Research Note

Efficacy of low-dose tylvalosin for the control of clostridiosis in broilers and its effect on productive parameters[1]

C. Garcés-Narro,* J. I. Barragán,* M. D. Soler,* M. Mateos,* M. C. López-Mendoza,* and J. Homedes†[2]

*Instituto de Ciencias Biomédicas, Facultad de Veterinaria, Universidad CEU Cardenal Herrera, Valencia, Spain; and †Esteve Veterinaria, Laboratorios Dr, Esteve S.A., Barcelona, Spain

ABSTRACT The study was carried out under field conditions in a commercial farm, and 1,440 as-hatched Ross-308 broilers were included. Broilers were randomly distributed into 24 experimental 4-m^2 pens (60 broilers/pen). Pens were randomized to the 3 treatment groups: a) tylvalosin 10 mg/kg of live BW during 2 d, b) positive control (tylosin during 2 d), and c) negative control (no treatment). The drugs were provided in the water supply. Mortality, individual BW, and feed intake were assessed. *Clostridium* presence was assessed in fecal and cecal samples, coccidian oocyst counts were assessed in fecal samples, and bacterial diversity was assessed in ileal content. Live BW at 42 d old was significantly better in the tylvalosin group than in tylosin and no-treatment groups, with tylvalosin-treated broilers reaching 80 to 100 g higher final live weight. Average daily gain results mirrored BW findings. The improvement of feed conversion rate with tylvalosin amounted to 0.13 and to 0.10 versus tylosin and no-treatment, respectively, with mortality being similar in all groups. Significantly reduced sulfite-reducing *Clostridium* and *Clostridium perfringens* counts in tylvalosin and tylosin groups versus the no-treatment group were observed in cecum content samples. In conclusion, according to the present study results, tylvalosin, at doses substantially lower than registered for poultry in Europe, has proven effective in controlling the colonization of the cecum by *Clostridium* ssp. in broilers, improving some productive performances.

Key words: tylvalosin, tylosin, *Clostridium*, broiler

2013 Poultry Science 92:975–978
http://dx.doi.org/10.3382/ps.2012-02750

INTRODUCTION

Enteric diseases have an increasingly severe impact on the broiler industry. They cause high economic losses due to poor performance, increased mortality rates, and increased medication costs, as well as reduced welfare and an increased risk of contamination of products for human consumption (Kaldhusdal, 2000). The global economic loss for the international poultry industry due to necrotic enteritis caused by *Clostridium perfringens* in broiler farms is estimated to be over $2 billion per year (Kaldhusdal, 2000). In addition, a recent analysis by Skinner et al. (2010) suggests that subclinical (mild) necrotic enteritis could cause a 12% reduction in BW and a 10.9% increase in feed conversion rate (**FCR**) in broiler farms. Several pathogens have been incriminated with *C. perfringens*-induced necrotic enteritis and related subclinical disease, having become economically significant problems (Timbermont et al., 2011). The acute clinical or subclinical disorder usually occurs at about 4 wk after hatching (Dahiya, 2006). Decreasing clostridia colonization has been shown to be beneficial for minimizing the onset of necrotic enteritis in birds (Collier et al., 2003), and current therapy to reduce clostridia in broilers is mainly based on tylosin, a macrolide antibiotic with a broad spectrum covering most *C. perfringens* strains (Martel et al., 2004). Tylosin has proved effective to reduce the concentration of *C. perfringens* (Collier et al., 2003) and to treat clinical outbreaks (Brennan et al., 2001).

Tylvalosin (INN; Aivlosin, Laboratorios Esteve, Barcelona, Spain; previously known as acetylisovaleryltylosin), also a macrolide, is a highly active agent against a wide range of bacteria (mainly gram-positive and mycoplasma, but also some gram-negative microbes) in many different veterinary species (EMA, 2004). In poultry, oral tylvalosin is rapidly absorbed and widely distributed to tissues, reaching very high levels in intestinal phagocytic and epithelial cells. Its main metabolite, 3-acetyltylosine is also microbiologically active. Terminal half-life is 1 to 1.45 h, and levels in intestinal

Received September 5, 2012.
Accepted December 23, 2012.
[1]Preliminary results of this paper were presented to the XLVII Symposium Científico de Avicultura, Barcelona 2010.
[2]Corresponding author: jhomedes@esteve.es

mucosa remain high 6 h after administration (EMA, 2004).

In vitro studies have clearly shown tylvalosin to enter and accumulate inside several cell types, including gut epithelial cells, whereas tylosin penetration in all cell types is relatively poor (Stuart et al., 2007). Such findings could mean a significantly improved effect of tylvalosin versus tylosin as a treatment for intestinal infections.

Anecdotal experiences in clinical practice suggest that lower tylvalosin doses than used against *Mycoplasma gallisepticum* (Stipkovits and Mockett, 2007) could be useful to control intestinal infections involving *C. perfringens*, a significant pathogen.

Therefore, our objective was to evaluate the efficacy of treatment with low doses of tylvalosin compared with standard therapy tylosin in clostridiosis control and improve production results in growing broiler chickens in a field study.

MATERIALS AND METHODS

One thousand four hundred forty 1-d-old as-hatched Ross 308 broilers were randomly assigned to 1 of 3 treatments (8 replicate pens per treatment; 60 birds per pen). To mimic real-world growing conditions according to current production standards, so that representative data could be obtained, the pens were uniformly distributed within a commercial farm facility, containing approximately 35,000 broilers of the same age. All the pens were covered with 10 cm of clean rice hulls as the rest of the farm.

Birds were provided ad libitum access to water and a standard wheat-soybean based meal diet that met or exceeded NRC (1994) nutrient requirements.

Pens were randomized to the following treatment groups: a) tylvalosin (Aivlosin, Laboratorios Esteve, Barcelona, Spain), 50 ppm (equivalent to 10 mg/kg of live BW) during 2 d (d 21, d 22); b) positive control (tylosin, Tailan soluble, Elanco Valquímica S.A., Madrid, Spain), 150 ppm (equivalent to 40 mg/kg of live BW) during 2 d (d 21, d 22); and c) negative control (no treatment). Drugs were administered via the water supply.

Individual BW and feed intake were assessed on d 1, 21, 28, and 42 (end of growing period). Mortality was checked daily throughout the study. The following production indexes were determined: ADG, FCR, and mortality.

Oocyst counts were assessed in fecal samples on d 21 and 28. Samples were obtained from all pens and pooled for groups of 4 pens receiving the same treatment, so that 6 pooled samples (2 samples per therapy group) were available for analysis.

Additionally, cecum and ileum contents were taken on d 28 from one broiler per pen to assess *Clostridium* presence and bacterial diversity respectively. For sample collection, birds were euthanized by an overdose (140 mg/kg of BW) of intravenous sodium pentobarbital (Dolethal, Vetoquinol, Madrid, Spain) and immediately dissected. Cecum and ileum were removed and an aliquot of cecal or ileal digesta was squeezed out and immediately transferred to a sterile vial. The contents corresponding to 2 broilers of different pens but receiving the same treatment were pooled. At the end, 12 pooled cecal samples (4 samples per therapy group) and 12 pooled ileal samples (4 samples per therapy group) were frozen at −20°C and available for analysis.

To assess the animal welfare, this trial was carried out according to the guidelines of the Federation of Animal Science Societies (2010). Total coccidian oocyst (*Eimeria* spp.) counts were carried out according to Hodgson (1970).

Clostridium perfringens counts were obtained by inoculation in tryptone sulfite neomycin agar (TSN-agar) at 42°C during 48 h in anaerobic conditions (Dafwang et al., 1987). Clostridia sulfite-reducing counts were obtained by incubation in sulfite polymyxin sulfadiazine agar (SPS-agar) at 37°C during 48 h in microaerophilic conditions (5% CO_2; Angelotti et al., 1962). Counts were expressed as log cfu/g of cecal or intestinal contents.

To determinate bacterial diversity, 16S rRNA gene-based terminal-RFLP was performed. Thus, bacterial DNA was extracted from the ileum content. Ileum samples were first defrosted. Extraction of DNA was conducted using a QIAamp DNA stool mini kit (Qiagen, Hilden, Germany) following the instructions of the manufacturer. After extraction, bacterial rRNA gene sequences were amplified by PCR using universal 16S primers 27F and 907R. The forward primer was 5′ labeled with 6-carboxyfluorescein (Lane, 1991). The PCR reactions were performed in 100 μL of final volume, containing 100 to 200 ng of DNA, 50 m*M* KCl, 10 m*M* Tris-HCl, 80 μ*M* of each dNTP, 1 μ*M* of each primer, 2 m*M* $MgCl_2$, and 1 U of DNA polymerase (Netzyme, Molecular Netline Bioproducts, N.E.E.D, SL, Valencia, Spain). The reaction mixtures were incubated in a thermalcycler (Techne TC-512) for 33 cycles consisting of 45 s at 95°C, 1 min at 48°C, and 1 min at 72°C. Approximately 200 ng of PCR products were digested with 2 U of the restriction enzyme *Hha*I (MBI Fermentans, Vilnius, Lithuania). The length of fluorescently labeled terminal restriction fragments (**T-RF**) were precisely measured after separation by capillary electrophoresis by using an automated DNA sequencer (model 373A; Applied Biosystems, Warrington, UK), and the fragment sizes were estimated using the Local Southern method of the GeneScan 3.7 software (Applied Biosystems). The percentage of each peak area compared with the total area (relative area) was calculated as described by Matsumoto and Benno (2007). Bacterial diversity was calculated according to the number of peaks obtained from each sample.

For statistics, data were analyzed as a GLM procedure of SPSS (SPSS Inc., 2007). A completely randomized model was used for performance parameters with pen as the experimental unit. For microbiology, a

Table 1. Production indexes

Treatment group	d 1 to 21[1]	d 21 to 28[1]	d 28 to 42[1]	d 21 to 42 (final period)[2]	d 1 to 42 (whole study)
Live BW (mean ± SE, g)					
No treatment	44.77 ± 0.40[a]	972.50 ± 18.94[a]	1,556.61 ± 9.19[a]	2,842.81 ± 17.02[a]	—
Tylosin	43.94 ± 0.40[a]	995.00 ± 18.94[a]	1,575.75 ± 9.15[a]	2,819.84 ± 17.06[a]	—
Tylvalosin	44.11 ± 0.40[a]	1,001.25 ± 18.94[a]	1,611.29 ± 9.13[b]	2,921.40 ± 16.72[b]	—
ADG (mean ± SE, g/d)					
No treatment	44.18 ± 0.90[a]	83.44 ± 3.26[a]	91.79 ± 1.56[a]	89.01 ± 1.44[a]	66.66 ± 0.70[a]
Tylosin	45.29 ± 0.90[a]	83.00 ± 3.26[a]	89.07 ± 1.56[a]	87.05 ± 1.44[a]	66.21 ± 0.70[a]
Tylvalosin	45.58 ± 0.90[a]	87.17 ± 3.26[a]	93.49 ± 1.56[a]	91.39 ± 1.44[b]	68.53 ± 0.70[b]
Feed conversion rate (mean ± SE)					
No treatment	1.573 ± 0.034[a]	1.691 ± 0.056[a]	2.175 ± 0.038[ab]	2.018 ± 0.028[a]	1.926 ± 0.026[a]
Tylosin	1.535 ± 0.034[a]	1.737 ± 0.056[a]	2.252 ± 0.038[a]	2.085 ± 0.028[a]	1.962 ± 0.026[a]
Tylvalosin	1.535 ± 0.034[a]	1.642 ± 0.056[a]	2.062 ± 0.038[b]	1.924 ± 0.028[b]	1.826 ± 0.026[b]

[a,b]Different superscripts in cells in the same column for a specific parameter indicate $P < 0.05$.

[1]Body weight the first day of the period.

[2]Body weight the last day of the period.

nested model was used with pens nested within treatments and birds as the subsamples. Treatment means were separated using the least squares means option of SPSS. Differences among treatment means were tested using Scheffe's multiple comparison tests, and statistical significance was declared at $P < 0.05$. All microbiological concentrations were subjected to $\log_{10}$ transformation before statistical analysis.

Mortality rates were compared using a chi-squared test. All analyses were performed with SPSS statistical software, and a P-value <0.05 was considered statistically significant.

RESULTS

Production rates were significantly better in tylvalosin group than in tylosin and no-treatment groups. Body weight changes during the study are shown in Table 1. Significant differences were found on d 28, and a maximal effect was observed on d 42, with a final live BW of 80 to 100 g higher in tylvalosin-treated broilers.

The ADG results mirrored BW findings. Differences did not reach statistical significance for either periods, from 21 to 28 d and from d 28 to 42; however, they were significant in the interval from d 21 to 42 as well as for the whole study period, due to a consistently increasing effect throughout the trial (Table 1). Feed intake did not show differences between treatment groups. As a consequence, FCR for the d 21 to 42 interval was significantly lower in tylvalosin-treated broilers. For the whole study period, FCR improvement with tylvalosin amounted to 0.13 versus tylosin and to 0.10 versus no-treatment (Table 1). Mortality was similar in all groups, and no treatment effect was observed on survival.

Microbiological findings did not show statistically significant differences between treatment groups in coccidial oocyst counts on d 21 (before treatment) and d 28 (after treatment) in fecal samples.

Samples obtained from cecum content in 28-d broilers showed significantly reduced sulfite-reducing *Clostridium* and *C. perfringens* counts in tylvalosin and tylosin groups versus the negative control (Table 2). Although bacterial diversity in ileal samples was similar among the different groups, microbial profiles were quite different (Table 3). Observing the distribution of bacterial populations based on their characterization by terminal-RFLP, we can see changes in microbial profiles, with a greater presence of about 400 T-RF in samples from the no-treatment group. The tylvalosin group has most bacteria with low T-RF, and the tylosin-treated group has a great number of bacteria in the environment of 150 to 250 T-RF. This suggests that the treatments do not reduce the number of bacteria species, but modify the profile of the bacterial population.

DISCUSSION

As suggested by Williams (2005), coccidiosis has an effect on *Clostridium* counts. But in this trial, the same level of oocysts has been detected among the treatments, so no effect of coccidiosis on the *Clostridium* counts has been registered. The use of tylosin reduces the *Clostridium* counts in the cecum, according to the results of Collier et al. (2003) and Martel et al. (2004). The use of tylvalosin also reduces the *Clostridium* counts, as EMA (2004) suggested. In fact, the effect on cecal *Clostridium* is not different between the 2 macrolides, but both are significantly different in comparison with the negative control.

Table 2. Sulfite-reducing *Clostridium* and *Clostridium perfringens* counts in cecum in different treatment groups on d 28 (mean ± SE)

Treatment	Sulfite-reducing *Clostridium* (log cfu/g)	*Clostridium perfringens* (log cfu/g)
No treatment	6.91 ± 0.46[a]	5.83 ± 0.73[a]
Tylosin	3.37 ± 0.46[b]	2.54 ± 0.73[b]
Tylvalosin	3.98 ± 0.46[b]	1.92 ± 0.73[b]

[a,b]Different superscripts in cells in the same column for a specific day indicate $P < 0.05$.

Table 3. Restriction fragments (T-RF)[1] obtained from ileal bacterial populations on d 28

Fragment sizes (bp)	No treatment (% of T-RF)	Tylosin (% of T-RF)	Tylvalosin (% of T-RF)
0 to 100	5.8	9.4	38.9
101 to 150	3.8	15.6	9.7
151 to 200	3.8	29.7	29.2
201 to 250	15.4	14.1	15.3
251 to 300	0.0	3.1	1.4
301 to 350	0.0	0.0	0.0
351 to 400	61.5	25.0	2.8
401 to 500	1.9	1.6	2.8
501 to 600	7.7	1.6	0.0

[1]After digestion with the restriction enzyme *Hha*I.

The effect of reduced proliferation of *Clostridium* in the cecum can help having a lower incidence of enteritis (Collier et al., 2003), improved gut health, and better nutrient absorption (Dahiya, 2006). This is usually associated to a better performance in animals. However, in this trial, the performance of animals with tylvalosin, such as BW, ADG, and FCR, is higher than those of the other groups. The difference in bacterial diversity could be responsible for the different capability of nutrient absorption and, hence, the assimilation of nutrients, as the bacterial population influences a variety of immunological, physiological, nutritional, and protective processes of the gut (Dibner and Richards, 2005). In this sense, the bacterial profile of the chicken gut is quite different. So, it is possible that the varying performance of broilers is produced due to this different bacterial diversity.

According to the present study results, tylvalosin, at doses substantially lower than registered for poultry in Europe, has proved effective to control *Clostridium* proliferation in broilers. Clostridial presence in cecum samples was clearly reduced, in a similar extent to the one achieved by tylosin, but the bacterial profile has been modified in a different sense among the treatments, and production indexes such as ADG and FCR were significantly improved versus tylosin and control groups.

REFERENCES

Angelotti, R., H. E. Hall, M. Foter, and K. H. Lewis. 1962. Quantitation of *Clostridium perfringens* in foods. Appl. Microbiol. 10:193–199.

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

Collier, C. T., J. D. van der Klis, B. Deplancke, D. B. Anderson, and H. R. Gaskins. 2003. Effects of tylosin on bacterial mucolysis, *Clostridium perfringens* colonization, and intestinal barrier function in a chick model of necrotic enteritis. Antimicrob. Agents Chemother. 47:3311–3317.

Dafwang, I. I., S. C. Ricke, D. M. Schaefer, P. G. Brotz, M. L. Sunde, and D. J. Pringle. 1987. Evaluation of some commercial media for the cultivation and enumeration of *Clostridium perfringens* from the chick intestine. Poult. Sci. 66:652–658.

Dahiya, J. P. 2006. Potential strategies for controlling necrotic enteritis in broiler chickens in post-antibiotic era. Anim. Feed Sci. Technol. 129:60–88.

Dibner, J. J., and J. D. Richards. 2005. Antobiotic growth promoters in agriculture: History and mode of action. Poult. Sci. 84:634–643.

EMA. 2004. Aivlosin Summary of Product Characteristics. European Medicines Agency. Accessed Dec. 2012. http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/veterinary/000083/WC500061063.pdf.

Federation of Animal Science Societies. 2010. Guide for the Care and Use of Agricultural Animals in Research and Teaching. 3rd ed. Fed. Anim. Sci. Soc., Champaign, IL.

Hodgson, J. N. 1970. Coccidiosis: Oocyst counting technique for coccidiostat evaluation. Exp. Parasitol. 28:99–102.

Kaldhusdal, M. 2000. The economic impact of *Clostridium perfringens* is greater than anticipated. World Poult. 16:50–51.

Lane, D. J. 1991. 16S/23S rRNA sequencing. E. Stackebrandt and M. Goodfellow, ed. Pages 115–175 in Nucleic Acid Techniques in Bacterial Systematics. 1st ed. John Wiley & Sons, Chichester, NY.

Martel, A., L. A. Devriese, K. Cauwerts, K. De Gussem, A. Decostere, and F. Haesebrouck. 2004. Susceptibility of *Clostridium perfringens* strains from broiler chickens to antibiotics and anticoccidials. Avian Pathol. 33:3–7.

Matsumoto, M., and Y. Benno. 2007. The relationship between microbiota and polyamine concentration in the human intestine: A pilot study. Microbiol. Immunol. 51:25–35.

NRC. 1994. Nutrient Requirements of Poultry. 9th rev. ed. Natl. Acad. Press, Washington, DC.

Skinner, J. T., S. Bauer, V. Young, G. Pauling, and J. Wilson. 2010. An economic analysis of the impact of subclinical (mild) necrotic enteritis in broiler chickens. Avian Dis. 54:1237–1240.

SPSS Inc. 2007. One-way ANOVA. Pages 314–319 in SPSS Statistics Base 17.0. User's Guide. SPSS Inc., Chicago, IL.

Stipkovits, L., and A. P. A. Mockett. 2007. Aivlosin granules for oral solution used for the treatment and prevention of mycoplasmosis due to *Mycoplasma gallisepticum*. Abstracts of the 15th Congress of the World Veterinary Poultry Association, Beijing, China: 537.

Stuart, A. D., T. D. K. Brown, G. Imrie, J. B. Tasker, and A. P. A. Mockett. 2007. Intra-cellular accumulation and trans-epithelial transport of aivlosin, tylosin and tilmicosin. The Pig Journal 60:26–35.

Timbermont, L., F. Haesebrouck, R. Ducatelle, and F. Van Immerseel. 2011. Necrotic enteritis in broilers: An updated review on the pathogenesis. Avian Pathol. 40:341–347.

Williams, R. B. 2005. Intercurrent coccidiosis and necrotic enteritis of chickens: Rational, integrated disease management by maintenance of gut integrity. Avian Pathol. 34:159–180.