgikon Scientific ConF.e, 3-4. october, p 397- 406. ISBN 978-963-396-130-8

Such N., Koltay I. A., Menyhárt L., M. A. Rawash, Molnár A., Mezőlaki Á., Pál L., Dublecz K., Farkas V. (2020): Búza és a kukorica alapú tápok etetésének hatása 7 és 14 napos brojlercsirkék bélbakterióta összetételére. XXVI. Ifjúsági Tudományos Fórum, május 21. Keszthely. konferenciakiadvány 1-7. o. ISBN 978-963-396-143-8

Publication in summary in a peer-reviewed conference volume:

Such, N., I. Koltay, L. Pál, L. Wágner, M. A. Rawash, Á. Mezőlaki, A. Márton, A. Molnár, K. Dublecz (2019): Feeding two single strain probiotic bacteria and wheat bran failed to modify the production traits but altered some gut characteristics in broiler chickens. 22nd European Symposium in Poultry Nutrition, 10-13 June.