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308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

Purified Cell Wall of *Saccharomyces cerevisiae* Increases Protection Against Intestinal Pathogens in Broiler Chickens

B. Baurhoo¹, F. Goldflus² and X. Zhao¹

¹Department of Animal Science, McGill University, Ste. Anne de Bellevue, H9X 3V9, QC, Canada

²Biorigin, São Paulo, SP, Brazil

Abstract: A study was conducted to determine effects of a mannanoligosaccharide prebiotic, derived from cell wall of the yeast *Saccharomyces cerevisiae*, on morphological development of the intestines and microbial populations of the ceca and litter. Dietary treatments included: antibiotic-free diet (CTL), diet 1 + virginiamycin (VIRG; 16.5 mg/kg feed) and diet 1 + ActiveMOS (MOS; 1.5 kg/T starter diet and 1 kg/T grower diet). Each treatment was assigned to 3 pen replicates (55 birds/pen). At day 14, 24 and 34, cecal contents were used for *Lactobacilli*, *Bifidobacteria*, *E. coli* and *Campylobacter* quantification whereas litter was analyzed for *Campylobacter* and *E. coli*. At same time points, jejunum samples were used in histological analysis. MOS significantly increased goblet cell number in the jejunum ($p < 0.05$) at day 24 and 34. In contrast to the CTL and VIRG diet, MOS consistently increased cecal populations of *Bifidobacteria* ($p < 0.05$) at all times. Moreover, at day 34, MOS increased cecal populations of *Lactobacilli* ($p < 0.05$) and reduced *E. coli* and *Campylobacter* concentrations ($p < 0.05$). None of the dietary treatments altered *E. coli* and *Campylobacter* concentrations in the litter. In comparison to antibiotics, MOS, therefore, improved intestinal health conditions by increasing goblet cell number into the villi membrane, stimulating growth of beneficial bacteria and reducing colonization by pathogenic bacteria.

Key words: Antibiotic, mannanoligosaccharides, bacteria, goblet cells, broiler

INTRODUCTION

Antibiotics as growth promoters have intensely been used for decades in poultry production to improve farm performance and in the control of intestinal pathogens. With increasing interests in discontinuing the use of antibiotics in livestock production, there is much concern about finding natural alternatives to antibiotics that would sustain sound chicken health. Several products with different modes of actions have been proposed, including prebiotics, probiotics, acidifiers and phenolic compounds.

Mannanoligosaccharide (MOS), a natural feed additive derived from yeast cell wall, has received profound scientific consideration in chickens due to its associated intestinal health benefits in the presence or absence of antibiotics. For instance, MOS competitively binds to mannose-specific lectin, namely FimH, of gram-negative pathogens that express Type-1 fimbriae such as *Salmonella* and *Escherichia coli* (Thomas *et al.*, 2004) and result in their excretion of the intestines (Spring *et al.*, 2000; Baurhoo *et al.*, 2007b). Moreover, MOS substantially increased the number of goblet cells in broiler intestines (Baurhoo *et al.*, 2007a). Goblet cells are specialized cells that produce and secrete mucins, glycoprotein compounds, which bind pathogenic microorganisms and reduce their adherence to the intestinal mucosa (Bloomberg *et al.*, 1993). These represent key mechanisms by which MOS reduce intestinal colonization of pathogens.

But, intestinal health responses vary among different MOS products due to differences in mannoproteins, mannose and glucose compositions which occur depending on the yeast strain used, growing conditions and product manufacturing. The present study aimed at determining effects of a less studied MOS product, derived from cell wall of the yeast *Saccharomyces cerevisiae*, on morphological development of the intestines and microbial populations of the intestines and litter, in comparison with those of an antibiotic-free diet and one containing the antibiotic, virginiamycin.

MATERIALS AND METHODS

Bird management and experimental design: Four-hundred-ninety-five 1-day-old male Cobb 500 broilers were randomly assigned to 3 dietary treatments (3 pen replicates; 55 birds per pen). Experimental diets included: antibiotic-free diet (CTL), VIRG (diet 1 + 16.5 mg/kg virginiamycin) and MOS (diet 1 + ActiveMOS³: 1.5 kg/T of starter diet and 1 kg/T of grower diet). Diets were formulated to be isonitrogenous, isoenergetic and to meet or exceed NRC (1994) requirements for macro and micronutrients. The basal diet is as previously described by Baurhoo *et al.* (2007a). A 2-phase feeding program was used including a starter diet starting from day 1-21 and a grower diet from day 22-38. Birds had access to water at all times.

Birds were grown on clean wood shavings in concrete-floor pens and each pen was equipped with 1 tube

feeder and 1 automatic waterer. Birds were grown under environmentally controlled conditions following standard temperature regimens which gradually decreased from 32-24°C by 0.5°C daily and a 20L:4D cycle. Procedures for bird management and care were approved by the Animal Care Committee of McGill University.

Microbiological analysis of ceca and litter: At day 24 and 34, birds were euthanized by electrical stunning and bleeding of the carotid artery. Fresh ceca were aseptically removed and used in microbiological analysis respective to birds and treatments (3 birds/pen). At day 14, considering limited amounts of digesta at the chick's young age, cecal contents from 3 birds were pooled per sample replicate to obtain enough digesta for enumeration of *Lactobacilli*, *Bifidobacteria*, *Campylobacter*, *Salmonella* and *E. coli*. Cecal samples were diluted 10-fold by weight in buffered peptone water⁴ followed by serial dilution in 0.85% sterile saline solution. All microbiological analyses were performed in duplicates and the average values were used for statistical analysis.

Lactobacilli were anaerobically assayed using Lactobacilli MRS Agar⁴ and incubated at 37°C for 48 h. Enumeration of *Bifidobacteria* was performed using Wilkins-Chalgren agar⁵ supplemented with glacial acetic acid⁴ (1 ml/L) and mupirocin⁵ (100 mg/L) and incubated at 37°C for 3 days. *E. coli* was assayed using Rapid *E. coli* 2 agar⁶ and *E. coli* supplement⁵ for 24 h at 37°C. *Salmonella* were assayed using Brilliant Green Agar⁵ for 24 h at 37°C. *Campylobacter* concentrations were determined using *Campylobacter* Agar Base⁵, Lysed Horse Blood⁵, Preston *Campylobacter* Selective Supplement⁵ and *Campylobacter* Growth Supplement⁵ as recommended by the supplier for 48 h at 42°C. Colonies of respective bacteria were counted after the incubation periods.

At same bird ages, litter was collected in the middle of each pen and equidistant from each other at each side end of the pen to the floor pen surface and away from the drinker region using examination gloves. Litter samples were thoroughly mixed and kept in sterile microbiological bags at -20°C until analysis. A 10 g sample was then used for subsequent enumeration of *Salmonella*, *Campylobacter* and *E. coli* as previously described.

Histological analysis: From each euthanized bird, at day 14, 24 and 34, a 1-cm segment of the jejunum was excised, washed in physiological saline solution and fixed in 10% buffered formalin. The tissue samples were then embedded in paraffin and a 2 µm section of each sample was placed on a glass slide and stained with haematoxylin and eosin for histological analysis. Parameters measured included villi height, goblet cell number into villi membrane, muscularis layer thickness

and crypt depth. Histological sections were examined using a phase contrast microscope⁷ with integrated image analysis NIS-Element BR v. 2.3 software. Ten measurements were recorded per bird for each variable and the average values used in statistical analysis.

Statistical analysis: All data were analyzed by a 1-way ANOVA using the MIXED procedure of SAS (SAS Institute, 2003) with pen nested within treatments. The Scheffe's Multiple Comparison test was used to test differences between treatment means and statistical significance was declared at p<0.05. All microbiological concentrations were subjected to log₁₀ transformation prior to statistical analysis.

RESULTS

Intestinal morphology: MOS caused major increases in goblet cell number when compared to CTL and VIRG fed birds at both day 24 and 34 (Table 1). There were, however, no differences in villi height, crypt depth and muscularis layer thickness among birds fed the CTL, VIRG and MOS diets at all time points.

Microbiology of ceca and litter: MOS consistently increased the cecal concentrations of *Bifidobacteria*, at day 14, 24 and 34, in comparison to CTL and VIRG fed birds (Fig. 1). On the other hand, *Bifidobacteria* seemed to be sensitive to antibiotic as demonstrated by its reduced concentrations when compared to CTL fed birds at all times. Additionally, MOS significantly increased *Lactobacilli* concentrations when compared to birds fed the CTL and VIRG diets at day 34; but, *Lactobacilli* counts were similar between birds fed CTL and VIRG (Fig. 2). There were also no treatment differences on *Lactobacilli* counts among birds in all treatment groups at day 14 and 24.

Birds were free of *Salmonella* similar to previous trials conducted in the same research facility. MOS significantly reduced cecal concentrations of *E. coli* and *Campylobacter* at day 34 only when compared to CTL fed birds (Fig. 3 and 4, respectively). When the same comparison was made, *Campylobacter* concentration was lower in VIRG fed birds at day 34. None of the

Table 1: Effects of antibiotics and MOS on goblet cells number per villus of broiler chickens¹

Age	Treatments ²			SEM
	CTL	VIRG	MOS	
Day 14	131.57	133.57	141.69	9.78
Day 24	135.59 ^b	127.54 ^b	174.22 ^a	8.30
Day 34	171.26 ^{ab}	140.10 ^b	196.52 ^a	10.84

¹Mean±SE of 9 replicates. ²CTL: antibiotic free diet; VIRG: CTL + 16.5 mg/kg virginiamycin; MOS: CTL + 0.15% and 0.1% ActiveMOS in starter (1-21 days) and grower (22-38 days) diet respectively. ^{a,b}Values with different superscript within same row are different (Scheffe t-test, p<0.05)

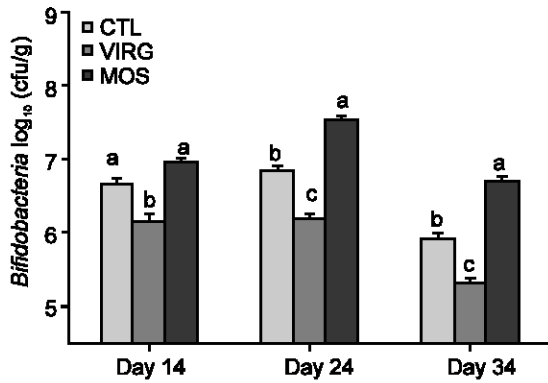


Fig. 1: Concentrations (log₁₀ CFU/g) of *Bifidobacteria* in the ceca of broiler chickens fed CTL (antibiotic free diet); VIRG (CTL + 16.5 mg/kg virginiamycin) and MOS: (CTL + 0.15% and 0.1% ActiveMOS in starter (1-21 days) and grower (22-34 days) diets respectively). ^{a,b,c} Values with different superscript within a group are different (Scheffe t-test, p<0.05)

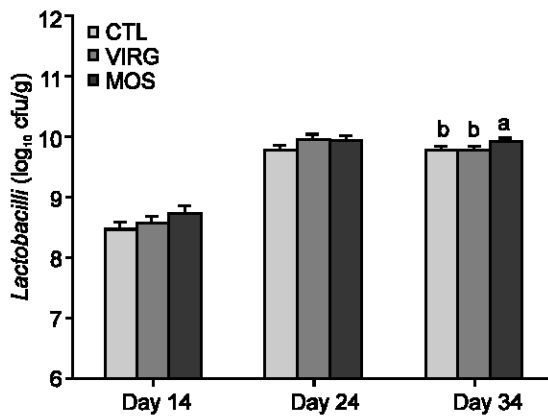


Fig. 2: Concentrations (log₁₀ CFU/g) of *Lactobacilli* in the ceca of broiler chickens fed CTL (antibiotic free diet); VIRG (CTL + 16.5 mg/kg virginiamycin) and MOS: (CTL + 0.15% and 0.1% ActiveMOS in starter (1-21 days) and grower (22-34 days) diets respectively). ^{a,b} Values with different superscripts within the same day are different (Scheffe t-test, p<0.05)

dietary treatment significantly altered concentration of these pathogenic bacteria at day 14 and 24. But, MOS tended to lower cecal loads of *E. coli* at day 24 and *Campylobacter* loads at day 14 and 24 when compared to CTL fed birds. Results of this study also demonstrate that neither MOS nor virginiamycin were effective in reducing *E. coli* and *Campylobacter* concentrations in the litter.

DISCUSSION

Our finding that MOS significantly increased the number of goblet cells into the villi membrane agrees with previous reports with broilers and turkeys (Baurhoo *et al.*, 2007a; Solis de los Santos *et al.*, 2007). The exact mechanism underlying such effect is still not clearly defined. Goblet cells, specialized cells residing in the villi membrane of the intestines, are responsible for the synthesis and secretion of high molecular weight glycoproteins, known as mucins. Mucin secretion is responsible for maintenance of the mucus blanket that plays important role in lubrication of the intestinal tract and transport of nutrients between luminal contents and epithelial lining. Most importantly, mucins represent the first line of host defense against invading pathogens or their associated toxins. Mucins mimic glycoprotein attachment sites of epithelial cell membrane and competitively bind to lectin receptors of pathogenic bacteria (Chadee *et al.*, 1987). Additionally, mucins possess specific mannosyl receptors in the oligosaccharide units that competitively attach to Type-I fimbriae of gram negative pathogens (Sajjan and Forstner, 1990); similar mechanism has been proposed for MOS actions in eliminating gram-negative pathogens. The continuous process of mucin secretion coupled with peristalsis movements allows excretion of trapped pathogens from the intestines. Increased mucin secretions due to MOS would, therefore, contribute to greater elimination of intestinal pathogens.

This study also demonstrates that MOS may favorably alter microbial ecology of the chicken intestines. MOS significantly increased intestinal concentrations of *Bifidobacteria* and *Lactobacilli*, but the increase was more pronounced in *Bifidobacteria*. On the other hand, virginiamycin inhibited growth of these beneficial bacteria due to its bactericidal properties against gram-positive bacteria. MOS, therefore, allow establishment of an intestinal population of beneficial bacteria that would limit growth of pathogens by competing for nutrients and bindings sites (Rolfe, 2000) and secreting antibacterial substances (Gibson and Wang, 1994; Jin *et al.*, 1996a,b). But, the mechanistic approach underlying MOS effects in favoring growth of beneficial bacteria is poorly understood. Our findings suggest that, through competitive exclusion of gram negative pathogenic bacteria possessing Type-I fimbriae, MOS and subsequent mucins secretion offer larger intestinal space for growth of beneficial bacteria. In addition, preferential colonization to glycoprotein receptor sites on the mucus blanket of the intestines by beneficial bacteria than Type-I pathogens such as *E. coli* has previously been demonstrated (Mack *et al.*, 1999). Our findings are in agreement with previous reports indicating significant increases in intestinal populations of beneficial bacteria when broilers were fed MOS diets (Fernandez *et al.*, 2002; Baurhoo *et al.*, 2007a,b). Unfortunately, MOS did

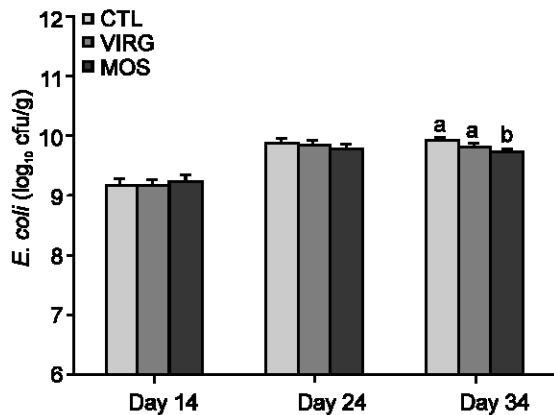


Fig. 3: Concentrations (log₁₀ CFU/g) of *E. coli* in the ceca of broiler chickens fed CTL (antibiotic free diet); VIRG (CTL + 16.5 mg/kg virginiamycin) and MOS: (CTL + 0.15% and 0.1% ActiveMOS in starter (1-21 days) and grower (22-38 days) diets respectively). ^{a,b}Values with different superscripts within the same day are different (Scheffe t-test, p<0.05)

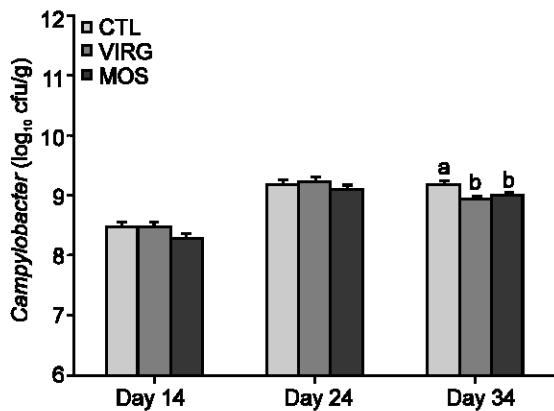


Fig. 4: Concentrations (log₁₀ CFU/g) of *Campylobacter* in the ceca of broiler chickens fed CTL (antibiotic free diet); VIRG (CTL + 16.5 mg/kg virginiamycin) and MOS: (CTL + 0.15% and 0.1% ActiveMOS in starter (1-21 days) and grower (22-38 days) diets respectively). ^{a,b}Values with different superscripts within the same day are different (Scheffe t-test, p<0.05)

not alter intestinal population of beneficial bacteria in other studies (Spring *et al.*, 2000; Fairchild *et al.*, 2001) despite significant reduction in pathogenic bacteria concentrations.

MOS successfully reduced *E. coli* and *Campylobacter* concentrations in the ceca, but these effects only occurred in the final stage of the study. Baurhoo *et al.* (2007a) and Fernandez *et al.* (2002) also observed a delay in MOS actions in challenge studies with

Salmonella enteritidis and *E. coli* in broilers. On the other hand, virginiamycin was only effective in reducing intestinal colonization of *Campylobacter* at day 34. Intestinal pathogens are known to compete with hosts for nutrients and cause overstimulation of the immune system (Bedford, 2000); these effects divert energy away from production, thereby depressing growth. Moreover, intestinal pathogens contaminate chicken carcasses as a consequence of accidental breakage of the intestines during processing (Heyndrickx *et al.*, 2002), thereby representing an important source of food-borne illnesses. MOS and to a lesser extent antibiotics can, therefore, minimize the harmful effects of intestinal pathogens and improve safety of poultry meat for human consumption.

The mechanistic action of MOS in attaching to Type-I fimbriae of gram negative pathogens and their elimination of the intestine has previously been proposed (Newman, 1994; Thomas *et al.*, 2004). Our findings suggest that increased mucin secretion and increased growth of beneficial bacteria represent additional key mechanisms underlying MOS actions against intestinal pathogens. The synergistic effects of these MOS effects would enable greater protection against intestinal pathogens. MOS is, thus, an effective dietary strategy that creates conditions of good health in the chicken intestines. A reduction in intestinal *E. coli* and *Campylobacter* concentrations was expected to cause less excretion of these pathogens and consequently less into the litter. But, such scenario was not evidenced in the present study when broilers were fed MOS and virginiamycin diets. The suitable environmental conditions and nutrient-rich contents of broiler litter (Kelleher *et al.*, 2002) might have favored microbial proliferation.

Our findings suggest that in addition to its direct effect of attaching to Type-I fimbriae of gram-negative pathogens, MOS action in eliminating intestinal pathogens synergistically occurs via increased mucin production and increased colonization by beneficial bacteria. In the absence of antibiotics, MOS can, therefore, confer greater protection against intestinal pathogens in broiler chickens.

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³Biorigin, São Paulo, Brazil.

⁴Fisher Scientific, ON, Canada.

⁵Oxoid, ON, Canada.

⁶Bio-Rad laboratories, ON, Canada.

⁷Nikon DXM 1200c, Nikon Corporation, Tokyo, Japan.