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Effects of *Clostridium butyricum* and *Lactobacillus plantarum* on growth performance, immune function and volatile fatty acid level of caecal digesta in broilers

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ABSTRACT

The study was conducted to investigate the effects of *Clostridium butyricum* and *Lactobacillus plantarum* on growth performance, immune function and volatile fatty acid (VFA) level of caecal digesta in broilers. One hundred and forty broilers were assigned to five groups (CON: basal diet; CB: basal diet+ *C. butyricum;* MLP: basal diet+ *L. plantarum;* MIX: basal diet+ *C. butyricum* + *L. plantarum;* ANT: basal diet + Aureomycin). The results showed that, birds in CB group had greater serum IgM level than that in control group at day 21 (*P* < .05). Birds in MIX group had greater serum IgM and IgA levels than those in MLP group at day 42 (*P* > .05). The current results indicated that dietary supplementation of *C. butyricum* increased serum immunoglobulin level and VFA level of caecal digesta in broilers, but a combination of supplementations with *C. butyricum* and *L. plantarum* had no significant effect on growth performance.

Introduction

At present, antibiotics cause a series of problems, such as antibiotic-resistant pathogens and antibiotic residues in animal products, so research on effective alternatives to antibiotic with no residue, no antibiotic-resistance become very important. There are many alternatives to antibiotics, such as probiotics, prebiotics, phyto-extractin fermented feed (Dong et al., 2016; Hu et al., 2016; Wang, Deng, et al., 2017; Wang et al., 2017). Probiotics can maintain a healthy gastrointestinal environment and improved intestinal function is pursued through the intake of sufficient quantity of live, benefical microorganisms. So as substitutes of antibiotics, probiotics were attracted extensive attention.

Lactobacillus, such as *Enterococcus faecalis, Lactobacillus plantarum, Lactobacillus acidophilus, Lactobacillus casei, etc.*, is being widely used. The metabolites of *L. plantarum* include lactic acid, bacteriocin and protease, which can maintain the balance of intestinal

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microorganisms, and promote the digestion and absorption of nutrients in animals (Mount-zouris et al., 2010; Zhao, Guo, Guo, & Tan, 2013). Besides, probiotics had positive effects on animals under stress environment. However, probiotics, especially *Lactobacillus,* are very sensitive to external environment, such as feed process, storage, and gastrointestinal tract of animal (Corona-Hernandez et al., 2013). So the microcapsule technology that probiotics are wrapped in coated material can improve the resistance of probiotic (Dong et al., 2016).

Clostridium butyricum, as a kind of intestinal probiotics, can effectively improve the functions of digestion and absorption of nutrients, inhibit the propagation of harmful microorganisms (Kong, Chen, Kweon, & Park, 2011; Okamoto, Abe, Shirai, & Ueda, 2000). The metabolite of *C. butyricum* contains amylase, which can hydrolyse starch and carbohydrates into oligosaccharides. The oligosaccharides can be absorbed by animal and utilized by *Bifidobacteria* and *Lactobacillus* in intestinal tract. Microbes in intestinal tract can also produce short chain fatty acids (SCFAs) by deconstruction of dietary indigestible carbohydrates. These SCFAs can decrease the intestinal pH and inhibit the growth of certain harmful microorganisms (Jozefiak, Kaczmarek, & Rutkowski, 2008). Moreover, these SCFAs play a critical role in host energetics and hydration, and the levels of butyrate are of particular physiological significance (Yeoman et al., 2012). *C. butyricum* can produce high proportion of butyric acid, so *C. butyricum* is of importance in host nutrition. However, the composition and proportions of these SCFAs are depending on microbial composition of gastrointestinal tract, although Zhao et al. (2013) reported that dietary supplementation of *C. butyricum* and *E. faecium* can regulate lipid metabolism and caecal microbiota in broilers, the effects on the composition of SCFAs is need to be studied.

Therefore, the aims of this study were to investigate the effects of dietary supplementation of *C. butyricum* and microencapsulated *L. plantarum* on growth performance, immune function, intestinal morphology, and volatile fatty acids (VFAs) levels of caecal digesta in broilers.

Materials and methods

Preparation of C. butyricum and Microencapsulated L. plantarum

The microencapsulated *L. plantarum*(10^{10}cfu/g) was prepared by our laboratory according to (Dong et al., 2016). The *C. butyricum*(10^9cfu/g)was provided by a company.

Experimental design

Birds care and handling were in compliance with the Animal Ethics Committee Guidelines of Academy of State Administration of Grain (Beijing, China). The animal experiment was performed in Shisanling experiment base of Academy of State Administration of Grain (Changping district, Beijing, China).

A total of 140 one-day-old male Arbor Acres broilers (initial body weight: 41.6 ± 5 g) were purchased from a commercial hatchery (Huadu Broiler Farms, China). Birds were raised in cages with wire mesh floor. The stocking density was 560 cm²/bird. Birds were randomly placed into five treatments with seven replicates each (four birds per replicate). Control group (CON), birds were fed a basal diet without antibiotics; *C. butyricum* group

(CB), birds were fed a basal diet containing 10^6 cfu *C. butyricum*/kg of diet; microencapsulated *L. plantarum* group (MLP), birds were fed a basal diet containing 10^7 cfu *L. plantarum*/kg of diet; *C. butyricum* and microencapsulated *L. plantarum* group (MIX), birds were fed a basal diet containing 10^6 cfu *C. butyricum*/kg of diet and 10^7 cfu *L. plantarum*/kg of diet; antibiotics group (ANT), birds were fed a basal diet containing 30 mg Aureomycin/kg of diet. Feeding trial consisted of feeding starter (Days 1–21) and feeding finisher (Days 22–42) periods (Table 1). Experimental diets, in mash form, were formulated to meet all nutrient requirements of Chinese feeding standard of chicken (NY/T 33-2004).

Bird management

The birds were housed in cages for 42 days in an environmentally controlled room. Environmental temperature in the room was maintained at 32–35°C in the first week and then gradually reduced to 25°C until the end of the experiment. The broilers had free access to feed and water with 23 h fluorescent illumination per day throughout the trial period. The broilers were vaccinated with ND-IB vaccine at day 7, and IBD vaccine at day 14.

Growth performance

At days 21 and 42, after fasted for 12 h, broilers were weighted by cage, and feed intake was recorded on a cage basis. Average daily gain (ADG), average daily intake (ADFI), and feed conversion ratio (FCR) were calculated during 0–21 days and 22–42 days. The mortality was counted during the whole feeding trial.

Table 1. Ingredients and nutrient composition of the basal diet (g/kg diet as fed basis).

	Composition	
I gredient	Starter (days 1–21)	Finisher (days 22–42)
Corn	557.50	571.10
Soybean meal	367.50	350.00
Soybean oil	29.60	41.70
Dicalcium phosphate	18.60	14.20
limestone	12.50	14.00
Salt	3.00	3.00
Choline chloride (50%)	2.60	2.00
Minerals premix[a]	2.00	2.00
Vitamin premix[b]	0.20	0.20
L-Methionine	2.87	0.10
L-Lysine HCl	3.09	1.20
L-Threonine	0.54	0.50
Total	1000	1000
Calculated chemical composition (g/kg diet as fed basis)		
ME (MJ/kg)	12.14	12.92
Crude protein	22.10	20.700
Calcium	1.09	1.01
Total Phosphorus	0.65	0.65
Lysine	1.20	1.11
Methionine	0.55	0.39

[a]Vitamin premix provided the following per kilogram of diet; vitamin A, 9500 IU; vitamin D_3, 62.50 µg; vitamin K_3, 2.65 mg; vitamin B_{12}, 0.025 mg; vitamin B_2, 6 mg; vitamin E, 30 IU; biotin, 0.0325 mg; folic acid, 1.25 mg; pantothenic acid, 12 mg; nicotinic acid, 50 mg.
[b]The mineral premix provided the following per kg of diet: Cu, 8 mg; Zn, 75 mg; Fe, 80 mg; Mn, 100 mg; Se, 0.15 mg; I, 0.35 mg.

Determination of serum immunoglobulin level

At day 21 and day 42, one bird was taken from each cage, and blood samples were collected from wing vein. The serum samples were harvested after centrifugation (3000 rpm, 10 min) at 4°C and stored at −20°C. The levels of serum IgA, IgM and IgG were measured using chicken-specific ELISA kits (Uscn Life Science Inc, Wuhan, China).

Intestinal morphometric measurement

At day 21 and day 42, after collecting serum samples, the birds were killed by jugular bleeding. The 1-cm segments of the jejunum were taken from the middle part of jejunum section. Tissue samples were rinsed in physiological saline solution, and fixed in 10% buffered formalin overnight, serially dehydrated in graded ethanol solutions (50%, 70%, 80%, 96%, and 100%), cleared with xylene and embedded in paraffin wax, then sectioned by a microtome at a thickness of 5 μm (three cross sections from each sample), placed on a glass slide, and stained with haematoxylin and eosin. Histological sections were examined by an image analyser (BOND-III LeicaMicrosystems, Leica Imaging Systems Ltd, Cambridge, UK) to measure villus height and crypt depth. Villus height was measured from the tip of the villus to the villus-crypt junction, and the crypt depth was defined as the depth of the invagination between adjacent villi (Jo et al., 2012). Seven replicates per treatment were chosen to determine the intestine morphology and nine intact, well-oriented villi were selected to measure the villus height and crypt depth. Morphological structure of villus height and crypt depth was measured at 40 × magnification.

VFA analysis

Caecal contents was aseptically collected into tubes and immediately put in liquid nitrogen. Then the samples were preserved at −80°C for VFAs analysis. The VFAs level was determined using the method described by Zhang, Li, Lu, and Yi (2003). Approximately 0.25 g of thawed caecal digesta was diluted with 2 ml distilled water in a screw-capped tube. After homogenization and centrifugation, 1 ml of supernatant was transferred into a centrifuge tube and 0.2 ml metaphosphoric acid solution was added. The sample was homogenized and placed in an ice bath for at least 30 min to allow the protein to settle completely. Finally, samples were centrifuged (10 min at 10,000 rpm), and the supernatant was analysed with gas chromatography analyser (Agilent 7980B, American).

Statistical analyses

All data were analysed with an ANOVA by SPSS for Windows version 20.0 (SPSS, Chicago, IL). The significant differences were determined by Duncan's new multiple range test. Statements of statistical significance were based on $P < .05$.

Table 2. Effects of *C. butyricum* and microencapsulated *L. plantarum* on growth performance in broiler.

Items	CON	CB	MLP	MIX	ANT	*P*-value
Days 1–21						
ADFI (g/d)	45.04 ± 0.71	45.69 ± 0.44	44.51 ± 0.97	46.38 ± 0.66	44.81 ± 0.70	0.392
ADG (g/d)	33.5 ± 0.57	33.61 ± 0.32	33.11 ± 0.83	34.08 ± 0.63	34.59 ± 0.75	0.557
Feed conversion ratio (g/g)	1.37 ± 0.03b	1.36 ± 0.01b	1.37 ± 0.01b	1.36 ± 0.01b	1.30 ± 0.02a	0.027
Days 22–42						
ADFI (g/d)	122.81 ± 1.57	125.34 ± 3.51	125.14 ± 3.48	132.9 ± 7.05	123.82 ± 3.06	0.436
ADG (g/d)	69.09 ± 1.91	71.24 ± 2.62	70.73 ± 0.94	72.06 ± 4.52	70.16 ± 1.63	0.904
FCR (g/g)	1.82 ± 0.04	1.76 ± 0.04	1.75 ± 0.04	1.8 ± 0.01	1.75 ± 0.02	0.25

Notes: Means within a row with different letters differ significantly ($P < .05$). CON = a basal diet; CB = a basal diet + *C. butyricum*; MLP = a basal diet + microencapsulated *L. plantarum*; MIX = a basal diet + *C. butyricum* + microencapsulated *L. plantarum*. ANT = a basal diet + *Aureomycin*.

Results

Growth performance

ANT groups had lower FCR than other groups at day 21 ($P < .05$). No differences were observed in growth performance among all the treatments ($P > .05$) (Table 2).

Serum immunoglobulin level

Birds in CB group had greater IgM level at day 21 than that in control group ($P < .05$) (Table 3). Birds in CB or/and MLP supplemented groups had greater IgA and IgG levels than those in control group at day 21 and day 42 ($P > .05$). Birds in MIX groups had greater IgM and IgA levels at days 21 and 42 than those in MLP group ($P > .05$).

Jejunum morphplogic parameters

Results of jejumum morphometric parameter were shown in Table 4. At day 42, crypt depth of birds in CB group were significantly lower than that in control group ($P < .05$). Villus height/crypt depth ratio was significantly increased in CB group compared with control group ($P < .05$).

VFAs level of caecal digesta

Results of VFAs level of caecal digesta were presented in Table 5. The results showed that at day 21, the levels of acetic acid and valerate acid of caecal digesta in broilers from MLP

Table 3. Effects of *C. butyricum* and microencapsulated *L. plantarum* on inmunoglobulins in broilers.

Items	CON	CB	MLP	MIX	ANT	*P*-values
21d						
IgA (µg/ml)	50.11 ± 2.06	55.69 ± 1.92	53.20 ± 1.76	53.40 ± 1.85	52.65 ± 2.38	0.555
IgM (mg/ml)	1.75 ± 0.16a	2.50 ± 0.08b	1.79 ± 0.07a	2.11 ± 0.19ab	2.03 ± 0.29ab	0.039
IgG (mg/ml)	1.00 ± 0.16	1.10 ± 0.29	1.28 ± 0.10	1.07 ± 0.13	1.01 ± 0.11	0.664
42d						
IgA (µg/ml)	68.38 ± 2.31	69.49 ± 2.94	68.65 ± 1.55	69.22 ± 1.50	67.55 ± 3.34	0.981
IgM (mg/ml)	1.71 ± 0.62	2.45 ± 1.17	1.86 ± 0.17	2.10 ± 0.43	2.35 ± 0.62	0.868
IgG (mg/ml)	1.09 ± 0.20	1.27 ± 0.33	1.25 ± 0.29	1.30 ± 0.58	1.07 ± 0.27	0.979

Notes: Means within a row with different letters differ significantly ($P < .05$). CON = a basal diet; CB = a basal diet + *C. butyricum*; MLP = a basal diet + microencapsulated *L. plantarum*; MIX = a basal diet + *C. butyricum* + microencapsulated *L. plantarum*. ANT = a basal diet + *Aureomycin*.

Table 4. Effect of *C. butyricum and* microencapsulated *L. plantarum* on jejunum morphology in broilers.

Items	Age (days)	CON	CB	MLP	MIX	ANT	P-values
Villus height	21	0.81 ± 0.03	0.93 ± 0.06	0.89 ± 0.05	0.90 ± 0.10	0.85 ± 0.07	0.588
(mm)	42	1.09 ± 0.11	1.18 ± 0.10	1.26 ± 0.06	1.12 ± 0.06	1.21 ± 0.12	0.695
Crypt depth	21	98.90 ± 2.92	89.10 ± 4.38	95.80 ± 7.39	92.20 ± 9.24	85.10 ± 3.80	0.567
(μm)	42	183.87 ± 14.25b	136.88 ± 19.78a	163.65 ± 10.53ab	144.33 ± 6.76ab	124.75 ± 11.62a	0.047
Villus height/crypt depth	21	7.91 ± 0.45	8.25 ± 0.59	7.46 ± 0.31	8.09 ± 0.65	8.91 ± 0.36	0.323
	42	6.19 ± 0.48a	9.16 ± 0.48b	6.36 ± 0.36a	7.63 ± 0.59ab	6.99 ± 0.93a	0.037

Notes: Means within a row with different letters differ significantly ($P < .05$). CON = a basal diet; CB = a basal diet + *C. butyricum*; MLP = a basal diet + microencapsulated *L. plantarum*; MIX = a basal diet + *C. butyricum* + microencapsulated *L. plantarum*. ANT = a basal diet + *Aureomycin*.

Table 5. Effect of *C. butyricum* and microencapsulated *L. plantarum* on short chain fatty acid (SCFA) concentrations in cecum digesta (mg/kg of wet ileal digesta).

Parameter (mM)	Age (days)	Dietary treatments					P-values
		CON	CB	MLP	MIX	ANT	
Acetic	21	4.06 ± 0.33a	5.17 ± 0.28b	5.37 ± 0.43b	5.09 ± 0.13b	4.73 ± 0.10ab	.033
(μg/μL)	42	4.52 ± 0.32	5.03 ± 0. 29	5.15 ± 0.20	4.58 ± 0.40	5.17 ± 0.59	.481
Propionic	21	0.79 ± 0.04	0.91 ± 0.04	0. 90 ± 0.05	0.87 ± 0.05	0.78 ± 0.03	.119
(μg/μL)	42	1.06 ± 0.04a	1.23 ± 0.09ab	1.45 ± 0.10b	1.27 ± 0.14ab	1.09 ± 0.07b	.047
Butyrate	21	1.81 ± 0.31	2.15 ± 0.13	2.08 ± 0.22	2.24 ± 0.11	2.18 ± 0.21	.666
(μg/μl)	42	1.08 ± 0.14	1.24 ± 0.12	1.29 ± 0.22	1.13 ± 0.23	1.25 ± 0.16	.905
Valerate	21	133.70 ± 10.24a	133.33 ± 2.29a	160.32 ± 5.18b	156.92 ± 6.51b	139.00 ± 9.05ab	.032
(ng/μL)	42	127.98 ± 8.70	158.81 ± 9.83	156.29 ± 14.79	132.13 ± 19.76	129.57 ± 10.07	.244
Total	21	7.80 ± 0.73	8.18 ± 0.36	9.20 ± 0.36	8.64 ± 0.23	7.74 ± 0.23	.057
(μg/μL)	42	7.85 ± 0.60	8.36 ± 0.63	8.44 ± 0.36	8.19 ± 1.19	8.30 ± 0.84	.966

Notes: Means within a row with different letters differ significantly ($P < .05$). CON = a basal diet; CB = a basal diet + *C. butyricum*; MLP = a basal diet + microencapsulated *L. plantarum*; MIX = a basal diet + *C. butyricum* + microencapsulated *L. plantarum*. ANT = a basal diet + *Aureomycin*.

group was significantly increased than that in the control group ($P < .05$). Birds in CB group had greater acetic acid level compared to birds in control groups ($P < .05$). At day 42, birds in MLP group and ANT group had the greater propionic acid level compared to birds in control group ($P < .05$).

Discussion

In recent years, many studies had shown that probiotics can promote growth performance in broiler chickens (Mountzouris et al., 2010; Zhao et al., 2013). Zhang, Yang, Guo, and Long (2011) observed that dietary supplementation of *C. butyricum* had no effect on growth performance in broilers. Yang et al. (2012) and Zhang et al. (2014) reported that dietary supplementation of *C. butyricum* significantly improved ADG in broilers, but no difference was observed between *C. butyricum*–supplemented group and the group receiving antibiotics. Cao, Chan, Chui, and Xiao (2012) also observed that dietary supplementation of *C. butyricum* improved growth performance of broilers. Peng et al. (2016) showed that *L. plantarum* B1 enhanced broiler ADG and FCR. In the present study, birds fed diets supplemented with CB、 MLP and MIX had lower FCR compared with that in control group ($P > .05$). The effect of CB and MLP supplementation on growth performance of broilers was consistent with the results of other studies (Cao et al., 2012; Peng et al., 2016).

Probiotics can benefit immune function and immune response in several animal models (Vanderpool, Yan, & Polk, 2008). The level of serum immunoglobulin is currently an important indicator. An increased immunoglobulin concentration has been associated with a benefit in the immune status, because IgM, IgG, and IgA are the main immunoglobulins protecting against pathogenic microorganisms (Fang et al., 2017; Herich, 2016). Yang et al. (2012) reported that diets supplemented with *C. butyricum* increased serum IgA, IgG, and IgM levels in broilers. The main effect of IgM is that when the body is violated by the pathogen, it is combined with the complement to dissolve the pathogenic bacteria, and finally the immune effect is achieved. IgG has a variety of antibacteria, viruses, and other exotoxin activity, especially plays an important role in humoral immunity. Wang et al. (2017) reported that diets supplemented with *L. plantarum* have higher IgG than the antibiotic. Wang et al. (2017) reported that diets supplemented with *L. plantarum* had higher serum IgG level than that in antibiotic group. Ren et al., (2017) reported that chitosan–alginate microcapsule would protect the IgG from severe gastric conditions and guarantee an efficient release in the intestinal tract. The results indicate that *C. butyricum* and *L. plantarum* can increase the level of serum immunoglobulin than the control group. The possible reason is that the metabolites of *C. butyricum* and *L. plantarum* are mainly protein and B vitamins, which can promote the resistance ability of poultry (Nakajima et al., 1999). But *C. butyricum* and *L. plantarum* did not show significant effect than the CB or the MLP group. The specific mechanism of action needs to be further studied.

The jejunum is the main region for nutrient absorption in broiler intestine, so the morphometric parameters can be used to evaluate intestinal function (Varel, Robinson, & Pond, 1987). Digestion and absorption of nutrients are accomplished by the action of a heterogeneous monolayer of columnar epithelial cells along the villus/crypt axis in small intestine. Healthy chickens tend to have longer villus, shallower crypts and larger

villus height/crypt depth ratio (Boka, Mahdavi, Samie, & Jahanian, 2014). In our study, the content of serum immunoglobulin in broilers is in accordance with the trend of intestinal morphological parameters, which is in accordance with the experimental results (Levkut et al., 2017). The current results indicated that the villus width, crypt depth, and the villus height/crypt depth ratio were greater in broilers received probiotics than those fed basal diet, which were in agreement with the result reported by Chichlowski et al. (2007), who reported that feeding of micro-direct microflora increased jejunal villus height and decreased crypt depth compared with the control group. The current results showed that the supplementation of microencapsulated *L. plantarum* and *C. butyricum* had no significant effect on morphometric parameters. The specific mechanism of action needs to be further studied. Similarly, the increased villus height and villus height-to-crypt depth ratio might be associated with the increased numbers of beneficial bacteria (Lactobacilli) (Xu, Hu, Xia, Zhan, & Wang, 2003).

VFAs mainly refer to acetic acid, propionic acid, butyric acid, which accounted for 95% of T-VFAs. One of the basic roles of VFAs in the animal body is to provide energy. Acetic acid, propionic acid and butyric acid are absorbed by the intestinal mucosal epithelial cells as energy source, which can promote gastrointestinal cell proliferation and maturation. Van der Wielen et al. (2000) reported that during growth of broiler chickens, VFAs are responsible for the reduction in numbers of *Enterobacteriaceae* in the caeca. The main fermentative chamber in broiler chickens is the caecum and this contains the largest number of bacteria compared with other gastrointestinal tract segment. Hence, the microbiota has high ability to ferment carbohydrates. *C. butyricum* can produce acetic acid, butyric acid and other organic acids in the intestine, which provides nutrients not only for animal body, but also reduce the intestinal pH, promote lactobacillus bacteria growth, inhibit the growth of pathogenic microorganisms, maintain the balance of intestinal flora and improve the animal health. Butyric acid is known to decrease virulence gene expression and invasion of *Salmonella* in epithelial cells in vitro; acetic acid has opposite effects (Lawhon, Maurer, Suyemoto, & Altier, 2002; Van Immerseel et al., 2004). In this study, we observed high concentrations of VFAs in caecal digesta of the broilers fed *C. butyricum*. The addition of *L. plantarum* and *C. butyricum* increased the concentration of SCFAs in caecal digesta compared with alone *L. plantarum* supplementation, which was consistent with the growth performance result. The results of the present study are also consistent with Sinha (1986), who reported that the major metabolites of lactobacillus bacteria were lactic acid and SCFAs, which are responsible for the antimicrobial activity against *E. coli* in the intestine. Hinton et al. (1992) also proved that lactobacillus bacteria can produce high concentrations of lactic acid, which decrease the pH of their environment and the growth of other bacteria.

Conclusions

In conclusion, the results suggest that addition of *C. butyricum* and *L. plantarum* in diets can increase serum immunoglobulin level, improve intestinal morphometric parameters, increase VFAs levels of cacal digeata, but this study did not show a significant synergistic effect between *C. butyricum* and *L. plantarum*. Therefore, it is necessary to further study the role of *C. butyricum* and *L. plantarum* in poultry diets and their interaction.

References

Boka, J., Mahdavi, A. H., Samie, A. H., & Jahanian, R. (2014). Effect of different levels of black cumin (nigella sativa l) on performance, intestinal escherichia coli colonization and jejunal morphology in laying hens. *Journal of Animal Physiology & Animal Nutrition*, 98(2), 373. doi:10.1111/jpn.12109

Cao, Y., Chan, F. D., Chui, Y., & Xiao, H. (2012). Characterization of flax fibres modified by alkaline, enzyme, and steam-heat treatments. *Bioresources*, 7(3), 4109–4121. doi:10.15376/biores.7.3.4109-412

Chichlowski, M., Croom, W., Edens, F., McBride, B., Qiu, R., & Chang, C. (2007). Microarchitecture and spatial relationship between bacteria and ileal, cecal, and colonic epithelium in chicks fed a direct-fed microbial, primalac, and salinomycin. *Poultry Science*, 86(6), 1121. doi:10.1093/ps/86.6.1121

Corona, P. S., Hemandez, A., Reverte-Vinaixa, M. M., Amat, C., & Flores, X. (2013). Outcome after knee arthrodesis for failed septic total knee replacement using a monolateral extermal fixator. *Journal of Orthopaedic Surgery*, 21(3), 275–80. doi:10.1177/230949901302100302

Dong, Z., Wang, Y., Song, D., Hou, Y., Wang, W., Qi, W., Yun, T., & Li, A. (2016). The effects of dietary supplementation of microencapsulated enterococcus fecalis and the extract of camellia oleifera seed on growth performance, intestinal morphology, and intestinal mucosal immune functions in broiler chickens. *Animal Feed Science & Technology*, 212, 42–51. doi:10.1016/j.anifeedsci.2015.11.014

Fang, J., Martínez, Y., Deng, C., Zhu, D., Peng, H., Jiang, H., & Li, A. (2017). Effects of dietary enzymolysis products of wheat gluten on the growth performance, serum biochemical, immune, and antioxidant status of broilers. *Food & Agricultural Immunology* (2), 1–13. doi:10.1080/09540105.2017.1332009

Herich, R. (2016). Is the role of iga in local immunity completely known? *Food & Agricultural Immunology*, 28(2), 223–237. doi:10.1080/09540105.2016.1258547

Hinton, A., Corrier, D. E., & Deloach, J. R. (1992). Inhibition of the Growth of Salmonella typhimurium and Escherichia coli 0157:H7 on Chicken Feed Media by Bacteria Isolated from the Intestinal Microflora of Chichens. *Journal of Food Protection*, 55(6), 419–423. doi:10.4315/0362-028X-55.6.419

Hu, Y., Wang, Y., Li, A., Wang, Z., Zhang, X., Yun, T., Qiu, H., & Yin, Y. (2016). Effects of fermented rapeseed meal on antioxidant functions, serum biochemical parameters and intestinal morphology in broilers. *Food & Agricultural Immunology*, 27(2), 182–193. doi:10.1080/09540105.2015.1079592

Jo, G., Choe, M., Lee, S., Park, W., Kahng, Y. H., & Lee, T. (2012). The application of graphene as electrodes in electrical and optical devices. *Nanotechnology*, 23(11), 112001. doi:10.1088/0957-4484/23/11/112001

Jozefiak, D., Kaczmarek, S., & Rutkowski, A. (2008). A note on the effects of selected prebiotics on the performance and ileal microbiota of broiler chickens. *Journal of Animal and Feed Sciences*, *17* (3), 392–397. doi:10.22358/jafs/66633/2008

Kong, M., Chen, X. G., Kweon, D. K, & Park, H. J. (2011). Investigations on skin permeation of hyaluronic acid based nanoemulsion as transdermal carrier. *Carbohydrate Polymers*, *86*(2), 837–843. doi:10.1016/j.carbpol.2011.05.027

Lawhon, S. D., Maurer, R., Suyemoto, M., & Altier, C. (2002). Intestinal short-chain fatty acids alter *Salmonella typhimurium* invasion gene expression and virulence through BarA/SirA. *Molecular Microbiology*, *46*(5), 1451–1464. doi:10.1046/j.1365-2958.2002.03268.x

Levkut, M., Husáková, E., Bobíková, K., Karaffová, V., Levkutová, M., & Ivanišinová, O. (2017). Inorganic or organic zinc and muc-2, iga, il-17, tgf-β4 gene expression and siga secretion in broiler chickens. *Food & Agricultural Immunology*, 1–11. doi:10.1080/09540105.2017.1313202

Mountzouris, K. C., Tsitrsikos, P., Palamidi, I., Arvaniti, A., Mohnl, M., Schatzmayr, G., & Fegeros, K. (2010). Effects of probiotic inclusion levels in broiler nutrition on growth performance, nutrient digestibility, plasma immunoglobulins, and cecal microflora composition. *Poultry Science*, *89* (1), 58–67. doi:10.3382/ps.2009-00308.

Nakajima, T., Koh, M., Singh, R. N., & Shimada, M. (1999). Electrochemical behavior of surface-fluprinated graphite. *Electrochimica Acta*, *44*(17), 2879–2888. doi:10.1016/S0013-4686 (99)00048-1

NY/T 33-2004. Nutrient requirements of Chinese feeding standard of chicken. Beijing: The Ministry of Agriculture of the People's Republic of China.

Okamoto, M., Abe, M., Shirai, H., & Ueda, N. (2000). Diagnostic ultrasonography of the ulnar nerve in cubital tunnel syndrome. *Journal of Hand Surgery (European Volume)*, *25*(5), 499–502. doi:10. 1054/jhsb.1999.0350

Peng, Q., Zeng, X., Zhu, J., Wang, S., Liu, X., Hou, C., Thacker, P., & Qiao, S. (2016). Effects of dietary lactobacillus plantarum B1 on growth performance, intestinal microbiota, and short chain fatty acid profiles in broiler chickens. *Poultry Science*, *95*, 893–900. doi:10.3382/ps/pev435

Ren, Z., Zhang, X., Guo, Y., Han, K., & Huo, N. (2017). Preparation and in vitro delivery performance of chitosan-alginate microcapsule for igg. *Food & Agricultural Immunology*, *28*(1), 1–13. doi:10.1080/09540105.2016.1202206

Sinha, R. P. (1986). Toxicity of organic acids for repair-deficient strains of *Escherichia coli*. *Applied & Environmental Microbiology*, *51*(6), 1364–1366.

Vanderpool, C., Yan, F., & Polk, D. (2008). Mechanisms of probiotic action: Implications for therapeutic applications in inflammatory bowel diseases. *Inflammatory Bowel Diseases*, *14*, 1585. doi:10.1002/ibd.20525

Van der Wielen, P. W., Biesterveld, S., Notermans, S., Hofstra, H., Urlings, B. A., & van Knapen, F. (2000). Role of volatile fatty acids in development of the cecal microflora in broiler chickens during growth. *Applied and Environmental Microbiology*, *66*, 2536–2540. doi:10.1128/AEM.66. 6.2536-2540.2000

Van Immerseel, F., De Buck, J., Pasmans, F., Bohez, L., Boyen, F., Haesebrouck, F., & Ducatelle, R. (2004). Intermittent long-term shedding and induction of carrier birds after infection of chickens early posthatch with a low or high dose of *Salmonella enteritidis*. *Poultry Science*, *83*, 1911–1916. doi:10.1093/ps/83.11.1911

Varel, V. H., Robinson, I. M., & Pond, W. G. (1987). Effect of dietary copper sulfate, Aureo SP250, or clinoptilolite on ureolytic bacteria found in the pig large intestine. *Applied and Environmental Microbiology*, *53*, 2009–2012.

Wang, W., Chen, J., Zhou, H., Wang, L., Ding, S., Wang, Y., Song, D., & Li, A. (2017). Effects of microencapsulated *Lactobacillus plantarum* and fructooligosaccharide on growth performance, blood immune parameters, and intestinal morphology in weaned piglets. *Food & Agricultural Immunology*, 1–11. doi:10.1080/09540105.2017.1360254

Wang, Y., Deng, Q., Song, D., Wang, W., Zhou, H., Wang, L., & Li, A. (2017). Effects of fermented cottonseed meal on growth performance, serum biochemical parameters, immune functions, antioxidative abilities, and cecal microflora in broilers. *Food & Agricultural Immunology*, 1–14. doi:10.1080/09540105.2017.1311308

Xu, Z., Hu, C., Xia, M., Zhan, X., & Wang, M. (2003). Effects of dietary fructooligosaccharide on digestive enzyme activities, intestinal microflora and morphology of malebroilers. *Poultry Science*, *82*(6), 1030. doi:10.1093/ps/82.6.1030

Yang, C., Cao, G., Ferket, P., Liu, T., Zhou, L., Zhang, L., Xiao, Y., & Chen, A. (2012). Effects of probiotic, *Clostridium butyricum*, on growth performance, immune function, and cecal microflora in broiler chickens. *Poultry Science*, *91*, 2121–2129. doi:10.3382/ps.2011-02131

Yeoman, C. J,, Chia, N., & Jeraldo, P. (2012). The microbiome of the chicken gastrointestinal tract. .*Anim Health Res Rev*, *13*(1), 89–99.

Zhang, L., Cao, G., Zeng, X., Zhou, L., Ferket, P., Xiao, Y., Chen, A., & Yang, C. (2014). Effects of *Clostridium butyricum* on growth performance, immune function, and cecal microflora in broiler chickens challenged with *Escherichia coli* K88. *Poultry Science*, *93*, 46–53. doi:103382/ps.2013-03412

Zhang, W., Li, D., Lu, W., & Yi, G. (2003). Effects of isomalto-oligosaccharides on broiler performance and intestinal microflora. *Poultry Science*, *82*, 657–663. doi:10.1093/ps/82.4.657

Zhang, B., Yang, X., Guo, Y., & Long, F. (2011). Effects of dietary lipids and *Clostridium butyricum* on the performance and the digestive tract of broiler chickens. *Archives of Animal Nutrition*, *65*, 329–339. doi:10.1080/1245039X.2011.568274

Zhao, X., Guo, Y., Guo, S., & Tan, J. (2013). Effects of *Clostridium butyricum* and enterococcus faecium on growth performance, lipid metabolism, and cecal microbiota of broiler chickens. *Applied Microbiology and Biotechnology*, *97*, 6477–6488. doi:10.1371/journal.pone.0010463