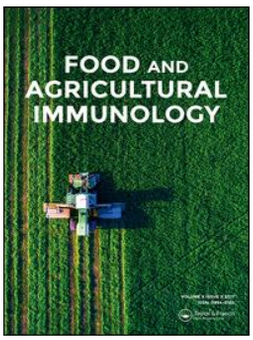

Food and Agricultural Immunology

ISSN: 0954-0105 (Print) 1465-3443 (Online) Journal homepage: https://www.tandfonline.com/loi/cfai20

Effects of microencapsulated probiotics and plant extract on antioxidant ability, immune status and caecal microflora in *Escherichia coli* K88-challenged broiler chickens

Z. L. Dong, Y. W. Wang, D. Song, W. W. Wang, K. B. Liu, L. Wang & A. K. Li

To cite this article: Z. L. Dong, Y. W. Wang, D. Song, W. W. Wang, K. B. Liu, L. Wang & A. K. Li (2019) Effects of microencapsulated probiotics and plant extract on antioxidant ability, immune status and caecal microflora in *Escherichia coli* K88-challenged broiler chickens, Food and Agricultural Immunology, 30:1, 1123-1134, DOI: 10.1080/09540105.2019.1664419

To link to this article: https://doi.org/10.1080/09540105.2019.1664419

Published online: 22 Sep 2019.

FOOD AND AGRICULTURAL IMMUNOLOGY
2019, VOL. 30, NO. 1, 1123–1134
https://doi.org/10.1080/09540105.2019.1664419

Effects of microencapsulated probiotics and plant extract on antioxidant ability, immune status and caecal microflora in *Escherichia coli* K88-challenged broiler chickens

Z. L. Dong[a,b], Y. W. Wang[a], D. Song[a], W. W. Wang[a], K. B. Liu[a], L. Wang[a] and A. K. Li[a]

[a]Academy of National Food and Strategical Reserves Administration, Beijing, People's Republic of China; [b]Hunan International Joint Laboratory of Animal Intestinal Ecology and Health, Laboratory of Animal Nutrition and Human Health, College of Life Sciences, Hunan Normal University, Changsha, Hunan, People's Republic of China

ABSTRACT

In this study, a total of 180 one-day-old male Arbor Acres broilers were randomly allotted to 5 groups with 6 replicates per group. Birds in negative control group were fed a corn-soybean meal-based diet, and birds in positive control group, MEF group, COSE group, MEF + COSE group were challenged with *E. coli* K88 and supplemented with 0, 1×10^{10} cfu MEF/kg of diets, 500 mg COSE/kg of diets or their combinations, respectively. Results showed that PC group had lower average body weight and greater *E. coli* counts in caecal contents than NC group. MEF and COSE significantly increased average body weight, serum IgA level, T-SOD activity compared with that of PC group. MEF and MIX group had less caecal *E. coli* counts than PC birds. The results indicated dietary supplementation of MEF and COSE had a positive moderating effect on *E. coli* K88-challenged broiler chickens.

ARTICLE HISTORY
Received 1 July 2019
Accepted 21 August 2019

KEYWORDS
Probiotics; plant extract; broiler; *E. coli* K88

Introduction

Enterotoxigenic *Escherichia coli* K88 is a major pathogen responsible for the diarrhoea and infection, which could result in poor growth, increased mortality and a great economic loss in poultry industry (Alonso, Padola, Parma, & Lucchesi, 2011). Although vaccination strategies include the antibiotics could control colibacillosis and promote the growth of broiler chickens, the excessive uses of antibiotics lead to antibiotic residues in animal products and the production of drug-resistant bacteria (Iii & Drew, 2000). The use of antibiotics has been banned in European Union in 2006 and limited in the United States of America by the Veterinary Feed Directive (Food and Drug Administration, 2000). So research aimed at identifying new antibiotic substitutes is very urgent to poultry industry.

Beneficial effects of probiotics highly depend on species- and strain-specific, tolerance and adhesion in gastrointestinal tract (Corona-Hernandez et al., 2013). Lactic acid

CONTACT Y. W. Wang wyw@chinagrain Academy of National Food and Strategical Reserves Administration, Beijing 100037, People's Republic of China; A. K. Li lak@chinagrain.org Academy of National Food and Strategical Reserves Administration, Beijing 100037, People's Republic of China

bacteria, such as *Enterococcus faecalis*, play an important role in intestinal tract by modulating gut microflora of animals (Han et al., 2018; Wang et al., 2018). However, lactic acid bacteria are very sensitive to the external environment, such as feed process, storage, and gastrointestinal tract of animal (Corona-Hernandez, Alvarez-Parrilla, Lizardi-Mendoza, Islas-Rubio, & de la Rosa, 2013; Prakash, Tomaro-Duchesneau, Saha, & Cantor, 2011). Microencapsulated probiotics have beneficial effects on enhancing the survivability of probiotics to the adverse environment in the gastrointestinal tract and on improving the overall health of broilers (Corona-Hernandez et al., 2008; Zhang, Li, Yun, Qi, et al., 2015b). Furthermore, the antimicrobial protein produced by *E. faecalis* showed antibacterial activity against *Escherichia coli*, so *E. faecalis* might be used for inhibition of the growth of pathogenic bacteria (Pantev et al., 2003; Shekh & Roy, 2012).

Plant extract is a class of new alternatives to antibiotics, and may act as prophylactic agent (Spišáková et al., 2013). *C. oleifera* seed is one of the important sources of high-quality edible oil in China (And & Yen, 2006). Saponins, oligosaccharides and polysaccharides extracted from *C. oleifera* seed have many biological effects, such as immunostimulant, hypocholesterolaemic and antimicrobial properties (Liu, Jia, Gao, Li, & Tu, 2014; Ye, Yang, Fang, & Li, 2015), but there were only a few reports on the effects of *C. oleifera* seed extract in poultry, especially under infections condition.

Therefore, this study was to investigate the effects of dietary microencapsulated *E. faecalis* or/and *C. oleifera* seed extract supplementation on growth performance, serum antioxidant ability, immune status and caecal microflora in broiler chickens infected with *E. coli* K88.

Materials and methods

Diets

Microencapsulated *E. faecalis* products were produced by our research group according to Zhang et al. (2015a). The microencapsulated product was analysed to contain 1×10^{10} cfu *E. faecalis*/g of product. The mean particle size of microencapsulated *E. faecalis* product was 631 μm (Mastersizer-2000 Laser Particle Analyzer, Malvern Instruments, Ltd., Malvern, UK). *C. oleifera* seed extract was prepared using aqueous enzymatic method, which can synchronously obtain high quality oil and unsaponifiable lipid by using enzymes (protease, amylase, pectinase, cellulase) to disintegrate the cell walls of oilseeds and hydrolyse the lipoprotein in the cells (De Moura & Johnson, 2009). The product is obtained with light brown colour, with 30% *C. Oleifera* polysaccharide and 30% saponin.

Experimental diets (Table 1), free of antibiotics, in mash form, were formulated to meet nutrient requirements of Chinese feeding standard of chicken (NY/T 33-2004). Experimental diets and water were provided *ad libitum*. Environmental temperature in the rooms was maintained at 32–35°C in the first week and then gradually reduced to 25°C until the end of the experiment.

Birds and experimental procedure

A total of 180 one-day-old male Arbor Acres broilers were obtained from a commercial hatchery (Huadu Broiler Breeding Farms, Beijing, China). The broilers were randomly

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

Ingredient	Day 0–28
Corn	557.50
Soybean meal	367.50
Soybean oil	29.60
Dicalcium phosphate	18.60
Limestone	12.00
Salt	3.50
L-Lysine HCl	3.09
L-Methionine	2.87
L-Threonine	0.50
Choline chloride (50%)	2.60
Vitamin premix[a]	0.20
Mineral premix[b]	2.00
Total	1000.00
Calculated chemical composition (g/kg diet as fed basis)	
ME (kcal/kg)	3200.00
Crude protein	230.00
Calcium	10.00
Total phosphorus	7.00
Available phosphorus	4.10
Lysine	11.00
Methionine	5.70

[a]Vitamin premix provided the following per kilogram of diet; vitamin A, 9500 IU; vitamin D_3, 62.5 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.

allotted to 5 groups with 6 replicates per group and there were 6 broilers per replicate. The treatments were as follows: negative control (NC), birds were fed a basal diet and not challenged with *E. coli* K88; positive control (PC), birds were fed a basal diet and challenged with *E. col*i K88; MEF group (MEF), birds were fed a diet containing 1×10^{10} cfu *E. faecalis*/kg of diet and challenged with *E. coli* K88; COSE group (COSE), birds were fed a diet containing 500 mg COSE/kg of diet and challenged with *E. coli* K88; MEF + COSE group (MIX), birds were fed a diet containing 1×10^{10} cfu *E. faecalis*/kg of diet and 500 mg COSE/kg of diet and challenged with *E. coli* K88.

Feeding experiment was from day 1 to day 28. The *E. coli* K88 strain was originally obtained from the China Institute of Veterinary Drug Control (Beijing, China). The frozen strain was thawed and 100 μL was inoculated into sterile tubes containing 10 mL of sterile Luria–Bertani (LB) broth. The inoculated broth was incubated at 37°C with orbital shaking for 24 h (HZQ incubator; Harbin Donglian Electronic Technology Co. Ltd., Heilongjiang, China). Subsequently, 1 mL of *E. coli* K88 preculture was transferred to 100 mL of LB broth and incubated with orbital shaking at 37°C for 18 h. The inoculum was diluted with sterile saline solution and plated on LB agar culture media, and the concentrations of viable *E. coli* K88 were counted after incubating for 24 h at 37°C. The stock culture was prepared in sterile saline solution and adjusted to 1×10^9 cfu/mL of *E. coli* K88 as the inoculum.

From day 7 to 14, each bird in challenged groups was orally dosed with 1 mL inoculum (10^9 cfu/mL of *E. coli* K88) per day using a polyethylene tube attached to a syringe. The birds in NC group were administered similarly with the same amount of saline solution.

Broilers care and handling were in compliance with the Animal Ethics Committee Guidelines of Academy of National Food and Strategical Reserves Administration

(Beijing, China) following guidelines recommended in the Guide for the Care and Use of Agricultural Animals in Research and Teaching (FASS, 2010).

Sampling

Birds fasted for 12 h were weighed on a cage basis at day 21 and day 28 to evaluate growth performance. At day 21 and day 28, one bird was randomly selected from each cage, and blood samples were taken from the wing vein. The serum samples were harvested after centrifugation (3000 g, 10 min) at 4°C and stored at −20°C. The concentrations of serum immunoglobulin levels were measured using chicken-specific ELISA kits (Uscn Life Science INC., Wuhan, China) according to the ELISA procedures described by the protocol. Another set of serum sample was collected for the determination of malondialdehyde (MDA) level (cat#: A003-1), glutathione peroxidase (GSH-Px) activity (cat#: A005), total superoxide dismutase T-SOD activity (cat#: A001-3). The enzyme activities were measured using commercially available colorimetric diagnostic kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

At day 21 and day 28, after collecting serum samples, the birds were killed by jugular bleeding. Liver, bursa of fabricius, spleen were weighed. Relative organ weight was expressed as the organ weight (g)/body weight (kg).

The caecal contents were removed and put in sterilized tubes, then stored at −20°C for subsequent enumeration of microbial population. 0.5 g caecal contents were diluted with 4.5 mL of phosphate buffer saline in a flask and then diluted 10-fold from 10^{-2} to 10^{-9}. Diluent then were plated on Eosin Methylene Blue agar at 37°C for 24 h to enumerate *E. coli*, and on *Lactobacillus* select agar to enumerate *Lactobacillus* incubating in an anaerobic incubator at 37°C for 48 h, respectively. Flat colony counting method by counting the bacteria was to determine caecal contents. Results were reported as $\log^{10}$ cfu/g of caecal *Lactobacillus* and *E. coli*. All agars were obtained from Hopebiol, Bio-technology Co., Ltd (Qingdao, China).

Statistical analysis

All data were analysed using SPSS 17.0 (SPSS INC., Chicago, IL) for one-way ANOVA. Differences among means of treatments were compared using Duncan's new multiple range test. Flora counts were transformed to logarithms before analysis. Differences were considered statistically significant at $P \leq .05$.

Results

Growth performance

The results of growth performance were shown in Table 2. Birds in PC group had lower average body weight than NC birds on day 21 ($P < .05$). Birds in MEF, MIX or COSE groups had greater average body weight than PC birds at day 21 ($P < .05$). No significant differences in average body weight were observed among birds in MEF, COSE and MIX at day 28. Feed consumption and feed-to-gain ratio were not calculated due to an indeterminate amount of feed wastage.

Table 2. Effects of microencapsulated *E. faecalis* and *C. oleifera* seed extract on growth performance in *E. coli* K88-challenged broilers.

Item	NC	PC	MEF	COSE	MIX	*P*-value
Day 21						
ABW (g/bird)	693 ± 47[b]	616 ± 43[a]	690 ± 40[b]	680 ± 51[b]	676 ± 24[b]	<.001
Day 28						
ABW (kg/bird)	1.10 ± 0.09	0.98 ± 0.05	1.08 ± 0.06	1.09 ± 0.10	1.08 ± 0.13	.219

[a,b]Means within a row with different letters differ significantly ($P < .05$).
ABW = average body weight.
Treatments: NC = a basal diet; PC = a basal diet + *E. coli* K88; MEF = a basal diet + microencapsulated *E. faecalis* + *E. coli* K88; COSE = a basal diet + *C. oleifera* seed extract + *E. coli* K88; MIX = a basal diet + microencapsulated *E. faecalis* + *C. oleifera* seed extract + *E. coli* K88.

Relative organ weight

The results of relative organ weights were shown in Table 3. Birds in MEF and MIX groups had greater relative liver weight than PC birds on day 21 and day 28 ($P < .05$). Birds in COSE or MIX groups had greater relative spleen weight ($P < .05$) and birds in COSE group had a greater relative weight of bursa of fabricius ($P < .05$) than NC and PC birds at day 21.

Serum paramaters

Birds in PC group had greater ($P < .05$) serum IgA level than birds in NC group at day 21 (Table 4). Birds in MEF and COSE groups had greater serum IgA level ($P < .05$), and birds in COSE or MIX groups had greater serum IgM level ($P < .05$) than NC and PC birds at day 21. Birds in MEF and MIX groups had greater serum IgA and birds in MIX group had greater serum IgM level ($P < .05$) than PC birds at day 28.

According to the results in Table 5, birds in PC group had greater serum MDA level than birds in NC group at day 21 ($P < .05$). Birds in MEF, COSE and MIX groups had lower serum MDA level, and greater serum T-SOD activity than PC birds ($P < .05$), and birds in COSE group had greater serum GSH-Px activity than NC and PC birds ($P < .05$) at day 21. Birds in MEF, COSE or MIX groups had lower serum MDA level, and greater serum GSH-Px activity ($P < .05$), and birds in MEF or COSE groups had greater serum T-SOD activity than PC birds ($P < .05$) at day 28.

Table 3. Effects of microencapsulated *E. faecalis* and *C. oleifera* seed extract on relative organ weight of broilers challenged with *E. coli* K88 (organ weight (g)/body weight (kg)).

Item	NC	PC	MEF	COSE	MIX	*P*-value
Day 21						
Liver	28.0 ± 3.4[a]	29.1 ± 3.1[a,b]	34.5 ± 4.2[c]	32.5 ± 2.3[b,c]	34.0 ± 3.8[c]	.002
Spleen	0.85 ± 0.1[a]	0.83 ± 0.1[a]	0.87 ± 0.1[a,b]	0.99 ± 0.1[b,c]	1.02 ± 0.2[c]	.014
Bursa of fabricius	1.78 ± 0.3[a]	1.91 ± 0.2[a,b]	2.16 ± 0.3[b]	2.52 ± 0.4[c]	2.20 ± 0.2[b]	.002
Day 28						
Liver	25.9 ± 4.4[a,b]	24.1 ± 2.8[a]	27.7 ± 1.7[bc]	30.0 ± 2.5[c]	28.4 ± 3.2[b,c]	.008
Spleen	1.09 ± 0.2	0.99 ± 0.1	1.04 ± 0.1	1.06 ± 0.2	1.06 ± 0.2	.816
Bursa of fabricius	2.12 ± 0.4	1.59 ± 0.2	1.82 ± 0.4	2.18 ± 0.5	2.21 ± 0.6	.061

[a,b,c] Means within a row with different letters differ significantly ($P < .05$).
Treatments: NC = a basal diet; PC = a basal diet + *E. coli* K88; MEF = a basal diet + microencapsulated *E. faecalis* + *E. coli* K88; COSE = a basal diet + *C. oleifera* seed extract + *E. coli* K88; MIX = a basal diet + microencapsulated *E. faecalis* + *C. oleifera* seed extract + *E. coli* K88.

Table 4. Effects of microencapsulated *E. faecalis* and *C. oleifera* seed extract on immunoglobulin level in *E. coli* K88-challenged broilers.

Item	NC	PC	MEF	COSE	MIX	*P*-value
Day 21						
IgA (μg/mL)	1.61 ± 0.2^{a}	1.95 ± 0.1^{b}	3.15 ± 0.2^{d}	2.71 ± 0.4^{c}	$1.79 \pm 0.2^{a,b}$	<.001
IgM (μg/mL)	29.4 ± 1.6^{a}	28.5 ± 3.4^{a}	$29.6 \pm 8.8^{a,b}$	43.4 ± 2.7^{b}	34.0 ± 0.9^{b}	<.001
IgG (mg/mL)	3.44 ± 0.1	3.15 ± 0.2	3.53 ± 0.3	3.24 ± 0.4	3.62 ± 0.4	.086
Day 28						
IgA (μg/mL)	1.21 ± 0.2^{a}	1.03 ± 0.2^{a}	1.7 ± 0.1^{b}	$1.44 \pm 0.1^{a,b}$	1.84 ± 0.6^{b}	.010
IgM (μg/mL)	27.5 ± 5.4^{a}	27.6 ± 4.0^{a}	$33.4 \pm 3.2^{a,b}$	$31.7 \pm 4.6^{a,b}$	35.1 ± 4.5^{b}	.044
IgG (mg/mL)	6.47 ± 1.8	6.17 ± 1.2	7.14 ± 1.4	7.69 ± 1.5	7.86 ± 0.9	.244

[a,b,c] Means within a row with different letters differ significantly ($P < .05$).

Treatments: NC = a basal diet; PC = a basal diet + *E. coli* K88; MEF = a basal diet + microencapsulated *E. faecalis* + *E. coli* K88; COSE = a basal diet + *C. oleifera* seed extract + *E. coli* K88; MIX = a basal diet + microencapsulated *E. faecalis* + *C. oleifera* seed extract + *E. coli* K88.

Microbiota of caecal contents

Results of caecal microbiota were shown in Figure 1. Birds in PC group had greater ($P < .05$) *E. coli* counts in caecal contents than NC birds at day 21 and 28. Birds in MEF group had greater caecal *Lactobacillus* counts ($P < .05$), and birds in MEF and MIX groups had less caecal *E. coli* counts than PC birds ($P < .05$) at day 21. Birds in COSE group had less caecal *E. coli* counts than PC birds ($P < .05$) at day 28.

Discussion

Enterotoxigenic *E. coli* K88 colonized in the intestine can cause diarrhoea, release enterotoxins to destroy the structure and function of intestinal epithelial cells in birds, and impair the growth of birds (Zhang et al., 2012). The current results showed that the average body weight of chickens was depressed by *E. coli* K88 challenging. Function disorder of intestinal tract may be due to that *E. coli* bind to ganglioside on the intestinal epithelial cell surface and reduce total percentage of intestinal mucosa, which therefore change the host metabolic pattern and decrease growth performance (Fairbrother, Nadeau, & Gyles, 2005; Sugiharto, Hedemann, Jensen, & Lauridsen, 2012).

Probiotics have positive influences on the modulation either innate or acquired immunity, or both (Fabricio, João, Z, & Gil-Turnes, 2002; Santiago-López, Hernández-

Table 5. Effects of microencapsulated *E. faecalis* and *C. oleifera* seed extract on anti-oxidative capacity *in E. coli* K88-challenged broilers.

Item	NC	PC	MEF	COSE	MIX	*P*-value
Day 21						
MDA (nmol/mL)	3.42 ± 0.5^{a}	6.28 ± 0.7^{c}	$3.38 \pm$?0.6^{a}	4.71 ± 0.7^{b}	3.83 ± 0.5^{a}	<.001
GSH-Px, U/mL	1793 ± 166^{a}	1695 ± 137^{a}	$1936 \pm 157^{a,b}$	2187 ± 246^{b}	1748 ± 258^{a}	.005
T-SOD (U/mL)	58.9 ± 7^{a}	60.5 ± 8^{a}	100 ± 21^{c}	90.7 ± 12^{bc}	81.6 ± 11^{b}	<.001
Day 28						
MDA (nmol/mL)	$6.19 \pm 0.8^{a,b}$	7.26 ± 1^{b}	5.94 ± 1.1^{a}	5.47 ± 0.7^{a}	5.11 ± 1.4^{a}	.007
GSH-Px (U/mL)	1658 ± 152^{b}	1360 ± 172^{a}	$1776 \pm 188^{b,c}$	1973 ± 145^{c}	$1881 \pm 176^{b,c}$	.001
T-SOD (U/mL)	76.8 ± 5^{a}	$85.5 \pm 10^{a,b}$	101 ± 11^{c}	102 ± 9^{c}	$93.2 \pm 10^{b,c}$	<.001

[a,b,c] Means within a row with different letters differ significantly ($P < .05$).

MDA = Malondialdehyde, GSH-Px = Glutathione peroxidase, T-SOD = Total superoxide dismutase.

Treatments: NC = a basal diet; PC = a basal diet + *E. coli* K88; MEF = a basal diet + microencapsulated *E. faecalis* + *E. coli* K88; COSE = a basal diet + *C. oleifera* seed extract + *E. coli* K88; MIX = a basal diet + microencapsulated *E. faecalis* + *C. oleifera* seed extract + *E. coli* K88.

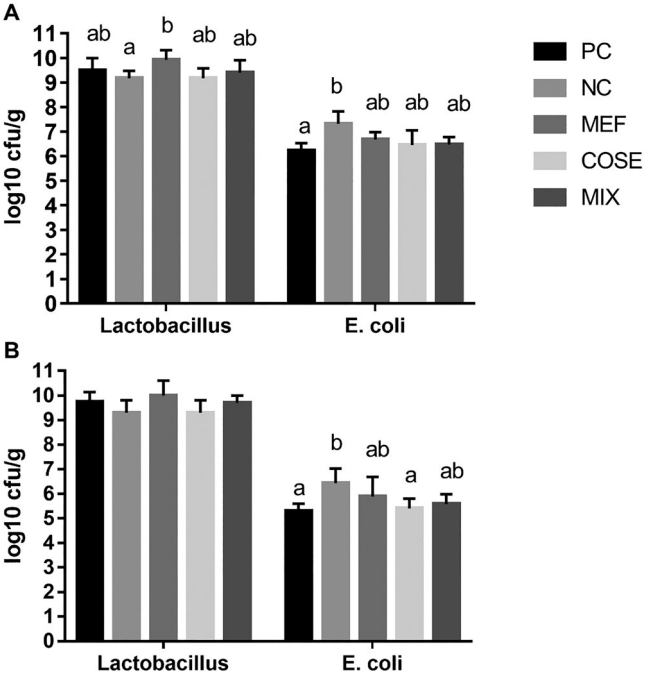

Figure 1. Effects of microencapsulated *E. faecalis* and *C. oleifera* seed extract on microflora of caecal content in *E. coli* K88-challenged broilers at 21 d (A) and 28 d (B). [a,b] Means within a row with different letters differ significantly ($P < .05$). Treatments: NC = a basal diet; PC = a basal diet + *E. coli* K88; MEF = a basal diet + microencapsulated *E. faecalis* + *E. coli* K88; COSE = a basal diet + *C. oleifera* seed extract + *E. coli* K88; MIX = a basal diet + microencapsulated *E. faecalis* + *C. oleifera* seed extract + *E. coli* K88.

Mendoza, Mata-Haro, Vallejo-Cordoba, & González-Córdova, 2018). Different strains of *Lactobacillus* may influence the immune system by promoting immune organ development (Brisbin, Gong, & Sharif, 2008), increasing serum cytokine levels (Brisbin et al., 2011). Haghighi, Gong, Hayes, Sanei, and Parvizi (2005, 2006) found that *Lactobacillus acidophilus, Bifidobacterium bifidum,* and *Streptococcus faecalis* enhanced the systemic antibody response in chickens. In the current study, microencapsulated *E. faecalis* significantly increased serum IgA level. Extrapolating from results of Brisbin et al. (2011) and Dong et al. (2016), *E. faecalis* mainly plays a fundamental role on protective or immune responses to intestinal homeostasis.

The antioxidative properties of bacteria, such as *Bifadobacterium longum*, *Lactobacillus acidophilus, Lactobacillus* were described (Amaretti et al., 2013). Serum MDA and T-SOD activities are the main parameters to assess oxidative status. Results of this study showed *E. faecalis* supplementation significantly reduced serum MDA level and enhanced serum T-SOD activity ($P < .05$), so it is suggested that dietary microencapsulated *E. faecalis* supplementation may be beneficial to alleviate lipid peroxidation and oxidative stress in *E. coli* K88-challenged broiler chickens.

Probiotics (especially *Lactobacillus* species) promote gut defence function and intestinal microflora balance (Stašová et al., 2015). The current study indicated microencapsulated *E. faecalis* effectively alleviated the growth suppression caused by *E. coli* K88 infection. It is

known that *E. faecium* strain significantly reduce the number of *E. coli* pathotypes adhering to the gut mucosa (Carmen, Sebastian, & Kathrin, 2013). Similar results reported by Cao et al. (2013) demonstrated that *E. faecium* supplementation improved growth performance by decreasing caecal *E. coli* counts in broilers. Our results suggest microencapsulated *E. faecalis* enhanced host health status through maintaining a beneficial intestinal microbiota, with caecal *E. coli* counts decreased and caecal *Lactobacillus* counts increased in challenged broilers. This effect might be attributed to that some products of lactic acid bacteria fermentation like lactic acid and short-chain fatty acids decrease the pH value of gut, thereby inhibiting the activity of pathogenic bacteria, and contributing to intestinal microbial balance (Pantev et al., 2003).

The current result indicated dietary supplementation of *C. oleifera* seed extract at 0.5 g/kg had positive effects on body weight of *E. coli* K88-challenged birds. However, some other studies reported that triterpenoids in *Camellia* plants were a class of compounds and could have positive or negative effects on animals. Khalaji et al. (2011) demonstrated that dietary supplementation with 0.3 g/kg *Camellia sinensis* L. plant extract had no effect on growth performance while the dose of diet at 0.5 g/kg decreased body weight and feed intake in broilers. Negative effects of *C. oleifera* may have been ascribed to negative properties of saponins, such as feed intake depression caused by the astringent and irritating taste of saponins (Oleszek, Nowacka, Gee, Wortley, & Johnson, 1994) and low ability of protein digestibility (Shimoyamada, Ikedo, Ootsubo, & Watanabe, 1998). On the other hand, similar result was observed by Ye et al. (2015) demonstrated that diet supplemented with 0.25 g/kg or 0.5 g/kg saponins increased body weight of broilers infected with *E. coli*. The variations results from different studies might be attributed to the source of saponins, additive amount of saponins, and saponins supplementation in different condition.

Many plant seed extracts have shown the beneficial effect on immune responses (Chen et al., 2013; Debnath et al., 2018). Zhai, Li, Wang, and Hu (2011, 2014) reported that saponins derived from ginseng could be used as immune enhancers against infectious diseases in poultry. Qiu, Hu, and Cui (2007) and Liang, Liu, and Zhao (2013) reported polysaccharides derived from plants extract had significant immunostimulating effects in broilers. Furthermore, Chen et al. (2013) showed saponins from radix trichosanthis had potential antioxidant activity both in *vitro* and in *vivo*, and similar findings have also been reported that saponins from ginseng stem-leaf could be a promising agent against oxidative stress (Yu et al., 2015). This study evaluated the effects of *C. oleifera* seed extract on immune function and antioxidative ability in broilers challenged with *E. coli* K88. Our results showed that 0.05% *C. oleifera* seed extract and combination with *E. faecalis* significantly increased relative spleen weight, serum IgA and IgM levels, T-SOD and GSH-Px activity. These results indicated that plant extracts containing high-level saponins might improve serum immunity and antioxidant capacity to defence their susceptibility to *E. coli* infection.

Francis, Kerem, Makkar, and Becker (2002) and Zhang, Yang, Han, and Zhao (2014) studied triterpene saponins obtained from the *C. oleifera Abel* seed pomace and found that triterpene saponins exhibited inhibitory effects against *Staphyloccocus aure*us and *E. coli*. Ye et al. (2015) also demonstrated that saponin identified as *camelliagenin* significantly inhibited the formation of the biofilm of *E. coli* and *S. aureus*. In this study, birds receiving *C. oleifera* seed extract had less caecal *E. coli* counts in caecal contents. The potential antibacterial activity may be associated with the interaction between saponins

and gram-negative cells components. Saponins binds to lipid A, which increases the permeability of bacterial cell wall, and then reduce motility and surface hydrophobicity of *E. coli* (Arabski, Wasik, Dworecki, & Kaca, 2009; Wojnicz, Kucharska, Kicia, & Tichaczek-goska, 2012).

Conclusion

The results indicated that dietary supplementation of microencapsulated *E. faecalis* and *C. oleifera* seed extract can improve growth performance, enhance serum immune and antioxidative functions, and benefit the caecal microflora in *E. coli* K88-challenged broiler chickens.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This research was financially supported by the National Key Research and Development Program of China [grant number 2018YFD0501401-5] and Beijing Agricultural Innovation Consortium 04-2019 [grant number BAIC04-2019].

References

Alonso, M. Z., Padola, N. L., Parma, A. E., & Lucchesi, A. (2011). Enteropathogenic *E. Coli* contamination at different stages of the chicken slaughtering process. *Poultry Science*, *90*(11), 2638–2641. doi:10.3382/ps.2011-01621

Amaretti, A., Nunzio, M. D., Pompei, A., Raimondi, S., Rossi, M., & Bordoni, A. (2013). Antioxidant properties of potentially probiotic bacteria: In vitro and in vivo activities. *Applied Microbiology & Biotechnology*, *97*(2), 809–817. doi:10.1007/s00253-012-4241-7

And, C. P. L., & Yen, G. C. (2006). Antioxidant activity and bioactive compounds of tea seed (*Camellia oleifera* Abel.) oil. *Journal Agricultural & Food Chemistry*, *54*(3), 779. doi:10.1021/jf052325a

Arabski, M., Wasik, S., Dworecki, K., & Kaca, W. (2009). Laser interferometric and cultivation methods for measurement of colistin/ampicillin and saponin interactions with smooth and rough of *Proteus mirabilis* lipopolysaccharides and cells. *Journal of Microbiological Methods*, *77*(2), 178–183. doi:10.1016/j.mimet.2009.01.020

Brisbin, J. T., Gong, J., Orouji, S., Esufali, J., Mallick, A. I., Parvizi, P., … Sharif, S. (2011). Oral treatment of chickens with lactobacilli influences elicitation of immune responses. *Clinical & Vaccine Immunology*, *18*(9), 1447–1455. doi:10.1128/CVI.05100-11

Brisbin, J. T., Gong, J., & Sharif, S. (2008). Interactions between commensal bacteria and the gut-associated immune system of the chicken. *Animal Health Research Reviews*, *9*(1), 101–110. doi:10.1017/S146625230800145X

Cao, G. T., Zeng, X. F., Chen, A. G., Zhou, L., Zhang, L., Xiao, Y. P., & Yang, C. M. (2013). Effects of a probiotic, *Enterococcus faecium*, on growth performance, intestinal morphology, immune response, and cecal microflora in broiler chickens challenged *with Escherichia coli* K88. *Poultry Science*, *92*(11), 2949–2955. doi:10.3382/ps.2013-03366

Carmen, B., Sebastian, O., & Kathrin, L. H. (2013). Feeding the probiotic *Enterococcus faecium* strain NCIMB 10415 to piglets specifically reduces the number of *Escherichia coli* pathotypes

that adhere to the gut mucosa. *Applied & Environmental Microbiology*, *79*(24), 7896–7904. doi:10.1128/AEM.03138-13

Chen, Y., Miao, Y., Huang, L., Li, J., Sun, H., Zhao, Y., … Zhou, W. K. (2013). Antioxidant activities of saponins extracted from radix trichosanthis: An in vivo, and in vitro, evaluation. *BMC Complementary & Alternative Medicine*, *14*(1), 86. doi:10.1186/1472-6882-14-86

Corona-Hernandez, R. I., Alvarez-Parrilla, E., Lizardi-Mendoza, J., Islas-Rubio, A. R., & de la Rosa, L. A. (2013). Structural stability and viability of microencapsulated probiotic bacteria: A Review. *Comprehensive Reviews in Food Science & Food Safety*, *12*(6), 14–628. doi:10.1111/1541-4337.12030

Debnath, T., Ghosh, M., Lee, Y. M., Nath, N. C. D., Lee, K. G., & Lim, B. O. (2018). Identification of phenolic constituents and antioxidant activity of *Aloe barbadensis* flower extracts. *Food & Agricultural Immunology*, *29*(1), 27–38. doi:10.1080/09540105.2017.1358254

De Moura, J. M. L. N., & Johnson, L. A. (2009). Two-stage countercurrent enzyme-assisted aqueous extraction processing of oil and protein from soybeans. *Journal of the American Oil Chemists' Society*, *86*(3), 283–289. doi:10.1007/s11746-008-1341-8

Dong, Z. L., Wang, Y. W., Song, D., Hou, Y. J., Wang, W. W., Qi, W. T., … Li, A. K. (2016). The effects of dietary supplementation of pre-microencapsulated *E. faecalis* 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

Fabricio, R., João, C., Z, L., & Gil-Turnes, C. (2002). Effect of the probiotic cenbiot on the humoral response to an *Escherichia coli* bacterin. *Food & Agricultural Immunology*, *14*(2), 133–138. doi:10.1080/09540100220145151

Fairbrother, J. M., Nadeau, E., & Gyles, C. L. (2005). *Escherichia coli* in postweaning diarrhea in pigs: An update on bacterial types, pathogenesis, and prevention strategies. *Animal Health Research Reviews*, *6*(01), 17–39. doi:10.1079/ahr2005105

FASS. (2010). *Guide for the care and use of agricultural animals in agricultural research and teaching*. Champaign, IL: Federation of Animal Science Societies.

Food and Drug Administration. (2000). Animal Drug Availability Act, Veterinary Feed Directive. 21 CFR part 510:415–558. 76924–76930.

Francis, G., Kerem, Z., Makkar, H. P., & Becker, S. (2002). The biological action of saponins in animal systems: A review. *British Journal of Nutrition*, *88*(06), 587–605. doi:10.1079/BJN2002725

Haghighi, H. R., Gong, J., Gyles, C. L., Hayes, M. A., Zhou, H., & Sanei, J. R. (2006). Probiotics stimulate production of natural antibodies in chickens. *Clinical & Vaccine Immunology*, *13*(9), 975–980. doi:10.1128/CVI.00161-06

Haghighi, H. R., Gong, J. C., Hayes, M. A., Sanei, B., & Parvizi, P. (2005). Modulation of antibody-mediated immune response by probiotics in chickens. *Clinical & Diagnostic Laboratory Immunology*, *12*(12), 1387–1392. doi:10.1128/CDLI.12.12.1387-1392.2005

Han, J., Wang, F., W, Y., Song, D., Lu, Z., Dong, X., … Li, A. K. (2018). Effects of *Clostridium butyricum* and *Lactobacillus plantarum* on growth performance, immune function and volatile fatty acid level of caecal digesta in broilers. *Food & Agricultural Immunology*, *29*(1), 797–807. doi:10.1080/09540105.2018.1457013

Iii, J. D. Q., & Drew, M. D. (2000). Effects of oral antibiotics or bovine plasma on survival, health and growth in dairy calves challenged with *Escherichia coli*. *Food & Agricultural Immunology*, *12*(4), 8311–8318. doi:10.1080/09540100020008173

Khalaji, S., Zaghari, M., Hatami, K., Hedari-Dastjerdi, S., Lotfi, L., & Nazarian, H. (2011). Black cumin seeds, artemisia leaves (*Artemisia sieberi*), and *Camellia L.* Plant extract as phytogenic products in broiler diets and their effects on performance, blood constituents, immunity, and cecal microbial population. *Poultry Science*, *90*(11), 2500–2510. doi:10.3382/ps.2011-01393

Liang, M. F., Liu, G. H., & Zhao, Q. Y. (2013). Effects of Taishan Robinia pseudoacacia polysaccharides on immune function in chickens. *International Immunopharmacology*, *15*(4), 661. doi:10.1016/j.intimp.2013.02.026

Liu, X., Jia, L., Gao, Y., Li, B., & Tu, Y. (2014). Anti-inflammatory activity of total flavonoids from seeds of *Camellia oleifera* Abel. *Acta Biochimica et Biophysica Sinica*, *46*(10), 920–922. doi:10.1093/abbs/gmu071

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

Oleszek, W., Nowacka, J., Gee, J. M., Wortley, G., & Johnson, I. T. (1994). Effects of some purified alfalfa (*Medicago sativa*) saponins on transmural potential difference in mammalian small intestine. *Journal of the Science of Food & Agriculture*, *65*(1), 35–39. doi:10.1002/jsfa.2740650107

Pantev, A., Valcheva, R., Danova, S., Ivanova, I., Minkov, I., & Haertlé, T. (2003). Effect of enterococcin a 2000 on biological and synthetic phospholipid membranes. *International Journal of Food Microbiology*, *80*(2), 145. doi:10.1016/s0168-1605(02)00143-5

Prakash, S., Tomaro-Duchesneau, C., Saha, S., & Cantor, A. (2011). The gut microbiota and human health an emphasis on the use of microencapsulated bacterial cells. *Journal of Biomedicine & Biotechnology*, *2011*(981214), 2–12. doi:10.1155/2011/981214

Qiu, Y., Hu, Y. L., & Cui, B. A. (2007). Immunopotentiating effects of four Chinese Herbal polysaccharides administered at vaccination in chickens. *Poultry Science*, *86*(12), 2530–2535. doi:10.3382/ps.2007-00076

Santiago-López, L., Hernández-Mendoza, A., Mata-Haro, V., Vallejo-Cordoba, B., & González-Córdova, A. F. (2018). Immune response induced by fermented milk with potential probiotic strains isolated from artisanal cocido cheese. *Food & Agricultural Immunology*, *29*(1), 911–929. doi:10.1080/09540105.2018.1485632

Shekh, R. M., & Roy, U. (2012). Biochemical characterization of an anti- candida, factor produced by *Enterococcus faecalis*. *BMC Microbiology*, *12*, 132. doi:10.1186/1471-2180-12-132

Shimoyamada, M., Ikedo, R., Ootsubo, S., & Watanabe, K. (1998). Effects of soybean saponins on chymotryptic hydrolyses of soybean proteins. *Journal of Agricultural & Food Chemistry*, *46*(12), 4793–4797. doi:10.1021/jf980694j

Spišáková, V., Levkutová, M., Revajová, V., Ševčíková, Z., Lauková, A., & Levkut, M. (2013). Leukocytic response and composition of enteral microbiota in chickens fed a sage extract supplemented diet and infected with *Salmonella* Enteritidis PT4. *Food & Agricultural Immunology*, *24*(1), 33–45. doi:10.1080/09540105.2011.640994

Stašová, D., Husáková, E., Bobíková, K., Karaffová, V., Levkutová, M., & Levkut, M. (2015). Expression of cytokines in chicken peripheral blood mononuclear cells after stimulation by probiotic bacteria and campylobacter jejuni in vitro. *Food & Agricultural Immunology*, *26*(6), 813–820. doi:10.1080/09540105.2015.1036356

Sugiharto, S., Hedemann, M. S., Jensen, B. B., & Lauridsen, C. (2012). Diarrhea-like condition and intestinal mucosal responses in susceptible homozygous and heterozygous f4r+ pigs challenged with enterotoxigenic *Escherichia coli*. *Journal of Animal Science*, *90*(Suppl 4), 281–283. doi:10.2527/jas.53840

Wang, Y. W., Dong, Z., Song, L., Zhou, D., Wang, H., W, W., & Li, A. K. (2018). Effects of microencapsulated probiotics and prebiotics on growth performance, antioxidative abilities, immune functions, and caecal microflora in broiler chickens. *Food & Agricultural Immunology*, *29*(1), 859–869. doi:10.1080/09540105.2018.1463972

Wojnicz, D., Kucharska, A. Z., Kicia, M., & Tichaczekgoska, D. (2012). Medicinal plants extracts affect virulence factors expression and biofilm formation by the uropathogenic *Escherichia coli*. *Urological Research*, *40*(6), 683. doi:10.1007/s00240-012-0499-6

Ye, Y., Yang, Q., Fang, F., & Li, Y. (2015). The camelliagenin from defatted seeds of *Camellia oleifera* as antibiotic substitute to treat chicken against infection of *E. Coli* and *Staphylococcus aureus*. *BMC Veterinary Research*, *11*(1), 1–10. doi:10.1186/s12917-015-0529-z

Yu, J., Chen, Y., Zhai, L., Zhang, L., Xu, Y., Wang, S., & Hu, S. (2015). Antioxidative effect of ginseng stem-leaf saponins on oxidative stress induced by cyclophosphamide in chickens. *Poultry Science*, *94*(5), 927–933. doi:10.3382/ps/pev055

Zhai, L., Li, Y., Wang, W., & Hu, S. (2011). Enhancement of humoral immune responses to inactivated newcastle disease and avian influenza vaccines by oral administration of ginseng stem-and-leaf saponins in chickens. *Poultry Science*, *90*(9), 1955–1959. doi:10.3382/ps.2011-01433

Zhai, L., Wang, Y., Yu, J., & Hu, S. (2014). Enhanced immune responses of chickens to oral vaccination against infectious bursal disease by ginseng stem-leaf saponins. *Poultry Science*, *93*(10), 2473–2481. doi:10.3382/ps.2014-04056

Zhang, L., Li, J., Yun, T. T., Li, A. K., Qi, W. T., & Li, A. K. (2015a). Evaluation of pilot-scale microencapsulation of probiotics and product effect on broilers. *Journal of Animal Science*, *93*(10), 4796–4807. doi:10.3382/ps.2012-02453.

Zhang, L., Li, J., Yun, T. T., Qi, W. T., Liang, X. X., Wang, Y. W., & Li, A. K. (2015b). Effects of pre-encapsulated and pro-encapsulated *Enterococcus faecalis* on growth performance, blood characteristics, and cecal microflora in broiler chickens. *Poultry Science*, *94*(11), 2821–2830. doi:10.3382/ps/pev262.

Zhang, T., Wang, C. G., Lv, J. C., Wang, R. S., & Zhong, X. H. (2012). Survey on tetracycline resistance and antibiotic-resistant genotype of avian Escherichia coli in North China. *Poultry Science*, *91*(11), 2774–2777. doi:10.3382/ps.2012-02453

Zhang, X. F., Yang, S. L., Han, Y. Y., & Zhao, L. (2014). Qualitative and quantitative analysis of triterpene saponins from tea seed pomace (*Camellia oleifera* Abel) and their activities against bacteria and fungi. *Molecules*, *19*(6), 7568–7580. doi:10.3390/molecules19067568