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The Role of Penicillin G Potassium in Managing *Clostridium perfringens* in Broiler Chickens

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SUMMARY. The efficacy of penicillin G potassium (Pot-Pen) administered via drinking water to manage necrotic enteritis (NE) was investigated in a *Clostridium perfringens* (CP) challenge study using 1600 broiler chickens assigned to one of four treatment groups: nonchallenged, nonmedicated; challenged, nonmedicated; challenged, Pot-Pen 0.2 g/L; challenged, Pot-Pen 0.4 g/L. Overall mortality due to NE was significantly reduced among Pot-Pen–treated pens; mortality due to other causes did not differ among the treatment groups. Among all birds, growth performance parameters were significantly improved among Pot-Pen–treated pens. When considering birds randomly sacrificed 4 days post-Pot-Pen initiation, mean NE lesion scores were greatest among the challenged, nonmedicated pens; only one of 80 randomly sacrificed birds treated with Pot-Pen had NE lesions. Among the nonmedicated control pens, body weight (BW) was significantly greater among birds that did not have NE-associated lesions. When sacrificed birds were stratified by NE lesion score, there were no significant differences in BW among the treatment groups. Results of this study suggest that CP-associated subclinical disease can significantly reduce broiler performance. Furthermore, the positive effects of treatment with Pot-Pen appeared to be associated with the prevention and/or treatment of NE-specific lesions.

RESUMEN. El papel de la penicilina G potásica en el manejo del *Clostridium perfringens* en pollos de engorde.

Mediante un estudio de desafío de *Clostridium perfringens*, se investigó la eficacia de la penicilina G potásica administrada en el agua de bebida para el manejo de la enteritis necrótica. Se utilizaron 1600 pollos de engorde asignados a cuatro tratamientos: 1) no desafiados / no medicados; 2) desafiados / no medicados; 3) desafiados / medicados con penicilina G 0.2 g/L; 4) desafiados / medicados con penicilina G 0.4 g/L. En general, la mortalidad debido a enteritis necrótica se redujo en los corrales tratados con penicilina G, mientras la mortalidad por otras causas no difirió entre tratamientos. Los parámetros productivos se incrementaron significativamente en los grupos tratados. Analizando aves sacrificadas aleatoriamente 4 días posteriores a la administración de la penicilina G, el promedio del registro de lesiones entre las aves desafiadas / no medicadas fue el más alto; solo una de 80 aves tratadas con penicilina G mostró lesiones de enteritis necrótica. Entre los corrales no medicados, el peso corporal fue mayor en las aves que no mostraron lesiones asociadas con enteritis necrótica. Una vez sacrificadas, las aves se clasificaron en base al registro de lesiones. No se observaron diferencias significativas en el peso corporal entre los tratamientos. Los resultados de este estudio sugieren que la enfermedad subclínica asociada con *Clostridium perfringens* puede reducir significativamente el rendimiento productivo de los pollos de engorde. Además, los efectos positivos del tratamiento con penicilina G están aparentemente asociados con la prevención y/o tratamiento de lesiones específicas de enteritis necrótica.

Key words: broiler chicken, *Clostridium perfringens*, necrotic enteritis, penicillin G potassium

Abbreviations: BW = body weight; CP = *Clostridium perfringens*; FCR = feed conversion ratio; NE = necrotic enteritis; Pot-Pen = penicillin G potassium

Necrotic enteritis (NE), an enteric disease of chickens caused by *Clostridium perfringens* (CP), was first described in 1961 by Parish (8). This pathogen is of great economic significance to the broiler industry, causing insidious morbidity that interferes with growth and feed efficiency and substantial mortality associated with the more fulminate form of the disease (13). The spectrum of disease associated with CP includes subclinical infection that affects performance (11), mild clinical infection characterized by diarrhea (5), hepatitis that affects performance (6) and results in condemnation of livers at slaughter, and sudden death with no premonitory signs (15).

Control of NE has traditionally depended upon a variety of strategies, including reducing exposure to dietary risk factors and concurrent enteric infections, particularly coccidiosis, and administering feed additives with activity against CP (13). Use of most antimicrobial growth promoters is associated with a dramatic decrease in the incidence of NE (13). It is believed that CP-associated clinical and subclinical infection will become an increasing problem among broiler chickens in the European Union as result of the ban of antimicrobial growth promoters (12). Evidence from Europe to date suggests that not only NE mortality but also subclinical NE is having severe economic consequences for the poultry industry (4). A higher rate of carcass contamination may also signal a higher risk of food-borne disease.

New control and prevention strategies for CP-associated manifestations are urgently needed because of the increasing restrictions being placed on the use of antimicrobial growth promoters. Together with measures to avoid risk factors predisposing to NE, targeted antimicrobial use is likely to be a key element in evolving CP management strategies. Little current published information exists on the efficacy of penicillin in the control of NE. This CP challenge study was undertaken to investigate the utility of penicillin G potassium (Pot-Pen Water Soluble Powder; Vétoquinol N.-A. Inc., Lavaltrie, Québec, Canada) administered via the drinking water for the management of clinical and subclinical CP infection in broilers under current conditions of poultry husbandry.

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Table 1. Treatment groups in a study to determine the effects of penicillin G potassium (Pot-Pen) administered in the drinking water to chicks inoculated with CP.

Treatment group	Challenged with CP	Pot-Pen dosage (g/L)	Penicillin activity (IU/L)
Negative control	No	0	0
Positive control	Yes	0	0
Pot-Pen 0.2 g/L	Yes	0.2	297,000
Pot-Pen 0.4 g/L	Yes	0.4	594,000

MATERIALS AND METHODS

Birds and housing. A total of 1600 1-day-old Cobb × Cobb broiler chickens (800 males and 800 females) obtained from a commercial hatchery were used in this study. Birds were vaccinated at the hatchery for Marek's disease and avian infectious bronchitis. The research facility was thoroughly cleaned and disinfected prior to bird placement. Birds were housed in 32 contiguous pens at a density of 50 birds per 4.2 m^2 pen. Pens had concrete floors bedded with new chopped straw. Pen barriers were solid plastic for 30 cm up from the floor, topped by 90 cm of welded 1-inch wire. Lighting program, heating, ventilation, and other management procedures were typical of modern intensive broiler farms in Ontario, Canada. Birds received no medications other than penicillin G potassium (Pot-Pen) throughout the study.

Study design. A randomized complete block design was used involving the four treatment groups described in Table 1. The four treatment groups were 1) challenged with *C. perfringens*, nonmedicated; 2) unchallenged, nonmedicated; 3) challenged with *C. perfringens*, treated with Pot-Pen at 0.2 g/L; and 4) challenged with *C. perfringens*, treated with Pot-Pen at 0.4 g/L. The 32 pens were divided into four blocks, with eight pens per block (Fig. 1). Treatment groups were randomly assigned across the four male and four female pens within each block. Birds from each hatchery box were randomly assigned to treatments such that boxes were approximately equally represented among the pens in each block. Birds were observed at least once daily, and observations were recorded, including the number of live birds per pen. Body weights (BWs) were measured at the beginning of the 28-day study period (day 0) and on days 15 and 28 (end of study). Feed consumed was recorded for days 0 to 15, days 16 to 28, and days 0 to 28.

Feed was withdrawn for 12 hours on day 15 immediately prior to inoculation with CP. The inoculum was administered via the feed for a 24-hr period beginning on day 16. For the treated groups, Pot-Pen was administered for 5 consecutive days via the drinking water, beginning on the day when clinical signs and/or mortality due to NE were first noted. Pen water intake was measured daily. On day 20, five birds randomly selected from each pen (total 160 birds) were euthanatized with carbon dioxide, weighed, and the intestinal tracts were scored for NE. All birds that either died or were euthanatized after day 5 were submitted to the study pathologist for gross necropsy to determine the likely cause of death.

Feed and water. Dry feed was provided *ad libitum* by one tub-type feeder per pen except on days 15 and 16. During the 12-hour prechallenge period on day 15 tub feeders were withdrawn, and during the 24-hour inoculation period on day 16 the inoculum-feed mixture was provided in trough-type feeders. All rations met or exceeded nutrient requirements of broiler chickens (7).

Water was provided *ad libitum* by four nipple-type drinkers per pen. For the two nonmedicated groups, water nipples were connected to the facility's main water line throughout the study period. For the two groups treated with Pot-Pen, water nipples were disconnected from the main water line during the 5-day medication period, and medicated water was delivered from a pail hanging above the pen. A fresh batch of medicated water was prepared once daily; unconsumed water from the previous day's batch was weighed. Treatment with Pot-Pen in the drinking water began when NE-associated clinical signs occurred (e.g., huddling, reduced feed intake, reduced response to stimuli) and continued for 5 days.

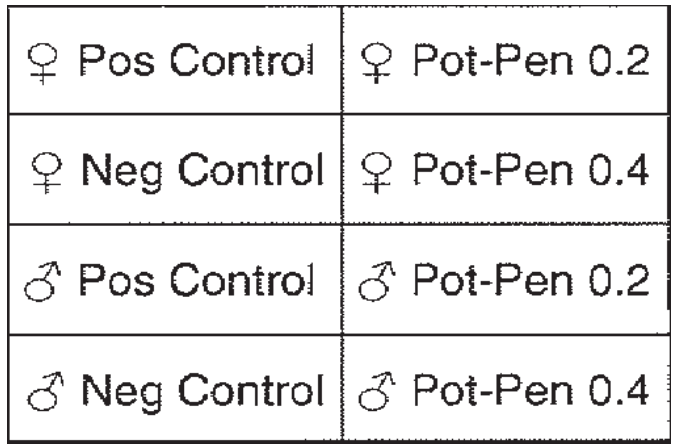

Fig. 1. Complete block study design for assignment of 1-day-old male and female broiler chicks to four treatment groups. Pos Control = challenged with CP, nonmedicated; Neg Control = unchallenged, nonmedicated; Pot-Pen 0.2 and Pot-Pen 0.4 = challenged with CP, treated with penicillin G potassium (Pot-Pen) at 0.2 g/L and 0.4 g/L of drinking water, respectively, for 5 days.

CP challenge. A CP challenge model, based on that developed originally by Prescott et al. (9), was used to initiate NE among the experimental animals. This model, with minor modifications, has been described in a number of subsequent publications (1,10). The isolate was obtained from a field case of necrotic enteritis in a nonmedicated broiler flock in Ontario in 2001. At the time of administration, the inoculum contained approximately 10^8 colony-forming units of CP per mL. The inoculum-feed mixture was provided in the morning and afternoon of day 16. Feeders were placed in all pens simultaneously and were removed and weighed at the end of each 12-hr period.

NE lesion scoring in sacrificed birds. A pathologist blinded to treatment group performed necropsies on all birds. Intestinal lesion scores were assigned for gross NE lesions as described by Prescott et al. (9): 0 = no lesions; 1 = thin friable small intestine; 2 = focal necrosis, ulceration, or both; 3 = patchy necrosis; 4 = severe extensive necrosis typical of that seen in birds that have died of NE.

Calculations and statistical analysis. Feed conversion ratio (FCR) was calculated on a pen basis as follows for each specified time period, feed conversion = total feed consumed ÷ (total weight of live birds + total weight of sacrificed birds + total weight of culled or dead birds − total weight of birds at placement). Pen-level BW was calculated by dividing the total weight of the live birds by the number of live birds. Necrotic enteritis mortality was calculated as the number of deaths confirmed due to NE, expressed as a percentage of the number of birds alive at the specified time period.

Except for analysis involving the randomly sacrificed birds on day 20, the pen was the experimental unit for all statistical analyses. Mortality was measured as a proportion at the pen level ($p = x/y$), then subjected to an empirical logit transformation of the form $\text{logit}(x) = \log((x + 0.1) \div ((y - x) + 0.1))$. All analyses were conducted using Proc Mixed in the SAS system (SAS v. 9.1; SAS Institute, Cary, NC), with block as a random effect. Statistical significance was set to P-value < 0.05.

RESULTS

Treatment with Pot-Pen in the drinking water was initiated on day 17 when signs of clinical NE were observed (huddling, reduced feed intake, reduced response to stimuli) and continued until day 22. By day 20, with the exception of expected production mortality, all remaining birds (including those sacrificed) appeared clinically normal.

Mortality. Mortality due to all causes and NE-associated mortality were significantly less in the negative control and the

Table 2. Pen-level mortality in broiler chicks inoculated with CP and treated with penicillin G potassium (Pot-Pen).[A]

	Mean mortality (%)				
	Day 17 (start of Pot-Pen administration)–28 (end of study)				Day 20–28
Treatment group	No. pens (birds)	All causes[B]	Non-NE[C]	NE only[D]	NE only
Negative control	8 (389)	2.62[a]	2.05[a]	0.5[a]	0.0
Positive control	8 (393)	10.12[b]	1.26[a]	8.86[b]	7.0
Pot-Pen 0.2 g/L	8 (389)	0.76[a]	0.51[a]	0.25[a]	0.0
Pot-Pen 0.4 g/L	8 (396)	2.0[a]	2.04[a]	0.00[a]	0.0

[A]Values with different lowercase superscript within a column are significantly different, P-value < 0.05.
[B]Test for overall significance of treatment group, P-value $= 0.0263$.
[C]Test for overall significance of treatment group, P-value $= 0.2656$.
[D]Test for overall significance of treatment group, P-value < 0.0001.

Pot-Pen (both doses) treated pens compared to the positive control pens (Table 2). The only NE-associated mortality observed after Pot-Pen treatment began (day 17) occurred in the positive control group (Figure 2). Mortality due to causes other than NE did not differ significantly among the four treatment groups (Table 2).

Lesions and body weight of birds sacrificed on day 20. Among the 80 birds sacrificed on day 20 from the two Pot-Pen treatment groups, only one had NE-associated lesions (Table 3). NE lesion scores were significantly less among birds treated with Pot-Pen compared to positive and negative control birds. Lesion scores of 2 and 3 (i.e., corresponding to mild and subclinical necrotic enteritis) were observed in 11 negative control birds (Table 3) indicating CP infections in the negative control group as well.

Among all sacrificed birds, BW was significantly greater for those treated with Pot-Pen compared to both the positive or negative control birds (Table 4). When birds were stratified by NE lesion score (0 and 2), BW did not significantly differ among treatment groups (Table 4). Among all sacrificed birds, mean BW declined with increasing NE lesion score severity (Table 5). When only control groups were considered (i.e., non-Pot-Pen–treated birds), the latter observation was again observed (Table 5).

Body weights and feed intake throughout the trial. Mean pen BW did not differ among treatment groups on day 15 before inoculation with CP (Table 6). By day 28, mean BW was highest in pens treated with Pot-Pen 0.4 g/l; mean BW was significantly greater in Pot-Pen–treated pens compared to positive control pens (Table 6). Mean BW did not significantly differ between negative and positive control pens or between 0.2 g/l and 0.4 g/l Pot-Pen–treated pens.

By day 28, both feed intake and FCR were improved among Pot-Pen–treated pens compared to positive control pens (Table 6). Mean feed intake was significantly greater among Pot-Pen 0.4 g/l–treated pens compared to positive and negative control pens. FCR was significantly improved among the Pot-Pen 0.2 g/l– and 0.4 g/l–treated pens compared to the positive control pens.

DISCUSSION

As concerns relating to antimicrobial resistance grow worldwide, the food animal industry is under increasing pressure to evolve antimicrobial usage practices away from broad growth promotion

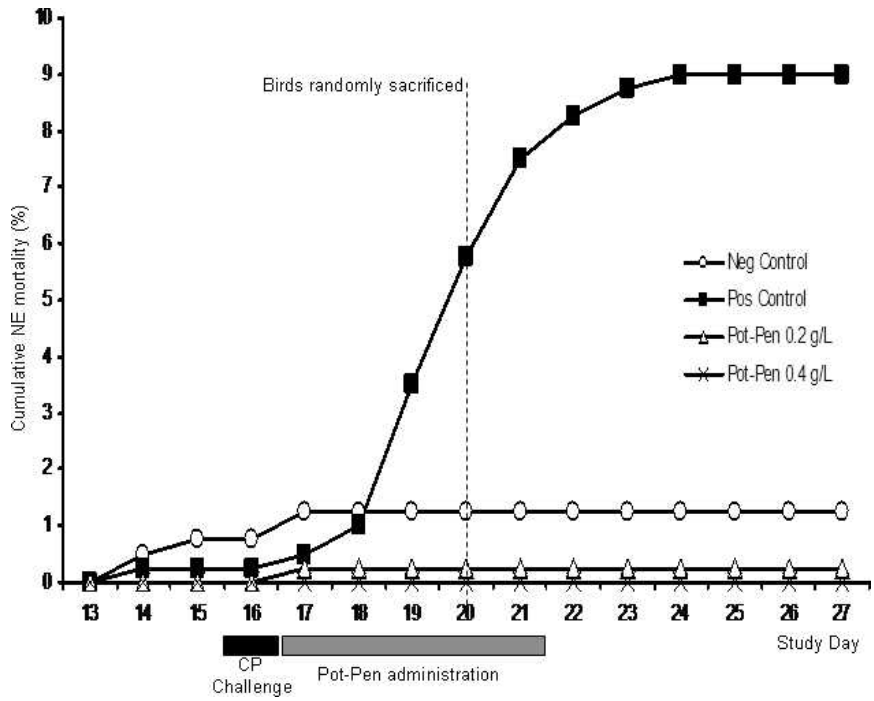

Fig. 2. Cumulative pen-level NE mortality among broiler chickens in a study to evaluate the effectiveness of penicillin G potassium (Pot-Pen) for the management of CP infections. Pos Control = challenged with CP, nonmedicated; Neg Control = unchallenged, nonmedicated; Pot-Pen 0.2 and Pot-Pen 0.4 = challenged with CP, treated with penicillin G potassium (Pot-Pen) at 0.2 g/l and 0.4 g/l of drinking water, respectively.

Table 3. Lesion scores in broiler chickens randomly sacrificed 4 days post–penicillin G potassium (Pot-Pen) treatment initiation (day 20).[A]

NE score	Negative control no. (%)	Positive control no. (%)	Pot-Pen 0.2 g/L no. (%)	Pot-Pen 0.4 g/L no. (%)
0	29 (72.5)	16 (40.0)	39 (97.5)	40 (100.0)
2	8 (20.0)	17 (42.5)	1 (2.5)	0 (0.0)
3	3 (7.5)	7 (17.5)	0 (0.0)	0 (0.0)
Average NE score[B]	0.625[a]	1.375[b]	0.050[c]	0.000[c]

[A]Values within a row with different lowercase superscripts are significantly different, *P*-value < 0.05.
[B]Overall significance of treatment group, *P*-value < 0.0001.

application and toward a targeted management and disease control role. For this latter approach to be successful, a much better understanding is required of disease-specific pathogenesis and antimicrobial-specific activities. This study provides important insights into the clinical manifestations and pathogenesis of *C. perfringens* infection in broilers and the prospective role for the targeted use of aqueous penicillin G potassium in the control and management of necrotic enteritis. For a comprehensive review of the literature related to the clinical and pathological manifestations, epidemiology, impact, diagnosis, and control of CP-associated enteric illness in broilers, readers are referred to Wilson *et al.* (15).

Results of this study demonstrated a variety of clinical manifestations associated with CP infection in broilers and support similar findings reported elsewhere (15). Together with overt mortality, many clinically normal birds had NE intestinal lesions. Among the 40 clinically normal birds randomly sampled from CP challenged, non-Pot-Pen–medicated pens on day 20, 18% demonstrated mild NE intestinal lesions and 43% demonstrated subclinical NE lesions. Even though 61% of randomly sacrificed Positive Control birds demonstrated NE lesions on day 20, only 7% of birds went on to die as a result of NE. These findings demonstrate that many broilers infected with CP can have pathological lesions consistent with NE and can survive with no obvious clinical signs.

This study demonstrated the impact of both mild and subclinical NE on bird growth and performance. Although clinically normal at the time of sacrifice on day 20, birds with mild (lesion score 3) and subclinical (lesion score 2) NE intestinal lesions had significantly reduced body weights. Reduction magnitudes in body weight appeared to correspond to NE lesion severity, and the degree of body weight reduction was the same whether all treatment groups were considered or only non-Pot-Pen–treated groups. The latter observation demonstrated the impacts of mild and subclinical NE on BW in isolation of any potential overall Pot-Pen growth

Table 4. Penicillin G potassium (Pot-Pen) treatment effect on BW among birds randomly sacrificed 4 days post-treatment initiation (day 20).[A]

	All birds		Birds with NE score 0		Birds with NE score 2	
Treatment group	No.	BW (kg)[B]	No.	BW (kg)[C]	No.	BW (kg)[D]
Negative control	40	0.605[a]	29	0.616[a]	8	0.544[a]
Positive control	40	0.576[a]	16	0.623[a]	17	0.561[a]
Pot-Pen 0.2 g/L	40	0.659[b]	39	0.656[a]	1	0.760[a]
Pot-Pen 0.4 g/L	40	0.661[b]	40	0.661[a]	0	NA

[A]Values within column with different lowercase superscript are significantly different, *P*-value < 0.05.
[B]Test for overall significance of treatment group, *P*-value $= 0.0002$.
[C]Test for overall significance of treatment group, *P*-value $= 0.0797$.
[D]Test for overall significance of treatment group, *P*-value $= 0.1172$.

Table 5. Effect of NE lesion score on BW among birds randomly sacrificed 4 days posttreatment initiation (day 20).[A]

	All birds			Untreated birds		
NE score	No.	BW (kg)[B]	Difference	No.	BW (kg)[C]	Difference
0	124	0.644[a]	Reference	45	0.619[a]	Reference
2	26	0.564[b]	−12%	25	0.556[b]	−10%
3	10	0.548[b]	−15%	10	0.548[b]	−13%

[A]Values within a column with different lowercase superscript are significantly different, *P*-value < 0.05.
[B]Test for overall significance of NE score, *P*-value < 0.001.
[C]Challenged and unchallenged control groups only (i.e., non-penicillin G potassium–treated birds); test for overall significance of NE score, *P*-value $= 0.007$.

promoter effect. Study findings also suggested that a low level of CP infection was present either in the hatchery that provided the chicks used in the study or in the research barn environment. Two birds of the negative control group died of NE before day 16 (i.e., before the challenged groups were inoculated with CP).

Together with successfully inducing a range of NE manifestations, this CP challenge study demonstrated the effectiveness of Pot-Pen administration in the management of CP-infected broilers and provided insights into Pot-Pen's mechanism of action. Stratification of the study results by treatment group and NE lesion score suggested that most, if not all, of the improvements noted above were related to the specific action of Pot-Pen on CP infection. Whereas NE-associated mortality was significantly reduced among Pot-Pen–treated birds, treatment had no significant effect on non-NE-associated mortality. The significant improvements in mean BW in the two groups treated with Pot-Pen might be interpreted as a nonspecific growth-promoting effect of Pot-Pen. However, when results of the randomly sacrificed birds were stratified by NE lesion score (0 and 2) mean BW did not differ among the four treatment groups. This suggested that mean BW was higher in groups treated with Pot-Pen because of Pot-Pen's specific antimicrobial action against CP.

At the time of writing, treatment of NE with penicillin G potassium constitutes extra-label use of the product. However, both the 0.2 and

Table 6. Body weight (BW), feed intake (FI), and feed conversion ratio (FCR) in broiler chickens challenged with *C. perfringens* on day 16 and treated with penicillin G potassium (Pot-Pen) in the drinking water days 17 to 22.[A]

		BW (kg)		FI (kg/day)	FCR (kg/kg)
	No. pens	Day 15[B]	Day 28[C]	Days 15–28[D]	Days 15–28[E]
Treatment group					
Negative control	8	0.367[a]	1.125[ab]	0.097[a]	1.70[a]
Positive control	8	0.366[a]	1.102[b]	0.097[a]	1.80[b]
Pot-Pen 0.2 g/L	8	0.349[a]	1.155[ac]	0.100[ab]	1.61[c]
Pot-Pen 0.4 g/L	8	0.362[a]	1.173[c]	0.102[b]	1.65[ac]
Gender					
Males	16	0.360[a]	1.166[a]	0.102[a]	1.679[a]
Females	16	0.362[a]	1.111[b]	0.096[b]	1.707[a]

[A]Values within treatment group or gender within column with different lowercase superscript are significantly different, *P*-value < 0.05.
[B]Test for overall significance of treatment group, *P*-value $= 0.0167$.
[C]Test for overall significance of treatment group, *P*-value $= 0.0064$.
[D]Test for overall significance of treatment group, *P*-value $= 0.0547$.
[E]Test for overall significance of treatment group, *P*-value < 0.0001.

0.4 g/L dosages of Pot-Pen were clearly effective in preventing NE-associated mortality and subclinical disease. The efficacy of various antimicrobials has been studied; however, recently published information exists on only a relatively small number. These include tylosin, bacitracin methylene disalicilate, and narasin (1,2,3,14). The ability to administer penicillin via the drinking water can result in increased drug intake in birds off-feed and provides flexibility in administration compared to in-feed administration of antimicrobials. In addition, penicillin is a relatively low-cost treatment option.

The data from the present investigation showed that the effect of Pot-Pen on mortality and growth parameters was specific, in that the positive effects of treatment with Pot-Pen appeared to be associated with the prevention and/or treatment of NE-associated lesions. As CP is a ubiquitous organism in broiler flocks (13), results of this study suggest that targeted treatment with aqueous Pot-Pen during the period of greatest susceptibility to NE offers a means of managing NE-associated mortality and subclinical disease.

REFERENCES

1. Brennan, J., R. Bagg, D. Barnum, J. Wilson, and P. Dick. Efficacy of narasin in the prevention of necrotic enteritis in broiler chickens. Avian Dis. 45:210–214. 2001.

2. Brennan, J., G. Moore, S. E. Poe, A. Zimmerman, G. Vessie, D. A. Barnum, and J. Wilson. Efficacy of in-feed tylosin phosphate for the treatment of necrotic enteritis in broiler chickens. Poult. Sci. 80:1451–1454. 2001.

3. Brennan, J., J. Skinner, D. A. Barnun, and J. Wilson. The efficacy of bacitracin methylene disalicylate when fed in combination with narasin in the management of necrotic enteritis in broiler chickens. Poult. Sci. 82:360–363. 2003.

4. Casewell, M., C. Friis, E. Marco, P. McMullin, and I. Phillips. The European ban on growth-promoting antibiotics and emerging consequences for human and animal health. J. Antimicrob. Chemother. 52:159–161. 2003.

5. Kaldhusdal, M., and M. Hofshagen. Barley inclusion and avoparcin supplementation in broiler diets. 2. Clinical, pathological, and bacteriological findings in a mild form of necrotic enteritis. Poult. Sci. 71:1145–1153. 1992.

6. Lovland, A., and M. Kaldhusdal. Severely impaired production performance in broiler flocks with high incidence of *Clostridium perfringens*–associated hepatitis. Avian Pathol. 30:73–81. 2001.

7. National Research Council. Nutrient requirements of poultry, 9th rev. ed. National Academies Press, Washington, D.C. 1994.

8. Parish, W. E. Necrotic enteritis in the fowl (*Gallus gallus domesticus*). I. Histopathology of the disease and isolation of a strain of Clostridium welchii. J. Comp. Pathol. 71:377–393. 1961.

9. Prescott, J. F., R. Sivendra, and D. A. Barnum. The use of bacitracin in the prevention and treatment of experimentally-induced necrotic enteritis in the chicken. Can. Vet. J. 19:181–183. 1978.

10. Skinner, J., and J. Brennan. Efficacy of bacitracin methylene disalicylate fed in combination with naracin for prevention of necrotic enteritis. Poult. Sci. 78(Suppl. 1):129. 1999.

11. Stutz, M. W., S. I. Johnson, and F. R. Judith. Effects of diet, bacitracin, and body weight restrictions on the intestine of broiler chicks. Poultry Sci. 62:1626–1632. 1983.

12. Van Immerseal, F., J. De Buck, F. Pasmans, G. Huyghebaert, F. Haesebrouck, and R. Ducatelle. *Clostridium perfringens* in poultry: an emerging threat for animal and public health. Avian Pathol. 33:537–549. 2004.

13. Wages, D. P., and K. Opengart. Necrotic enteritis. In: Diseases of poultry, 11th ed. Y. M. Saif, H. J. Barnes, J. R. Glission, A. M. Fadly, L. R. McDougald, and D. E. Swayne, eds. Iowa State University Press, Ames, Iowa. pp. 781–785. 2003.

14. Wilson, J. Efficacy of tylosin tartrate administered in drinking water for the treatment of necrotic enteritis in broiler chickens. In: Elanco Global Enteritidis Symposium, Cambridge, England. pp. 3–17. July 9–11, 2002.

15. Wilson, J., G. Tice, M. L. Brash, and S. St. Hilaire. Manifestations of Clostridium perfringens and related bacterial enteritides in broiler chickens. World's Poult. Sci. J. 61:435–449. 2005.

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