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Effect of Dietary $Al_2(SO_4)_3$ on *Salmonella* Transmission, Feed Consumption and Growth in Immature Replacement Breeders

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Abstract: Alternate-day feed restriction of broiler breeders is known to reduce adult gonadal function and increase ingestion of litter and feces, thereby increasing horizontal transmission of *Salmonella*. Similarly, preslaughter feed withdrawal increases crop contamination with *Salmonella*. We hypothesized that dietary $Al_2(SO_4)_3$ (alum) would reduce voluntary consumption of feed, providing an alternative method of restriction (or preslaughter feed withdrawal). When alum was fed at 10% of the broiler starter diet, body weights were reduced by 67%, with a 60% reduction in feed consumption. *In vitro*, alum (10%) caused a 3.5 log reduction in *Salmonella enteritidis* (SE) recovery as compared to controls. In broilers 8 h prior to slaughter using a SE horizontal transmission model, alum (10%) had no effect on recovery of SE from crop or cecal tonsils. In additional experiments, alum (10%) significantly increased SE recovery from cecal tonsils and significantly elevated heterophil to lymphocyte ratios by 14 days of treatment as compared to either pair-fed broilers or controls. In conclusion, these data indicate that dietary alum causes increased stress as compared to daily dietary restriction, does not reduce horizontal transmission of SE, and is not a useful alternative to physical feed restriction.

Key words: Aluminum sulfate, broiler breeder, feed restriction

Introduction

Prior to sexual maturity, broiler breeders on *ad libitum* feeding become obese and express decreased fertility at reproductive age (Goerzen *et al*., 1996; Robinson *et al*., 1991; Robinson and Wilson, 1996). Therefore, body weight of growing and reproductively active broiler breeders must be regulated. Traditionally, obesity and growth rate are modified by feed restriction programs that may involve limited daily access to feed or alternate day *ad libitum* access to feed. While feed restriction is necessary in many commercial breeder-grower operations, such restriction programs are now known to reduce reproductive potential as birds sexually mature. Furthermore, conventional feed restriction programs may enhance size heterogeneity within the flock and cause behavioral/aggression problems. In the absence of feed, broilers exhibit increased "grazing" behavior with the ingestion of litter and feces, thereby increasing horizontal transmission of *Salmonella* (Corrier *et al*. 1999a). Presently, we examined the use of dietary $Al_2(SO_4)_3$ (alum) as a method of reducing voluntary consumption of feed, and the potential of dietary alum as an alternative method of reducing feed intake, growth rate, and obesity.

Materials and Methods

Experiment 1: To determine the effect of selected concentrations of dietary alum on broiler growth, 200 straight run day-of-hatch broiler chicks were obtained from a local hatchery. Chicks were randomly assigned to 12 round pens (1 m^2) with 20 chicks per pen and 2 replicate pens per treatment. Chicks were fed alum at 0, 1.25, 2.50, 5.00, or 10.00% of the broiler starter diet. Chicks were allowed *ad libitum* access to feed for 11 days. Body weights were recorded at 1, 8 and 11 days of age. Cumulative feed consumption was also recorded.

Experiment 2: In order to evaluate the relative antimicrobial properties of selected alum concentrations, an assay system was used to simulate the organic matter found in the broiler crop. For this purpose, an autoclaved unmedicated corn-soybean meal-based broiler ration, formulated to meet or exceed the levels of critical nutrients for growing broilers was used (NRC, 1994). The test system consisted of sterile 12 x 75 mm borosilicate tubes containing 0.5 g sterile feed, 1.9 mL alum solution and 100 µL 0.9% sterile saline containing 5 x 10^5 cfu/mL SE. Alum concentrations of 0, 4.56, 9.12, 12.8, 36.5, 73, 146 and 292 mM were prepared. For screening, the test tubes and contents were vortexed for 2 s and incubated for 1 h at 37°C. Following incubation, the samples were serially diluted and 100 µL of each dilution was spread on three brilliant green agar (BGA) plates containing 25 µg/mL novobiocin (NO) and 20 µg/mL nalidixic Acid (NA). The plates were incubated at 37°C for 24 hours, after which colony-forming units per milliliter were determined (Barnhart *et al*., 1999).

Experiment 3: To evaluate the effect of alum on recovery of SE in crops and cecal tonsils in market age broilers fed 10% dietary alum was compared to broilers

Table 1: Average body weight and feed consumption of broiler chick when fed an *ad libitum* diet (control) or selected concentrations of dietary alum diet on days 1, 8, and 11 of age

Group	Body Weight (g)			Feed Consumed per Chick (g)	FCR
	Day 1	Day 8	Day 11	Day 1-11	Day 11
Control	41.8 ± 0.5	130.5 ± 3.5[a]	207.7 ± 4.0[a]	269.8	1.30
1.25% $Al_2(SO_4)_3$	42.2 ± 0.6	111.9 ± 3.0[b]	164.2 ± 4.3[b]	259.1	1.58
2.50%$Al_2(SO_4)_3$	41.9 ± 0.6	95.9 ± 2.6[c]	133.7 ± 4.5[c]	176.2	1.32
5.0%$Al_2(SO_4)_3$	41.6 ± 0.6	69.1 ± 3.5[d]	92.0 ± 3.5[d]	190.3	2.07
10.0%$Al_2(SO_4)_3$	41.8 ± 0.6	54.3 ± 2.7[e]	67.5 ± 2.7[e]	119.4	1.77

subjected to a simulated conventional 8 h feed withdrawal. Commercial broiler chickens were obtained from the loading dock of a local processing plant (n=135) and were randomly assigned to 9 different pens (n=15 per pen). Broilers were housed in floor pens (2.2 m^2) on new pine shavings and provided *ad libitum* access to feed and water and allowed to acclimate for six days after transport. Five broilers from each pen were challenged with 1 x 10^8 cfu/mL SE in 1 mL saline by oral gavage. Seven days following the SE challenge, broilers in the control pens were subjected to feed withdrawal for 8 h. Simultaneously, 10% alum was incorporated into the diet of the principles and feed was not removed. At termination, all broilers were humanely killed and crops and cecal tonsils were collected aseptically. The crop was collected aseptically in individual sample bags. Following crop removal, 20 mL of tetrathionate broth base was added to each sample bag. The samples were subsequently stomached for 30 s. Cecal tonsils were aseptically removed and enriched in 20 mL of tetrathionate broth base. Crop and cecal tonsil samples were incubated for approximately 24 h at 37^{o}C. Following enrichment, each sample was streaked for isolation on BGA plates containing 25 µg/mL NO and 20 µg/mL NA. The plates were incubated at 37^{o}C for 24 h and examined for the presence or absence of the antibiotic resistant challenge isolate.

Experiment 4: For this experiment, the effects of alum on the horizontal transmission of SE and broiler growth was compared to *ad libitum* controls and pair-fed controls. Market age broilers (n=96) were obtained and randomly assigned to 6 pens (n=16 per pen) as described above. The next day, 30 broilers (n=5 per pen) were challenged with SE, as described above, and placed in a separate holding pen. Two days following the SE challenge two replicate pens were assigned to each feed treatment. Control broilers were provided a balanced broiler ration *ad libitum*, two pens were fed 10% dietary alum and two pens were pair-fed the average amount of feed the two 10% alum groups consumed daily. Twelve days following the SE challenge all broilers experienced an 8 h feed withdrawal period. Crop and cecal samples were obtained, enriched, plated, and evaluated as described above.

Experiment 5: Experiment 4 was repeated with the addition of two 5% dietary alum groups and two groups pair-fed according to the 5% alum groups. Seven and fourteen days post SE challenge, blood samples were taken from all broilers in Vaccutainer tubes containing K_3 EDTA and heterophil to lymphocyte ratios were determined. Broilers were subjected to 8 h feed withdrawal 15 days post SE challenge. Broilers were then humanely killed and crop and cecal samples were obtained, enriched and plated as above.

Results and Discussion

Experiment 1: The inclusion of 10% dietary aluminum sulfate in this first experiment allowed for only a 61% increase in body weight between day 1 and day 11, similar to the desired weight gain for broiler breeders between broilerization and sexual maturity (Table 1). All body weight data were analyzed by SAS software using ANOVA and further separated using General Linear Models procedure ($P < 0.05$; SAS, 2003). At 11 days-of-age, chicks fed 10% dietary alum weighed 67% less and consumed 60% less feed than *ad libitum* controls (Table 1). This data led us to believe that growth effects were possibly related to feed refusal rather than direct alum toxicity.

Experiment 2: Antimicrobial properties of alum in a simulated crop environment were examined *in vitro*. We hypothesized that the known antimicrobial properties of alum might chemically help prevent infection with this critical growth stage during a time that feed-restricted birds are commonly infected with *Salmonella*. Indeed, this *in vitro* assay suggested that such an effect might be possible as a 3.5 log_{10} drop in recoverable SE was observed with 292 mM alum (~10% wt:v) in this crop simulated assay (Fig. 1).

Experiment 3: Previous research has indicated that crops more frequently become *Salmonella* contaminated during preharvest feed restriction (Ramirez *et al.*, 1997; Corrier *et al.*, 1999b). Dietary alum (10% wt:wt) was evaluated as a potential alternative to conventional feed restriction. Inclusion of 10% dietary alum did not significantly alter recovery of SE from the crops or cecal tonsils as compared to controls that were also subjected to a simulated conventional preslaughter

Table 2: Effect of selected diets on the horizontal transmission of *Salmonella enteritidis* (SE) and subsequent colonization of crop or cecal tonsils in market age broilers subjected to a simulated 8 h feed withdrawal

Treatment	Crop		Cecal Tonsil	
	Seeders	Contacts	Seeders	Contacts
Experiment 3				
Control	15/24 (63%)	25/46 (54%)	24/24 (100%)	23/46 (50%)
Alum 10%	9/20 (45%)	16/36 (44%)	19/20 (95%)	20/36 (56%)
Experiment 4				
Control	ND	ND	8/10 (80%)^{ab}	4/22 (18%)^{b}
Alum 10%	ND	ND	10/10 (100%)^{a}	18/21 (86%)^{a}
Pair-Fed 10%	ND	ND	6/10 (60%)^{b}	8/22 (36%)^{b}
Experiment 5				
Control	4/10 (40%)^{b}	5/22 (23%)^{c}	5/10 (50%)^{bc}	9/22 (41%)^{b}
Alum 5%	9/9 (100%)^{a}	20/20 (100%)^{a}	9/9 (100%)^{a}	20/20 (100%)^{a}
Pair 5%	9/10 (90%)^{a}	17/21 (81%)^{b}	4/10 (40%)^{c}	12/21 (57%)^{b}
Alum 10%	9/9 (100%)^{a}	20/20 (100%)^{a}	8/9 (89%)^{ab}	20/20 (100%)^{a}
Pair 10%	8/10 (80%)^{ab}	15/22 (68%)^{b}	5/10 (50%)^{b}	8/22 (36%)^{b}

ND = Not Determined. a-c Values within columns of same experiment with different superscripts are significantly ($p<0.05$) different.

Table 3: Average heterophil to lymphocyte ratios of market aged broilers fed an *ad libitum* diet (control), 10% dietary alum diet, 10% pair-fed diet, 5% dietary alum diet, or 5% pair-fed diet for 7 d or 14 d post *Salmonella enteritidis* challenge

Group	Heterophil to Lymphocyte Ratio	
	7 d post challenge	14 d post challenge
Control	0.45 ± 0.09^{b}	0.53 ± 0.14^{bc}
10% $Al2(SO_4)_3$	0.79 ± 0.13^{a}	1.29 ± 0.17^{a}
10% Pair Fed	0.53 ± 0.10^{b}	0.70 ± 0.80^{b}
5% $Al2(SO_4)_3$	0.52 ± 0.08^{b}	0.93 ± 0.17^{ab}
5% Pair Fed	0.52 ± 0.11^{b}	0.35 ± 0.08^{c}

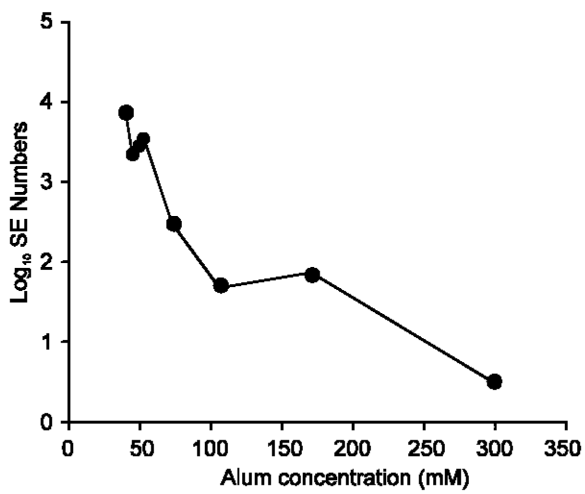

Fig. 1: Recovery of *Salmonella enteritidis* (SE) from aqueous feed samples contaminated with SE and treated with 8 selected concentrations of $Al_2(SO_4)_3$

8 h feed withdrawal (Table 2). All possible combinations of incidence of *Salmonella* recovery within experiments were compared using the chi-square test of independence to determine significant ($P < 0.05$) differences (Zar, 1984).

Experiment 4: This experiment explored the ability of 10% dietary alum to reduce the horizontal transmission of SE as compared to pair-fed or *ad libitum*-fed controls. However, inclusion of 10% dietary alum significantly increased the positive isolation of SE from the cecal tonsils of contact broilers as compared to pair-fed or *ad libitum*-fed controls (Table 2). The positive isolation of SE from the cecal tonsils of pair-fed contacts was significantly higher than the *ad libitum* controls. The behavior and condition of the broilers in this experiment was also observed. As expected, *ad libitum*-fed controls remained calm and appeared healthy. Pair-fed controls appeared nervous, were more active, and were flighty when a person approach would their pen. Other than these behavioral changes and low body weight, pair-fed controls appeared normal. In contrast, alum-fed broilers appeared lethargic, stunted, unthrifty and sometimes moribund. These observations, coupled with the reduction in body weight of the alum-treated broilers, and significantly increased SE recovery from 10% alum-fed broilers, led us to hypothesize that 10% dietary alum may be causing physical or chemical damage beyond that expected from voluntary refusal of feed (Fig. 2).

Experiment 5: To evaluate the possibility that a lower level of dietary alum (5%) would reduce the apparent toxicity observed in Experiment 4, we explored the ability of 10% dietary alum to reduce the horizontal transmission of SE as compared to pair-fed or *ad libitum*-fed controls. Similar to the results of Experiment 4, inclusion of either 5 or 10% dietary alum significantly increased the positive isolation of SE from the cecal tonsils or crops as compared to pair-fed or *ad libitum*-fed controls. Furthermore, SE was isolated more frequently from the crops of pair-fed contacts than *ad libitum*-fed control contacts (Table 2). Inclusion of 10% dietary alum significantly increased circulating heterophil to lymphocyte ratios after 14 days of treatment as

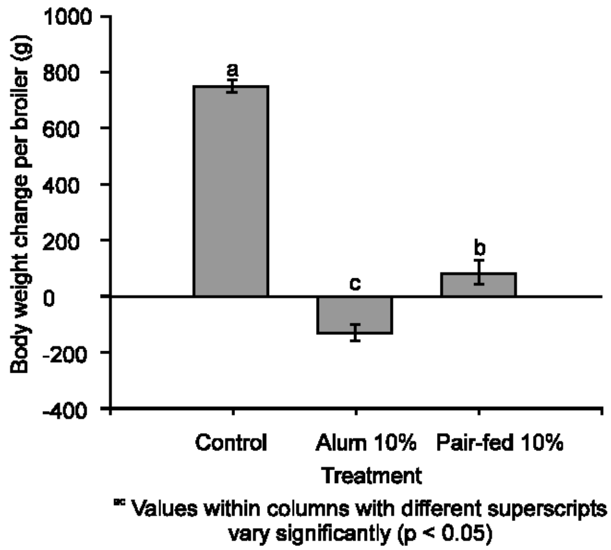

Fig. 2: Average body weight change of market aged broilers fed an *ad libitum* diet (control), 10% dietary alum diet, or 10% pair fed diet for 15 d post *Salmonella enteritidis* challenge

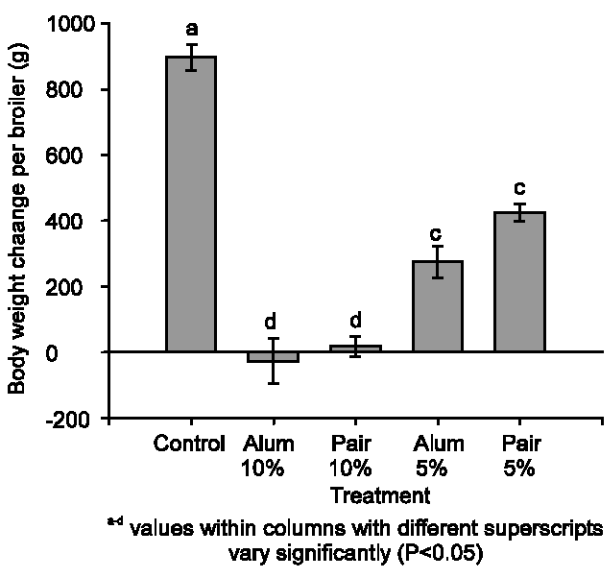

Fig. 3: Average body weight change of market aged broilers fed an *ad libitum* diet (control),10% dietary alum diet, 10% pair-fed diet, 5% dietary alum diet, or 5% pair-fed diet for 15 d post *Salmonella enteritidis* challenge

compared to controls, suggesting that 10% dietary alum caused a toxic or physical stress (Table 3). Inclusion of 5% dietary alum caused less reduction in broiler weight and does not appear to be an alternative to conventional feed restriction regimes for this reason (Fig. 3). Altogether, these data suggests that dietary alum causes toxic or physical damage as compared to more conventional dietary restriction, does not reduce horizontal transmission of SE, and is not a useful alternative to physical feed restriction.

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