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ORIGINAL ARTICLE

Efficacy of avilamycin for the prevention of necrotic enteritis caused by a pathogenic strain of *Clostridium perfringens* in broiler chickens

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ABSTRACT

The efficacy of avilamycin for the prevention of necrotic enteritis (NE) was investigated in a 35-day floor pen study of 2200 broiler cockerels using a *Clostridium perfringens* (*Cp*) feed inoculum challenge model. Treatments consisted of (1) nonmedicated, nonchallenged; (2) nonmedicated, challenged; (3) avilamycin at 15 ppm, challenged; (4) avilamycin at 30 ppm, challenged. Avilamycin was administered in the feed from day 7 to day 30 of the study. Challenge inoculum was administered on day 14 and delivered approximately 10^9 CFU *Cp*/bird. NE mortality rates from day 14–35 were significantly ($P < 0.0001$) lower in birds treated with avilamycin at 15 and 30 ppm when compared to nonmedicated, challenged birds. Treatment with avilamycin also resulted in a significant reduction in ileal *Cp* count on day 21 ($P < 0.0001$) and NE lesion scores on day 17 ($P < 0.006$) when compared to nonmedicated, challenged birds. The performance of birds treated with avilamycin was also improved when compared to nonmedicated, challenged birds. Cockerels that received either 15 or 30 ppm avilamycin had a significantly ($P < 0.0001$) increased body weight on day 35 and average daily gain from days 0–35 than nonmedicated, challenged birds. Furthermore, birds treated with avilamycin had an improved feed conversion rate from days 0–35 compared to both nonmedicated, nonchallenged birds and nonmedicated, challenged birds. This study confirms that avilamycin is effective at controlling mortality related to NE in growing broiler chickens.

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Necrotic enteritis; broiler chicken; efficacy; avilamycin; *Clostridium perfringens*

Introduction

Necrotic enteritis (NE), caused by the Gram-positive bacillus, *Clostridium perfringens* (*Cp*), leads to significant morbidity and mortality in broiler chickens worldwide. NE can present as both a clinical or subclinical disease with signs ranging from depression, decreased performance, and anorexia to death (Kaldhusdal & Hofshagen, 1992; Løvland & Kaldhusdal, 2001). Disease is most often associated with outbreaks in broiler chickens between two and six weeks of age (Long, 1973; Engstrom *et al.*, 2003; Løvland *et al.*, 2004) and mortality can range from 2% to 50% in untreated flocks (Long, 1973; Shane *et al.*, 1985; Craven *et al.*, 1999). Risk factors for development of NE include co-infection with other enteric pathogens such as *Eimeria* spp. (Williams, 2005), diets high in fishmeal, rapidly fermentable carbohydrates, and/or protein (i.e. wheat or barley) (Truscott & Al-Sheikhly, 1977; Prescott, 1979; Cooper *et al.*, 2009) or supplemented with zinc (Baba *et al.*, 1992).

NE can be induced predictably in a model, where a known pathogenic strain of *netB* positive *Cp* is administered orally to broiler chickens fed a diet with no antibiotic activity against *Cp* (Shojadoost *et al.*, 2012). Comparing mortality rates and intestinal lesion prevalence between groups of birds fed a non-medicated ration and birds fed a test diet can demonstrate the effectiveness of the test article in controlling NE outbreaks. Furthermore, a correlation between performance measures such as feed conversion rate (FCR) and weight gain with lesion scores has previously been demonstrated (Cooper *et al.*, 2009). Therefore, these parameters are also an important measure of the effect of an intervention against NE (Brennan *et al.*, 2001a, b, 2003; Dahiya *et al.*, 2007), especially when evaluating subclinical disease (Shojadoost *et al.*, 2012).

A variety of strategies have been used to control and prevent NE. Administering antibiotic feed additives that have recognized activity against *Cp* in the broiler ration has been shown to prevent losses from NE (Ficken & Wages, 1997; Elwinger *et al.*, 1998; Brennan *et al.*, 2001a, b, 2003). Avilamycin is an antibiotic of the orthosomycin family and is primarily active against Gram-positive bacteria. Additionally, avilamycin has been shown to have strong bactericidal effects on *Cp* *in vitro* (Devriese *et al.*, 1993; Watkins *et al.*, 1997). In Canada, avilamycin is licensed under the trade name Surmax100™ for the prevention of NE due to *Cp* in growing broiler chickens. The product is

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available by veterinary prescription only and licensed for use for 21 days with zero-day withdrawal period.

In 2000, Vissienon *et al.* demonstrated the effect of avilamycin in a *Cp* challenge trial, showing that chickens treated with avilamycin had a mortality rate of 0–8% versus 16–36% in nonmedicated birds. However, this study investigated a lower, previously approved growth promotion dose of 10 ppm during the entire grow-out period and utilized an intraduodenal infection model rather than an oral challenge route (Vissienon *et al.*, 2000). The ability of avilamycin at various doses to control NE mortality was previously tested in an unpublished dose-titration oral challenge study involving 648 birds by the current group of researchers (Bagg *et al.*, unpublished data). The treatment groups were: (1) nonmedicated, nonchallenged; (2) nonmedicated, challenged; (3–5) challenged and fed avilamycin at 15 ppm, 30 ppm, and 45 ppm, respectively, for the full duration of the 21-day study period; and (6) nonmedicated control until day 14, challenged, then fed avilamycin at 45 ppm until end of study (days 15–21). Results of this unpublished study showed a linear relationship between the level of avilamycin in the feed and a reduction in NE mortality, NE lesion scores, and *Cp* counts. Compared to a NE mortality rate of 47.22% in the nonmedicated, challenged birds, birds in treatment groups 3 through 6 showed significantly ($P < 0.001$) decreased mortality rates (26.85%, 14.82%, 9.26%, and 22.22%, respectively).

The objective of this study was to confirm, on a larger scale, the results of the previous unpublished dose titration study by Bagg *et al.* investigating the effective dosage of avilamycin used as a feed medication for the control of mortality and lesions associated with *Cp* in broiler chickens during an induced outbreak of NE.

Materials and methods

Birds and experimental facilities

A total of 2200 male broiler chicks (Ross 708 strain) from a commercial hatchery in Ontario, Canada were used in the study. Chicks were vaccinated with Marek's disease virus vaccine and infectious bronchitis virus vaccine at the hatchery then vaccinated orally against coccidiosis on the day of placement. The lighting programme (birds received 23 hours of light at 20 lux from days 0–7, 20 hours of light at 20 lux from days 7–10, followed by 20 hours of light at 5 lux from day 10 until market), heating, and ventilation were typical of a modern commercial broiler barn in Ontario, Canada. Pens were bedded with new wood shavings and separated by solid plastic barriers extending 1 foot up from floor level. Water was provided *ad libitum* from two nipple drinkers per pen. Feed was delivered via one tube-type feeder in each pen. Other than avilamycin, no growth promotants, antibiotics, or anticoccidials were included in or added to the feed. Prior to study initiation, nonmedicated feed was submitted to an external laboratory for analysis. Nutrient analysis of feed was determined using proximate analyses and avilamycin content was determined using a drug assay method. Tolerance nutrient specifications including upper and lower limits were calculated using the CVM Guidance Document # 40 (FDA, 1992) and the tolerance limit for avilamycin was set at ±25%.

Experimental design

A randomized complete block design with 10 replicate blocks of four pens (40 pens total) was used in the study. Blocking factor was by location within the facility and treatments were randomly assigned to each pen within each block, with each pen as an experimental unit. Treatment groups consisted of: (1) nonmedicated, nonchallenged; (2) nonmedicated, challenged; (3) avilamycin at 15 ppm, challenged; and (4) avilamycin at 30 ppm, challenged. Study personnel responsible for ileal *Cp* counts, scoring intestinal lesions, and for conducting necropsy analysis on mortalities were masked to treatment groups.

Study procedure

Birds were placed in each pen within 24 hours of hatch at an initial stocking density of 0.82 sq feet/bird. Birds were observed twice daily throughout the duration of the study, and dead or removed birds were not replaced. All treatment groups were fed a non-medicated starter diet from days 0–7. A treatment starter diet was fed for days 7–21 except for day 14 when a non-medicated starter diet was fed so as not to interfere with the *Cp* challenge. A treatment grower diet was fed from days 21–30 followed by a non-medicated finisher diet on days 30–35.

Animal weight and feed weight were recorded on days 7, 14 (challenge initiation), 21, 30, and 35 (end of study). On days 17 and 21, three birds were randomly selected from each pen and humanely sacrificed and scored for NE and coccidiosis lesions. The NE lesion scoring system was based on the criteria of Prescott, (1979) and ranged from 0 (normal, no evidence of gross lesions) to 4 (severe, extensive necrosis). The small intestine was also examined for evidence of coccidiosis and scored based on the system described by Johnson and Reid (1970). On day 14, prior to challenge, one randomly selected bird per pen was humanely sacrificed and the intestinal contents collected from the ileocolonic junction to the open end of the intestine into a sterile container. Ileal digesta samples were shipped for same day plating and *Cp* enumeration. The same procedure was repeated on two birds per pen on day 21 of the study period.

Bird mortalities or culls prior to the challenge were weighed, submitted for necropsy and a presumptive diagnosis of cause was made. Removals and deaths during this period were considered as normal illness and mortality. Birds that died or were euthanized following administration of challenge material were treated as study mortalities. These birds were weighed and subjected to a necropsy evaluation to determine the presumptive cause of death or disease. Three criteria were used to classify a mortality caused by *Cp*: (1) death/euthanasia following challenge on day 14; (2) gross necropsy diagnosis of NE; (3) NE lesion score greater than or equal to 1. Necropsied birds with concurrent NE and coccidiosis were included in the data analysis as a mortality associated with NE.

Challenge with Cp bacteria

The *Cp* challenge model was based on a previous model developed by Prescott, (1979). Treatment groups 2, 3, and 4 received an oral *Cp* challenge on day 14 using a *netB* positive *Cp* isolate from a field case of NE (CP33) from Ontario, Canada. The isolate was previously confirmed to be sensitive to avilamycin using MIC testing (Broth dilution method; Nakamura *et al.*, 1999). An inoculum was prepared to contain approximately 10^7 colony forming units of *Cp* per ml and was administered at a ratio of 1:1.5 feed-to-broth. Treatment feed was removed from the pen 8 hours prior to challenge diet being offered. The challenge feed was available for 16 hours before being replaced by the treatment diet.

Statistical analysis

A Linear Mixed Model fit using the Residual Maximum Likelihood (REML) method, with block as a random effect and treatment as a fixed effect was used to analyze the data. For the principle variable, per cent mortality, the data were analysed using the raw data means and also transformed means (arcsine square root transformation). Data related to NE lesion score and *Cp* enumeration at day 21 were averaged by pen prior to analysis. For any variable that had a significant treatment effect ($P < 0.05$), a Least Squares Means test was used to compare between treatment groups. When treatment effects were significant, linear contrasts were used to compare the combined effect of both avilamycin treatments versus the non-challenged nonmedicated group and the combined effect of both avilamycin treatments versus the challenged nonmedicated group. The analysis was conducted using JMP 8.0 (SAS Institute Inc., Cary, NC, USA).

Ethical statement

The protocols used in this study received Animal Care Committee approval and met the standards of the Canadian Council on Animal Care and Elanco Animal Care standards.

Results and discussion

The nutrient and avilamycin content of feed were found to fall within the recommended tolerance ranges (data not shown).

Table 1 shows the results of key parameters measured. The overall mortality due to NE in the nonmedicated, challenged birds was 31.04% versus 1.88% in the nonmedicated, nonchallenged birds ($P < 0.0001$). Likewise, average day 17 lesion scores were significantly higher in nonmedicated, challenged birds compared to nonmedicated, nonchallenged birds (Table 1). Nonmedicated, challenged birds also had decreased performance, with significantly reduced growth rates and increased FCR from day 14 to 21 (Table 1). These results confirm that the *Cp* challenge model was effective and that the disease was responsible for the performance losses. On day 21 there was evidence of *Cp* infection in the nonmedicated, nonchallenged birds. Birds in this treatment group had a similar *Cp* count and NE lesion score to nonmedicated, challenged cockerels

Table 1. Measures of growth performance, mortality, lesion score and *Clostridium perfringens* counts (*Cp*) from a study on the ability of avilamycin to control mortality due to NE in broiler chickens.

	Treatment group					
	1	2	3	4		
Avilamycin level (ppm)	0	0	15	30		
Cp challenge	No	Yes	Yes	Yes	*P*-value	*P*-value contrast[c]
Body weight day 35 (g)	1970^{AB}	1913^{A}	2035^{B}	2027^{B}	<0.0001	<0.0001
Average daily gain[a] d 14–21 (g)	55.8^{B}	38.3^{C}	58.4^{AB}	61.4^{A}	<0.0001	<0.0001
Feed conversion rate[ab] d 14–21	1.600^{A}	1.982^{B}	1.560^{A}	1.494^{A}	<0.0001	<0.0001
Average daily gain[a] d 0–35 (g)	52.5^{A}	44.9^{B}	53.3^{A}	54.3^{A}	<0.0001	<0.0001
Feed conversion rate[ab] d 0–35	1.661^{B}	1.690^{A}	1.615^{C}	1.608^{C}	<0.0001	<0.0001
NE mortality (%)	1.88^{B}	31.04^{A}	7.53^{B}	3.02^{B}	<0.0001	<0.0001
NE lesion score d 17	0.47^{A}	1.83^{B}	1.23^{B}	1.23^{B}	<0.0001	0.006
NE lesion score d 21	0.80^{AB}	1.04^{A}	0.27^{B}	0.50^{AB}	0.02	0.005
Cp count d 21 (log CFU/g digesta)	6.29^{A}	6.84^{A}	2.22^{B}	1.41^{B}	<0.0001	<0.0001

[A,B,C]Indicate a statistically significant difference in data in the same row.
[a]Adjusted for mortalities and removals due to morbidity.
[b]Calculated as g body mass gain per g feed intake.
[c]Comparison of the combined effect of both avilamycin treatment groups (3 and 4) versus the nonmedicated, challenged group.

although their mortality rate due to NE remained very low. Infection in this group was not unexpected and likely resulted from environmental contamination, feed contamination, or cross-contamination from challenged groups in adjacent pens.

The addition of avilamycin to the feed significantly ($P < 0.0001$) reduced mortality due to NE at both dose levels when compared to nonmedicated, challenged birds (Table 1). Furthermore, there was a trend to lower NE mortality in birds treated with 30 ppm avilamycin compared to 15 ppm at 3.02% and 7.53%, respectively. Although the difference was not statistically significant, this trend towards a lower mortality rate at the 30 ppm dose is supported by a previous dose titration study showing a linear decline in mortality rate as the avilamycin content of the feed increased up to 45 ppm (Bagg *et al.*, unpublished data). As expected, lesion scores in all challenged birds were significantly higher than lesion scores in nonmedicated, nonchallenged birds on day 17 (Table 1). However, when comparing lesion scores on both days 17 and 21, birds that were challenged but treated with avilamycin had significantly lower lesion scores than nonmedicated, challenged birds (Table 1). The improvement in NE lesion score for medicated, challenged birds shows a potential welfare benefit from avilamycin treatment. Traditionally, measures used to assess welfare in the poultry industry have included mortality and morbidity indicators such as contact dermatitis or abnormal leg rotation (Manning *et al.*, 2007). As intestinal lesion scores are a measure of NE morbidity, the reduction in score with treatment should also correlate with improved bird welfare. Avilamycin also had a significant ($P < 0.0001$) positive impact on day 21 ileal *Cp* counts compared to nonmedicated birds (Table 1). Similar to bird mortality, there was a trend toward lower *Cp* counts with a dose of 30 ppm versus 15 ppm, with counts of 2.22 and 1.41 log CFU/g digesta, respectively.

From day 0 to 35, average daily gain was not significantly different between nonmedicated, nonchallenged birds and medicated challenged birds. Furthermore, body weight on day 35 was not significantly different between these groups, showing that avilamycin was effective at reducing the negative impact of *Cp* infection on bird performance. Treatment with avilamycin significantly ($P < 0.0001$) improved FCR over the duration of the study (day 0–35). Feed production is an important factor in both environmental and economic sustainability in animal agriculture (Goodland, 1997; Tilman *et al.*, 2002; Castellini *et al.*, 2006).

At the time of writing, avilamycin has not been categorized by the World Health Organization as part of Medically Important Antimicrobials for Human Health nor is it used in human medicine. Currently, resistance levels of *Cp* to avilamycin are very low. Williams (2005) summarized the results from four studies testing the *in vitro* sensitivity of *Cp* to avilamycin and found no reports of resistance in any of the strains tested. The *in vivo* effect of avilamycin has also been demonstrated in efficacy studies (Elwinger *et al.*, 1998; Vissienon *et al.*, 2000). Moreover, it is expected that the requirement of a veterinary prescription and treatment duration of 21 days will lead to more prudent and targeted use of avilamycin, thereby slowing development of antimicrobial resistance in the future. These factors are important considerations as the use of antibiotics in food production and antibiotic resistance has become a prominent issue in the poultry industry worldwide. Current views and regulations regarding antibiotic use in food animals, including poultry production, vary globally. For example, while various European countries have banned the prophylactic use of certain antibiotics, other countries support the judicious use of antibiotics in-feed for disease prevention.

The results of this study provide evidence to support the use of avilamycin for control of mortality and lesions associated with *Cp* in growing chickens during a period of risk of NE. The results are supported by other studies that have investigated the effectiveness of avilamycin treatment against NE (Vissienon *et al.*, 2000) and confirm the results of a previously unpublished dose-titration study by the current researchers (Bagg *et al.*, unpublished data). In addition to the effects on morbidity and mortality due to NE, treatment with avilamycin resulted in an improved growth rate and FCR compared to nonchallenged cockerels that were fed a nonmedicated diet. These results support the targeted use of avilamycin to control mortality and improve performance of birds in the face of a *Cp* challenge.

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