Effect of a direct-fed microbial and prebiotic on performance and intestinal histomorphology of turkey poults challenged with *Salmonella* and *Campylobacter*

Shaban Rahimi,† Sophia Kathariou,‡ Oscar Fletcher,§ and Jesse L Grimes∗,1

*Prestage Department of Poultry Science, College of Agriculture and Life Sciences, North Carolina State University, Raleigh, NC 27695; †Faculty of Agriculture, Department of Poultry Science, Tarbiat Modares University, Tehran 1411713116, Iran; ‡Department of Food Bioprocessing and Nutrition Sciences, North Carolina State University, Raleigh, NC 27695; and §Department of Population Health and Pathobiology, College of Veterinary Medicine, North Carolina State University, Raleigh, NC 27607

ABSTRACT *Salmonella* and *Campylobacter* are leading human foodborne pathogens commonly associated with poultry and poultry products, and several methods to control these pathogens have been applied to poultry production. This study was conducted to evaluate the effect of CALSPORIN, (CSP), a direct-fed microbial (DFM), and yeast cell wall (*Saccharomyces cerevisiae*, IMW50, a mannanoligosaccharide (MOS)-based prebiotic, on performance, levels of *Salmonella* and *Campylobacter* in the feces, and intestinal histomorphometry in turkey poults. A 21-day battery cage study was conducted using 4 dietary treatments, including: an unsupplemented basal diet (corn and soybean-based) as negative control (NC); basal diet supplemented with 0.05% DFM; basal diet supplemented with 0.05% MOS; and basal diet supplemented with 0.05% mixture of DFM and MOS at equal proportions. Female Large White turkey poults (n = 336) were randomly distributed in 6 electrically-heated battery cages with 4 treatments and 12 replicates per treatment (7 poults per replicate pen). The first 16 pens were not inoculated with bacteria, while poults in pens 17 to 32 were orally challenged at day 7 with 10⁵ CFU *Salmonella* Heidelberg and the poults in pens 33 to 48 were orally challenged at day 7 with 10⁵ CFU *Campylobacter jejuni*. Feed consumption, body weight, and feed conversion ratio were measured weekly and at the end of the experiment. At day 21, fresh fecal samples from each pen were collected for *Salmonella* and *Campylobacter* enumeration and ileal tissue samples were collected from 1 bird per pen for histomorphology examination. DFM and MOS supplementation was accompanied with reduced levels of *Salmonella* shed by the treated birds compared to the control group, and with increased body weight (P ≤ 0.05). The surface area of villi increased in the MOS-supplemented group compared to the control group (P ≤ 0.05). There was a significant difference in V:C ratio between supplemented groups and control group (P ≤ 0.05). Based on these results, there is potential for CALSPORIN and IMW50 to reduce *Salmonella* shedding in feces, enhance ileal mucosal health, and improve growth performance of turkey poults.

Key words: direct-fed microbial, mannanoligosaccharide, *Salmonella*, *Campylobacter*, poults

INTRODUCTION

Salmonellosis and campylobacteriosis are leading foodborne zoonotic diseases worldwide, and are significantly associated with contaminated poultry products (Balan and Babu, 2017; Thomassen, 2019). The burden of foodborne diseases, including Campylobacteriosis, is substantial: every year almost 1 in 10 people become ill and 33 million healthy life years are lost. Foodborne diseases can be severe, especially for young children. Diarrheal diseases are the most common illnesses resulting from unsafe food, with 550 million people falling ill yearly (including 220 million children under the age of 5 years). *Campylobacter* is 1 of the 4 key global causes of diarrheal diseases (WHO, 2018). In developing countries, approximately 40 to 60% of young children become infected with *Campylobacter* annually and high numbers of asymptomatic carriers are reported (Coker et al., 2002; Samuel et al., 2004; Baker et al., 2006). Altogether, *Campylobacter* spp. adversely affects the health of millions of people worldwide with an estimated annual economical burden of up to $8 billion in the US alone. Although *C. jejuni* and *C. coli* are frequently isolated from the
digestive tract of a wide variety of animals, broiler chickens are considered the most important source of human infection (Grant et al., 1980; Lee and Newell, 2006). As much as 70% of raw poultry meat products sold in the US in 1999/2000 was found to be contaminated with high levels of viable Campylobacter (Zhao et al., 2001).

The Foodborne Disease Burden Epidemiology Reference Group of the WHO reported that foodborne diarrheal disease agents caused 230,000 global deaths in 2010, of which non-typhoidal Salmonella accounted for 59,000 (Havelaar et al., 2015). According to the European Food Safety Authority (EFSA), each year 90,000 salmonellosis cases are reported in the European Union, while the Centers for Disease Control and Prevention (CDC) estimates that about 1.2 million illnesses and 450 deaths occur every year in the United States (Thomassen, 2019). The CDC has estimated that nontyphoidal Salmonella species are second only to norovirus as cause of foodborne illness in the United States, causing approximately 11% of all domestically-acquired foodborne illnesses, and that Salmonella species are the leading cause of hospitalizations (35%) and deaths (28%) from foodborne illnesses (Scallan et al., 2011). USDA-FSIS and FDA NARMS 2002-2012 reported that Salmonella Enteritidis and S. Heidelberg were the 2 most common Salmonella serovars associated with poultry-associated salmonellosis (Hofacre et al., 2019). Incidence of human infections by Salmonella Enteritidis in the United States increased by 3% from 2006 to 2017 (Marder et al., 2018).

New regulations by the US Food and Drug Administration (FDA) that went into effect on January 1, 2017, banned the use of antibiotics as feed supplements to help livestock and poultry grow faster. According to the FDA, by 2014 17,000 tons of antibiotics were sold in the United States for livestock. This figure represented 80% of all US antibiotics sales (FDA, 2017). The European Union banned the use of antibiotic growth promoters (AGPs) in animal feed in 2006. At the same time, the problem with the Salmonella has been increasing and 24% of broilers raised were positive for colonization (EFSA, 2007). The latest data published by the EFSA show an increase in Salmonella Enteritidis prevalence in laying hen flocks (Navarro et al., 2018). The rise of antibiotic resistant bacterial strains has resulted in an increased interest to use of antibiotic alternatives such as probiotics, prebiotics, acidifiers, enzymes, and bacteriophage therapy in poultry production (Nilsson, 2014; Ahmadi et al., 2016).

Probiotics or Direct- Fed Microbial (DFM) are live microorganisms that, when administered in adequate amounts, confer a health benefit on the host (Sanders, 2008). Use of DFMs and prebiotics to prevent poultry intestinal colonization by Salmonella and Campylobacter and to reduce shedding of the organisms may effectively control the spread and prevalence of these bacteria in poultry. The inhibition effect produced by DFM on the population of Salmonella and Campylobacter through the competitive exclusion mechanism has been extensively documented (Reid and Friendship, 2002; Harirahan et al., 2004; Dahiya et al., 2006; Callaway et al., 2008; Grimes et al., 2008).

Prebiotics are defined as food ingredients that selectively stimulate the growth and activity of beneficial microorganisms such as Bifidobacterium and Lactobacillus in the gut and thereby benefit health. In addition, prebiotics can reduce the numbers of pathogenic microorganisms and increase colonization resistance to these pathogens (Cummings and McFarlane, 2002). Prebiotics are assumed to be non-digestible by digestive enzymes and thus can serve as substrate for beneficial bacteria, mainly located in the hind gut (Steiner, 2006; Ferket, 2011). Several carbohydrates that may be fermented by intestinal microorganisms can be classified as prebiotics (Bauer et al., 2006), including non-starch polysaccharides, resistant starch, and nondigestible oligosaccharides. Prebiotics can stimulate the enteric colonization of non-cultururable bacteria that discourage colonization by Salmonella and other pathogens, and they have the advantage of being stable to the elevated heat and pressure incurred during feed processing (Konstantinov et al., 2003; Rastall et al., 2005; Micciche et al., 2018).

The administration of Bacillus spores as feed additives as opposed to vegetative cells clearly distinguishes Bacillus from other bacterial probiotic formulations and offers a number of clear advantages. These include low cost of production, ease of preparation, resistance to production processes, and extended shelf life over a wide range of temperatures (La Ragione et al., 2001; La Ragione and Woodward, 2003; Upadhyaya et al., 2018). From recent studies, it was reported that spores of laboratory strains of Bacillus subtilis decrease colonization of young chicks by Escherichia coli 078:K80, Salmonella enterica serotype Enteritidis, and Clostridium perfringens (La Ragione et al., 2001; La Ragione and Woodward, 2003).

CALSPORIN (CSP, a DFM, Quality Technology International, Inc., QTII Elgin, IL) contains the naturally-occurring Bacillus subtilis strain C-1302. Yeast cell wall (Saccharomyces cervisiae, IMW50 is a mannanoligosaccharide (MOS)-based prebiotic derived from yeast and is produced by Quality Technology International (QTI) Inc., Elgin, IL. The MOS is not a substrate in microbial fermentation but exerts a significant growth-promoting effect by enhancing the animal’s resistance to enteric pathogens (Ferket, 2011). Most studies of these compounds were done with broilers, but little is known about their efficacy on animal health and colonization by bacterial foodborne pathogens in turkeys.

The aim of the present study was to investigate the influence of CALSPORIN and IMW50 on growth performance of turkey pouls, morphology of the ileum, and levels of Salmonella and Campylobacter shedding by the birds.
MATERIALS AND METHODS

Day-of-hatch female Large White turkey poultés (n = 336) of strain 85 × 700, Nicolas (Aviagen Turkeys, Lewisburg, WV) were obtained from a commercial hatchery and maintained in a battery cage system in an environmentally controlled room at the Talley Turkey Education Unit, North Carolina State University (NCSU). All bird handling procedures were approved by the NCSU Institutional Animal Care and Use Committee.

Poults received 1 of 4 dietary treatments: unsupplemented basal diet (corn and soybean-based) as control (NC); DFM (CALSPORIN) 0.05% in basal diet feed; MOS (IMW50) 0.05% in basal diet feed; or with 0.05% mixture of both DFM and MOS at equal proportions in basal diet feed for a period of 21 D using a completely randomized design. The DFM and MOS were obtained from Quality Technology International (QTI) Inc., Elgin, IL. The DFM and MOS-supplemented diets were mixed after all control feed was mixed and bagged. Poults were weighed individually, wing-banded, and randomly segregated into 3 groups. The experimental design included 3 groups with the 4 dietary treatments in each group with 4 replicates per treatment within each group and with 7 birds in each replicate (a total of 48 replicate pens and 336 poultés). One-third within each group and with 4 replicates per treatment.

Parameters Analyzed

Performance The performance parameters that were observed and measured were feed consumption (FC), BW, and feed conversion ratio (FCR).

Salmonella and Campylobacter Detection and Enumeration Fresh fecal samples were collected from all pens at day 21, for Salmonella and Campylobacter enumeration. The fecal samples were kept on ice and transferred to the laboratory for further processing. Sample suspensions in sterile water were serially diluted and plated on xylose lysine deoxycholate (XLD) agar (the United States Pharmacopeial Convention, Rockville, MD) for Salmonella and mCCDA agar (Oxoid) for Campylobacter isolation and identification. The XLD plates were incubated at 37°C for 24 h and CCDA plates were incubated microaerobically at 42°C for 48 h.

Intestine Histomorphology At day 21, tissue samples from the ileum were taken from one bird per cage for histomorphometric analysis. The tissue samples from just below Meckel’s diverticulum were fixed in 10% neutral buffered formalin and transferred to the histopathology laboratory in the College of Veterinary Medicine, North Carolina State University, for further processing. The tissue samples were trimmed and transverse sections of 5 microns thickness were stained with hematoxylin and eosin. The slides were digitalized using ImageJ software (ImageJ, US National Institutes of Health, Bethesda, MD, http://rsb.info.nih.gov/ij/).

Photomicrographs using a 4X objective resulting in magnification of 60X for the final displayed image were evaluated to obtain measurements at 5 locations per slide. Measurements of mucusal thickness (total thickness) and villus height was made and the crypt depth and surface area of villus were calculated (Iji et al., 2001). The villus length was measured from the villus tip to the junction of the intestinal crypt.

To estimate the number of mucusal glands, 10 photomicrographs were prepared for each treatment group.

Table 1. Composition of the ration for rearing turkey poultés to 21 D.

| Ingredient                  | %       |
|-----------------------------|---------|
| Item                        |         |
| Corn                        | 43.40   |
| Soybean meal                | 46.00   |
| Poultry fat                 | 4.00    |
| Dicalcium phosphate         | 3.80    |
| Limestone                   | 1.00    |
| Choline chloride            | 0.25    |
| DL-Methionine               | 0.25    |
| Niacin                      | 0.45    |
| DL-Methionine               | 0.25    |
| Choline chloride            | 0.25    |
| Minerals                    | 0.10    |
| Selenium premix             | 0.20    |
| Vitamins                    | 0.20    |
| MOS (IMW50)                 | 0.50    |
| Probiotic Calsporin (CSP)    | 0.20    |
| Prebiotic IMW50 (IMW)        | 0.20    |
| Mixture (CSP + IMW)         | 0.50    |

1Minerals mix supplied the following per kilogram of diet: 120 mg of Zn as ZnSO₄·H₂O; 120 mg of Mn as MnSO₄·H₂O; 80 mg of Fe as FeSO₄·H₂O; 10 mg of Cu as CuSO₄·5H₂O; 2.5 mg of I as I₂; 1.0 mg of Co as CoSO₄·5H₂O.
2Vitamin mix supplied the following per kilogram of diet when added at 0.2%: vitamin A, 6,600 IU; vitamin D₃, 2,000 IU; vitamin E, 33 IU; vitamin B₁₂, 19.8 µg; riboflavin, 6.6 mg; niacin, 55 mg; D-pantothenate, 11 mg; menadione, 2 mg; folic acid, 1.1 mg; thiamine, 2 mg; pyridoxine, 4 mg; deoxycholate, 126 µg; ethoxyquin, 50 mg.
3Selenium premix supplied 0.21 mg Se, as Na₂SeO₃.
4Probiotic Calsporin (CSP), probiotic IMW50 (IMW) and mixture of probiotic and prebiotic (CSP+IMW) (QIT, Inc, Elgin, IL) provided at 500 g/ton of feed in different treatments based on the experiment design.
RESULTS AND DISCUSSION

Performance

The initial BW of poults did not differ \((P > 0.05)\) between the treatment groups. However, there was a difference between control and treatment groups in BW and FC during the experiment. At last day of study (day-21), BW and FC in CSP and IMW50 supplemented groups were significantly \((P \leq 0.05)\) higher than control group (Table 2). In the other hand, Salmonella challenged group showed the lowest BW and FC compared to control group (Table 2). There was no mortality in any of the treatment groups throughout the experiment.

Lohmann and Sims (2012) reported that broilers fed diets supplemented with CSP or CSP + Q-MOS had significantly improved BW and FCR adjusted using a common BW of negative control (NC) compared with broilers fed NC or BMD diets, while broilers fed diets supplemented with both Q-MOS and CSP had the best calorie conversion. Several researchers—suggest that MOS, when added to poultry diets, allows the birds to perform at a similar level as when fed a diet supplemented with AGPs (Parks et al., 2001; Sims et al., 2004; Hooge, 2004a, b; Parks et al., 2005; Rosen, 2007). Supplementation of \(B.\) subtilis PB6 improved performance of broiler chickens (Teo and Tan, 2006; Upadhaya et al., 2018), which is in agreement with our finding in present study.

Salmonella and Campylobacter Challenge

The fresh fecal samples from all the cages were collected at day 21, and analyzed for \(Salmonella\) and \(Campylobacter\). Poults that were not inoculated with \(Salmonella\) had no detectable \(Salmonella\) in the feces (Table 3). Thus, no \(Salmonella\) was detected in the control treatment. \(Salmonella\) was recovered only from poults that were inoculated with \(Salmonella\). Dietary supplementation of DFM and MOS significantly
Table 4. Effect of 1CALSPORIN and 2IMW50 on histomorphology of intestine in turkey 3poults challenged with Salmonella and Campylobacter.

|                      | Crypt (µ) | Mucosa (µ) | Muscularis (µ) | Surface Area (µ ²) | Basal (µ) | Apical (µ) | Villous (µ) | V: C |
|----------------------|-----------|------------|----------------|--------------------|-----------|------------|-------------|------|
| **Bacteria**         |           |            |                |                    |           |            |             |      |
| Control              | 161b      | 760        | 217            | 6426a              | 126a      | 81c        | 599         | 3.72 |
| Salmonella           | 178b      | 771        | 214            | 6266a              | 127a      | 89b        | 594         | 3.34 |
| Campylobacter        | 169b      | 761        | 217            | 57689b             | 111b      | 87b        | 595         | 3.58 |
| SEM                  | 3         | 10         | 5              | 1368               | 3         | 2          |             |      |
| **Feed**             |           |            |                |                    |           |            |             |      |
| Control              | 163b      | 754b       | 239            | 66599b             | 119       | 88a        | 651         | 3.72 |
| IMW                  | 175a      | 825a       | 225            | 58820b             | 125       | 88a        | 559         | 3.41 |
| CSP                  | 164b      | 722c       | 201            | 61486b             | 122       | 92a        | 583b,c      | 3.41 |
| IMW+CSP              | 171b,a,b  | 755b       | 201            | 1580               | 3         | 2          |             |      |
| SEM                  | 4         | 11         | 5              | 1580               | 3         | 2          |             |      |
| **P-value**          |           |            |                |                    |           |            |             |      |
| Bacteria             | 0.0005    | NS         | NS             | 0.002              | 0.0001    | 0.0001     | NS          | NS   |
| Feed                 | 0.06      | 0.0001     | 0.0001         | 0.002              | 0.0001    | 0.0001     | 0.0001      | 0.002|
| BXF                  | 0.0001    | 0.0001     | 0.007          | 0.0001             | 0.0001    | 0.0001     | 0.0001      | 1.00 |
| Control              | 142c      | 505c       | 243b           | 51078c             | 122d      | 90c        | 452         | 3.18 |
| IMW                  | 173b,c,d  | 860a       | 214b           | 67806b             | 122d      | 71d        | 687         | 3.97 |
| CSP                  | 145b      | 747b,c     | 216b           | 63742b             | 128a      | 84d        | 602         | 4.15 |
| IMW+CSP              | 183b,c,e  | 836a       | 196d           | 68025b             | 130c      | 79e        | 654b,c      | 3.57 |
| Salmonella           | 180a,c    | 832a       | 218b           | 62100b             | 116d,e    | 69f        | 653b,c      | 3.63 |
| IMW                  | 194a      | 849a       | 248a           | 69496a             | 115d,c    | 97d        | 658b        | 3.39 |
| CSP                  | 180b,c    | 719b,c,d   | 189d           | 63496b             | 142a      | 100a       | 536         | 2.88 |
| IMW+CSP              | 153d      | 683d       | 202d           | 61956b             | 135a,b    | 102a       | 530d        | 3.46 |
| Campylobacter        | 168b,c,e  | 836a       | 256a           | 64562b             | 117e      | 77d        | 668         | 3.98 |
| IMW                  | 155d,f    | 765b       | 212d           | 62494b             | 120d      | 96d        | 606b,c      | 3.83 |
| CSP                  | 159d,f    | 698d,e     | 197d           | 49222e             | 106d      | 79e        | 539         | 3.39 |
| IMW+CSP              | 178b,c,e  | 744b,c     | 204d           | 54478c             | 107d      | 94b        | 566d        | 3.18 |

a–fMeans within a column lacking a common superscripts differ (P < 0.05).
1Calsporin (DFM) provided at 0.5 g/kg feed.
2IMW (MOS) provided at 0.5 g/kg feed.
3Poults were gavaged at 7 D with 10⁵ CFU Salmonella Heidelberg and Campylobacter jejuni.

reduced the Salmonella population in fecal samples of 21 D old poults compared to the Salmonella-inoculated group on the unsupplemented diet (P ≤ 0.05). The findings are in agreement with the reports by Knap et al. (2011) and Teo and Tan (2006) that Calsporin significantly reduced colonization of Salmonella Heidelberg in poults and broilers.

Analysis of feces at day 21 on Campylobacter-selective revealed that all poults, regardless of treatment, yielded Campylobacter from the fresh fecal droppings, suggesting environmental contamination of the poultry house by Campylobacter. Therefore, the potential impact of the diet supplementation on Campylobacter levels in the feces was not further assessed.

The observed reduction of Salmonella levels in in the fecal droppings can be attributed to the production of anti-Salmonella factors by B. subtilis and is in agreement with reports demonstrating that B. subtilis was successful in reducing the average cecal load of Salmonella in broiler chickens (Maruta et al., 1996; Fritts et al, 2000; La Ragione and Woodward, 2003; Knap et al. 2011). Reduction in Salmonella was also previously observed in broilers via the use of MOS (Spring et al., 2000).

Prebiotics such as MOS derived from the cell wall of the yeast Saccharomyces cerevisiae are thought to enhance the growth of beneficial bacteria while maintaining stability when subjected to pelleting processes temperatures up to 90°C and expansion conditions up to 105°C (Nollet, 2005; Kampf and Van der Aa, 2010). Beirao et al. (2019) reported that cell wall preparations from Saccharomyces cerevisiae IMW50 improved immune parameters in broilers challenged with Salmonella enteritidis.

**Intestine Histomorphology**

Effects of feed supplementation with DFM and MOS, alone or in combination were compared to controls on an unsupplemented diet, including poults challenged with Salmonella and Campylobacter and birds that were not inoculated with either pathogen (Table 4). Therefore, the morphometric data were organized into 3 groups: poults not bacterial challenged, those with Salmonella challenged and those challenged with Campylobacter. As shown in Table 4, mucosal thickness in the uninoculated group on the MOS-supplemented diet was higher than other groups (P < 0.05). The villous surface area in poults on the MOS-supplemented diet were higher than those on the control group (P < 0.05). The villus crypt (V:C) ratio in feed supplemented groups were significantly (P < 0.05) higher than control group (Table 4). The combination of both DFM and MOS resulted in no significant differences from the control diet. The DFM alone or the 1:1 mixture of DFM and MOS did not result in significant measurable impacts on the morphology of the intestine in the Salmonella-challenged group.
The ratio between the length of the villi and depth of the crypt is considered as an important parameter for intestinal health. A high ratio indicates a long villous in which the epithelium is mature and functionally active, in combination with a shallow crypt providing constant replacement of enterocytes lost from tips of villi as part of the normal physiological process.

In the present study, supplementation of MOS influenced the villous histomorphological changes in the intestine of poults. Improvement in villi length and depth of crypt in MOS supplemented groups indicated and functionally active epithelium and slower epithelial turnover rate and lower mucosal distress due to healthier gastrointestinal tract than controls in spite of the bacterial challenge. Therefore, there may be an increased surface area which could result in improved absorption of available nutrients (Caspari, 1992). It could also be possible that MOS act independent of the infection in improving the gut health. The beneficial effects of dietary MOS on the gut microflora, nutrient utilization, and growth performance may be associated with changes in brush border morphology and how it influences enteric disease resistance. Ferket (2003) reported dietary supplementation of MOS had a significant effect on intestinal villi morphology of turkey poults in comparison to those fed non-medicated control or virginiamycin-supplemented diets. This assumption is supported by the observed improvement in the length over the control groups. Supplementation of \textit{B. subtilis} to chickens has been observed to improved intestinal morphology as part of the normal physiological process.

Antibiotic Growth Promoters have been traditionally used to counter microbial infections in poultry. However, due to public health concerns, the use of AGP in poultry is either restricted or banned in several countries. Hence, the objective of this study was to ascertain if DFM or MOS, as alternative feed supplements, could enhance performance and protect the turkey poults from microbial colonization. The dietary supplements, DFM and MOS, resulted in enhanced performance while protecting the poults from microbial colonization. The DFM, \textit{Bacillus subtilis} C-3102, reduced the \textit{Salmonella} shedding in the feces of the turkey poults. Therefore, there is the potential for this DFM to reduce the risk of colonization of the birds or reduced amount of \textit{Salmonella} entering the processing phase. This would potentially improve food safety. Supplementation of feed with this MOS, not only affected \textit{Salmonella} colonization in turkey poults but also improved the gut health and gut integrity. Under the condition of this study, MOS conferred intestinal health benefits to the poults by improving its morphological development and microbial ecology.

ACKNOWLEDGMENTS

Kind cooperation of the technicians at the Histopathology Laboratory, Department of Population Health and Pathobiology, College of Veterinary Medicine, NCSU has been greatly appreciated.

REFERENCES

Ahmadi, M., M. Amir Karimi Torshizi, S. Rahimi, and J. J. Dennehy. (2016). Prophylactic bacteriophage administration more effective than post-infection administration in reducing \textit{Salmonella enteritica} serovar Enteritidis shedding in quail. Front. Microbial. 7:1253.

Baker, M. G., E. Sneyd, and N. A. Wilson. 2006. Is the major increase in notified \textit{Campylobacter} in New Zealand real? Epidemiol. Infect. 135:163–170.

Balan, K., and U. Babu. 2017. Comparative response of chicken macrophores to infection with \textit{Salmonella enterica} serovars. Poult. Sci. 96:1849–1854.

Bauer, E., B. A. Williams, M. W. A. Verstegen, and R. Mosenthin. 2006. Fermentable carbohydrates: potential dietary modulators of intestinal physiology, microbiology and immunity in pigs. In R. Mosenthin, J. Zentek, and T. Zebroska, eds., Biology of Growing Animals Series: Biology of Nutrition in Growing Animals. Elsevier Limited, Edinburgh, United Kingdom, 4:33–63.

Beiraio, B. M. Ingberman, M. Bonato, L. Borges, and R. Barbalho. 2019. Yeast cell wall immunomodulatory and intestinal integrity effects on broiler challenged with \textit{Salmonella Enteritidis}. Int. Poult. Sci. Forum, February 11–12, Atlanta, Georgia. 57 (Abstr.).

Callaway, T. R., T. E. Edrington, R. C. Anderson, J. A. Byrd, and D. J. Nisbet. 2008 Gastrointestinal microbial ecology and the safety of our food supply as related to \textit{Salmonella}. J. Anim. Sci. 86:E163–E172.

Caspari, W. F. 1992. Physiology and pathophysiology of intestinal absorption. Am. J. Clin. Nutr. 55:299S–308S.

Coker, A. O., R. D. Isokpehi, B. N. Thomas, K. O. Amisu, and C. L. Obi. 2002. Human \textit{Campylobacter} infection: a review. Vet Med-Czech. 49:441–446.

Causse, M., J. L. Grimes, S. Rahimi, E. Oviedo, B. W. Sheldon, and F. B. O. Santos. 2008. Effect of direct- feed microbial (Primalac) on growth of broiler chickens. J. of Appl. Poult. Res. 9:149–155.

Cummings, J. H., and G. T. McFarlane. 2002. Gastrointestinal effects of prebiotics. Br. J. Nutr. 87:145–151.

Dahiyat, P. O. D. Kilkie, A. G. Vankessel, and M. D. Drew. 2006. Potential strategies for controlling necrotic enteritis in broiler chickens in post-antibiotic era. Anim. Feed Sci. Technol. 129:60–68.

Dutta, V., E. Altermann, J. Olson, G. A. Wray, R. M. Siletzky, and S. Kathaiou. 2016. Whole-genome sequences of agricultural, host-associated \textit{C. coli} and \textit{C. jejuni} strains. Genome Announce 4:e00833–16.

EFSA (European Food Safety Authority). 2007. Report of the Task Force on Zoonoses Data Collection on the analysis of the baseline prevalence survey on the prevalence \textit{Salmonella} in broiler flocks of \textit{Gallus gallus}, in the EU. 2005:2006 [1]- part A: \textit{Salmonella} prevalence estimates. EFSA J. 98:1–85.

Ferket, P. R. 2003. Managing gut health in a world without antibiotics. In Proceedings Altech’s 17th European, Middle Eastern and African Lecture Tour. Alltech, Ireland.

Ferket, P. R. 2011. Strategies for finding alternatives to growth promoters. XXII Latin American Poultry Congress 2011.

Fritts, C., J. Kersey, M. Molt, E. Kroger, F. Yan, J. Jiang, M. Campos, L. Waldroup, and P. Waldroup. (2000): \textit{Bacillus subtilis} C-3102 (Calsporin) improves live performance and microbial status of broiler chickens. J. of Appl. Poult. Res. 9:149–155.

Food and Drug Administration (FDA). 2017. U S bans antibiotics use for enhancing growth in livestock. Food and Drug Administration (FDA). doi: 10.1036/1097-8542.BR0125171.

Grimes, J. L., S. Rahimi, E. Oviedo, B. W. Sheldon, and F. B. O. Santos. 2008. Effect of direct- feed microbial (Primalac) on turkey poults performance and susceptibility to oral \textit{salmonella} challenge. Poult. Sci. 87:1464–1470.

Grant, I. H., N. J. Richardson, and V. D. Bokkenheuser. 1980. Broiler chickens as potential source of \textit{Campylobacter} infections in humans. J. Clin. Microbiol. 11:508–510.

Hariharan, H., G. A. Morphy, and I. Kempe. 2004. \textit{Campylobacter jejuni}: public health hazards and potential control methods in poultry: a review. Vet Med-Czech. 49:441–446.
Havelaar, A. H., M. D. Kirk, P. R. Torgerson, H. J. Gibb, T. Hald, R. J. Lake, N. Prate, D. C. Bellinger, N. R. de Silva, N. Gargouri, N. Speybroeck, A. Cawthorne, C. Mathers, C. Stein, F. J. Angulo, and B. Devleeschauwer. World Health Organization Foodborne Disease Burden Epidemiology Reference. 2015. World Health Organization Global estimates and regional comparisons of the burden of foodborne disease in 2010. PLOS Med. 12: e1001923.

Hofacre, C., R. Berghaus, D. Cosby, M. Berrang, A. Hinton, Jr., K. Cookson, and M. Da Costa. 2019. Evaluating effectiveness of the Salmonella vaccination of broilers from day of age to the carcass rinse. Abstracts of the Int. Poultry Sci. Forum. USPEA. Atlanta GA. p15.

Hooge, D. M. (2004a). “Meta-analysis of Broiler Chicken Pen Trials Evaluating Dietary Mannan Oligosaccharide, 1993–2003”. Int. J. Poult. Sci. 3:163–174.

Hooge, D. M. (2004b). turkey pen trials with dietary mannan oligosaccharide: meta-analysis, 1993–2003. J. Poult. Sci. 3:179–188.

Iji, P. A., A. Saki, and D. R. Tieyv. 2001. Body and intestinal growth of broiler chicks on a commercial starter diet. Br. Poult. Sci. 42:505–513.

Kampf, D., and A. van der Aa. 2010. Mode of action of Bacillus subtilis and efficiency in piglet feeding, in Proc. 11. TagungSchweine-und Geflügelernährung, 23–25 November 2010, Institut für Agrar-und Ernährungswissenschaften, Universität Hallo-wittenberg, M. Gierusetal. (Hrsg.). ISBN: 978-3-86829-250-3. 28: 30.

Knap, I., A. B. Kehlet, M. Bennedsen, G. F. Mathi, C. L. Horacre, B. S. Lumkins, M. M. Jensen, M. Raun, and A. Lay. 2011. Bacillus subtilis (DSM17299) significantly reduces Salmonella in broilers. Poult. Sci. 90:1690–1691.

Konstantinov, S. R., W. Y. Zhu, B. A. Williams, S. Tammenga, W. M. Vos, and A. D. L. Akkermans. 2003. Effect of fermentable carbohydrates on piglet faecal bacterial communities as revealed by denaturing gradient gel electrophoresis analysis of 16S ribosomal DNA. FEMS Microbiol. Ecol. 43:225–235.

La Ragione, R. M., G. Calsula, S. M. Cutting, and M. J. Woodward, 2001. Bacillus subtilis spores competitively exclude Escherichia coli 078: K80 in poultry. Vet. Microbiol. 79:133–142.

La Ragione, R. M., and M. J. Woodward. 2003. Competitive exclusion by Bacillus subtilis of Salmonella enteric serotype Enteritidis and Clostridium perfringens in young chickens, Vet. Microbiol. 94:245–256.

Lee, M. D., and D. G. Newell. 2006. Campylobacter in poultry: filling an ecological niche. Avian Dis. 50:1–9.

Lohmann, T., and M. D. Sims. 2012. Effect of supplementing diets with CALSPORIN, BMD or Q-MOS plus CALSPORIN on live performance of broiler chicks. in Poultry Science Association 101st Annual Meeting. July 09–12, 2012, Athens, Georgia.

Marder, E. P., M. Griffin, P. R. Cleslac, J. Dunn, S. Hard, R. Jervis, A. S. Lathrop, A. Muse, P. Ryan, K. Smith, M. Tobin-D’Angelo, D. J. Vugia, K. G. Holt, B. J. Wolpert, R. Tauxe, and A. L. Gessler. 2018. Preliminary incidence and trends of infections with pathogens transmitted commonly through food-foodborne disease active surveillance network, 10 U.S. Sites, 2006–2017. MMWR Morb. Mortal. Wkly. Rep. 67:324–328.

Maruta, K., H. Miyazaki, S. Masuda, M. Takahashi, T. Maruhashi, Y. Radano, and H. Takashi. 1996. Exclusion of intestinal pathogens by continuous feeding with Bacillus subtilis C-3102 and its influence on the intestinal microflora in broilers. Anim. Feed Sci. Technol. 67:273–280.

Miccichie, A. C., S. L. Foley, H. O. Pavlidis, D. R. Mchtyre, and S. C. Ricke. 2018. A review of prebiotics against salmonella in poultry: current and future potential for microbiome research applications. Front. Vet. Sci. 5:1–11

Navarro, S. S., C. Marin, V. Cortes, C. Garcia, S. Vega, and P. Catala-Gregori. 2018. Aotophagia as a control measure for Salmonella in laying hens. Poult. Sci. 97:4367–4373.

Nilsson, A. 2014. Phage therapy constraints and possibilities. Ups J. Med. Sci. 119:192–198.

Nollet, L. 2005. Stability of Calsporin during pelleting of broiler feeds at 70, 80 or 90°C. CLO-INVE, Dendermonde, Belgium. Study code: Pelline Cal 0204/Broiler feeds.

Parks, C. W., J. L. Grimes, P. R. Ferket, and A. S. Fairchild. 2001. The effect of mannanoligosaccharides, bambermycins, and virginiamycin on performance of large white male market turkeys. Poult. Sci. 80:718–723.

Parks, C. W., J. L. Grimes, and P. R. Ferket. 2005. Effects of virginiamycin and a mannanoligosaccharide-virginiamycin shuttle program on the growth and performance of large white female turkeys. Poult. Sci. 84:1967–1973.

Rastall, R. A., G. R. Gibson, H. S. Gill, F. Guarner, T. R. Kleiham-mer, B. Pot, G. Reid, I. R. Rowland, and M. E. Sanders. 2005. Modulation of the microbial ecology of the human colon by probiotics, prebiotics and symbiotics to enhance human health: an overview of enabling science and potential applications. FEMS Microbiol. Ecol. 52:145–152.

Reid, G., and R. Friendship. 2002. Alternative to antibiotic use: probiotics for the gut. Anim. Biotechnol. 13:97-112.

Rosen, G. D. 2007. Holo-analysis of the efficacy of Bio-Mos® in turkey nutrition. Br. Poult. Sci. 48:27–32.

Samuel, M. C., D. J. Vugia, S. Shallow, R. Marcus, S. Segler, and T. McGivern. 2004. Campylobacter infection in the United States and declining trend in incidence. FoodNet 1996–1999. Clin. Infect. Dis. 38:S165–174.

Sanders, M. L. 2008. Probiotics: definition, sources, selection and uses. Clin. Infect. Dis. 46 Suppl 2:S58–S61.

SAS Institute. 1998. SAS/STAT Guide for Personal Computers. 8th ed. SAS Institute, Inc. Cary, NC.

Scallan, E., R. M. Hoekstra, F. J. Angulo, R. V. Tauxe, M-A Wid-dowson, S. L. Roy, J. L. Jones, and P. M. Griffin. 2011. Foodborne illness acquired in the United States major pathogens. Emerg. Infect. Dis. 17:7–15.

Simms, M. D., K. A. Dawson, K. E. Newman, P. Spring, and D. M. Hooge. 2004. Effects of dietary mannan oligosaccharide, bac-tracin methyle thiola culate, or both on the live performance and intestinal microbiology of turkeys. Poult. Sci. 83:1148–1154.

Smanya, M., and K. E. Yamauchi. 2002. Histological alterations of intestinal villi in chickens fed dried Bacillus subtilis varnantatto. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 133:95–104.

Spring, P., C. Wenk, K. A. Dawson, and K. E. Newman. 2000. The effect of dietary mannan oligosaccharides an actual parameters and the concentration of enteric bacteria in the ceca of Salmonella-challenged broiler chickens. Poult. Sci. 79:205–211.

Steiner, T. 2006. Managing Gut Health: Natural Growth Promoters as a Key to Animal Performance. Nottingham University Press, Nottingham, UK.

Teo, A. Y., and H. M. Tan. 2006. Effect of Bacillus subtilis PB6 (CLOSTAT) on broilers infected with a pathogenic strain of Escherichia coli. J. Appl. Poult. Res. 15:229–235.

Thomassen, F. 2019. The importance of preventing Salmonella in laying hens. Int. Hatch. Prac. 33:7–9.

Upadhaya, S. D., F. Rudeaux, and I. H. Kim. 2018. Effects of inclu-sion of Bacillus subtilis (Glassippo) to energy and protein-reduced diet on growth performance, nutrient digestibility and meat quality and gas emission in broilers. Poult. Sci. 98:2168–2178.

World Health Organization (WHO). 2018. https://www.who.int.