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Effects of Calsporin® on Turkey Performance, Carcass Yield and Nitrogen Reduction

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Abstract: Calsporin®, a probiotic that contains a strain of *Bacillus subtilus* in a spore form, has been used to increase performance of several species. An experiment was conducted to determine the effects of Calsporin® addition on gain, feed conversion, nitrogen excretion, and *salmonella spp*. count from the intestinal tract compared to a commercial antibiotic (Bacitracin-Zinc) in male turkeys to market age. Six hundred Hybrid large white tom poults were purchased from a commercial hatchery at day of hatch and randomly allotted into 24 pens, blocked by treatment. Treatments consisted of Calsporin® added at 30 grams per ton of the diet , Bacitracin-zinc at 50 grams per ton or no additive. Birds were housed in an industry standard curtain-sided barn on used pine shavings. Standard corn, soybean meal, animal by-product diets where used for all treatments with nutrient levels based on the 1994 NRC requirements for turkeys. Individual bird weights and pen feed consumption where collected at 21, 42, 63, 84, 105, 126 days. Daily mortality was collected and feed:gain was adjusted to account for mortality. Litter samples where taken from ten random pens at day 63. At 126 days of age three toms per pen where selected based on average pen weight and processed the following day to determine carcass and parts yield. Data were analyzed by analysis of variance (ANOVA) with a randomized block design using the general linear model. By week 12 the treatments that contained either Calsporin® or Bacitracin-zinc had a significantly ($p < 0.05$) improved gain compared to the treatment without either of the products . For the 0 to 15 and 0 to 18 week phases the birds without additives gained significantly ($p< 0.05$) less weight compared to all other treatments. No differences in feed efficiency, yield or mortality were observed. Litter samples indicated a reduction in ammonia volitization in Calsporin® fed birds. These data would indicate that Calsporin® may be used successfully as an antibiotic replacement in market turkeys.

Key words: Turkeys, probiotics, lactobacillus, antibiotic use

Introduction

A variety of feed additives are available for use by the turkey industry. One of the major classes of feed additives is the growth promoting antibiotics. The beneficial effects of subtheraputic doses of antibiotics have been well documented (Buresh *et al*., 1986; Watkins *et al*., 1982). Even with the beneficial effects of antibiotics such as improved gain and feed conversion, the use of antibiotics in subtheraputic doses is beginning to fall under heavy criticism. This criticism comes from the potential for creating strains of resistant, pathogenic bacteria (Kelly *et al*., 1998; Gustafson and Bowen, 1997; Gast and Stephens, 1986). A potential alternative to antibiotics as growth promoters is the use of probiotics. Probiotics occur naturally in the animal and act by modifying the ability of pathogenic bacteria to colonize and proliferate in the intestinal tract. For years the beneficial effects of probiotics have been studied. Fulton *et al*. (2002) used a "cocktail" of several beneficial bacterial commonly found in the micro flora of the intestine as a probiotic and found that it's use allowed for equal gain and feed conversion compared to a commercial antibiotic in ducks (Fulton *et al*., 2002). Parks and others found similar results using mannanoligosaccharide (MOS) in the turkey (Parks *et al*., 2001). The modification of pathogenic bacteria's ability to colonize due to the action of probiotics may occur in several ways. The probiotic's mechanism of action may include lower intestinal pH, low redox potential, miscellaneous inhibitory substances (e.g., fatty acids, deconjugated bile acids), competition for nutrients, adhesion to receptor sites (i.e. competitive exclusion) or even substitute as a receptor site (Yeo and Kim, 1997; Parks *et al*., 2001). Calsporin® contains *Bacillus subtilis* C-3102. The goal of this study was to evaluate the effects of Calsporin® addition on gain, feed conversion, nitrogen excretion, and *salmonella spp*. count from the intestinal tract compared to a commercial antibiotic (Bacitracin-Zinc) in male turkeys.

Materials and Methods

Six hundred Hybrid large white tom poults were purchased from a commercial hatchery at day of hatch. The poults were randomly placed into 24 pens, with 25 poults per pen. The study was set up as a randomized complete block design with each treatment allocated to

Table 1: Diet Composition for each phase of growth

Ingredients	0-3 (%)	3-6 (%)	6-9 (%)	9-12 (%)	12-15 (%)	15-18 (%)
Corn	47.029	48.279	59.429	67.009	72.248	78.286
Soy (48)	43.379	41.285	30.790	22.493	17.419	11.421
Pork Meal	7.000	5.000	5.000	6.000	5.000	4.800
Lard	-	3.270	2.970	3.400	4.260	4.430
Dical Phos	0.989	0.902	0.554	0.195	0.126	-
Limestone	0.535	0.344	0.241	-	0.075	-
Salt	0.250	0.250	0.200	0.200	0.200	0.200
Na Bicarb	0.250	0.250	0.250	0.250	0.250	0.400
DL Methionine	0.143	0.099	0.088	0.022	0.056	0.037
Lysine Hcl	0.033	-	0.173	-	0.044	0.049
Trace mineral mix[1]	0.100	0.100	0.100	0.100	0.100	0.100
Vitamin mix[2]	0.075	0.075	0.075	0.075	0.075	0.075
Choline Chloride	0.074	0.053	0.037	0.067	0.053	0.059
Avatec[4]	0.050	0.050	0.050	0.050	0.050	0.050
Selenium Mix[3]	0.030	0.030	0.030	0.030	0.030	0.030
Copper Sulfate	0.013	0.013	0.013	0.013	0.013	0.013
Filler (add back Corn)[5]						
Baciferm[5,6]						
Calsporin[5]						
Calculated Composition						
ME (kcal/kg)	2800.00	3000.00	3100.00	3200.00	3300.00	3400.00
Crude Protein (%)	28.00	26.00	22.00	19.00	16.50	14.00
Lysine (%)	1.70	1.55	1.40	1.00	0.92	0.75
TSA (%)	1.10	1.00	0.88	0.74	0.70	0.61
Threonine (%)	1.04	0.97	0.81	0.75	0.61	0.52
Valine (%)	1.46	1.37	1.15	1.00	0.87	0.74
Arginine (%)	2.00	1.85	1.53	1.30	1.11	0.92
Histidine (%)	0.76	0.72	0.61	0.53	0.47	0.40
Isoleucine (%)	1.28	1.20	1.00	0.83	0.71	0.59
Leucine (%)	2.36	2.22	1.94	1.74	1.56	1.39
TAA (%)	0.60	2.23	0.42	1.59	1.38	1.15
Tryptophan (%)	0.38	0.36	0.29	0.24	0.20	0.16
Calcium (%)	1.30	1.00	0.85	0.75	0.65	0.56
Available Phos (%)	0.60	0.50	0.42	0.38	0.32	0.28
Crude Fat (%)	2.92	6.02	6.03	6.77	7.68	8.00

[1]The mineral premix provided the following amounts per kilogram of diet: manganese 110 mg, iron 60 mg, iodine 2 mg, magnesium 27 mg. [2]The vitamin premix provided the following amounts per kilogram diet: vitamin A 13,200 mg, Vitamin D_3 5,775 mg, Vitamin E 21 mg, Niacin 82.5 mg, d-Panothenic acid 25 mg, Riboflavin 10 mg, Vitamin B_6 3.3 mg, Menadione 2.5 mg, Folic Acid 21 mg, Thiamin 1.7 mg, Biotin 0.33 mg, Vitamin B_{12} 0.02 mg. [3]The Selenium premix provided selenium at 0.18 mg. [4]Avatec provides lasalocid at 90g/ton of diet. [5] Based on treatment (Table 2) the diets received one of 4 combinations of the Filler, Baciferm, or Calsporin: 1. Filler 0.05%, Baciferm 0.0%, Calsporin 0.0%. 2. Filler 0.0%, Baciferm 0.05%, Calsporin 0.0%. 3. Filler 0.0%, Baciferm 0.05%, Calsporin 0.0003%. 4. Filler 0.0%, Baciferm 0.0%, Calsporin 0.0003%. [6]Bacifer provides bacitracin at 50g/ton of diet.

8 blocks of 3 pens such that each treatment is allotted to each block one time. The birds were housed in a climate controlled, curtain sided barn on used pine shavings. Common industry husbandry practices were used throughout the trial. Feed and water were made available *ad libtum* throughout the trial. Due to the potential for cross contamination of the bacterial product, treatments containing Calsporin® were randomized side by side as a single unit (Table 2). Plastic sheets where hung between pens and the aisle was cleaned daily with a bleach solution. Throughout the trial those pens fed treatments lacking Calsporin® (Table 2) where cared for first followed immediately by those pens that did receive Calsporin® treatments, upon exiting these pens shoes where dipped in bleach baths before reentering the aisle. Industry standard corn, soybean meal, animal by-product diets where used for all treatments (Table 1). Nutrient levels where based on the

Table 2: Dietary treatment codes used in turkeys fed either Calsporin® or Baciferm®

Treatment A: With Baciferm added at 0.05% of the diet (C-/B+)
Treatment B: With Calsporin added at 30 gms/ton of the diet (C+/B-)
Treatment C: No additives (C-/B-)

Table 3: Average Gain (kg) for each growth period for birds fed Calsporin (C) or Bacitracin (B)

Treatment	Phase (weeks)					
	0-3	0-6	0-9	0-12	0-15	0-18
C+/B-	0.53	2.16	4.74	7.90[b]	10.95[b]	14.32[b]
C-/B+	0.53	2.13	4.67	7.94[b]	11.00[b]	14.15[b]
C-/B-	0.51	2.12	4.51	7.37[a]	10.36[a]	13.41[a]
Std.Err.	0.014	0.041	0.062	0.129	0.141	0.172

Values with different superscripts are significantly ($p < 0.05$) different from each other. + or - designates addition or deletion of Calsporin® or bacitracin

Table 4: Average Gain (kg) for each growth phase for birds fed Calsporin (C) or Bacitracin (B)

Treatment	Phase (weeks)					
	0-3	3-6	6-9	9-12	12-15	15-18
C+/B-	0.53	1.61	2.59	3.16[ab]	3.06	3.36
C-/B+	0.53	1.60	2.54	3.27[b]	3.06	3.15
C-/B-	0.51	1.62	2.38	2.86[a]	2.99	3.06
Std.Err.	0.01	0.04	0.07	0.10	0.07	0.08

Values with different superscripts are significantly ($p < 0.05$) different from each other + or - designates addition or deletion of Calsporin® or bacitracin

1994 NRC requirements given for turkeys. To those treatments containing Calsporin®, Calsporin® was added at 30 grams per ton of the diet (Table 1) and levels were verified by assay. Individual bird weights and pen feed consumption where collected at 21, 42, 63, 84, 105 and 126 days. Daily mortality was collected and feed: gain was adjusted to account for mortality. Litter samples where taken from ten random pens at day 63. On day 63 the birds were moved from the brooder house to the grow-out house. The birds had been on the brooder litter for the 63 days allowing ample nitrogen excretion to occur for sampling. The litter samples were gathered from the four corners and center of each of the 32 pens. About 30 grams of each sample was collected. The samples were then incubated for one hour at 37 °C (99 °F), the concentration of ammonia in the container was detected using a Gastec air-sampling pump (absorbed 100 ml, one minute for each sample). At 126 days of age three toms per pen where selected based on average pen weight and processed the following day to determine carcass and parts yield.

Statistical Analysis: Data were analyzed by analysis of variance (ANOVA) with a randomized block design using the general linear model. Block effects were not significant and this portion of the variance was added into the error mean square. Following ANOVA, means were separated where appropriate using the least significant differences test. All data were analyzed with the JMP version of SAS. The level of significance was set at 0.05.

Results and Discussion

Table 3 and 4 summarize the average gain for all treatments for the duration of the study. For the 0 to 9 week phase there was no significant ($p < 0.05$) improvement in the average bird gain for any treatment although a trend appeared to occur that became statistically valid at later ages. At week 12 the treatments that contained either Calsporin® (treatment A) or Baciferm (treatment B) separately had a significantly ($p < 0.05$) improved gain compared to the treatment without either of the products (treatment C). For the 0 to 15 and 0 to 18 week phases the birds without additives gained significantly ($p< 0.05$) less weight compared to all other treatments.

For the duration of the trial there was no significant ($p < 0.05$) difference in feed efficiency between any of the treatments (Table 5 and Table 6.) although birds without additives were less efficient given their lower weight . There was no significant ($p < 0.05$) difference in the mortality observed between the treatments for the duration of the trial.

At day 63 litter samples were taken from pens that had diets with Calsporin® or without. The amount of ammonia determined in ~ 30 grams of litter is shown in

Table 5: Average adjusted Feed/Gain for each growth period for birds fed Calsporin (C) or Bacitracin (B)

Treatment	Phase (weeks)					
	0-3	0-6	0-9	0-12	0-15	0-18
C+/B-	1.48	1.61	1.81	2.01	2.33	2.42
C-/B+	1.37	1.63	1.81	1.94	2.27	2.39
C-/B-	1.37	1.53	1.77	1.99	2.28	2.42
Std. Error	0.03	0.03	0.04	0.04	0.05	0.05

Values with different values are significantly ($p < 0.05$) different from each other. + or - designates addition or deletion of Calsporin® or bacitracin

Table 6: Average adjusted Feed/Gain for each growth phase for birds fed Calsporin (C) or Bacitracin (B)

Treatment	Phase (weeks)					
	0-3	3-6	6-9	9-12	12-15	15-18
C+/B-	1.48	1.70	1.96	2.32	3.21	2.72
C-/B+	1.37	1.72	1.97	2.14	3.15	2.84
C-/B-	1.37	1.57	2.00	2.34	3.02	2.92
Std. Error	0.03	0.05	0.06	0.07	0.05	0.05

Values with different values are significantly ($p < 0.05$) different from each other. + or - designates addition or deletion of Calsporin® or bacitracin

Table 7: Ammonia volatilization from 10 random pens with or without Calsporin® at day 63 of the trial

	Treatment	
	Control	Calsporin®
	Ammonia (ppm)/Sample Size (g)	
	36 ppm/32.411g	5 ppm/32.966g
	23 ppm/30.425g	9ppm/31.222g
	17 ppm/30.675g	15ppm/30.086g
	32 ppm/30.809g	2ppm/30.147g
	18 ppm/30.334g	8ppm/30.206g
Mean ± Standard Deviation	25.2 ± 8.47 ppm	7.80 ± 4.87 ppm

Above means represent the ammonia present in ~30 grams of litter randomly collected from pens with or without Calsporin® at day 63 of the trial. A significant ($p > 0.001$) reduction in the ammonia volatilization was observed in treatments with Calsporin®.

Table 8: Average carcass yield of turkeys fed Calsporin® or bacitracin at 126 days of age (expressed as percentage of chilled carcass weight)

Trmt	Major	Minor	Breast	Wing	Thigh	Leg	Fat	Yield
C+/B-	23.47	5.42	28.89	11.67	14.23	12.56	1.75	79.74
C-/B+	23.36	5.59	28.95	11.81	13.84	12.59	1.64	79.40
C-/B-	23.34	5.55	28.89	11.99	14.72	12.41	1.67	79.53
Std.Err.	0.36	0.10	0.37	0.17	0.29	0.15	0.12	0.34

No significant differences were found

Table 7. On average birds treated with Calsporin® excreted significantly ($p < 0.001$) lower amounts of ammonia compared to those birds not treated with Calsporin®, 7.80 ppm and 25.2 ppm respectively. At day 126 three birds per pen where selected based on average bird weight per pen and processed the following day to determine carcass yield. On day of processing the pectoralis major and minor, leg, thigh, wing and fat pad where collected from each bird and weighed to determine any differences between treatments. No significant ($p < 0.05$) differences where found among any of the parts between any of the treatments (Table 8). On day of processing, carcass rinse samples where taken from each bird and analyzed for *Salmonella spp*. No *Salmonella* were detected in any of the birds (data not presented).

The beneficial effects of subtheraputic doses of antibiotics have been well documented (Buresh *et al.*,

1986; Watkins *et al.*, 1982), but the use of antibiotics in feed is being criticized. This criticism comes from the potential for creating strains of resistant, pathogenic bacteria (Kelly *et al.*, 1998; Gustafson and Bowen, 1997; Gast and Stephens, 1986) as well as pressure from the public for natural products. Therefore, alternatives to antibiotics must be developed. Fulton *et al.* (2002) used a "cocktail" of several beneficial bacteria commonly found in the micro flora of the intestine with positive results. The modification of pathogenic bacteria's ability to colonize due to the action of probiotics can occur in several ways which may include lower intestinal pH, low redox potential, miscellaneous inhibitory substances (e.g., fatty acids, deconjugated bile acids), competition for nutrients, adhesion to receptor sites (i.e. competitive exclusion) or even substitute as a receptor site (Yeo and Kim, 1997; Parks *et al.*, 2001). Calsporin® contains *Bacillus subtilis* C-3102 a strain of bacteria in sporulated form originally found in soil. When fed to poultry, Calsporin® increases the incidence of beneficial bacteria such as *Lactobaccillus* and *Bifidobacterium* and decreases the levels of harmful bacteria such as *Camphylobacter* and *Salmonella* (Maruta *et al.*, 1996) while having the additional benefit of being able to go through most pelleting systems at temperatures up to 90 °C. This effect in many ways resembles the effects of the growth promoting antibiotics currently in use. Use of various *Bacillus* strains has been shown to improve broiler performance (Van Wambeke and Peeters, 1995; Nguyen *et al.*, 1988). Calsporin® has also been shown to improve broiler performance (Fritts *et al.*, 2000) similarly to the performance enhancement seen in the current study. Although more work is needed in the turkey, this data would suggest that the addition of Calsporin® is a viable alternative to use of a growth promoting antibiotics in pelleted turkey rations.

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