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Meta-Analysis Summary of Broiler Chicken Trials with Dietary Actigen® (2009-2011)

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Abstract: Statistical meta-analyses of results from broiler trials in 2009-2011 using dietary Actigen® were conducted. Actigen® (Alltech, Inc., Nicholasville, Kentucky, USA) is a second-generation, yeast cell wall product considered to be a "growth permitter" through its roles in immune modulation and improved intestinal health. Parameters evaluated were dietary inclusion rates for Actigen®, age of birds, Body Weight (BW), feed conversion ratio or feed/gain ratio and mortality %. Nine reports were collected allowing 15 comparisons of negative control (nCON) diets and Actigen® supplemented diets fed during the entire trials. Similarly, 9 reports were collected allowing 11 comparisons of positive control (antibiotic supplemented) diets and Actigen® supplemented diets. When added to basal diets, Actigen® at average inclusion rates by phases of 520/400/347 g/tonne (n = 15) and broiler age of 41.87 days (n = 15) significantly and beneficially changed body weight by +0.129 kg (+5.41%), feed conversion ratio or feed/gain ratio by -0.046 (-2.54%) and mortality % by -0.76 (-10.5% relative to nCON). Compared with positive control (antibiotic) results, dietary Actigen® at average inclusion rates by phases of 535/331/238 g/tonne (n = 11) and broiler age of 43.64 days (n = 11) non significantly changed body weight by +0.016 kg (+0.62%), feed conversion ratio or feed/gain ratio by -0.003 (-0.17%) and mortality % by +0.57 (+7.97% relative to positive control). Broiler results for antibiotic or Actigen® supplemented diets were statistically equivalent. Comparisons between this present meta-analysis and those done previously on another yeast cell wall compound (Bio-Mos®) suggest that the second generation product Actigen® may be more effective in terms of growth promotion.

Key words: Actigen, antibiotic, broiler chicken, mannan oligosaccharide, meta-analysis

INTRODUCTION

To keep coccidiosis and bacterial diseases such as necrotic enteritis under control when broiler chickens are produced on litter, dietary coccidiostats or vaccines and various antibiotic growth promoters or alternative products are used. Bacitracin methylene disalicylate (BMD®, Alpharma, Inc., Fort Lee, New Jersey, USA) is selective in its action in that it affects only Gram positive microbes such as streptococci, staphylococci, and clostridia. Bacitracin suppresses synthesis and induces lysis of bacterial cell walls, interferes with synthesis of protoplasmic proteins within the cells, and reduces toxin production by certain bacteria resulting in less inflammation of the gut (International Minerals and Chemicals Corporation, ca. 1978). Virginiamycin (Stafac®, Phibro Animal Health, 2010) is a streptogramin antibiotic approved for broiler chickens at 5 g/ton (2,000 lb) to improve feed conversion ratio, 5-15 g/ton to improve weight gain, and 20 g/ton for prevention of necrotic enteritis caused by *Clostridium prefringens* susceptible to virginiamycin. The use of low-level antibiotics such as bacitracin and virginiamycin have come under pressure from consumers of chicken due to concern about antibiotic resistant bacteria and the potential diminished effectiveness of antibiotics used for humans.

Mannan oligosaccharide from yeast outer cell wall has been used as a dietary supplement in broiler feeds (Kumprecht *et al.*, 1997; Waldroup *et al.*, 2003; Rosen, 2007) since about 1993 when Alltech, Inc., Nicholasville, Kentucky, USA introduced the first generation commercial product (Bio-Mos®). Oligosaccharides are carbohydrates which on hydrolysis yield about 2 to 10 monosaccharides. The idea to use yeast mannan oligosaccharide in poultry feeds evolved from the concept that certain sugars, particularly mannose, largely block the colonization of intestinal pathogens such as *Salmonella* species and *Escherichia coli*, which contain type 1 fimbriae, from attaching to intestinal mannose, proliferating, and producing toxins and damage.

Some of the demonstrated modes of action of dietary mannan oligosaccharide include: 1) binding of enteric pathogens with Type 1 fimbriae (mannose seeking lectins) and thus blocking their adhesion to the gut lining (Eshdat *et al.*, 1978; Spring *et al.*, 2000); 2) modifying microflora fermentation (less propionic acid and ammonia production) to conserve nutrients for the bird to utilize (Ferket *et al.*, 2005); 3) maintaining integrity of gut lining and improved gut health such as greater villi height (Iji, 2001), more goblet cells/mm villus height thus increasing the brush border mucin barrier, and thinner

muscularis layer (Ferket *et al.*, 2005); and 4) immune modulation enhancing immune responses (adjuvant effect and liver secretion of mannose-binding protein to bind bacteria and initiate the complement cascade of the bird's immune system) (Savage *et al.*, 1996; Cotter *et al.*, 2002; Shashidhara *et al.*, 2003; Ferket *et al.*, 2005). The presence of dietary mannan oligosaccharide and pathogens together in the lumen of the intestine functions as an adjuvant and antigen system, allowing enhanced antigenicity and superior immune response (Shafey *et al.*, 2001).

A second-generation yeast outer cell wall specific compound derived product is Actigen® (Alltech, Inc., Nicholasville, Kentucky, USA). This was developed using nutrigenomics technology which allows detection of changes in expression of genes in intestinal cells. Internal trials by Alltech confirmed this to be at least 2.5 more times more concentrated in biological activity than the first generation mannan product (Bio-Mos®). The higher activity is clearly what makes this compound unique. Actigen® is typically supplemented to broiler chicken feeds at 200 to 800 g/tonne which is about 40% of the original product's inclusion rate. Actigen® is considered to be a growth promoter through its roles in immune modulation and improved intestinal health. The units of activity in the commercial product may be determined by rapid kit test developed by Alltech and a separate in-feed assay is also available to confirm quantitatively and qualitatively the presence of the product.

The objective of this summary article was to collect and statistically analyze by paired t-test (Statistix 8®, Analytical Software, Inc., Tallahassee, Florida, USA) broiler feeding trial results worldwide from 2009-2011 to compare negative control (basal diets) or positive control (antibiotic-supplemented) diets with Actigen®-supplemented diets.

MATERIALS AND METHODS

Worldwide broiler chicken pen trial reports (2009-2011) were analyzed statistically to determine effects of *Saccharomyces cerevisiae* var. *boulardii* yeast outer cell wall specific-compound derived product (Actigen®, Alltech, Inc., Nicholasville, Kentucky, USA) on live performance. Actigen® was developed using nutrigenomics technology which allows detecting changes in expression of genes in intestinal cells. Criteria for selecting studies were: 1) written report, 2) antibiotic(s) or Actigen® were fed for the entire trial, 3) age of birds (duration of trial), 4) negative (basal) and/or positive (antibiotic) control, 5) antibiotic stated for positive control, 6) replication, and 7) body weight and feed conversion ratio or feed/gain ratio were given (and mortality % was included when reported). Results were analyzed by treatments using paired t-test in Statistix 8® software (Analytical Software, Inc., Tallahassee, Florida, USA).

RESULTS

Negative control (nCON) diets versus Actigen® (ACT) diets: Presented in Table 1 is a summary of liver performance results from broiler chicken trials with negative control (nCON) versus Actigen®-supplemented (ACT) diets. There were 9 written reports providing 15 comparisons of results using these 2 treatments. The average age (and trial duration) was 41.87 days. Average inclusion rates for Actigen® by phases were 520/400/347 g/tonne, and it was fed during the entire trial in each case. Body weight was very highly significantly ($p < 0.001$) greater for the ACT birds than for nCON birds (+0.129 kg; +5.41%). The feed conversion ratio or feed/gain ratio was very highly significantly ($p < 0.001$) lower for ACT birds than for nCON birds (-0.046; -2.54). Mortality % was not significantly ($p = 0.153$) affected by dietary treatment; however, the ACT birds had 0.76% lower mortality which amounted to 10.5% relative reduction in mortality % compared to nCON birds.

Antibiotic growth promoter (AGP) diets versus Actigen® (ACT) diets:

Listed in Table 2a are the broiler chicken live performance results from 9 written reports providing 11 comparisons of antibiotic growth promoter supplemented (AGP) diets versus Actigen® supplemented (ACT) diets. Average age (and trial duration) was 43.64 days. Average inclusion rates for Actigen® by phases (starter/grower/finisher) were 535/331/238 g/tonne, and it was included in the diets during the entire trial in each case. There was a nonsignificant ($p = 0.548$) difference of +0.016 kg (+0.65%) in body weight in favor of the ACT treatment compared to the AGP treatment. There was a slight, nonsignificant ($p = 0.687$) difference of -0.003 (0.17%) in feed conversion ratio or feed/gain ratio in favor of the ACT group. On the other hand, there was a nonsignificant ($p = 0.551$) increase in mortality % of 0.57% (7.97% relative to AGP) attributable to the ACT diets. All of the live performance results for AGP and ACT were statistically equivalent (within the same statistical groups).

The antibiotic growth promoters and dietary inclusion rates pertaining to results in Table 2a are given in Table 2b. The antibiotic growth promoters used included Avilamycin, BMD®, BMD®/ Stafac®, and Surmax.

DISCUSSION

Actigen® is a second generation product containing mannan oligosaccharide (MOS), a specific compound derived from the cell wall of yeast (*Saccharomyces cerevisiae* var. *boulardii*). In-feed assays and product assays allow the confirmation of the active ingredient quantitatively and qualitatively through ELISA technology. These are key points in differentiating Actigen® from the MOS feed additive products which have gone before it.

Table 1: Summary of live performance results from broiler trials with negative control (nCON) versus Actigen®-supplemented (ACT) diets

Age, days ¹	Actigen®, g/tonne ²	Body weight (kg)		FCR or F/G ratio		Mortality (%)		Reference (Year)
		nCON	ACT	nCON	ACT	nCON	ACT	
42	800/400/200	2.382	2.501	1.947	1.852	4.83	4.46	Mathis (2009)
42	800/400/200	2.081	2.134	1.825	1.784	3.69	4.77	Mathis (2011a)
52	400/400/400	2.763	2.865	1.872	1.820	5.60	3.80	Mathis (2011b)
42	400/400/400	2.370	2.516	1.740	1.660	13.9	12.5	Kill <i>et al.</i> (2010)
42	400/200/200	2.370	2.552	1.740	1.660	13.9	11.5	Kill <i>et al.</i> (2010)
42	200/200/200	2.370	2.441	1.740	1.700	13.9	17.4	Kill <i>et al.</i> (2010)
42	800/400/200	3.317	3.437	1.746	1.708	5.56	3.89	Munyaka <i>et al.</i> (2011)
42	200/200/200	2.066	2.065	2.020	2.010	6.25	6.25	Peric <i>et al.</i> (2010)
42	400/400/400	2.066	2.234	2.020	1.950	6.25	2.30	Peric <i>et al.</i> (2010)
42	800/800/800	2.066	2.151	2.020	1.960	6.25	4.93	Peric <i>et al.</i> (2010)
40	800/400/200 ³	2.521	2.657	1.636	1.603	4.30	6.20	Nollet and Kay (2010)
32	400/400/400	1.877	1.901	1.658	1.654	4.00	4.00	Nollet (2011)
42	200/200/200	2.515	2.847	1.741	1.694	6.67	6.67	Lea <i>et al.</i> (2011)
42	400/400/400	2.515	2.677	1.741	1.729	6.67	3.33	Lea <i>et al.</i> (2011)
42	800/800/800	2.515	2.749	1.741	1.725	6.67	5.00	Lea <i>et al.</i> (2011)
Comparisons (n=)		15	15	15	15	15	15	
Mean		2.386 ^b	2.515 ^a	1.813 ^a	1.767 ^b	7.23	6.47	
p-value			<0.001		<0.001		0.153	
Difference			+0.129		-0.046		-0.76	
Diff. from nCON (%)			+5.41		-2.54		-10.5	

¹Average age was 41.87 days (number = 15).

²Actigen® in starter 0-21 days, grower 21-35 days and finisher 35-42 days unless otherwise stated. Average inclusion rates for Actigen by phases were 520/400/347 g/tonne (number = 15).

³Actigen in starter 0-10 days, grower 10-25 days and finisher 25-40 days

Table 2a: Summary of live performance results from broiler chicken trials with antibiotic growth promoter-supplemented positive control (AGP) versus Actigen®-supplemented (ACT) diets

Age, days ¹	Actigen®, g/tonne ²	Body weight (kg)		FCR or F/G ratio		Mortality (%)		Reference (Year)
		AGP	ACT	AGP	ACT	AGP	ACT	
42	800/400/200	2.530	2.501	1.843	1.852	4.17	4.46	Mathis (2009)
42	800/400/200	2.124	2.134	1.803	1.784	3.85	4.77	Mathis (2011a)
52	400/400/400	2.900	2.865	1.807	1.820	4.00	3.80	Mathis (2011b)
42	400/400/400	2.551	2.516	1.690	1.660	9.70	12.5	Kill <i>et al.</i> (2010)
42	400/200/200	2.551	2.552	1.690	1.660	9.70	11.5	Kill <i>et al.</i> (2010)
42	200/200/200	2.551	2.441	1.690	1.700	9.70	17.4	Kill <i>et al.</i> (2010)
42	800/400/200	3.430	3.437	1.720	1.708	6.39	3.89	Munyaka <i>et al.</i> (2011)
42	400/400/200 ³	1.831	2.037	1.840	1.840	21.0	16.3	Hitech Hatch Fresh (2009)
35	400/200/200 ⁴	1.652	1.636	1.502	1.513	4.87	4.95	Philippines (2009)
38	882/441/220 ⁵	1.857	1.955	1.681	1.680	2.92	3.01	U.S. Integrator (2010)
61	400/200/200 ⁶	4.300	4.377	1.980	2.004	2.33	2.39	N.C. A&T Res. (2011)
Comparisons (n=)		11	11	11	11	11	11	
Mean		2.571	2.587	1.750	1.747	7.15	7.72	
p-value			0.548		0.687		0.551	
Difference			+0.016		-0.003		+0.57	
Diff. from AGP (%)			+0.62		-0.17		+7.97	

¹Average age was 43.64 days (number = 11).

²Actigen® in starter 0-21 days, grower 21-35 days and finisher 35-42 days unless otherwise stated. Average inclusion rates for Actigen by phases were 535/331/238 g/tonne (number = 11).

³Actigen® in starter 0-10 days, grower 10-24 and finisher 24-42 days.

⁴Actigen® in starter 0-21 days, grower 21-28 days and finisher 28-35 days.

⁵Actigen® in starter 0-15 days, grower 15-28 days and finisher 28-38 days.

⁶Actigen® in starter 0-18 days, grower 18-35 days and finisher 35-61 days

Hooge (2004) conducted meta-analyses of broiler chicken pen trials evaluating dietary mannan oligosaccharide from 1993-2003, and all trials involved supplementation of feeds with the first generation product Bio-Mos® (Alltech, Inc., Nicholasville, Kentucky,

USA) from which Actigen® was derived. Compared to broiler results using negative control diets, Bio-Mos® diets significantly (p=0.020) improved body weight by 0.038 kg or +1.75%, feed conversion ratio by -0.035 or -1.89%, and mortality % by -0.759% actual or -16.4%

Table 2b: Antibiotics programs used in broiler trial results presented in Table 2a

Reference (Year)	Antibiotic	Inclusion rate by days of age
Mathis (2009)	BMD®	0-21 days 50 g/ton, 21-42 days 25 g/ton
Mathis (2011a,b)	BMD®	0-21 days 50 g/ton, 21-42 days 25 g/ton
Mathis (2011a)	BMD®/Stafac®	0-31 days BMD® 50 g/ton, 31-52 days Stafac® 20 g/ton
Kill <i>et al.</i> (2010)	Avilamycin	0-42 days 100 g/tonne
Munyaka <i>et al.</i> (2011)	BMD®	0-42 days 100 g/ton
Hitech Hatch Fresh (2009)	BMD®	0-42 days 350/tonne
Philippines Field Trial (2009)	Sumax	0-28 days
U.S. Integrator (2010)	BMD®	0-28 days
N.C. A&T Research (2011)	BMD®/Stafac®	0-18 days BMD® 50 g/ton, 18-35 days BMD® 25 g/ton; 35-61 days Stafac® 10 g/ton

Table 3: Comparison of Hooge (2004) meta-analyses and Rosen (2007) holo-analysis of broiler trial results using dietary Bio-Mos® with the present meta-analysis results herein

Reference	Statistical analysis	Additive	Difference due to Bio-Mos® or Actigen® vs. control		
			Body weight, kg (%)	FCR or F/G (%)	Mortality, % actual (% relative) ¹
Bio-Mos® or Actigen® diets vs. negative control					
Hooge (2004)	meta-analyses	Bio-Mos®	+0.038 (+1.75)	-0.035 (-1.89)	-0.759 (-16.4)
Rosen (2007)	holo-analysis	Bio-Mos®	+0.0276 (+1.48)	-0.0391 (-2.11)	+0.0311 (+0.43)
Present report	meta-analyses	Actigen®	+0.129 (+5.41)	-0.046 (+2.54)	-0.76 (-10.5)
Bio-Mos® or Actigen® diets vs. antibiotic diets					
Hooge (2004)	meta-analyses	Bio-Mos®	-0.007 (-0.32)	-0.008 (-0.11)	-0.83 (-18.1)
Present report	meta-analyses	Actigen®	+0.016 (+0.65)	-0.003 (-0.17)	+0.57 (+7.97)

¹Mortality % difference relative to the respective negative control or antibiotic control

relative to negative control. Nonsignificant ($p=0.408$) changes using Bio-Mos® diets compared to antibiotic growth promoter diets were -0.007 kg or -0.32% relative to antibiotic control in body weight and -0.008 or -0.11% in feed conversion ratio. The Bio-Mos® diets highly significantly ($p=0.007$) changed mortality % by -0.83% actual and -18.1% relative to the antibiotic growth promoter diets, indicating a strong beneficial effect. Rosen (2007) reported a meta-analysis of broiler chicken trials from 1997-2003 evaluating dietary mannan oligosaccharide product Bio-Mos® versus negative control. Body weight was 0.0276 kg (1.48%) greater, feed conversion ratio was 0.0391 (2.11%) lower, and mortality was 0.0311% (actual) (0.43% relative to negative control) higher with dietary Bio-Mos®. Beneficial response frequencies were 65% , 70% , and 52% , respectively, for these 3 parameters. In the present summary, body weight was greater ($p<0.001$) for the Actigen® (ACT) birds than for the negative control (nCON) birds ($+0.129$ kg; $+5.41\%$), feed conversion ratio or feed/gain ratio was lower ($p<0.001$) for ACT birds than for nCON birds (-0.046 ; -2.54), but mortality % was not significantly ($p=0.153$) affected by dietary treatment. The ACT birds had 0.76% lower mortality which amounted to 10.5% relative reduction in mortality % compared to nCON birds. There was a nonsignificant ($p=0.548$) difference of $+0.016$ kg ($+0.65\%$) in body weight in favor of the ACT treatment compared to the AGP treatment. There was a slight, nonsignificant ($p=0.687$) difference of -0.003 (0.17%) in feed conversion ratio or feed/gain ratio in favor of the

ACT group. On the other hand, there was a nonsignificant ($p=0.551$) increase in mortality % of 0.57% (7.97% relative to AGP) attributable to the ACT diets. All of the live performance results for AGP and ACT were statistically equivalent (within the same statistical groups).

Comparing the Hooge (2004) and Rosen (2007) dietary Bio-Mos® performance change results against their negative controls with the performance change results herein using ACT diets against nCON diets (see Table 3) shows greater body weight improvement ($+0.038$ and $+0.0276$ kg vs. $+0.129$ kg, respectively), with feed conversion ratio improvement slightly better, in the case of ACT diets (-0.035 and -0.0391 vs. -0.046 , respectively). Mortality findings in the 3 meta-analysis summary reports were variable with Hooge (2004) showing -0.759% actual and Rosen (2007) showing $+0.0311\%$ actual for Bio-Mos® diets vs. negative control, and the present summary finding -0.76% actual lower mortality in the ACT treatment compared to nCON.

Similarly, the dietary Bio-Mos® performance change results against antibiotic growth promoter diets in Hooge (2004) may be compared with the performance change results herein using ACT diets against AGP diets (Table 3). Hooge (2004) found nonsignificant ($p=0.408$) changes using Bio-Mos® diets compared to antibiotic growth promoter diets of -0.007 kg or -0.32% relative to antibiotic growth promoter diets in body weight and -0.008 or -0.11% in feed conversion ratio. In the present summary, there was a nonsignificant ($p=0.548$) difference of $+0.016$ kg ($+0.65\%$) in body weight in favor of the

of the ACT treatment compared to the AGP treatment, contradicting Hooge (2004), and there was a slight, nonsignificant ($p=0.687$) difference of -0.003 (0.17%) in feed conversion ratio or feed/gain ratio in favor of the ACT group, agreeing with Hooge (2004). Hooge (2004) observed that the Bio-Mos® diets highly significantly ($p=0.007$) changed mortality % by -0.83% actual and -18.1% relative to the antibiotic growth promoter diets, indicating a strong beneficial effect. Contrarily, in the present trial, there was a nonsignificant ($p=0.551$) increase in mortality % of 0.57% (7.97% relative to AGP) attributable to the ACT diets.

Bozkurt *et al.* (2009) found significantly improved body weight and feed conversion ratio at 21 and 42 d in male broilers fed a mannan oligosaccharide product (Bio-Mos® 0.1%) compared to negative control broilers. de Oliveira *et al.* (2009) fed dietary mannan oligosaccharide at 0.1% from 0-21 d and 0.05% from 21-42 d and found that litter ammonia volatilization decreased when mannan oligosaccharide diets were fed versus unsupplemented diets.

Mathis (2011b) reported results of a pen trial conducted on 10.2 cm (4 in.) built-up litter topped with pine shavings and using straight-run Cobb chicks. Three dietary treatments were: 1) negative control; 2) Actigen® at 800 g/tonne 0-7 d, 400 g/tonne 7-21 d, and 200 g/tonne 21-42 d; and 3) BMD® at 50 g/(U.S.) ton 0-21 d and 25 g/ton 21-42 d. Salinomycin at 50 g/ton (0-21 d) and 60 g/ton (21-35 d) was the coccidiostat. Intestinal villus height, villus height: crypt depth ratio, and goblet cell count were increased ($p<0.010$), and litter scores were improved ($p<0.001$), when birds were fed ACT or BMD® vs. nCON. Improved intestinal morphology of broilers fed dietary mannan oligosaccharide has been observed by several other researchers as well (for example: Iji, 2001; Yang *et al.*, 2008; de Oliveira *et al.*, 2009).

Conclusion: This meta-analysis summary of the 15 trials suggests that Actigen® may be effective as a dietary growth promoter/ permitter for broilers. The body weight and feed conversion ratio improvement responses seen in this meta-analysis agree with the earlier reviews of Bio-Mos® by Hooge (2004) and Rosen (2007) although numerically the magnitude of the responses are larger for Actigen® than they were for Bio-Mos®, compared to respective negative controls. Overall average reduction in mortality % (-0.76% actual) compared to negative control diets was the same when using Actigen® diets herein or for Bio-Mos® diets in the Hooge (2004) meta-analysis. Rosen (2007) found very little change in mortality % in his holo-analysis with Bio-Mos® diets ($+0.0311\%$ actual) compared to negative control diets. There was a nonsignificant difference in body weight in favor of the ACT treatment ($+0.016$ kg) compared to the AGP treatment, differing from Bio-Mos® treatment in Hooge (2004), and there was a slight, nonsignificant

difference in feed conversion ratio or feed/gain ratio in favor of the ACT group (-0.003), agreeing with Bio-Mos® treatment in Hooge (2004). Hooge (2004) observed that the Bio-Mos® diets highly significantly reduced mortality % (-0.83% actual) compared to AGP diets, indicating a strong beneficial effect. Contrarily, in the present meta-analysis summary, there was a nonsignificant increase in mortality ($+0.57\%$ actual) attributable to the ACT diets. These are preliminary results which give an indication of the effects of diets containing Actigen® on broiler live performance.

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