A 52-day pen trial on 10.2 cm built-up litter topped with pine shavings used a randomized complete block design (Tukey's HSD \( p \leq 0.05 \)). A total of 2,160 Cobb male chicks were randomly assigned to 48 pens (12 blocks) at 45 chicks/pen providing 0.0771 m\(^2\) per chick. Four phase feeds were used (0–17, 17–31, 31–42, and 42–52 days). Salinomycin (66.14 mg/kg) and 3-Nitro\(^6\) at 20 (50.0 mg/kg) were added from 0–31 days. Dietary treatments were: 1) negative control (nCON); 2) BMD\(^6\) at 55.12 mg/kg 0–31 days, Stafac\(^6\) at 22.05 mg/kg 31–52 days (AGP); 3) Actigen\(^6\) (ACT) at 400 mg/kg; and 4) BMD\(^6\) at 55.12 mg/kg 0–31 days, Stafac\(^6\) at 22.05 mg/kg 31–52 days plus Actigen\(^6\) (ACT) at 400 mg/kg (AGP + ACT). From 0–17 days, feed conversion ratio (FCR; \( p = 0.026 \)) and mortality-adjusted FCR (MAFCR; \( p = 0.027 \)) were lower for AGP and AGP + ACT than nCON, with ACT intermediate and statistically separate from other groups. At 31 days, body weight (BW; \( p = 0.043 \)) was greater for AGP + ACT than nCON, with AGP and ACT intermediate. From 31–52 days, FCR (\( p < 0.001 \)) and MAFCR (\( p < 0.001 \)) were lower for AGP, ACT, and AGP + ACT than nCON. At 42 days, BW was greater (\( p = 0.006 \)) for AGP and AGP + ACT than nCON, with ACT intermediate. The 0–42 day FCR (\( p = 0.002 \)) and MAFCR (\( p = 0.006 \)) were lower for AGP and AGP + ACT than nCON, with ACT intermediate. At 52 days, BW were greater (\( p < 0.001 \)) for AGP, ACT, and AGP + ACT than nCON. From 0–52 days, FCR (\( p < 0.001 \)) and MAFCR (\( p < 0.001 \)) were lower for AGP + ACT than nCON, with AGP and ACT intermediate, and mortality percent was lower (\( p = 0.096 \)) for AGP + ACT than nCON, with AGP and ACT in between. Based on performance, Actigen\(^6\) at 400 mg/kg was statistically equivalent to BMD\(^6\) /Stafac\(^6\) from 0–52 days and BMD\(^6\) /Stafac\(^6\) plus Actigen\(^6\) was the most effective treatment.

**Key words**: actigen, bacitracin, broiler chicken, mannan oligosaccharide, yeast cell wall


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**Introduction**

Widespread use of dietary low-level antibiotics has come under pressure from consumers of chicken due to concern about development of antibiotic-resistant bacteria and the potential of diminished effectiveness of antibiotics used for humans. For this reason, alternative feed additives have been sought to help maintain gut health and to improve live performance and processing characteristics of broiler chickens. One of the most prominent classes of feed additives for these purposes is yeast cell wall mannan oligosaccharide.

Certain components of pathogenic bacteria surfaces, called lectins, are involved in the onset of enteric and urinary disease by allowing adherence of the bacteria to epithelial cells at these sites (Freter and Jones, 1976; Salit and Gotschlich, 1977; Eshdat et al., 1978; Firon et al., 1983; Ruggieri et al., 1985). The use of oligosaccharides such as mannan oligosaccharide for pathogen control evolved from the discovery that simple sugars such as galactose, fucose, lactose, or mannosamine could reduce pathogen infection. Although there are a number of lectins specific for different sugars, lectins specific for mannose predominate in intestinal pathogens. The mannosamine sugar occupies the mannose-specific receptors that exist on certain pathogens for adherence to the intestinal epithelium (Oyofo et al., 1989a; Oyofo et al., 1989b; Corrier et al., 1990).

Dietary mannan oligosaccharide from *Saccharomyces cerevisiae* (yeast) outer cell wall acts as a high-affinity ligand and provides a competitive binding site for the harmful bacteria (Ofek *et al.*, 1977). Intestinal pathogens such as...
Salmonella species and Escherichia coli, which contain mannose-specific (Type 1) fimbriae, are therefore prohibited from attaching to intestinal epithelial cells, proliferating, and producing toxins (Spring et al., 2000; Edelman et al., 2003). In vitro studies examining E. coli adhering to epithelial cells revealed that E. coli could be displaced from these epithelial cells within 30 minutes when exposed to a mannan derivative whereas galactose and glucose derivatives (galactan and glucan) had no effect on attached bacteria (Ofek and Beachey, 1978).

Besides binding to enteric pathogens to prevent their adhesion to the gut wall (Eshdat et al., 1978; Spring et al., 2000), some of the other effects of dietary mannan oligosaccharide include: 1) in some cases, beneficially modifying microflora fermentation and intestinal bacterial population (Fernández et al., 2002; Baurhoo et al., 2007a; Baurhoo et al., 2007b; Rehman et al., 2009); 2) maintaining integrity of gut lining and improving gut health such as greater villus height (Ji et al., 2001; Zhang et al., 2005; Baurhoo et al., 2007b; de Oliveira et al., 2008; Morales-López et al., 2009), crypt depth (de Oliveira et al., 2008), and villus height/crypt depth ratio (Zhang et al., 2005), and greater goblet cell densities (Baurhoo et al., 2007b; Brümmer et al., 2010) and sizes (Brümmer et al., 2010); and 3) enhancing immune responses by immune modulation (Gómez-Verduzco et al., 2009). The presence of dietary mannooligosaccharide and pathogens together the lumen of the intestine functions as an adjuvant and antigen system, allowing enhanced antigenicity and superior immune response (Shafey et al., 2001).

Mannan oligosaccharide from yeast has been used as a dietary supplement in broiler feeds since about 1993, and several different commercial products are available. These additives typically contain mannan oligosaccharide and β-glucans from yeast cell walls. A holo-analysis of the efficacy of a commercial mannan oligosaccharide product in broiler nutrition was conducted using 32 publications containing results from 82 experiments in 21 countries and involving a total of 85,142 broiler chickens (Rosen, 2007). Calculated improvements using the mannan oligosaccharide supplemented diets compared to negative control diets were: body weight gain, +27.6 g/bird; feed conversion ratio, −0.0391, and mortality, +0.0311%. The respective beneficial response frequencies for body weight gain, feed conversion ratio, and mortality were 65, 70 (52% jointly), and 52%.

Hooge et al. (2003) reported that in a 49-day broiler pen trial, negative control diets, bacitracin methylene disalicylate diets (55/55/27.5 mg/kg from 0–21, 21–42, and 42–49 d, respectively), and mannan oligosaccharide diets (0.1, 0.05, and 0.05% by phases) were compared using lasalocid as a coccidiostat throughout. In a second 49-day pen trial, negative control diets, bacitracin methylene disalicylate (55 mg/kg from 0–42 days) and virginiamycin (11 mg/kg from 42–49 days) diets, and mannan oligosaccharide (0.1% from 0–21 days and 0.05% from 21–49 days) diets were compared using nicarbazin in starter and monensin in grower and finisher feeds as coccidiostats. The antibiotic or mannan oligosaccharide supplemented diets significantly increased body weight and decreased feed conversion ratio versus negative control diets. Mortality percentages were not significantly different by treatments.

de Oliveira et al. (2007) observed in a trial using broilers 13–23 day of age with excreta collection from 20–22 days and digesta collection at 23 days, diets with mannan oligosaccharide (0.1%) significantly increased ileal digestibility coefficients for dry matter, crude protein, Ca, P, and Ca retention versus unsupplemented or antibiotic supplemented diets. Diets with mannan oligosaccharide significantly increased digestible energy values versus unsupplemented diets and AME values versus diets containing antibiotics.

Gómez-Verduzco et al. (2009) found in a pen trial in which broilers were inoculated with live coccidial vaccine (Paracox-5) on d 1 and challenged with 3 Eimeria strains on d 15 that dietary mannan oligosaccharide (2, 1, and 0.5 kg/tonne in phase feeds) significantly improved 15–42 and 22–42 day feed conversion ratio versus no supplementation (Nollet et al., 2007). Supplementation of mannan oligosaccharide (0.05%) alone or in combination with coccidiostats (125 ppm nicarbazin 0–21 days and 65 ppm salinomycin 21–49 days) to broiler diets significantly reduced oocysts in feces (cells/g). Dietary mannan oligosaccharide significantly increased intestinal IgA (ng/mL), tracheal IgA (ng/mL), antibody titers against Newcastle Disease Virus at 17 or 24 days, and basophilic hypersensitivity (mm²) compared to unsupplemented controls.

In a 42-day pen trial in which broiler chickens were fed basal diets or mannan oligosaccharide supplemented diets (0.2, 0.1, 0.05% by phases) and subjected to a Clostridium perfringens challenge at 21–23 days, the mannan oligosaccharide diets were effective at hindering the ulcerative (necrotic enteritis) effect of pathogenic Clostridium perfringens on the intestines of the chickens. The dietary mannan oligosaccharide also resembled the zinc bacitracin (0.0333%) treatment in thinning the villi so as to improve the digestive/absorptive site (Ngxumeshe and Gous, 2009). Cecal E. coli and Campylobacter counts were significantly lowered at 34 days in broilers fed diets supplemented with a high level of mannan oligosaccharide (0.5%) (Baurhoo et al., 2009). In a broiler pen trial in which dietary mannan oligosaccharide (0% versus 0.2% 0–21 days and 0.1% 21–42 days) was fed, there was a significantly lower population of E. coli in the litter of supplemented birds (Baurhoo et al., 2007b).

A second generation yeast outer cell wall derived product called Actigen® (ACT; Alltech, Inc., Nicholasville, Kentucky, USA) was developed using nutrigenomics technology which studies changes in expression of genes in intestinal cells. This specific component is extracted from, but is about 2.5 more times more concentrated than, the first generation yeast outer cell wall product (Bio-Mos®). Product assays and in-feed assays allow the confirmation of the active ingredient qualitatively and quantitatively through ELISA technology. Thus ACT is definitely different than the mannan oligosaccharide products that have gone before it. The ACT can be
supplemented to broiler chicken feeds at 200 to 800 g/tonne which is about 40% of the original product’s inclusion rate. It is considered to be a growth permitter through its roles in immune modulation and improved intestinal health.

The objective of this built-up litter pen trial was to compare the efficacy of diets supplemented with antibiotics, Actigen®, or both to negative control basal diets for improving broiler performance of broiler chickens in late spring and summer in Georgia, USA. It should be noted here that the addition of antibiotics to broiler chicken feeds in Japan is permitted only for growth promotion and not for prevention of diseases as in the USA (where lower or higher levels are allowed for growth promotion or disease prevention).

Materials and Methods

The litter floor pen trial was carried out according to standard operating procedures at Southern Poultry Research, Inc., Athens, Georgia, USA. The broiler chicks were acquired, housed, retained, and used in compliance with these guidelines and procedures for ethical treatment of experimental broiler chickens.

A 52-day broiler pen trial (May 16–July 7, 2011) was conducted on approximately 10.2 cm (4 in.) built-up litter with a coating of fresh pine shavings to compare the efficacy of antibiotic, Actigen®, antibiotic plus Actigen® supplemented diets with unsupplemented negative control diets for improving broiler performance. Two tube feeders and 1 bell-shaped drinker were used per pen. From placement until day 7, feed was also supplied on feeder trays placed on the litter. Gas heaters were the primary heat source with a heat lamp in each pen for supplemental heat as required. Fans and side-wall curtain manipulation were used for ventilation. Lighting was provided continuously 24 hours a day. Relative humidity was ambient.

A total of 2,160 day-old Cobb male broiler chicks from Cobb-Vantress Hatchery, Cleveland, Georgia, USA were randomly assigned to 48 pens, thus providing 45 chicks per pen. There were 12 blocks of pens with 4 pens per block, so each of the 4 dietary treatments was replicated 12 times in those blocks in a randomized complete block design. Stocking density was 0.0771 m² (0.83 ft²) per chick, and no birds were replaced during the trial.

Commercial type corn-soy basal diets containing animal protein were used (Table 1). Calculated analyses met or exceeded National Research Council (1994) standards except that starter feed metabolizable energy, crude protein, and calcium were lower and in line with current industry standards. Calculated total phosphorus has been reported. Experimental feeds were prepared as aliquots from a uniform basal diet by phases. The 4 dietary treatments included: 1)
Results

The broiler live performance results at 17, 31, 42, and 52 days of age in the built-up litter pen trial are shown in Table 2. Feed conversion ratio was calculated as feed weight/survivor weights, and mortality-adjusted feed conversion ratio was calculated as feed weight/survivor weights + dead bird weights. From 0–17 days, feed conversion ratio ($p=0.026$) and mortality-adjusted feed conversion ratio ($p=0.027$) were significantly better for AGP and AGP+ACT than nCON with ACT intermediate and statistically separate from other groups.

At 31 days, significant improvement in body weight ($p=0.043$) was observed using AGP+ACT compared to nCON, with AGP and ACT groups intermediate. The feed conversion ratio ($p<0.001$) and mortality-adjusted feed conversion ratio ($p<0.001$) from 0–31 days were significantly lower for AGP, ACT, and AGP+ACT groups compared to nCON.

At 42 days, body weight was significantly greater ($p=0.006$) for AGP and AGP+ACT groups than for nCON, with ACT intermediate. The 0–42 day feed conversion ratio ($p=0.002$) and mortality-adjusted feed conversion ratio ($p=0.006$) were significantly reduced in AGP and AGP+ACT groups compared to nCON, with ACT intermediate.

At 52 days (final results), significant ($p<0.001$) improvements in body weight were found using AGP, ACT, and AGP+ACT compared to nCON. The 0–52 day feed conversion ratio ($p<0.001$) and mortality-adjusted feed conversion ratio ($p<0.001$) were significantly lower for AGP+ACT than for nCON, with AGP and ACT intermediate. No significant differences in mortality percent were found between treatments at any age ($p \leq 0.05$). However, from 0–52 days mortality percent was lower for AGP+ACT than for nCON, and approaching significance ($p=0.096$), with AGP and ACT in between.

Discussion

Built-up litter offers a built-in disease challenge which tends to accentuate differences between nCON diets and diets supplemented with growth promoters (AGP; BMD® and Stafac®) or growth permitter (ACT; Actigen®). These differences were detected with the use of a single gender (male) pen trial to reduce variability and 12 replicate pens per treatment for good accuracy in determining treatment means and significant differences.

The antibiotics and yeast cell wall product have different modes of action with the antibiotics being effective against Gram-positive microorganisms, especially *Clostridium perfringens*, and the yeast cell wall product providing immune modulation (Shafey et al., 2001; Shashidhara et al., 2003), selective pathogen binding (Eshdat et al., 1978; Spring et al., 2000), and beneficially modifying microflora fermentation and intestinal bacterial population (Fernandez et al., 2002; Baurhoo et al., 2007a; Baurhoo et al., 2007b; Rehman et al., 2009). Despite their different modes of action, their subsequent beneficial effects on performance were similar. This makes the yeast cell wall an alternative product for consideration when antibiotic-free broiler production is required. Interesting also was the additive effect of antibiotics plus yeast cell wall product which make this a promising commercial treatment to enhance performance in situations where antibiotics continue to be used.

Litter conditions deteriorated as birds grew with the hot and very humid conditions. Thus a stronger response to AGP, ACT, and AGP+ACT above nCON group was seen as the litter became less favorable with age of the birds. One day a few birds were lost to heat, but not more than one out of any pen. More fans were used on the birds and the heat stress mortality stopped immediately.

Compared to the holo-analysis results using 82 experiments (85,142 broiler chickens) involving a dietary commercial mannan oligosaccharide product (Bio-Mos®, Alltech, Inc., Nicholasville, Kentucky, USA; Rosen, 2007) in which improvements were $+27.6\text{g/bird in weight and }−0.0391$ in feed conversion ratio (with a slight worsening in mortality of $+0.0311\%$), results of the trial reported herein at 52 days were $+102\text{g/bird, }−0.052$ in feed conversion ratio, and $−1.482\%$ in mortality for ACT compared to nCON groups. In two 49-day broiler pen trials, improvements in a mannan oligosaccharide (Bio-Mos®) supplemented diets were $+115\text{g/bird and }+70\text{g/bird in weight, }−0.180$ and $−0.060$ in feed conversion ratio, and $−2.50\%$ and $−0.42\%$ mortality compared to negative control diets (Hooge et al., 2003).

In conclusion, a built-up litter pen trial was conducted from May-July at Southern Poultry Research, Athens, Georgia, USA to evaluate live performance benefits of antibiotic growth promoters (BMD® 55.12 mg/kg 0–31 days and Stafac® 22.05 mg/kg 31–52 days), a yeast cell wall product (Actigen® 400 mg/kg 0–52 days) considered to be a growth permitter, or both types of additives in broiler diets versus....
basal diets. Based on performance, Actigen® at 400 mg/kg was statistically equivalent to BMD®/Stafac® from 0–52 days and BMD®/Stafac® plus Actigen® was the most effective treatment.

References


