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

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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* cervisiae, 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 10^5 CFU *Salmonella* Heidelberg and the poults in pens 33 to 48 were orally challenged at day 7 with 10^5 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 ($P \leq 0.05$). The surface area of villi increased in the MOS-supplemented group compared to the control group ($P \leq 0.05$). There was a significant difference in V:C ratio between supplemented groups and control group ($P \leq 0.05$). 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*, poult

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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

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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; Hariharan 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-culturable 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; Miccichie 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; Upadhaya 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 perfrigens* (La Ragione et al., 2001; La Ragione and Woodward, 2003).

CALSPORIN [**CSP**, a DFM, Quality Technology International, Inc., (QTI) 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 poults, morphology of the ileum, and levels of *Salmonella* and *Campylobacter* shedding by the birds.

MATERIALS AND METHODS

Day-of-hatch female Large White turkey poults (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 poults). One-third of the birds (pens 1 to 16) were not inoculated with either *Salmonella* or *Campylobacter*. All poults in pens 17 to 32 were orally challenged with 10^5 CFU *Salmonella* Heidelberg and all poults in pens 33 to 48 were orally gavaged with 10^5 CFU C*ampylobacter jejuni* 11601MD (Dutta et al., 2016*)* at 7 D of age. All bird care tasks were performed with control birds first, then with the inoculated birds. A corn soybean meal based pelleted and crumbled turkey starter diet was formulated and is presented in Table 1. This feed was fed to d21. Feed and water were provided ad libitum throughout the study. Biosafety Level 2 practices were used during the experiment, and all bacterial cultures and inoculum preparation work was performed in a biosafety hood. The lighting program was scheduled according to Management Guidelines for Growing Commercial Turkey (www.aviagenturkeys.com).

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

Ingredient	%
Item	
Corn	43.40
Soybean meal	46.00
Poultry fat	4.00
Dicalcium phosphate	3.80
Limestone	1.00
Lysine	0.40
Salt	0.45
DL-Methionine	0.25
Choline chloride	0.20
Minerals[1]	0.20
Vitamins[2]	0.20
Selenium premix[3]	0.10
CSP, IMW, (CSP+IMW)[4]	0.50
Calculated nutrient content	
Crude protein	27.00
ME (kcal/kg)	2925.00
Fat (%)	6.10
Methionine (%)	0.65
TSAA (%)	1.04
Lysine (%)	1.81
Calcium (%)	1.34
Available P (%)	0.73

[1]Minerals mix supplied the following per kilogram of diet: 120 mg of Zn as $ZnSO_4$, H_2O; 120 mg of Mn as $MnSO_4$ H_2O; 80 mg of Fe as $FeSO_4.H_2O$; 10 mg of Cu as $CuSO_4$; 2.5 mg of I as $Cu(IO_3)_2$; 1.0 mg of Co as $CoSO_4$.

[2]Vitamin mix supplied the following per kilogram of diet when added at 0.2%: vitamin A, 6,600 IU; vitamin D_3, 2,000 ICU; vitamin E, 33 IU; vitamin B_{12}, 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; D- biotin, 126 µg; ethoxyquin, 50 mg.

[3]Slenium premix supplied 0.21 mg Se, as Na_2SeO_3.

[4]Probiotic Calsporin (CSP), prebiotic IMW50 (IMW) and mixture of probiotic and prebiotic (CSP+IMW) (QIT, Inc, Elign, IL) provided at 500 g/ton of feed in different treatments based on the experiment design.

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 Histomorphologyy 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 mucosal 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 mucous glands, 10 photomicrographs were prepared for each treatment group

Table 2. Effect of [1]direct fed microbial (DFM) and [2]prebiotic on performance of turkey poults challenged with S*almonella* and *Campylobacter*.

	Body weight (g)				Feed consumption (g/b)					Feed conversion				
	0 D	7 D	14 D	21 D	0–7 D	7–14 D	0–14 D	14–21 D	0–21 D	0–7 D	7–14 D	0–14 D	14–21 D	0–21 D
Bacteria														
Control	62	165	329	548[a]	100	230	330	347	676	1.0	1.38	1.23	1.59	1.39
Salmonella	62	161	320	527[b]	99	227	323	327	649	1.0	1.40	1.25	1.59	1.40
Campylobacter	61	164	328	535[a,b]	103	230	333	340	674	1.0	1.41	1.25	1.60	1.41
SEM*	0.5	2.6	3.7	6	2.5	5	5	6	9	0.02	0.02	0.02	0.03	0.02
Feed														
Control	61	156[b]	316	520[b]	96	224	315[b]	321	636[b]	0.98	1.38	1.23	1.58	1.39
IMW	61	161[a,b]	330	545[a]	103	232	335[a]	343	677[a]	1.03	1.38	1.24	1.60	1.40
CSP	62	170[a]	330	546[a]	107	229	336[a]	344	680[a]	1.00	1.43	1.26	1.60	1.41
IMW+CSP	62	165[a,b]	326	537[a,b]	99	230	329[a,b]	344	673[a]	1.00	1.40	1.25	1.59	1.41
SEM*	0.5	3	4	6	3	6	5	7	10	0.03	0.03	0.02	0.04	0.02
P														
Bacteria	NS	NS	NS	0.04	NS	NS	NS	0.08	0.07	NS	NS	NS	NS	NS
Feed	NS	0.03	NS	0.04	0.06	NS	0.05	0.09	0.02	NS	NS	NS	NS	NS
BXF	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[1]Direct fed Microbial (CSP, Calsprin).
[2]Mannanoligosaccharide (IMW50).
[a,b]Means within a column lacking a common superscripts differ ($P < 0.05$).
*Standard error of means.

using a 10X objective (final magnification at the projected on-computer screen image was 360X). Images were converted to 8-bit and the threshold was adjusted using the auto command of ImageJ resulting in segmentation of vacuoles (goblet cells, GC) that were black against a white background. Particles were analyzed using the limits to threshold box checked in the set measurements of ImageJ (measurement set to 130 to 1,500) and shape limits (circularity) set to 0.40 to 1.00.

Statistical Analysis All the data were analyzed by two-way ANOVA (JMP 8.0. SAS, 1998) within a completely randomized design in a 3 (Unchallenged-control, *Salmonell*-challenged, and *C. jejuni* challenged) × 4 (control, DFM, MOS, and DFM+MOS) factorial arrangement. Differences between treatment means were considered significant at $P \leq 0.05$.

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.

Table 3. Effect of [1]direct fed microbial (DFM) and [2]prebiotic on *Salmonella* in fecal samples of 21-d-old[3] poults.

Treatments	S. Heidelberg (log_{10} cfu/g)	Without *Salmonella* challenge
PC[4]	3.99[a]	0
DFM	2.81[b]	0
MOS	2.60[b]	0
DFM+MOS	2.60[b]	0

[a,b]Means within a column lacking a common superscripts differ ($P < 0.05$).
[1]Calsporin (DFM).
[2]IMW (MOS) provided at 0 .5 g/kg feed.
[3]Poults were gavaged at 7 D with *Salmonella* Heidelberg 10^5CFU.
[4]PC = Positive control (challenged with *Salmonella*, and received unsuplemented diet).

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 [1]CALSPORIN and [2]IMW50 on histomorphology of intestine in turkey [3]poults 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	161^{b}	760	217	64262^{a}	126^{a}	81^{c}	599	3.72
	Salmonella	178^{a}	771	214	62663^{a}	127^{a}	92^{a}	594	3.34
	Campylobacter	166^{b}	761	217	57689^{b}	111^{b}	87^{b}	595	3.58
	SEM	3	10	5	1368	3	2		
Feed	Control	163^{b}	754^{b}	239^{a}	59247^{b}	118	79^{b}	591^{b}	3.62
	IMW	175^{a}	825^{a}	225^{a}	66599^{a}	119	88^{a}	651^{a}	3.72
	CSP	164^{b}	722^{c}	201^{b}	58820^{b}	125	88a	559^{c}	3.41
	IMW+CSP	171^{a,b}	755^{b}	201^{b}	61486^{b}	122	92^{a}	583^{b,c}	3.41
	SEM	4	11	5	1580	3	2	10	
P-value	Bacteria	0.0005	NS	NS	0.002	0.0001	0.0001	NS	NS
	Feed	0.06	0.0001	0.0001	0.002	NS	0.0001	0.0001	0.002
	BXF	0.0001	0.0001	0.007	0.0001	0.0001	0.0001	0.0001	1.00
	Control	142^{f}	595^{e}	243^{a,b}	51078^{c}	122^{b-d}	90^{b,c}	452^{e}	3.18^{d}
	IMW	173^{b-d}	860^{a}	214^{c,d}	67806^{a,b}	122^{b-d}	71^{e,f}	687^{a}	3.97^{b}
	CSP	145^{f}	747^{b,c}	216^{b,c}	63742^{a,b}	128^{b-d}	84^{c,d}	602^{c}	4.15^{a}
	IMW+CSP	183^{a-c}	836^{a}	196^{c,d}	68025^{a,b}	130^{a-c}	79^{d,e}	654^{a,b}	3.57^{c}
Salmonella	Control	180^{a-c}	832^{a}	218^{b,c}	62100^{a,b}	116^{d,e}	69^{f}	653^{a,b}	3.63
	IMW	194^{a}	849^{a}	248^{a}	69496^{a}	115^{d-f}	97^{a,b}	658^{a}	3.39
	CSP	186^{a,b}	719^{b-d}	189^{d}	63496^{a,b}	142^{a}	100^{a}	536^{d}	2.88
	IMW+CSP	153^{e,f}	683^{d}	202^{c,d}	61956^{b}	135^{a,b}	102^{a}	530^{d}	3.46
Campylobacter	Control	168^{c-e}	836^{a}	256^{a}	64562^{a,b}	117^{c-e}	77^{d-f}	668^{a}	3.98
	IMW	158^{d-f}	765^{b}	212^{c,d}	62494^{a,b}	120^{c,d}	96^{a,b}	606^{b,c}	3.83
	CSP	159^{d-f}	698^{c,d}	197^{c,d}	49222^{c}	106^{e,f}	79^{d,e}	539^{d}	3.39
	IMW+CSP	178^{a-c}	744^{b,c}	204^{c,d}	54478^{c}	102^{f}	94^{a,b}	566^{c,d}	3.18

[a–f]Means within a column lacking a common superscripts differ ($P < 0.05$).
[1]Calsporin (DFM) provided at 0.5 g/kg feed.
[2]IMW (MOS) provided at 0.5 g/kg feed.
[3]Poults were gavaged at 7 D with 10^5 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 ($P \leq 0.05$). 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* cervisiae 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 Histomorphologyy

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 (Caspary, 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 *B. subtilis* to chickens has been observed to improved intestinal histology, such as villus length, cell area, and cell mitosis (Smanya and Yamauchi, 2002).

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, *Bacillus subtilis* C-3102, reduced the *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 *Salmonella* entering the processing phase. This would potentially improve food safety. Supplementation of feed with this MOS, not only affected *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.

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