Effect of a direct-fed microbial (Primalac) on structure and ultrastructure of small intestine in turkey poults[1]

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ABSTRACT The effects of dietary supplementation of the direct-fed microbial (DFM) Primalac in mash or crumbled feed on histological and ultrastructural changes of intestinal mucosa was determined in 2 populations of poults; 1 with and 1 without a *Salmonella* spp. challenge. Three hundred thirty-six 1-d-old female Large White turkey poults were randomly distributed into 8 treatment groups with 6 replicates of 7 poults in each pen. The poults were placed on 1 of 4 dietary treatments in a 2 × 2 × 2 factorial arrangement (mash or crumble feed, with or without DFM, not-challenged or challenged at 3 d of age). The DFM groups were fed a Primalac-supplemented diet from d 1 until the last day of the experiment (d 21). At 3 d of age, 50% of the poults were challenged with 1 mL of 10^{10} cfu/mL of *Salmonella* spp. (*Salmonella enterica* serovar Typhimurium, *Salmonella* Heidelberg, and *Salmonella* Kentucky) by oral gavage. The inoculated poults were housed in a separate room from nonchallenged controls. Feed and water were provided ad libitum for all birds. At d 21, 1 poult per pen (total of 6 poults per treatment) was randomly selected and killed humanely by cervical dislocation. After necropsy, the small intestine was removed, and tissue samples from duodenum, jejunum, and ileum were taken for light and electron microscopic evaluation. The DFM birds showed increased goblet cell (GC) numbers, total GC area, GC mean size, mucosal thickness, and a greater number of segmented filamentous bacteria compared with controls. Changes in intestinal morphology as observed in this study support the concept that poultry gut health and function, and ultimately bird performance, can be improved by dietary supplementation with DFM products such as Primalac as used in this study.

Key words: probiotic, turkey, intestine, morphology, *Salmonella*

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INTRODUCTION

Salmonellosis is a common gastrointestinal disorder in humans and is predominantly caused by *Salmonella*-contaminated foods. Poultry is considered a reservoir for *Salmonella* bacteria and a potential cause of outbreaks of disease in the human population. *Salmonella* spp. colonize the intestines of poultry and can cause foodborne illness in humans. Reduction of *Salmonella* colonization in the intestinal tract of poultry decreases the risk of potential carcass contamination during slaughter.

Salmonella colonization in the intestine of fowl may be effectively controlled or inhibited by oral ingestion of probiotics (Alvarez et al., 2003; Rahimi et al., 2007). Tellez et al. (2001) demonstrated that avian-specific probiotics and anti-*Salmonella enteritidis*-specific IgY inhibited *S. enteritidis* and *Salmonella typhimurium* colonization and organ invasion of market-aged chickens. Corrier et al. (1991) reported a decrease of *Salmonella* colonization in turkey poults inoculated with cecal anaerobic bacteria and dietary lactose. Oral inoculation of young chicks with anaerobic bacterial cultures, also known as probiotics, has been proven to effectively reduce *Salmonella* colonization (Snoeyenbos et al., 1979).

The term "probiotic" (from Greek meaning "for life") describes the living microorganism as having a positive influence on improving the intestinal microbial balance. Fuller (1989) describes probiotics as live microbial feed supplements that beneficially affect the host animal by improving its intestinal microbial balance. Probiotics are defined by the World Health Organization as a

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preparation of only one or a few strains of microorganisms, the primary purpose of which is to improve animal performance. According to the Food and Agriculture Organization of the United Nations, probiotics are live microorganisms administered in adequate amounts that confer beneficial health effects on the host. The US National Food Ingredient Association defined probiotics (direct-fed microbial, **DFM**) as a source of live naturally occurring microorganisms that includes bacteria, fungi, and yeast (Miles and Bootwalla, 1991).

Probiotics favorably alter the balance of intestinal microflora by inhibiting the growth of harmful bacteria, promoting good digestion, and increasing resistance to infection. Probiotics promote a balance of intestinal flora that produce organic compounds such as lactic acid, hydrogen peroxidase, and acetic acid. These products increase the acidity of the intestine, which inhibits the reproduction of harmful bacteria. Probiotic bacteria also produce bacteriocins which are natural antibiotics that kill undesirable microorganisms (Nava et al., 2005).

In poultry, probiotics are used to improve general health, increase growth, increase meat and egg production, improve the feed conversion ratio, and suppress pathogens. It has been observed that probiotics affect gastrointestinal (**GI**) tract histology and ultrastructure (Awad et al., 2006) and the regulation of mucus synthesis and secretion (Deplancke and Gaskins, 2001). Mucus is secreted by the goblet cells throughout the GI tract and forms an adherent gel on the mucosal surface (Sklan, 2004). Probiotics may also enhance the integrity of the tight junctions between the intestinal epithelial cells during infections or inflammatory conditions (Montalto et al., 2004; Shen et al., 2006).

Probiotics as feed additives must be chosen carefully and tested before market approval, and their safety, strain types, and specific efficacy should be determined. Today, the probiotic bacteria used in food and natural supplements are harvested via a highly controlled fermentation process. This process results in high populations of bacteria and ensures quality and purity of bacteria.

The objective of this study was to evaluate the histological and ultrastructural changes of intestinal mucosa of turkey poults fed a starter diet with or without a DFM (probiotic) in 2 groups of birds; 1 with and 1 without a challenge with 3 strains of *Salmonella* (*Salmonella* Typhimurium, *Salmonella* Heidelberg, and *Salmonella* Kentucky). This is a companion paper to that of Grimes et al. (2008) who reported a 1 log reduction of a *Salmonella* population and improvement of bird performance by feeding a DFM.

MATERIALS AND METHODS

The study was carried out according to the guidelines of North Carolina State University's Animal Care and Use Committee. Day-of-hatch Large White female poults (85x700, Nicholas Turkey Breeding Farms, Lewisburg, WV) were obtained from a commercial hatchery (Sleepy Creek Hatchery, Goldsboro, NC) and placed in 2 rooms (A and B), with each room containing 2 Petersime batteries (Petersime Incubator Co., Gettysburg, OH) with wire mesh floors. Twelve pens of 7 birds each were used in each battery (24 pens per room, 336 birds total). One of 4 dietary feed treatments was assigned to each pen (6 pens per room for each diet). One room (A) housed nonchallenged poults and, in the other room (B), poults were challenged with an oral gavage of *Salmonella*. The feeding and management of these poults reared to 21 d were as described previously (Grimes et al., 2008). The 4 dietary treatments were 1) mash feed with no DFM, 2) mash feed with DFM, 3) pelleted (20-s steam conditioning at 80°C) and crumbled feed with no DFM, and 4) pelleted and crumbled feed with DFM. The DFM (Primalac, Star Labs Inc., Clarksdale, MO) was added at 1 g/kg and contained primarily *Lactobacillus acidophilus* and *Lactobacillus casei* (as well as other genera); the microbial blends and concentrations are proprietary. To reduce the chance of cross contamination of the DFM, the DFM treatment pens were kept separate from non-DFM pens so there were no shared water troughs and no shared pen dividers (Angel et al., 2005).

A standard management procedure was used throughout the experiment. Feed and water were provided ad libitum. The illumination was 23L:1D (0000 to 0100 h). The poults were observed 3 times a day for any sign of illness; any unusual finding was recorded. Strict biosecurity procedures were maintained between treatment groups. Separate boots and lab coats were used for each treatment room. Caretaking was conducted on a treatment basis to minimize cross contamination between pens of different treatment groups.

At 3 d of age, each poult in the *Salmonella* challenge group was orally gavaged with 1 mL of a 10^{10} cfu/mL cocktail containing approximately equivalent populations of *Salmonella* Typhimurium, and 2 field isolates of *Salmonella* Heidelberg and *Salmonella* Kentucky as described by Grimes et al. (2008). At d 21, after 12 h of feed withdrawal, 1 poult per pen (6 poults per treatment group) was randomly selected, removed, weighed, and then humanely killed by cervical dislocation. After necropsy, the intestines were removed and sampled for light and electron microscopic evaluation. The birds were also sampled to make sure the challenge in the intestine of these birds was successful (Grimes et al., 2008).

Light Microscopy

Approximately 2 cm from each segment of duodenum, jejunum, and ileum were cut and placed separately in a 10% formalin solution for further processing to examine histomorphological changes of the intestinal mucosa. In the histopathology laboratory of the College of Veterinary Medicine (North Carolina State University, Raleigh), each sample was cut into 5-mm sec-

tions and placed into tissue cassettes. The tissues were processed, embedded in paraffin, and subsequently cut into 5-μm-thick slices that were placed onto slides. The tissues were stained with hematoxylin-eosin for light microscopy evaluation and measurement of intestinal mucosal villus height and crypt depth.

Sections from 6 birds per treatment group were placed on each slide. A series of 4 to 6 digital images were taken from each tissue and evaluated using ImageJ (ImageJ, US National Institutes of Health, Bethesda, MD; http://rsb.info.nih.gov/ij/).

Measurements of mucosal thickness (total thickness), villus height (villi), and crypt depth were made, and villus:crypt ratios were calculated. Photomicrographs taken with the 4× objective (magnification of 60× for the final displayed image) were evaluated to obtain measurements at 5 locations in each of 6 sections (1 section per poult) for each region of the intestine. This yielded 30 measurements per intestinal region per treatment group. Slides were oriented so that flat or relatively flat areas of serosa were evident and villi were distinctive.

To estimate the mucus layer thickness, 10 photomicrographs were prepared for each treatment group using the 10× objective (final magnification at the projected on-computer screen image was 360×). 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 command of ImageJ (measurement set to 130–1500) and shape limits (circularity) set to 0.40–1.00. ImageJ creates a mask of counted particles (vacuoles in this case). This mask was saved as a jpeg file and opened in Photoshop Elements; the background was selected using the magic wand tool and inverted to create an overlay for the original jpeg image. The overlaid original image was evaluated for goodness of fit to GC in enterocytes. Images in which good fit could not be obtained were not used in the analysis.

All data were analyzed using the GLM procedure (SAS Institute, 1998). The effects of *Salmonella* challenge, feed processing (feed form), and DFM on villus height, crypt depth, villus height plus crypt depth and GC number, area, and mean size in the duodenum, jejunum, and ileum were determined in a 2 × 2 × 2 factorial arrangement. The pen was used as the experimental unit. Treatment means were separated using the least squares means procedure of SAS with the level of significance of $P \leq 0.05$ unless otherwise stated (SAS Institute, 1998).

Scanning Electron Microscopy

Scanning electron microscopy was accomplished in 1-cm segments of duodenum, jejunum, and ileum. These sections were washed in 0.1 *M* phosphate buffer (pH 7.4) and fixed in 3% buffered glutaraldehyde (in 100 m*M* phosphate buffer). In the Center for Electron Microscopy (North Carolina State University, Raleigh), the samples were washed 3 times in the buffer and post-fixed in buffered 2% osmium tetroxide (in 100 m*M* phosphate buffer) for 2 h, and washed again in phosphate buffer. Then, samples were dehydrated in graded ethanol serial washings, dried in a CO_2 critical point dryer (Samdri-795, Tousimis, Rockville, MD), secured to stubs with silver paint with approximately 25 nm gold/palladium (Anatech Hummer 6.2, Anatech Ltd., Hayward, CA) and viewed at 15 kV. Electron micrographs were taken using a Jeol 5900 LV scanning electron microscope (Jeol Ltd., Rockville, MD) from different areas of the samples for estimating villi alterations, mucus secretion, density of GC, and bacterial colonization.

Transmission Electron Microscopy

Samples of duodenum, jejunum, and ileum were washed in 0.1 *M* phosphate buffer (pH 7.4), fixed in 3% buffered glutaraldehyde, and then washed 3 times in the buffer and post-fixed in buffered 2% osmium tetroxide (in 100 m*M* phosphate buffer) for 2 h, and washed again in phosphate buffer. The samples were washed in the same buffer and dehydrated in serial ethanol solutions (30, 50, 70, 95, and 100%).

The samples were vacuum filtered (to remove the bubbles) in 24 h changes of ethanol:resin (Spurr's), 1:3 ethanol:resin, and 3 changes of 100% resin, for 24 h each. For infiltration of resin, the concentration of resin was gradually increased and stored overnight for 3 d. Samples were embedded in fresh resin in flat embedding molds for orientation purposes and cured at 70°C overnight for polymerization. Blocks were trimmed to remove excess resin. Ultra-thin sections were cut at approximately 75 nm using an LKB Nova ultra microtome (Leica, Deerfield, IL) fitted with a diamond knife (Diatome) on copper grids, and stained with urany1 acetate and Reynold's lead citrate. Electron micrographs (4,500× and 10,000×) of intestinal mucosal cells and microvilli were taken using the Jeol JEM-100s transmission electron microscope (Jeol Ltd.).

RESULTS AND DISCUSSION

Histomorphometric analysis alterations of intestinal mucosa and GC are shown in Table 1. There was no main effect of *Salmonella* challenge in any of the tissues. In the duodenum, there was a significant increase in the GC count, area, and mean size due to feeding of DFM. There was no significant effect on these parameters in the duodenum due to feed form, nor were there any interaction effects. In the jejunum there was a feed form × DFM interaction for GC number, where DFM in mash feed increased the GC number but had no effect in crumbled feed. There was a *Salmonella* × feed ×

DFM interaction in the jejunum for both GC total area and mean size, where in birds challenged with *Salmonella*, the DFM increased GC total area and mean size for birds fed mash feed but not for birds fed crumbled feed. There were no treatment effects in the ileum for GC number, total area, or mean size.

Alterations in villus height (**VH**), crypt depth (**CD**), VH + CD (**L**), and VH:CD ratio (**V:C**) due to treatments are presented in Table 2. There was no main effect due to *Salmonella* or DFM on any parameter in the duodenum. There was a *Salmonella* × feed × DFM interaction on L and VH, where *Salmonella*-challenged birds fed mash feed with no DFM had decreased VH and L compared with birds on the other treatments. There was a *Salmonella* × feed interaction effect for V:C where birds challenged with *Salmonella* and fed mash feed had decreased V:C compared with birds on the other treatments. There was no significant treatment effect on CD in the duodenum. However, the *Salmonella* × DFM effect on CD in the duodenum approached significance ($P = 0.09$) where birds that were not challenged with *Salmonella* and fed DFM had reduced CD (0.13 vs. 0.16 ± 0.007 μm), whereas there was no difference for birds challenged with *Salmonella* and fed DFM (0.15 vs. 0.15 ± 0.007 μm; means not shown). There were no treatment effects for L, VH, CD, or V:C in the jejunum. In the ileum, birds challenged with *Salmonella* had increased L and VH. Although these differences are significant, they are relatively small and it is not unusual to observe some variable responses to

Table 1. Effect of *Salmonella*, direct-fed microbial (DFM)[1], and form of feed on the number, area, and mean size of intestinal mucosal goblet cells[2]

Intestinal segment and feed form	DFM	No *Salmonella*			*Salmonella*		
		Number	Area (μm)	Mean size (μm)	Count	Area (μm)	Mean size (μm)
Duodenum							
Crumble	No	230.6[b]	23,991[b]	103.7[b]	226.0[b]	26,785[b]	120.4[ab]
	Yes	269.8[a]	34,146[a]	125.2[a]	239.0[a]	27,695[a]	113.9[ab]
Mash	No	212.0[b]	26,082[b]	118.8[b]	231.7[b]	25,191[b]	108.0[b]
	Yes	236.0[a]	32,133[a]	135.7[a]	242.7[a]	32,659[a]	135.3[a]
Mean		237.1	29,088	120.8	234.8	28,083	119.4
SEM		15.4	3,328	9.6	15.4	2,794	7.2
Salmonella (S)	NS	NS	NS				
Feed (F)	NS	NS	NS				
Direct-fed microbial (D)	0.05	0.01	0.02				
S × F	NS	NS	NS				
S × D	NS	NS	NS				
F × D	NS	NS	NS				
S × F × D	NS	NS	NS				
Jejunum							
Crumble	No	332.4[b]	29,704[b]	88.8[b]	342.3[b]	38,809[ab]	110.4[a]
	Yes	348.0[b]	31,580[b]	88.4[b]	290.0[b]	28,287[b]	95.8[ab]
Mash	No	390.8[b]	42,429[a]	107.7[a]	288.0[b]	25,980[b]	86.9[b]
	Yes	409.5[a]	38,486[ab]	91.8[b]	408.0[a]	44,721[a]	107.9[a]
Mean		370.2	35,550	94.2	332.1	34,450	100.2
SEM		22.3	3,393	4.5	22.3	3,756	6.8
S	NS	NS	NS				
F	0.05	0.05	NS				
D	NS	NS	NS				
S × F	NS	NS	0.04				
S × D	NS	NS	NS				
F × D	0.05	0.05	NS				
S × F × D	NS	0.01	0.004				
Ileum							
Crumble	No	332.0	29,390	89.4	482.2	46,925	96.9
	Yes	456.5	41,366	92.0	450.8	42,376	93.7
Mash	No	392.0	35,197	88.4	430.7	40,353	92.8
	Yes	371.0	34,935	91.2	321.8	34,739	104.2
Mean		387.9	35,222	90.2	421.4	41,098	96.9
SEM		49.4	5,254	6.2	38.8	5,261	7.4
S	NS	NS	NS				
F	NS	NS	NS				
D	NS	NS	NS				
S × F	NS	NS	NS				
S × D	NS	NS	NS				
F × D	NS	NS	NS				
S × F × D	NS	NS	NS				

[a,b]Means within columns with different superscript letters are different ($P \leq 0.05$). NS = not significant.

[1]Primalac (Star Labs Inc., Clarksdale, MO).

[2]Total of 48 poults (1 bird per pen) from 8 treatment groups (6 replicates per treatment) were killed for histopathology examination. Then, 6 sections of small intestine (similar segments from duodenum, jejunum, and ileum from each bird) were placed onto each respective slide. There were a total of 24 slides (8 slides for each segment of small intestine) with 6 sections onto each slide.

Table 2. Effect of *Salmonella*, direct-fed microbial (DFM)[1], and form of feed on villus height (V), crypt depth (C), length (L = V + C), and V:C ratio of poult intestine[2]

Intestinal segment and feed form	DFM	No *Salmonella* (μm)				*Salmonella* (μm)			
		L	Villus	Crypt	V:C	L	Villus	Crypt	V:C
Duodenum									
Crumble	No	2.32[a]	2.16[a]	0.16	13.48[a]	2.54[a]	2.39[a]	0.15	16.47[a]
	Yes	2.24[a]	2.11[a]	0.13	16.24[a]	2.41[a]	2.26[a]	0.14	15.95[a]
Mash	No	2.40[a]	2.25[a]	0.15	15.00[a]	1.84[b]	1.70[b]	0.15	12.13[b]
	Yes	2.29[a]	2.16[a]	0.13	16.90[a]	2.26[a]	2.10[a]	0.16	13.58[b]
Mean		2.31[x]	2.17[x]	0.14	15.4[x]	2.26[x]	2.11[x]	0.15	14.5[x]
SEM		0.07	0.07	0.005	1.34	0.06	0.07	0.005	1.34
Salmonella (S)	NS	NS	NS	NS					
Feed (F)	0.001	0.001	NS	NS					
Direct-fed microbial (D)	NS	NS	NS	NS					
S × F	0.001	0.001	NS	0.03					
S × D	0.03	0.05	0.09	NS					
F × D	0.01	0.02	NS	NS					
S × F × D	0.01	0.01	NS	NS					
Jejunum									
Crumble	No	1.27	1.14	0.12	8.44	1.28	1.11	0.13	8.33
	Yes	1.30	1.17	0.13	9.40	1.22	1.09	0.13	8.49
Mash	No	1.18	1.08	0.11	9.99	1.20	1.06	0.14	7.75
	Yes	1.23	1.11	0.12	9.61	1.29	1.16	0.12	9.40
Mean		1.25	1.12	0.12	9.36	1.24	1.11	0.13	8.49
SEM		0.06	0.06	0.007	0.83	0.06	0.03	0.007	0.62
S	NS	NS	NS	NS					
F	NS	NS	NS	NS					
D	NS	NS	NS	NS					
S × F	NS	NS	NS	NS					
S × D	NS	NS	NS	NS					
F × D	NS	NS	NS	NS					
S × F × D	NS	NS	NS	NS					
Ileum									
Crumble	No	0.84	0.72[b]	0.12	5.87	1.04	0.91[a]	0.14	6.89[a]
	Yes	0.98	0.90[a]	0.14	6.08	1.13	1.01[a]	0.12	7.68[a]
Mash	No	1.02	0.91[a]	0.10	7.96	0.94	0.80[a]	0.14	5.74[b]
	Yes	0.85	0.73[b]	0.12	6.13	1.04	0.90[a]	0.14	6.38[b]
Mean		0.92[x]	0.82[x]	0.13	6.51	1.04[y]	0.90[y]	0.14	6.67
SEM		0.03	0.05	0.01	0.61	0.03	0.03	0.01	0.59
S	0.01	0.04	NS	NS					
F	NS	NS	NS	NS					
D	NS	NS	NS	NS					
S × F	NS	NS	NS	0.01					
S × D	NS	NS	NS	NS					
F × D	NS	0.03	NS	NS					
S × F × D	NS	0.04	NS	NS					

[a,b]Means within columns with different superscript letters are different ($P \leq 0.05$). NS = not significant.

[x,y]Means among *Salmonella* within tissue with different superscript letters are different ($P \leq 0.05$).

[1]Primalac (Star Labs Inc., Clarksdale, MO).

[2]Total of 48 poults (1 bird per pen) from 8 treatment groups (6 replicates per treatment) were killed for histopathology examination. Then, 6 sections of small intestine (similar segments from duodenum, jejunum, and ileum from each bird) were placed onto each respective slide. There were a total of 24 slides (8 slides for each segment of small intestine) with 6 sections onto each slide.

microfloral presence in the digestive tract (Budino et al., 2005). Whereas there was no feed or DFM effect in birds challenged with *Salmonella*, unchallenged birds fed crumbled feed with DFM had greater VH compared with birds fed mash feed with DFM.

Light photomicrographs of VH and density of vacuoles (GC) in ileum of nonchallenged poults fed or not fed DFM are shown in Figure 1. It is possible that the increments in VH were a result of increased numbers of epithelial cells and perhaps, increments in their size. Although modest, the slight changes in intestinal morphology observed in the present study are in agreement with the findings of Rahimi and Karimi (2005) who reported that the supplementation of probiotic (Bioplus 2B, Razak Pharmaceutical and Biotechnology Laboratories Co., Tehran, Iran) in broiler diets improved VH and CD in the small intestines of treated birds compared with the control birds. Pelicano et al. (2005) reported that probiotic supplementation had a significant influence on increasing VH, CD, and density of glands throughout the small intestine in chickens. Gunal et al. (2006) reported that the increments of VH, CD, and V:C in jejunum and ileum of probiotic-fed broilers were greater compared with the control groups. In addition, Chichlowski et al. (2007) reported increased VH and CD in the jejunum of broiler chicks fed the same DFM used in the current study compared with control chicks fed no DFM. The increment in the size and number of

intestinal glands and villi could result in greater enzyme production resulting in better digestion and absorption of nutrients (Mohan et al., 1996).

Increments in VH and V:C ratio are directly correlated with enhanced epithelial cell turnover (Fan et al., 1997). This could mean that the increments of VH that were observed with DFM treatment are associated with increased enterocyte turnover rates. Increments in VH and CD of the DFM-treated poults could improve the absorptive surface area in the GI tract of these poults, potentially leading to better performance. These results support the concept that DFM treatment of poultry increases VH. Greater CD is related to higher cell proliferative activity for allowing adequate epithelial turnover rate and compensating for losses in VH (Pluske et al., 1997).

According to Cera et al. (1988), maximum absorption and digestion capacity is provided by a large luminal area with VH and mature enterocytes and is essential to animal development. Santos et al. (2002) examined the effect of a probiotic on the intestinal mucosa of piglets and reported that a high V:C ratio was evident when villi were long (finger-like) and crypts were flat (little depth); consequently, nutrient absorption was

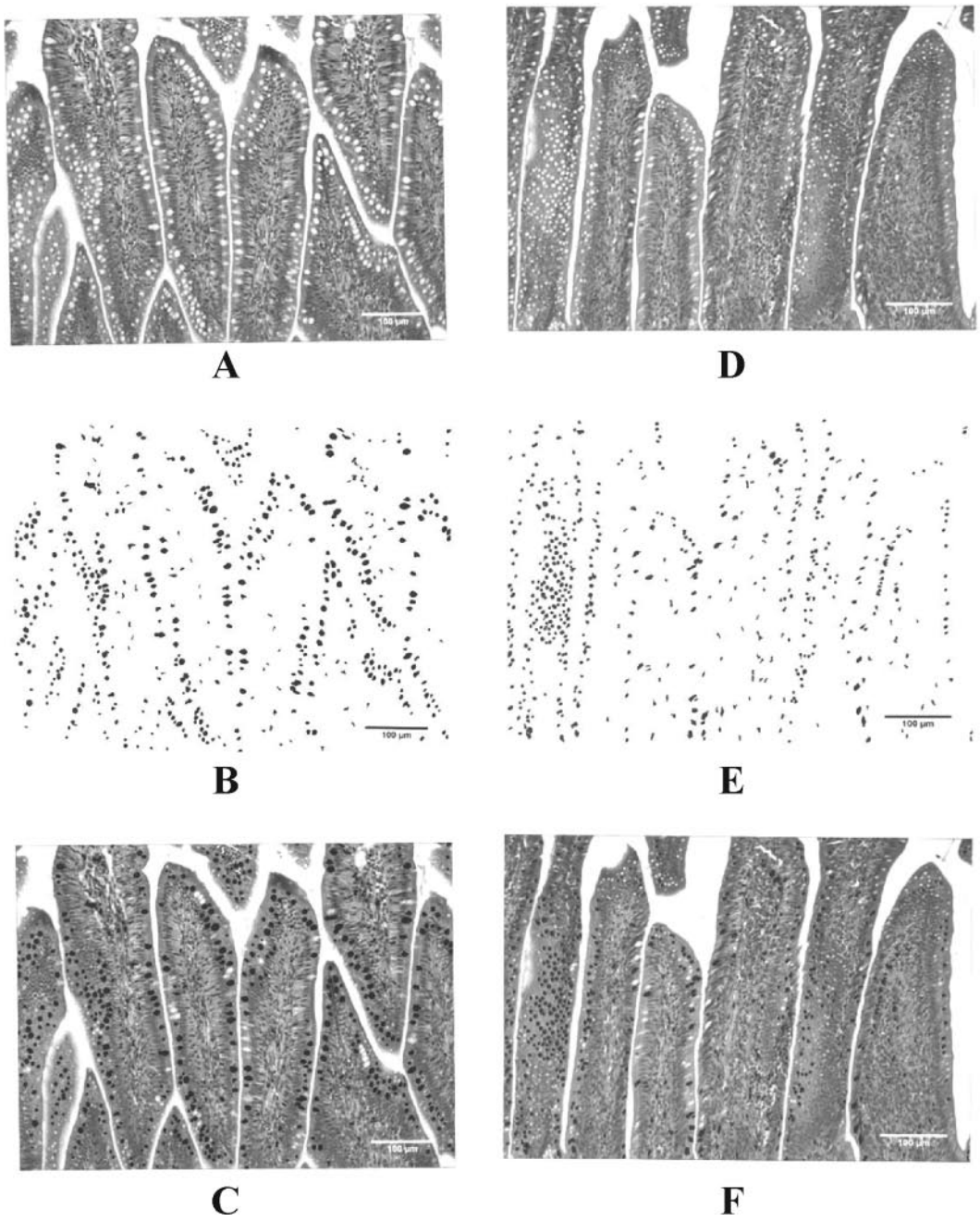


Figure 1. Light micrographs of villus height and density of goblet cells in ileum of poults with direct-fed microbial (panels A, B, C) and without direct-fed microbial (panels D, E, F). None of these birds were challenged with *Salmonella*. Panels A and D are original images of villi, panels B and E are processed from A and D showing segmentation of vacuoles (goblet cells) that appear black against the white background, and panels C and F are the mask of counted particles.

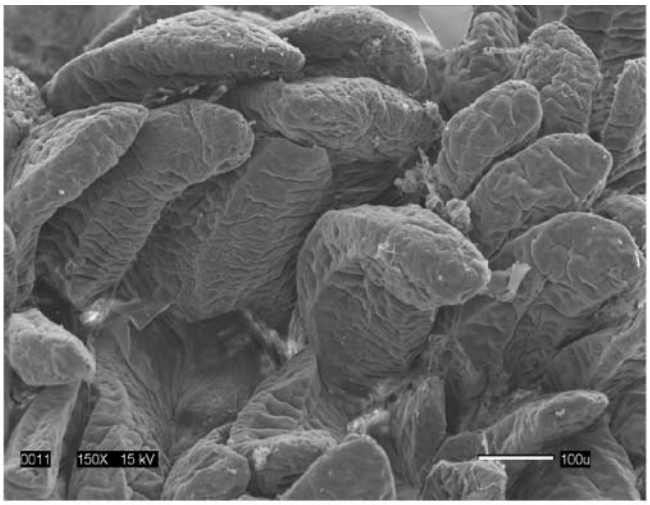


Figure 2. Scanning electron micrographs of ileum of 21-d-old poults fed diets with or without a direct-fed microbial (DFM), showing a higher density of villi in DFM-fed poults B and C compared with control poult A.

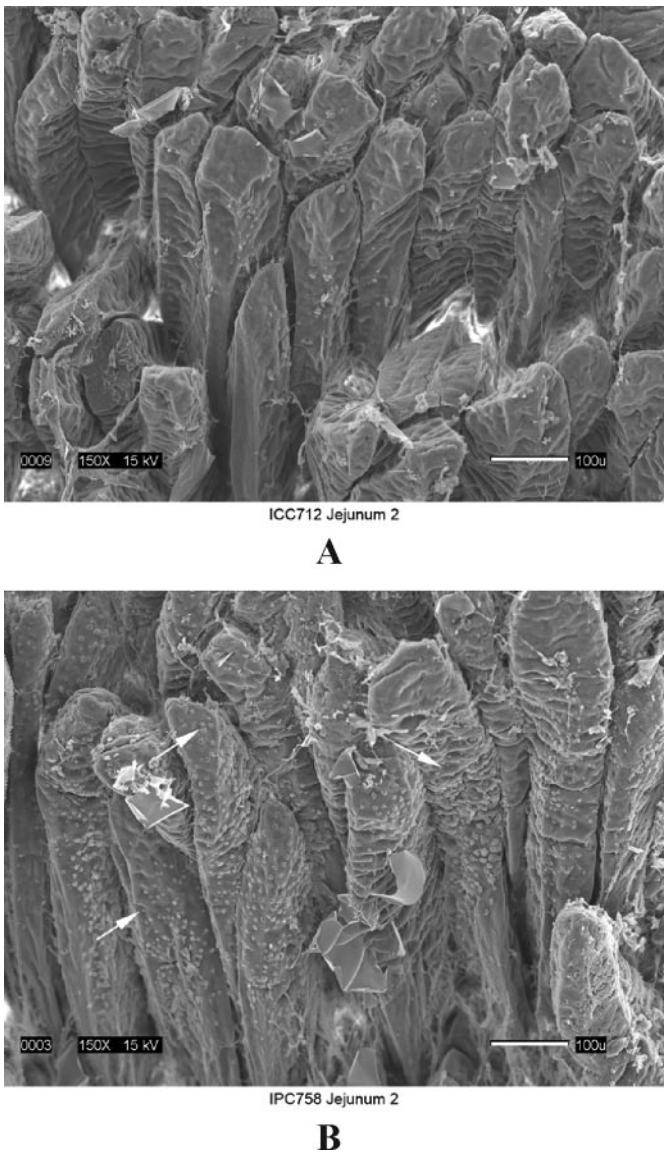


Figure 3. Scanning electron micrographs of jejunum of 21-d-old poults fed diets with or without a direct-fed microbial (DFM), showing a higher density of goblet cells (arrow) in the DFM-fed bird (B) compared with the control bird (A).

improved. Pelicano et al. (2005) reported that birds fed a *Bacillus subtilis*-based probiotic had greater villus length ($P < 0.1$) in the jejunum and ileum, whereas greater CD ($P < 0.01$) was observed in the duodenum, jejunum, and ileum of broilers receiving *B. subtilis*.

Smirov et al. (2005) indicated that a dietary probiotic increased the proportion of *Lactobacillus* spp. in the ileum compared with the controls ($P < 0.05$) and significantly enlarged the GC "cup" area throughout the small intestine compared with the control groups. The GC cup area was increased by 18% in the duodenum, 82% in the jejunum, and 40% in the ileum compared with control chicks (Smirov et al., 2005).

Iji et al. (2001) reported that VH of birds fed diets supplemented with mannan-oligosaccharide from d 1 to 21 increased in the duodenum, jejunum, and ileum with age, whereas CD increased in the duodenum and jeju-

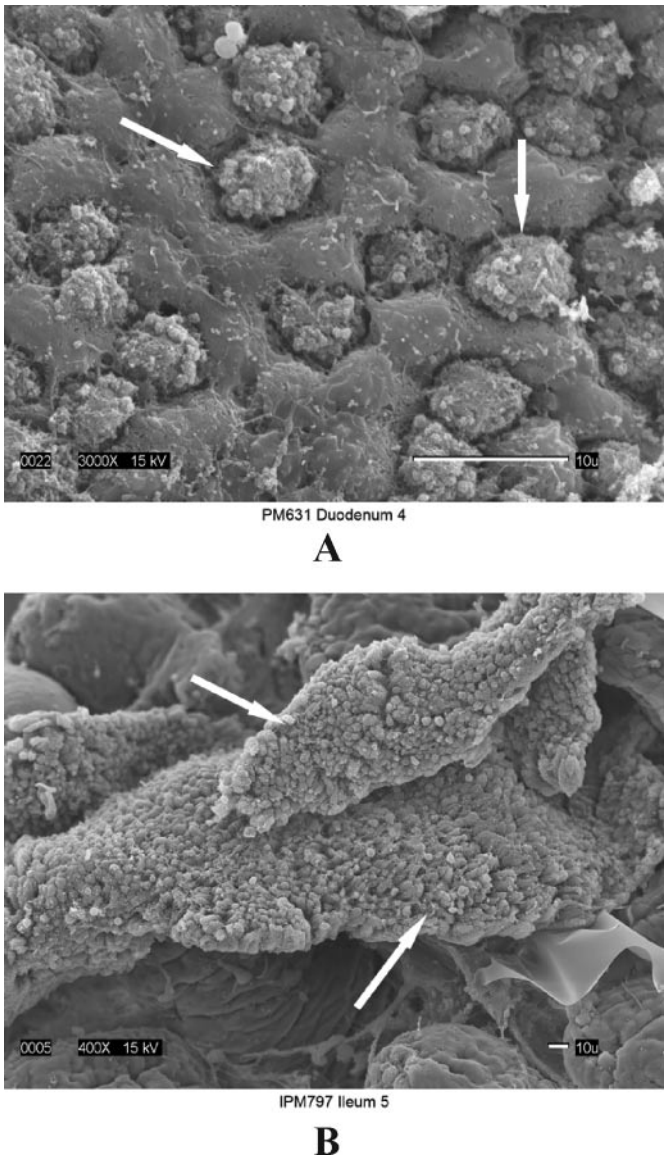


Figure 4. Scanning electron micrographs of duodenum (A) and ileum (B) of 21-d-old poults fed diets with a direct-fed microbial, showing a high density of goblet cells and mucus secretion (arrows, A) and a high amount of mucus blanket layer (arrow) covering the structural detail of the intestinal surface (B).

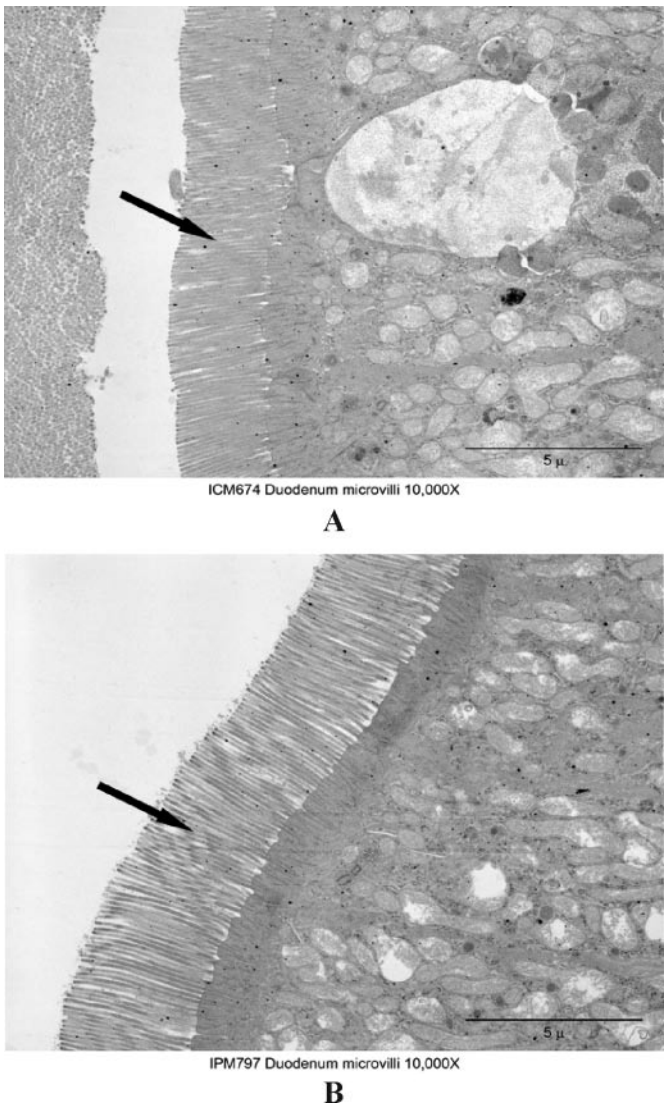


Figure 5. Transmission electron micrographs of enterocytes in the duodenum of 21-d-old poults fed diets with or without a direct-fed microbial (DFM) and challenged with *Salmonella*. The height of microvilli (arrow) in the DFM-fed poult (B) seems slightly greater than in the control poult (A).

num. They also reported that micronutrients influenced the morphology of the intestine.

The density of villi and number of GC were increased throughout the intestines of DFM-fed birds when samples were examined using scanning electron microscopy (Figures 2, 3, and 4). In Figure 2, the length of villi in the nontreated birds seemed slightly longer than ones in the DFM-fed birds, which can affect the increment of surface area in the intestine. In addition, a greater amount of mucus covering the intestines of DFM-fed birds could be observed (Figure 4). Although a clear distinction is difficult to discern, it appears that one can observe, using transmission electron microscopy, slightly greater microvillus length and density of GC due to DFM compared with the control diet (Figures 5 and 6). In addition, the DFM supplementation in feed was associated with increased mucin layer thickness in intestinal lumen as observed using transmission electron microscopy (Figure 7). Chichlowski et al. (2007) reported increased goblet cells in broiler chicks fed the same DFM used in the current study compared with control birds not fed DFM. Ikeda et al. (2002) reported that goblet cells may play an important role in epithelial cell repair following damage to the GI mucosa. Caballero-Franco et al. (2007) reported a 60% increase in basal luminal mucin content with a probiotic treatment. The mucus layer acts as a barrier between the luminal contents and intestinal nutrient transporters and it protects the mucosal surface from exogenous and endogenous irritants such as bile salts (Yagi et al., 1990). The increase in size and number of intestinal glands and villi could result in increased enzyme pro-

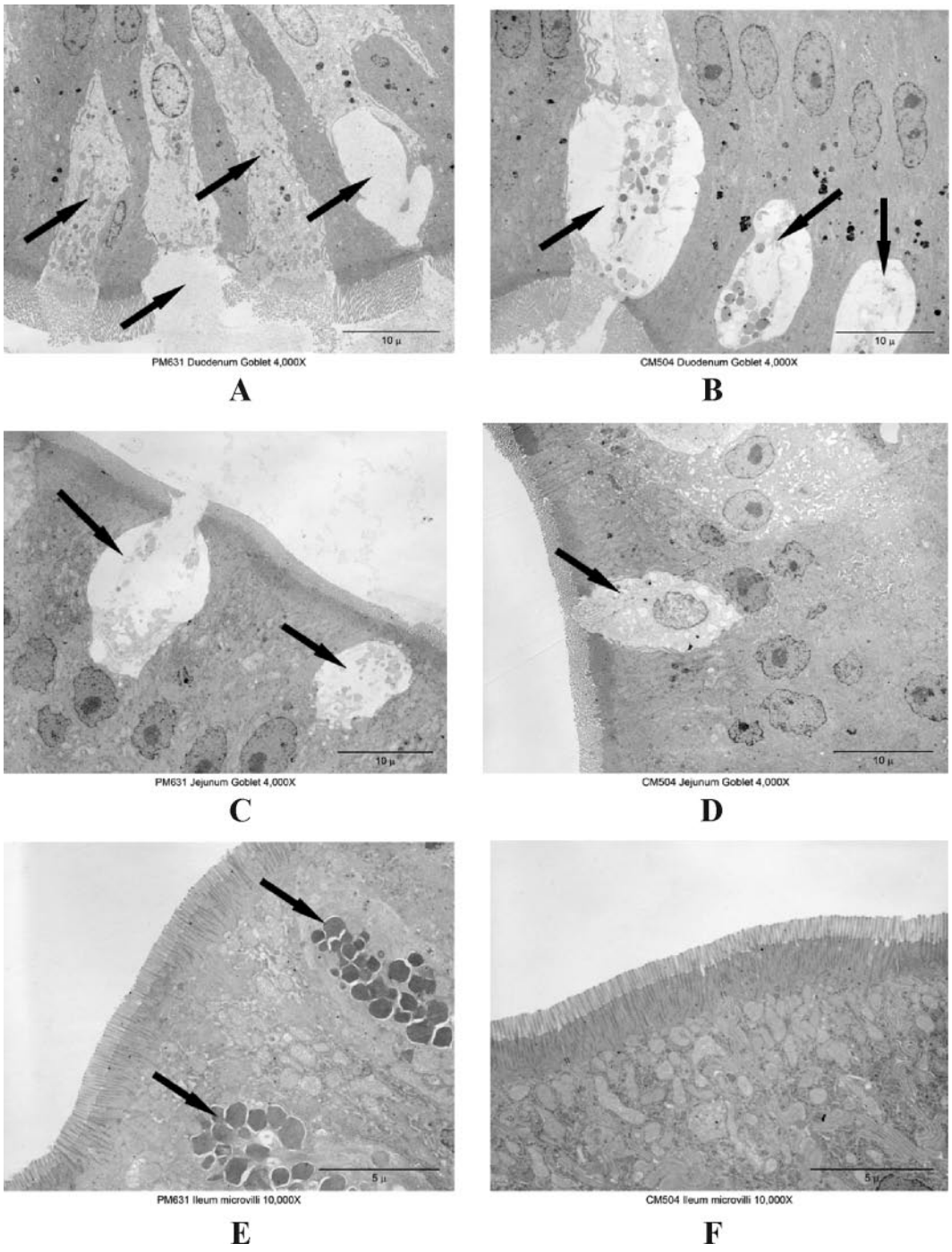
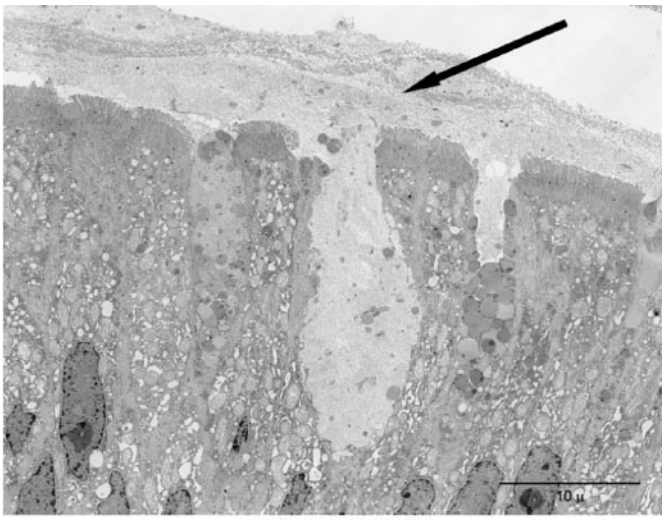


Figure 6. Transmission electron micrographs of duodenum, jejunum and ileum of 21-d-old poults showing a higher density of goblet cells (arrow) in poults fed a direct-fed microbial (panels A, C, and E) compared with the control-fed poults (panels B, D, and F).

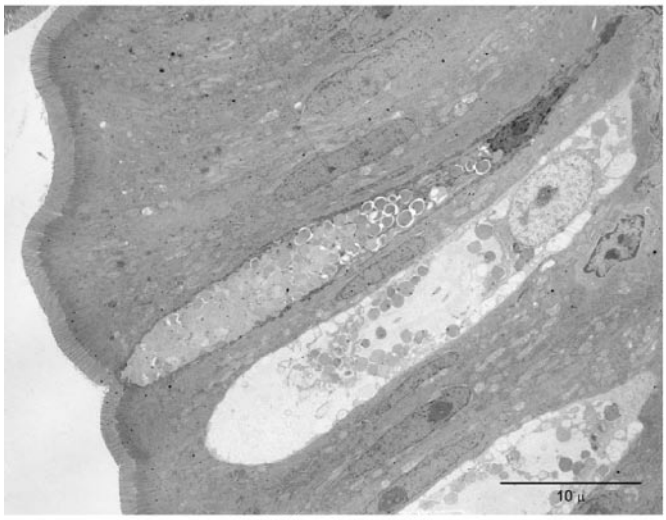
duction, resulting in better digestion and absorption of nutrients.

The dietary DFM used in this study reduced *Salmonella* colonization and improved turkey poult performance (Grimes et al., 2008). In the scanning electron microscopic evaluation of the samples, rod-shaped bacteria (possibly *Salmonella*) were observed on the mucosal surface of the intestine of *Salmonella*-challenged control groups (Figure 8). Most of these bacteria were positioned on the tissue surface rather than embedded in the mucus blanket. In *Salmonella*-inoculated birds, histomorphological changes in intestinal mucosa such as shortening, atrophy, and reduced density of villi were observed (Figure 9). It appears that feeding DFM may restore some of the villi loss or damage associated with *Salmonella* challenge. These results are in agreement with the findings of Ghabdan (1998) who observed that spray application of a probiotic in water reduced *Salmonella* and *Escherichia coli* colonization in the ceca of chickens from 38.8 to 9.72% and from 51.4 to 22.2%,



IPM797 Ileum Goblet 4,000X

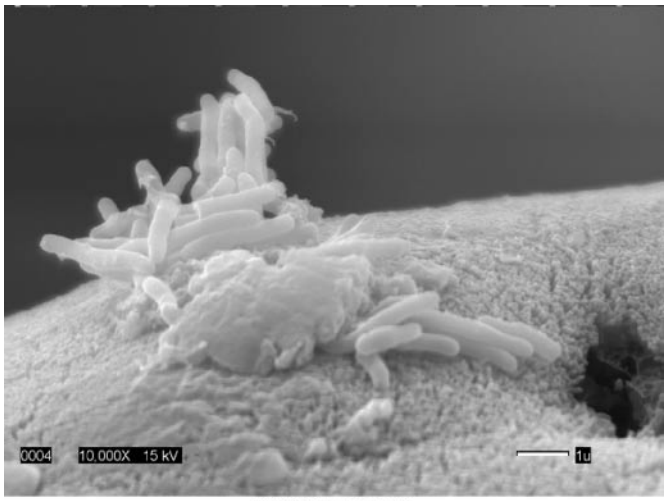
A



ICC712 Ileum Goblet 4,000X

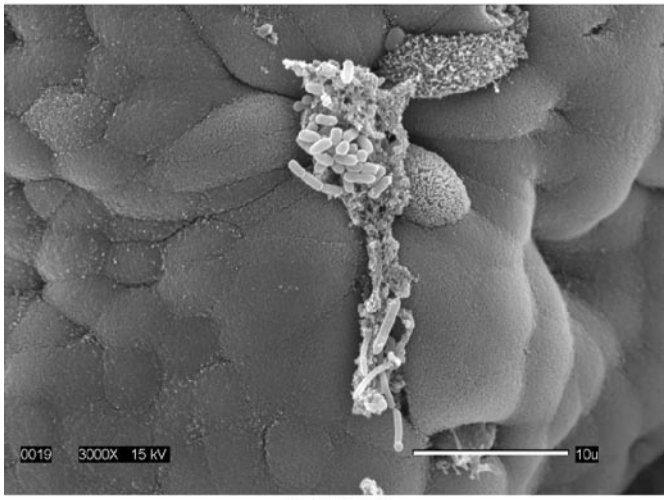
B

Figure 7. Transmission electron micrographs of ileum of 21-d-old poults fed diets with or without a direct-fed microbial (DFM), showing a higher amount of mucus secretion (arrow) in the intestinal lumen of the bird fed DFM (A) compared with the control-fed bird (B).



ICC712 Duodenum B4

A



PC591 Ileum 3

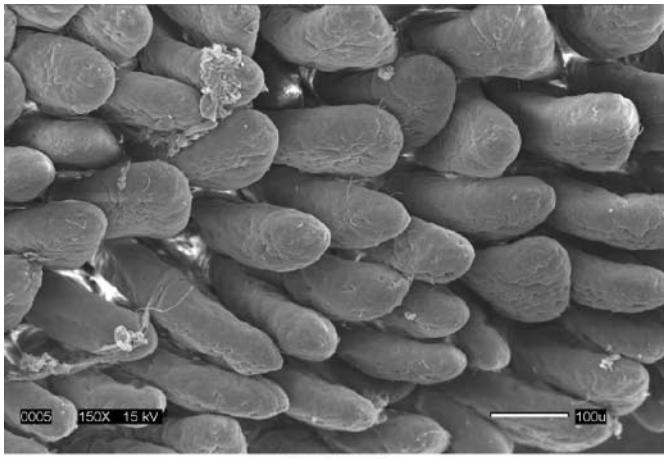
B

Figure 8. A) Scanning electron micrograph of duodenum of a 21-d-old poult fed a diet without a direct-fed microbial (DFM) and inoculated with *Salmonella*, showing colonization of rod-shape bacteria (*Salmonella*-like organism) on mucosal surface of intestine; B) the DFM-fed poult has colonization of cocci-like microorganisms on the surface of the intestinal mucosa.

respectively. However, Ozurk and Yildrim (2004) found that a probiotic-based *Lactobacillus* treatment had no effect on ileal and cecal gram-negative bacteria counts.

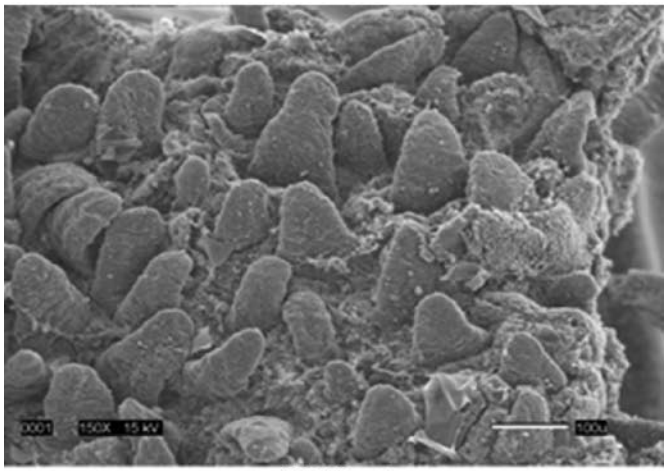
Although the segmented filamentous-like bacteria were not quantified, it appeared that the DFM-fed birds had a large number of these organisms (Figure 10). This is in contrast to the report by Chichlowski et al. (2007) who reported that segmented filamentous-like bacteria were less numerous in DFM-fed chicks compared with control-fed chicks. These types of bacteria are important to induce the development of an immune response (Meyerholz et al., 2002) and to have a potential antagonistic effect against GI bacterial pathogens (Heczko et al., 2000). The components of the bacterial cell wall such as peptidoglycan and lipopolysaccharide have been reported to play an important role in the activation of the immune system (Hamman et al., 1998). These nonpathogenic bacteria strongly stimulate the mucosal immune system and induce intestinal epithelial cells to express major histocompatibility complex class II molecules. These bacteria may have potential antagonistic effects against GI bacterial pathogens (Heczko et al., 2000).

Interaction between mucin and bacteria play a role in the integrity of the mucus barrier and thus may influence its protective properties (Gotteland et al., 2001). Gunal et al. (2006) reported that supplementation of a probiotic in broiler diets increased the mucin glycoprotein concentration by 110% in the jejunum compared with the control ($P < 0.05$). Kunikata et al. (2002) reported that probiotic bacterial strains act on mucin secretion and synthesis via prostaglandin production.



CM504 Ileum 1

A



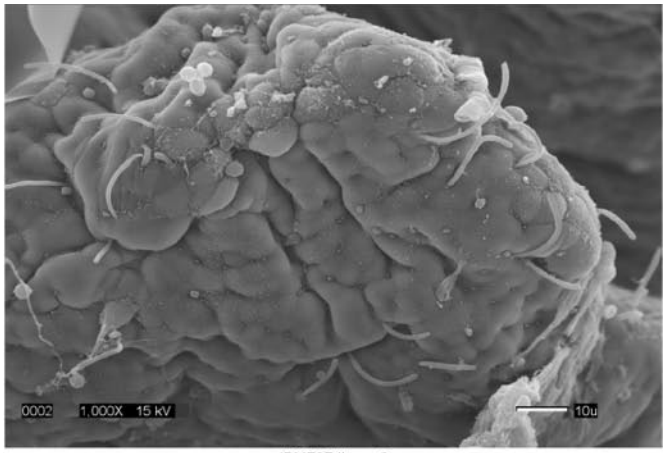
ICM674 Ileum 1

B

IPM797 Ileum 4

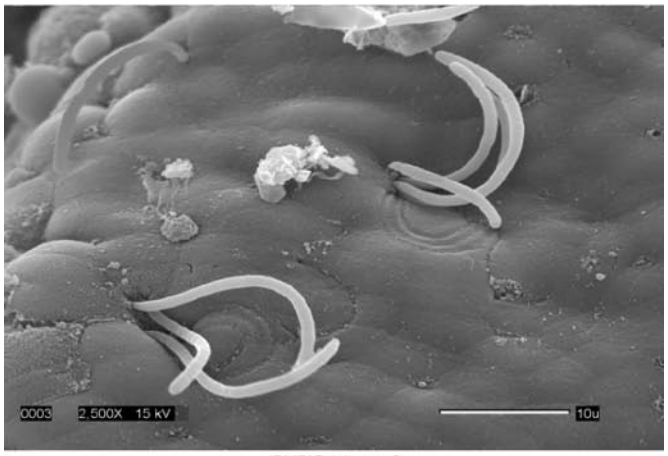
C

Figure 9. Scanning electron micrographs of ileum of 21-d-old poults fed diets with a direct-fed microbial (DFM, panel C) or without DFM (panels A and B). A) Not challenged with *Salmonella*; B) and C) challenged with *Salmonella*. Panel B shows shortening, thickening, and atrophy of the villi compared with panels A and C.



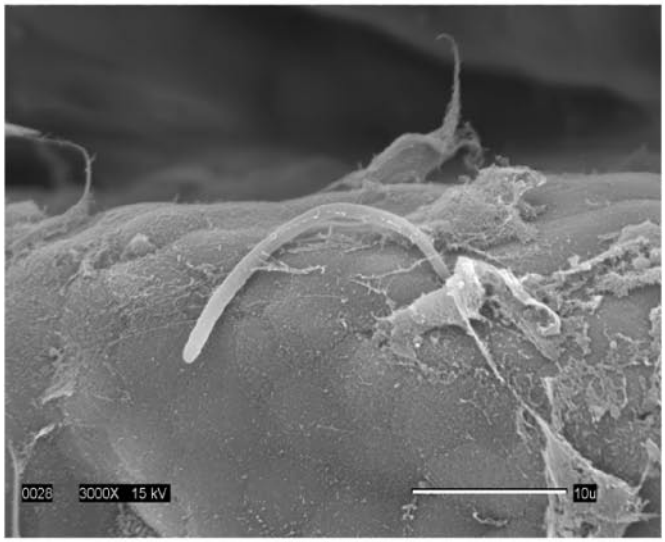
IPM797 Ileum 2

A



IPM797 Jejunum 5

B



PM631 Ileum 3

C

Figure 10. Scanning electron micrographs of ileum (A) and jejunum (B) of 21-d-old poults fed diets with a direct-fed microbial (DFM) and challenged with *Salmonella*, and ileum (C) of a poult fed a DFM and not challenged with *Salmonella*, showing colonization of segmented filamentous-like bacteria on mucosal surface of intestine.

Because of the resistance of mucin to proteolytic enzymes of the GI tract, the role of microflora is very important in mucin degradation. There are many bac-

terial species that possess mucin-degrading glycosidases and glycosulfatases (Robertson and Wright, 1997).

Changes in intestinal morphology as observed in this study support the concept that poultry gut health and function, and ultimately bird performance, can be improved by dietary supplementation with DFM products such as Primalac as used in this study.

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