Efficacy of In-Feed Tylosin Phosphate for the Treatment of Necrotic Enteritis in Broiler Chickens

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ABSTRACT The efficacy of tylosin phosphate for the treatment of necrotic enteritis (NE) was investigated in a floor pen study of 2,000 broiler chickens. A model in which Clostridium perfringens was administered in the feed on Days 14 to 16 was used to initiate an outbreak of NE. Treatments, allocated at the pen level in a randomized complete block design, consisted of five levels of tylosin phosphate (0, 50, 100, 200, or 300 ppm) administered in the feed on Days 15 to 22, following the identification of an outbreak of NE on Day 15. Mortality due to NE was significantly reduced (P < 0.05) for medicated birds at all dose levels of tylosin phosphate compared to unmedicated birds. Mean NE lesion scores on Day 17 were significantly reduced (P < 0.05) by all levels of tylosin treatment compared to those of unmedicated birds, decreasing linearly from 2.66 at 0 ppm to 0.38 at 100 ppm and 0 at higher doses. Tylosin at all levels provided improvement in Day 29 body weight, average daily gain, feed to gain ratio, and average daily feed intake compared to unmedicated birds. The results of this study provide evidence that tylosin phosphate, when administered in feed, is effective in the treatment of clinical outbreaks of NE in broiler chickens and suggest that the optimal dose for this purpose is 100 ppm.

(Key words: tylosin phosphate, necrotic enteritis, broiler chicken, efficacy)

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INTRODUCTION

Necrotic enteritis (NE), caused by the Gram-positive bacillus Clostridium perfringens is an important disease of broiler chickens, causing significant morbidity and mortality worldwide. The disease frequently occurs as outbreaks characterized by depression, anorexia, and sudden death. Control of this condition is dependent upon a variety of elements including reducing exposure to potential dietary risk factors, reduction of concurrent enteric infections, particularly coccidiosis, and the use of feed additives with activity against C. perfringens (Ficken and Wages, 1997).

Although studies have demonstrated the efficacy of certain drugs in the prevention of NE (Long and Truscott, 1976; Prescott et al., 1978; George et al., 1982), few have been demonstrated in feed to be effective in the treatment of NE once clinical signs and intestinal lesions have become apparent in affected birds (Hamdy et al., 1983a,b).

The macrolide antibiotic tylosin has been shown to have in vitro activity against C. perfringens (Stutz and Lawton, 1984; Kondo, 1988; Watkins et al., 1997) and to reduce the incidence of mortality associated with C. perfringens enterotoxaemia in an intraduodenal C. perfringens inoculation model (Vissiennon et al., 2000). The objective of this study was to evaluate the efficacy of tylosin phosphate administered in the feed for the treatment of NE in broiler chickens during an induced clinical outbreak mimicking typical conditions of modern poultry husbandry.

MATERIALS AND METHODS

A total of 2,000 1-d-old Cobb × Cobb broiler chicks obtained from a commercial hatchery were used in this study. Birds were vaccinated for Marek’s disease at the hatchery but received no other medications throughout the study, other than tylosin phosphate. Replacements were not made for early mortality. The research facility was thoroughly cleaned and disinfected prior to bird placement. Treatment groups were randomly assigned to 40 pens on concrete floors with new wood shavings for bedding. The area of each pen was 4.2 m², and each contained 25 male and 25 female birds at trial commence-
ment. Pens were separated by solid plastic barriers extending 30 cm up from floor level followed by 90 cm of welded wire. Lighting program, heating, ventilation, and other management procedures were typical of modern intensive broiler farms in Ontario, Canada. Water was provided ad libitum by four nipple-type drinkers per pen. Dry feed was provided ad libitum by one tube-type feeder per pen, except for a 12-h period of feed withdrawal immediately prior to C. perfringens inoculum administration and during the challenge period itself, when inoculum-feed mixture was provided in trough-type feeders. All experimental procedures were conducted in accordance with the guidelines of the Canadian Council on Animal Care (1993).

A C. perfringens challenge model, based on that developed originally by Prescott et al. (1978), was used to initiate an outbreak of NE among the experimental animals. The model, with minor modifications, has been described in a number of subsequent publications (Brennan et al., 1996; Skinner and Brennan, 1999). Inoculum was administered via the feed commencing Day 14 and ending Day 16. Birds were fed a commerical wheat-based broiler starter diet from Days 0 to 21, except for Days 8 to 13 when a high protein starter containing 50% fishmeal (a recognized risk factor for naturally occurring outbreaks of NE) (Truscott and Al-Sheikhly, 1977) was fed. A commercial broiler grower diet was fed following the termination of the treatment period on Day 22 until trial termination on Day 29.

The challenge inoculum contained between 2 × 10^8 and 3 × 10^8 cfu C. perfringens/mL at administration. The inoculum was prepared from a culture of C. perfringens isolated from a bird displaying severe lesions of NE.

Birds that were moribund and unable to reach food or water were culled and euthanized by cervical dislocation. All dead or culled birds were necropsied to determine the cause of morbidity or death. A gross pathologic diagnosis of NE was based on the presence of intestinal lesions typical of naturally occurring and experimentally produced NE. These lesions consisted of focal to confluent areas of bland mucosal necrosis of the small intestine, often forming a pseudomembrane with no gross evidence of inflammatory reaction or hemorrhage. All dead or culled birds were also scored for intestinal lesions of NE as follows: 0 = no gross lesions; 1 = thin walled or friable; 2 = focal necrosis or ulceration; 3 = large patches of necrosis; 4 = severe extensive necrosis (Prescott et al., 1978). In addition, on Day 15 one male bird per pen was euthanized with inhaled carbon dioxide gas, subjected to necropsy, and scored for gross lesions of NE and coccidiosis, the latter according to criteria established for floor-pen studies in chickens (Johnson and Reid, 1970).

Criteria for declaring an NE outbreak were established prior to commencement of the study and consisted of the following: 1) observation of clinical signs in the flock consistent with naturally occurring and experimentally produced outbreaks of NE (consisting of some combination of depression, huddling, reduced feed intake, and changes in fecal consistency), and 2) at least 30% of birds randomly selected on Day 15 for euthanizing having gross intestinal lesions typical of NE as defined above, or 3) at least 30% of randomly selected and euthanized birds and spontaneous mortalities postinoculation (and prior to initiation of treatments) combined having gross intestinal lesions of NE.

On Day 15 an outbreak of NE was declared based on the following criteria: 1) the flock had clinical signs (as described above) consistent with an outbreak of NE, 2) 36/40 (90%) randomly selected and euthanized birds had gross intestinal lesions consistent with NE and no gross evidence of coccidiosis, and 3) 5/6 (83%) birds found dead on Day 15 had gross intestinal lesions consistent with NE.

Following identification of the outbreak on Day 15, treatments consisting of five levels of tylosin phosphate (0, 50, 100, 200, or 300 ppm), administered in the feed for seven consecutive days (Days 15 to 22), were randomly allocated at the pen level in a randomized complete block design. Each of the eight blocks consisted of five adjacent pens within the trial facility. The investigators and technical staff were not informed of the treatment assignment until the termination of the study.

Four birds were randomly selected from each pen (two of each sex, total 160 birds) on each of d 17 and 22, euthanized, and scored for gross lesions of NE and coccidiosis, as described above. Birds that died or were euthanized for humane purposes postchallenge (Day 14) were submitted for detection of C. perfringens. Gram-stained smears of intestine were examined for the presence of typical organisms; samples of the same tissues were cultured anaerobically on blood agar at 37°C and examined after 20 h for the presence of colonies exhibiting typical growth characteristics (smooth, entire, and with a double zone of hemolysis) (Ficken and Wages, 1997). Birds that died or were euthanized for humane purposes after Day 14 were classified as positive for NE if they had gross pathologic and microbiologic findings consistent with that diagnosis.

Body weights were measured on a pen basis on Days 0 and 29. Feed consumption was measured on a pen basis twice daily during the 3-d challenge and over the entire study. One sample of each feed was analyzed for moisture, crude protein, ash, fat, calcium, and phosphorus. A

### Table 1: Mortality and lesion scores in a study of the efficacy of tylosin phosphate in the treatment of necrotic enteritis (NE) in broiler chickens

<table>
<thead>
<tr>
<th>Tylosin phosphate (ppm)</th>
<th>NE mortality, Days 15–29 (%)</th>
<th>Mean NE lesion score, Days 17</th>
<th>Mean NE lesion score, Days 22</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>3.25a</td>
<td>2.66a</td>
<td>0.63a</td>
</tr>
<tr>
<td>50</td>
<td>0</td>
<td>1.41b</td>
<td>0.32b</td>
</tr>
<tr>
<td>100</td>
<td>0.25a</td>
<td>0.38a</td>
<td>0.06a,b</td>
</tr>
<tr>
<td>200</td>
<td>0.5b</td>
<td>0a</td>
<td>0.006a,b</td>
</tr>
<tr>
<td>300</td>
<td>0.25b</td>
<td>0b</td>
<td>0</td>
</tr>
</tbody>
</table>

*Means within a given column with no common superscript are significantly different (P < 0.05).  
1Day 22 mean NE lesion scores at 100 and 200 ppm are not significantly different from 0 ppm (P = 0.08).
sample of each feed was also analyzed for tylosin content using HPLC. Permitted analytical variation of drug content for all medicated feeds was ±25% of the calculated drug content.

Statistical analyses were conducted using the SAS® System (SAS Institute, 1997). Necrotic enteritis pen mortality data were analyzed by a series of pairwise treatment comparisons using an exact P-value for the median test (SAS Proc NPAR1WAY). For the purposes of this analysis, spontaneous mortalities and moribund birds that were euthanized for humane purposes and that had a final diagnosis of NE were treated identically. The Mann-Whitney-Wilcoxon test (exact P-value from SAS Proc StatXact) was used to conduct pairwise comparisons by treatment of mean pen lesion scores. Analysis of variance was used to test the effect of treatment on mean final body weight, average daily gain, ratio of feed to gain, and average daily feed intake. The pen was considered the experimental unit for all analyses.

**RESULTS**

All feeds were within tolerance for analyzed drug and nutrient content. The addition of tylosin phosphate to the feed significantly reduced mortality (P < 0.05) due to NE at all dose levels when compared to unmedicated birds (Table 1).

Mean NE lesion scores on Day 17 were significantly reduced (P < 0.05) by all levels of tylosin compared to unmedicated birds, decreasing linearly from 2.66 at 0 ppm to 0.38 at 100 ppm and 0 at higher doses (Table 1).

On Day 22, mean NE lesion scores were essentially equal to or lower than those observed at corresponding dose levels on Day 17. The mean NE lesion score observed on Day 22 was significantly lower (P < 0.05) for birds administered tylosin at 300 ppm compared to unmedicated birds (Table 1). Birds administered tylosin at 100 and 200 ppm had mean NE scores that were lower than unmedicated birds but were not significantly different (P = 0.08). Coccidiosis lesion scores were 0 on Days 17 and 22 for all euthanized birds.

Tylosin at all levels provided improvement in Day 29 body weight, average daily gain, feed to gain ratio, and average daily feed intake compared to unmedicated birds (Table 2).

**DISCUSSION**

The results of this study demonstrate that tylosin phosphate is effective in reducing morbidity, mortality, and suppression of growth and feed efficiency associated with NE in broiler chickens, when administered in the feed after onset of an outbreak.

These findings are consistent with previous studies of the activity of tylosin against *C. perfringens* in vitro (Stutz and Lawton, 1984; Kondo, 1988; Watkins et al., 1997) and support the finding of Vissiennon et al. (2000) that tylosin is effective in reducing mortality associated with this disease.

Significantly reduced mean lesion scores among medicated birds on Days 17 and 22 indicate that, in addition to reducing mortality, administration of tylosin significantly reduced the clinical impact of challenge among surviving birds. This result suggests that the positive effect of medication on growth parameters (Table 2) was related to control of NE and not simply a result of a growth promoter effect.

All dose levels of tylosin significantly reduced mortality and mean lesion scores on Day 17 and improved growth parameters. For Day 17, birds receiving 100 ppm had significantly reduced mean lesion scores compared to those receiving 50 ppm. Although on Day 17, the birds receiving 200 ppm dose had significantly lower mean lesion scores compared to those receiving 100 ppm, the magnitude of the effect is not considered by the authors to be of clinical relevance.

The results of this study thus provide evidence that tylosin phosphate, when administered in feed, is effective in the treatment of clinical outbreaks of NE in broiler chickens and suggest that the optimal dose for this purpose is 100 ppm.

**REFERENCES**


*Table 2. Growth performance measures in a study of the efficacy of tylosin phosphate in the treatment of necrotic enteritis (NE) in broiler chickens*


