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Feeding two single strain probiotic bacteria and wheat bran failed to modify the production traits but altered some gut characteristics in broiler chickens

Búzakorpa, valamint két egytényezős probiotikum etetése nem befolyásolta a termelési paramétereket, azonban megváltoztatta a bél egyes paramétereit brojlercsirkék esetében

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ABSTRACT

The effects of a single strain lactic acid producing bacteria (LAB) (*Lactobacillus farciminis* 5x10^9 CFU/kg) and a single strain butyric acid producing bacteria (BAB) (*Clostridium butyricum* 2.5x10^9 CFU/kg) with or without wheat bran supplementation (WB), were investigated on the production traits and on several gut characteristics of broiler chickens. In total, 576 male Ross 308 day-old chickens were divided into 24 floor pens and fed a corn-soybean based control diet (C) and five other probiotic or wheat bran supplemented diets (LAB, BAB, LAB+WB, BAB+WB, C+WB) in 4 replicates. The wheat bran content of the starter, grower and finisher diets were 3, 6 and 6%, respectively. During the 37 day long fattening period, growth rate, feed intake were recorded and feed conversion was calculated. At the end of the trial, 8 chickens per treatment were slaughtered and the following parameters investigated: trypsin, lipase and amylase activity of the jejunal chyme, ileal histomorphology and *Lactobacillus* load. None of the treatments resulted significant differences in the production traits (P>0.1). BAB supplementation tended to decrease digestive enzyme activity. Feeding WB in all combination increased crypt depth (P=0.002), ileal muscle layer thickness (P=0.001) and decreased the villi: crypt ratio (P=0.037) in the ileum.

Keywords: *Clostridium butyricum*, gut health, *Lactobacillus farciminis*, poultry, wheat bran

ÖSSZEFOGLALÓ

Kutatásunk során egy tejsavtermelő (LAB) (*Lactobacillus farciminis* 5x10^9 CFU/kg) és egy vajsavtermelő baktériumtörzset tartalmazó (BAB) (*Clostridium butyricum* 2.5x10^9 CFU/kg) probiotikum készítmény hatását vizsgáltunk önmagában, valamint búzakorpa kiegészítéssel (WB) a termelési paraméterekre és a bél néhány morfológiai tulajdonságára baromfi esetében. Ennek során 576 Ross 308 típusú napos kakast osztottunk hat kezelési csoportra, 4 ismétlésben, csoportonként 24 állatot beállítva, kukorica alapú tápot alkalmazva (C, LAB, BAB, LAB+WB, BAB+WB, C+WB). Az indító, nevelő és befejező táp búzakorpa tartalma 3, 6 and 6% volt. A 37 napos nevelés alatt mértük a csirkék testtömeg-gyarapodását, takarmányfogyasztását és kiszámításra került a takarmányértékesítés. A hízlalást követően kezelésenként 8 állat került levágásra, majd az alábbi paraméterek vizsgálatára került sor: tripszin, lipáz és amiláz aktivitás a jejunumból, ileális hisztomorfológiai paramáterek és ileális *Lactobacillus* szám. Egyik kezelés sem eredményezett szignifikáns különbségeket a termelési paraméterekben (P>0.1). A BAB kezelés tendenciálisan csökkentette az emésztőenzim aktivitást. A búzakorpa

kiegészítés hatására minden kombinációban nőtt az ileális kriptamélység (P=0.002), az izomvastagság (P=0.001) és csökkent a boholy-kripta arány (P=0.037).

Kulcsszavak: búzakorpa; broilercsirke; bélegészség; *Clostridium butyricum*, *Lactobacillus farciminis*

INTRODUCTION

It is generally known that the diverse gut microbiota play an important role in the, digestion, metabolism, growth performance and health of the host (Wang et al., 2017). Some bacteria play important role in the metabolism of the nutrients both of feed and endogenous origin. They can also degrade indigestible compounds, synthetize proteins and vitamins and stimulate the gut associated immune system (Fuller et al., 1983). Recent research results suggest that not really the differences in the microbe composition in the different digestive tract parts affect the physiological processes of birds, but the changes in the metabolic activity of microbes (Sánchez et al., 2017). Among the metabolites, the well-known short chain fatty acids (SCFA-s), acetic acid, propionic acid butyric acid and lactic acid, have crucial role. Among them lactic acid can decrease the pH in the different gut segments, while butyric acid plays as an energy source of the epithelial cells and also as a signal molecule (Soomro et al., 2002; Hu et al., 2008). Beside carbohydrates, some butyrate producing bacteria can use lactic acid as substrate (Belenguer et al., 2011). It is the reason, that probiotic feed additives recently contain several strains of butyric and lactic acid producing bacteria.

The main precursors of the bacterial fermentation in the gastrointestinal tract are different digestible carbohydrates or different non-digestible oligosaccharides like the soluble arabinoxylan and beta-glucan of cereal grains, the mannan oligosaccharides of yeast cell wall or the fructose polymer inuline (Hamer et al., 2008). Wheat bran contains high amount of soluble arabinoxylan (AX), which can splatted by exogenous xylanase enzyme to shorter chain arabinoxylan oligosaccharides (AXOS). Increasing the AXOS content of diets, results in a growing number of butyric acid producing bacteria in the caeca of broiler chickens. It decreases the abundance of potential pathogenic groups such as salmonella, campylobacter or clostridia (Van Immerseel et al., 2017). Increasing the butyric acid concentration in the small intestine or in the caeca could improve gut structure, the absorption of nutrients and this way even the production traits (Schneeman, 2002; James et al., 2003). The aim of this study was to investigate the single and combined effects of feeding wheat bran, with or without *Clostridium butyricum* and *Lactobacillus farciminis* supplementation on the production traits, ileal microflora, gut histology and digestive enzyme secretion of broiler chickens.

MATERIAL AND METHODS

Birds and experimental design

A total of 576 ROSS 308 day-old male broiler chickens were randomly assorted into six groups of 24 birds each in 4 replicated: control (C), lactic acid producing bacteria supplemented with *Lactobacillus farciminis* in $5x10^9$ CFU/kg by Chemnet (LAB), butyric acid producing bacteria with *Clostridium butyricum* in $2.5x10^9$ CFU/kg by Huvepharma (BAB), wheat bran supplemented (WB), wheat bran and LAB supplemented (LAB+WB) and wheat bran and BAB supplemented (BAB+WB). The animals were kept in pen at a stocking density of 10 birds/m^2, which meets the criteria of the national and EU standards.

Feed

Feed and water were available ad libitum. The basal diet was commercial feed for chickens of a nutrient content conforming to the recommendations of the breeder (Aviagen, 2014; Table 1; Table 2). Starter, grower and finisher diets were fed between day 1–10, day 11-24, and day 25-37, respectively. The wheat bran content of the starter, grower and finisher diets were 3, 6 and 6%, respectively.

Performance parameters

During the 37 day long fattening period, the growth rate, feed intake, and feed conversion were measured at pen basis at the end of each period (10^{th}, 24^{th}, 37^{th} day).

Table 1. Composition of experimental diets (g/kg as fed)

Ingredient	Starter (day 1 to 10 of life)		Grower (day 11 to 24 of life)		Finisher (day 25 to 40 of life)	
	Control	WB	Control	WB	Control	WB
Maize	466	434	534	469	589	524
Wheat bran	0	30	0	60	0	60
Extracted soybean meal	338	333	361	352	310	300
Sunflower oil	63	70	62	76	60	74
Limestone	19	19	15	15	15	15
Monocalcium phosphate	80	80	0	0	0	0
Lysine	5	5	2	2	2	2
Methionine	4	4	3	3	3	3
Threonine	1	1	1	1	0	1
L-Valine	1	1	0	0	0	0
NaCl	3	3	3	3	3	3
$NaHCO_3$	1	1	1	1	1	1
Premix†	4	4	4	4	3.5	3.5
Phytase	0	0	0.1	0.1	0.1	0.1
NSP enzyme	0	0	0.1	0.1	0.1	0.1
Total	1000	1000	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):

Starter and grower premixes – 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;

Finisher premix - retinyl acetate – 3.4 mg, cholecalciferol – 97 µg, dl-alpha- tocopherol-acetate – 45.5 mg, menadione – 2.7 mg, thiamin – 1.9 mg, riboflavin – 5.0 mg, pyridoxin HCL – 3.2 mg, cyanocobalamin – 19 µg, niacin – 28.5 mg, pantothenic acid – 10 mg, folic acid – 1.3 mg, biotin – 140 µg, l-ascorbic acid – 40 mg, betaine – 193 mg, antioxidant – 25 mg, Zn (as $ZnSO_4 \cdot H_2O$) – 96 mg, Cu – 9.6 mg, Fe (as $FeSO_4 \cdot H_2O$) – 29 mg, Mn (as MnO) – 29 mg, I (as KI) – 1.2 mg, Se (as Na_2SeO_3) – 350 µg;

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

Ingredient	Starter (day 1 to 10 of life)		Grower (day 11 to 24 of life)		Finisher (day 25 to 40 of life)	
	Control	WB	Control	WB	Control	WB
AME_n (MJ/kg)	12,1	12,2	13,1	13,0	13,0	13,1
Dry matter	88,8	89,0	88,5	88,8	88,2	88,8
Crude protein	22,9	23,0	20,7	21,2	18,8	19,1
Crude fat	8,3	9,2	9,1	10,1	8,9	10,0
Crude fibre	4,02	4,575	3,77	4,18	3,63	4,33
Ash	6,69	6,83	5,61	5,96	5,43	5,69
Ca	1,07	1,08	0,94	0,94	0,89	0,89
P	0,80	0,81	0,67	0,71	0,66	0,7
Starch	30,5	29,4	36,9	33,6	38,7	36,4

Ileal morphology, ileal Lactobacillus counts and digestive enzyme activity

On day 37 of life, 2 chickens per pen, 8 birds per treatment were slaughtered and the following parameters investigated: trypsin, lipase and amylase activity of the jejunal chyme, histomorphology of the ileum and Lactobacillus content of the ileum. The microbial composition was determined with classical agar culturing. MRS (de Man, Rogosa and Sharpe) broth was used for the lactobacillus. For the histomorphological examination ileal tissue samples were taken close to the junction of Meckel's diverticulum. Tissue sections were washed with 2% PBS (phosphate buffered saline) and fixed in 10% phosphate buffered formalin. Samples were embedded in paraffin blocks and sectioned (4 µM in thickness). A routine staining procedure was carried out using haematoxylin and eosin. Ileum sections were measured using a microscope (Leica DMi8 Microscope, Leica Microsystems CMS GmbH, Germany 2015). Villus height, muscle layer thickness and crypt depth were determined with ImageJ software (Version 1.47) developed by National Institutes of Health (Maryland, USA).

Statistical analyses

All data were analysed using the SPSS 16.0 software. The analysis was carried out with two way ANOVA. Differences were considered significant at a level of $p \leq 0.05$. Where Levene's test was significant, Mann-Whitney test was used.

RESULTS

None of the treatments resulted significant differences in the production traits (Table 3), and the ileal Lactobacillus counts (Table 4).

BAB supplementation tended to decrease digestive enzyme activity (Table 5). The histomorphology parameters are reported in Table 5. Feeding WB in all combination increased crypt depth in ileum (P=0.002), increased the ileal muscle layer thickness (P=0.002) and decreased the villi: crypt ratio (P=0.037) in the ileum.

Table 3. Effect of wheat bran, *Lactobacillus farciminis* and *Clostridium butyricum* supplementation on performance parameters of broiler chickens at 37 days of age

Dietary treatments*		Body weight, (g)	Feed intake, (g)	Gain, (g)	Feed conversion ratio (g/g)
Control		2,468	4,036	2,427	1.66
BAB		2,515	4,141	2,474	1.67
LAB		2,460	4,037	2,419	1.66
WB		2,481	3,982	2,440	1.63
BAB+WB		2,516	4,070	2,475	1.64
LAB+WB		2,493	4,016	2,452	1.63
Wheat bran					
	No	2,481	4,071	2440	1.66
	Yes	2,497	4,023	2456	1.63
BAB					
	No	2474	4,009	2434	1.64
	Yes	2,515	4,105	2474	1.66

Table 3. Continued

Dietary treatments*		Body weight, (g)	Feed intake, (g)	Gain, (g)	Feed conversion ratio (g/g)
LAB					
	No	2,474	4,009	2434	1.64
	Yes	2,476	4,027	2436	1.65
Pooled SEM		14,281	42.123	14.317	0.020
Wheat bran		0.605	0.289	0.608	0.253
Probiotic		0.482	0.279	0.487	0.867
Wheat bran x probiotic		0.909	0.872	0.911	0.978

Control – commercial maize-based diet; LAB - Control group supplemented with $5x10^9$ colony forming units/kg *Lactobacillus farciminis* spores BAB – Control group supplemented with $2.5x10^9$ colony forming units/kg *Clostridium butyricum* spores; WB – Control group supplemented with 6% of wheat bran; SEM – standard error of the mean

Table 4. Effect of wheat bran, *Lactobacillus farciminis* and *Clostridium butyricum* supplementation on ileal *Lactobacillus* numbers in broiler chickens at 37 days of age

Dietary treatments		*Lactobacillus counts (CFU/g)*
Control		5,65
BAB		6,12
LAB		5,37
WB		5,79
BAB+WB		5,82
LAB+WB		5,86
Wheat bran		
	No	5,71
	Yes	5,82
BAB		
	No	5,72
	Yes	5,97
	No	5,72
LAB	Yes	5,62
Pooled SEM		0.130
Wheat bran		0.685
Probiotic		0.547
Wheat bran x probiotic		0.494

Control – commercial maize-based diet; LAB - Control group supplemented with $5x10^9$ colony forming units/kg *Lactobacillus farciminis* spores BAB – Control group supplemented with $2.5x10^9$ colony forming units/kg *Clostridium butyricum* spores; WB – Control group supplemented with 6% of wheat bran; SEM – standard error of the mean; CFU: colony forming units

Table 5. Effect of wheat bran, *Lactobacillus farciminis* and *Clostridium butyricum* supplementation on ileal histological and enzyme activity in broiler chickens at 37 days of age

Dietary treatments		Villus length µm	Crypt depth µm	Villi:crypt ratio µm	Muscle layer thicknes s µm	Trypsin mE/ mg protein	Lipase mE/ mg protein	Amilase mE/ mg protein
Control		765.8	170.3	4.4	161	81.5	0.11	5.72
BAB		874.2	151.4	5.8	127	52.7	0.08	4.09
LAB		745.0	159.2	4.7	132	83.4	0.18	5.53
WB		753.0	137.5	5.6	119.6	79.9	0.16	7.18
BAB+WB		869.4	141.3	6.3	111.3	63.1	0.13	3.39
LAB+WB		745.6	129.0	5.8	109.2	95.79	0.16	3.58
Wheat bran								
	No	797.9	136.3b	5.9a	113.6b	72.5	0.12	5.11
	Yes	787.0	160.9a	4.9b	141.3a	78.9	0.15	4.77
BAB								
	No	759.4	153.9	5.0	140.3	80.7	0.13	6.45
	Yes	872.0	145.9	6.0	118.4	57.9	0.10	3.74
LAB								
	No	759.4	153.9	5.04	140.3	80.7	0.13	6.45
	Yes	745.3	144.1	5.25	120.6	89.2	0.17	4.62
Pooled SEM		29.76	4.08	0.22	4.56	5,83	0,01	0,63
Wheat bran		0.927	**0.002**	**0.037**	**0.001**	0.548	0.320	0.758
Probiotic		0.203	0.496	0.165	0.102	0.084	0.168	0.084
Wheat bran x probiotic		0.996	0.369	0.758	0.323	0.868	0.509	0.548

Control – commercial maize-based diet; LAB - Control group supplemented with 5x10^9 colony forming units/kg *Lactobacillus farciminis* spores BAB – Control group supplemented with 2.5x10^9 colony forming units/kg *Clostridium butyricum* spores; WB – Control group supplemented with 6% of wheat bran; SEM – standard error of the mean

DISCUSSION

In some previous studies, when *Clostridium butyricum* was added to broiler diets, growth performance, ileal *Lactobacillus* load, ileal histomorphology, meat quality, fatty acid profile, and the immune system were positively affected (Zhang et al., 2011A; Zhang et al., 2011B., Gao et al., 2012; Yang et al., 2012, Zhao et al., 2013). Earlier experiments have shown that *C. butyricum* increases the concentration of n- butyric acid in the avian caeca (Zhang et al., 2011A) and survives extreme low pH values, so it can be used as a feed supplement (Kong et al., 2011). This bacterial strain is a Gram-positive anaerobic producer of butyric acid found in both soil and intestinal tract of healthy animals. In present experiment, *C. butyricum* supplementation had no effect on the production traits but tended to decrease the digestive enzyme activity. A recent study has reported that *Lactobacillus farciminis* treatment abolished the hyperalgesia to colorectal distension (CRD) induced by acute stress. in rats (Ait-Belgnaoui et al., 2009). The present study indicated that *L. farciminis* in the diet couldn't improve body weight, feed intake, feed conversion ratio, ileal *Lactobacillus* counts and gut morphology of broilers. In a previous study, wheat bran derived polysaccharides, arabinoxylans

(AXs), were evaluated for their immunostimulatory and protective efficacy against Eimeria infection in chickens. Humoral response revealed significantly higher (P<0.05) total Igs, IgG and IgM titres at day 7th and 14th post primary and secondary injections of sheep red blood cells in the experimental chickens administered with AXs as compared to those of control group. The protection against Eimeria and daily weight gain were significantly higher (P<0.05) in the chickens of experimental groups as compared to control; whereas, mean oocyst per gram of droppings and lesion scores were significantly higher (P<0.05) in control group as compared to chickens in the experimental groups. In conclusion, AXs showed both immune stimulatory and protective effects against coccidiosis in broiler chickens (Akhtar et al., 2012). In another study, the supplementation of bran AXOS at either 0.5% (w/w) to the wheat-based diet or at 0.25% (w/w) to the maize-based diet significantly (P<0.05) improved the feed conversion rate without increasing the body weight of the animals, thus pointing out to an improved nutrient utilization. The positive effect of bran AXOS supplementation on feed utilization was similar to that obtained by adding an AX-degrading xylanase directly to the wheat-based diet. No significant effect on feed utilization was obtained with another type of non-digestible oligosaccharides such as fructo-oligosacharides (FOS) derived from chicory roots. Bran AXOS significantly increased the level of *bifidobacteria* but not total bacteria in the caeca of chickens, an effect not observed with either xylanase or FOS addition. These data suggest that bran AXOS have beneficial nutritional effects and may act as prebiotics (Courtin et al., 2008). Any alteration in the diet and the intestinal microflora can alter the morphology of gastrointestinal tract of broilers (Yang et al. 2007). The histomorphological changes in the ileum of broiler chickens reported in this study provide information regarding the potential for using wheat bran and probiotics in broiler feed. In the present experiment none of the probiotics influenced the histomorphological parameters. The reason for this could be at least partly the optimal keeping conditions. In the present study, supplementation of broilers with wheat bran decreased villus height: crypt depth ratio, increased the chrypth depth and increased the muscle layer thickness in the ileum significantly (P<0.05). Contrary to the current results, in a previous study the inclusion of 10% wheat bran in the diet did not influence the gut morphology results (Li et al., 2018). There are similar to those of Chen et al. (2013), who found no effect on gut morphology when 10% wheat bran was added to the diet of pigs. The deeper crypts indicate faster tissue turnover to permit renewal of the villus as needed in response to normal sloughing or inflammation from pathogens or their toxins and high demands for tissue (Yason et al., 1987). The crypt can be thought of as the villus factory; a large crypt suggests faster tissue turnover and more energy demands for histogenesis (Awad et al. 2009). According to Montagne (2003) the effect of dietary fiber on intestinal epithelial anatomy and structural development seems to be dependent on the ability of dietary fiber to increase digesta viscosities. The presence in the lumen of high viscosity digesta may increases the rate of villus cell losses, leading to villus atrophy, a phenomena associated with an increased crypt-cell. Chiou (1996) carried out an experiment to study the effect of different dietary fibre sources on the intestinal morphology of geese. The thickness of ileal and caecal muscle layer were significantly thicker in the geese fed with cellulose supplemented diets than in those fed with the alfalfa meal, barley hull, rice hull, lignin, or pectin treatment diets.

According to Han (2017) the 7.52% dietary fibre content caused more thick muscle layer than the 1.46% or 9.03% fibre containing diets in ducks. Feed passage rate generally increases as dietary fibre content increases (Ferket and Veldkamp, 1999). Thinner muscle layers have been observed with growth promoting antibiotic supplementation (Ferket et al., 2002) or with fibres of various sources (Molnár et al., 2015). In this experiment, the 6% wheat bran supplementation caused more thick muscle layer than the control group. This may indicate an increase in intestinal peristalsis.

CONCLUSION

According to the results of this study wheat bran and probiotics could modify slightly the different gut characteristics of broilers, but it do not necessarily mean improved production traits.

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