**In vivo anticoccidial activity of quinfamide in broilers: a preliminary report**

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**ABSTRACT**

The aim of this trial was to evaluate the anticoccidial efficacy of quinfamide in broilers. Three different treatments were implemented over a 49-day period as follows: quinfamide; quinfamide plus carbopol and decoquinate, all prepared as small pellets and mixed with feed at a final dose of either active principle of 30 ppm. Parameters measured were: weight gain, number of oocysts shedding per gram of litter and degree of gross lesions caused by coccidia. Body weight gain was statistically greater for quinfamide and quinfamide-carbopol groups in comparison to other groups. However, only the quinfamide-carbopol group showed similar efficacy in the oocyst counts as compared to the decoquinate group. Statistically significant differences were observed when intestinal lesions score were compared and the less affected group was quinfamide-carbopol. Based on these results, it is concluded that quinfamide possesses a low anticoccidial activity. However, this is noticeably improved when it is prepared as pellets with carbopol. The adhesion of carbopol to intestinal mucosa may influence residence time of quinfamide in the gastrointestinal tract, thus enhancing efficacy.

**Introduction**

Coccidiosis, a disease caused by the coccidian protozoan *Eimeria*, is one of the most common and economically important diseases of chicken and has a major economic impact on the global poultry industry (Williams 1999; Tenter et al. 2002; Chapman et al. 2013). All *Eimeria* species tend to develop drug resistance to most anticoccidial drugs. Consequently, the search for new drugs is always welcomed (Guo et al. 2007; Fei et al. 2013).

Quinfamide, a dichloroacetyl derivative of quinolinol, is widely used to treat intestinal *Entamoeba* infections in humans at a dose of 300 mg/adult (Padilla et al. 1995; Padilla et al. 1998; Romero et al. 2005). Quinfamide has low bioavailability and acts at the luminal level, immobilising trophozoites of *Entamoeba histolytica* causing somehow their destruction and their elimination with faeces (Morales et al. 2000; Romero et al. 2005).

As more becomes known about parasite biochemical pathways, new speculations regarding a similar antiparasitic activity of antiprotozoan drugs arise. For example, virulence of *Entamoeba histolytica* relies on several factors, such as the presence of an N-acetyl-D-galactosamjne (Gal/GalNAc), a surface lectin that binds galactose and N-acetylgalactosamine. Invasion of the host cells occurs when trophozoites of *E. histolytica* adhere and penetrate the colonic tissue through a galactose-binding lectin present on the surface of the target cells (Bernal et al. 2006; Baxt et al. 2008).

Furthermore, it was recently reported that an apicomplexan parasite, *Toxoplasma gondii*, invades the host cell and modulates immunity through a Gal-specific lectin activity that is related to the attachment of the protozoan to the mammalian cell, and the carbohydrate profile of this microneme protein complex, namely TgMIC4, resembles that of the galectins family as a reminiscent of the Gal/GalNAc-binding surface protein from *E. histolytica* (Marchant et al. 2012). As *Toxoplasma* and *Entamoeba* share molecular similarities, potential targets may also be shared by coccidian. Hence the previous investigation of the activity of quinfamide against *Eimeria* (Aquino et al. 2013), where good results were obtained. Amoebas...
and coccidia do not belong to the same taxonomic phylum. Yet, interestingly, the anticoccidial effect of quinfamide on *Eimeria*-infected sheep was reported as moderate and enhanced by chitosan (Aquino et al. 2013). This effect due to the vehicle anticipated that efficacy of quinfamide could be affected by formulation. For this reason, the aim of this trial was to evaluate the effectiveness of quinfamide compared with that of quinfamide formulated with a polymer matrix-vehicle (carbopol) and utilising decoquinate as a gold standard in the treatment of avian coccidiosis caused by *Eimeria maxima*, *E. tenella* and *E. acervulina*.

**Materials and methods**

**Experimental compounds**

Quinfamide (kindly donated by *Laboratorios Aranda S.A. de C.V., México*) was pelleted with or without carbopol as suggested in Patent: MX/a/2012/013222 and PCT/MX2013/000137. This latter carrier was added because it has mucoadhesive properties and slows down the transit time of quinfamide in the gut (Qi et al. 2007; Paker & Neau 2009; Singh et al. 2014; Surassmo et al. 2015). Feed was first prepared without the experimental compounds. The necessary amount of pellets to reach 30 ppm of quinfamide or decoquinate was added to the daily required quantity of food and then homogenised using a small conical mixer, for no more than 3 min.

**Experimental design of the floor-pen trial**

The floor-pen trial was performed according to the guidelines of the World Association for the Advancement of Veterinary Parasitology (WAAVP), for the evaluation of anticoccidial efficacy in chickens and turkeys (Holdsworth et al. 2004). Two-week old, coccidian-free Cobb-500 seeder chickens were prepared as previously described (Mathis et al. 2004). Briefly, all seeder chickens were inoculated with a mixture containing $5 \times 10^4$ sporulated oocysts of *Eimeria acervulina*, $2 \times 10^4$ sporulated oocysts of *E. maxima* and $5 \times 10^3$ sporulated oocysts of *E. tenella* (Cornelissen et al. 2009). Ten seeder animals were placed into each pen, except in the uninfected-group pens, on days 4, 5 and 6 after their inoculation. Water was added daily to each pen to maintain litter moisture to increase uniform and rapid sporulation of shed oocysts. Litter and fresh droppings were randomly sampled daily over the course of the study to determine *Eimeria* exposure by estimating the mean amount of oocysts per pen, which resulted in 420,000 oocysts of *E. acervulina*, 70,000 oocysts of *E. maxima*, and 420,000 oocysts of *E. tenella* per pen.

For this study, sample size was calculated using the GPower programme for repeated measures (Faul et al., 2007), with a statistical significance ≤ 0.05 and 0.80 power for the test (effect size = 0.4). Hence, three hundred one-day old broiler chickens were weighed and randomly allotted to one of three experimental treatments and two control groups in separated pens (20 chickens/replicate and 3 replicates per group), with seven weekly measurements. The starter feeding programme was carried out from days 1 to 21, grower from days 22 to 35, and finishing from days 36–49. Diets compositions are shown in Table 1 (NRC 1994).

In order to determine quinfamide anticoccidial efficacy in broilers, three different treatments for experimentally infected broilers were assayed throughout the duration of this trial, i.e. from day one to 49. Treated groups were designated as: Q (quinamide-treated), QC (quinamide–carbopol-treated), and D (decoquinate-treated). Two control groups were also included in this study: an uninfected and untreated control group (UU) and an infected and untreated group (IU). The UU group was set aside in a clean separated space. Treatments were provided as an in-feed mix, using the following inclusion rates: 30 ppm of quinfamide at 0.1% (group Q); 30 ppm of quinfamide at 0.1% mixed with carbopol at 0.1% (group QC). The third experimental group received 30 ppm of decoquinate prepared at 0.1% (group D) and was considered the golden standard. In all of these 3 groups,
medication was prepared as pellets as described in Patent: MX/a/2012/013222 and PCT/MX2013/000137.

Body weight gain

Body weight gain was measured and regarded as the end point because is a parameter directly linked to anticoccidial activity and measurements were carried out individually on days 1, 7, 14, 21, 28, 35, 42 and 49.

Oocyst faecal output quantification

On days 1, 7, 14, 21, 28, 35, 42 and 49, litter samples were collected from each pen in order to quantify the number of oocysts per gram of litter. Oocysts analysis were performed according to the guidelines of the World Association for the Advancement of Veterinary Parasitology (WAAVP), for the evaluation of anticoccidial efficacy in chickens and turkeys (Holdsworth et al. 2004). Composite litter samples were collected from the front, middle and back of each pen (Long & Rowell 1975; Conway & McKenzie 2007). Oocysts counts were expressed as oocyst per gram of litter and were determined for each pen obtaining three repetitions per group. The McMaster technique was carried out to smooth variation in such a way that the overall variance in oocyst counts approaches an irreducible minimum for a particular sample size and degree of aggregation (Long & Rowell 1975; Mathis et al. 2004; Morgan et al. 2005).

Mortality rate

The mortality rate was determined using the following formula: percent mortality = \( \frac{(\text{total number of dead birds in the group/initial number of birds in group})\times 100}{\text{after being exposed to the infectious agent in the absence or presence of the treatments.}} \)

Lesion scoring

On days 21, 35 and 49, four broiler chickens were randomly selected from each pen and were humanely euthanized, as laid out by the bioethical committee of the institution where the study was carried out (UNAM), based on international normativity and Mexican regulations NOM-062-ZOO-1999 (SAGARPA 1999). Numerical ranking of gross lesions was developed, setting a score of 0 (no lesions), 1 (mild lesions), 2 (moderate lesions), 3 (severe lesions) and 4 (extremely severe lesions) (Johnson & Reid 1970). Coccidial intestinal lesions were scored in the upper, middle, and caecal regions that correspond to the natural predilection site for *E. acervulina, E. maxima* and *E. tenella*, respectively.

Statistical analysis

Statistical analysis was carried out based on a Generalised Linear Model (GLM). Thus body weight gain was linked to a Gaussian distribution, oocyst per gram of litter was also linked to a Poisson distribution and lesion score was linked to logit distribution. All models were analysed by Maximum likelihood (Liang & Zeger 1986). Body weight gain and oocyst per gram of litter means were compared by the Bonferroni method. All significant differences were based on \( p < .05 \). The analyses were performed with package software IBM SPSS® Statistics 20.

Results

Body weight gain

Table 2 shows the mean±SE body weight gains recorded weekly from day 1 to day 49 and statistically significant differences in this parameter are highlighted. At the end of this trial, body weight gain from

| Table 1. Diet composition and nutritional values for starting (1 to 21 days), growing (22 to 35 days) and finishing (36 to 49 days) broilers. |
|---|---|---|
| Ingredient, kg | Starting | Growing | Finishing |
| Corn | 590.50 | 618.65 | 663.00 |
| Soybean meal | 344.80 | 308.90 | 269.70 |
| Soybean oil | 23.80 | 33.50 | 32.10 |
| Salt | 2.40 | 2.30 | 2.10 |
| Calcium limestone | 9.50 | 8.80 | 6.50 |
| Dicalcium phosphate | 18.00 | 16.70 | 15.20 |
| DL-methionine | 1.75 | 1.75 | 1.65 |
| L-lysine | 2.15 | 2.30 | 2.75 |
| Vitaminsa | 2.50 | 2.50 | 2.5 |
| Mineralsb | 1 | 1 | 1 |
| Sodium bicarbonate | 3.60 | 3.60 | 3.50 |
| Total | 1000 | 1000 | 1000 |
| Calculated analysis, g/kg | | | |
| Crude protein | 207.90 | 194.10 | 183.00 |
| Calcium | 8.80 | 8.20 | 7.60 |
| Available phosphorus | 4.40 | 4.10 | 3.80 |
| Methionine | 4.90 | 4.80 | 4.50 |
| Sulfur amino acids | 8.20 | 7.90 | 7.40 |
| Lysine | 12.70 | 11.90 | 11.20 |
| Potassium | 8.00 | 7.40 | 6.80 |
| Sodium | 2.20 | 2.10 | 2.00 |
| Chlorine | 1.90 | 1.80 | 1.70 |
| Linoleic acid | 26.00 | 31.50 | 31.30 |
| Metabolizable energy, MJ/Kg | 12.56 | 12.97 | 13.13 |

<sup>a</sup>Amount/kg: Retinol 0.9 g, cholecalciferol 0.019 g, d-alpha-tocopherol 0.004 g, phylloquinone 1.0 g, riboflavin 4.0 g, cyanocobalamin 0.060 g, pyridoxine 3.0 g, calcium pantothenate 13.0 g, niacin 25 g, biotin 0.063 g, choline chloride 250 g.

<sup>b</sup>Amount/kg: selenium 0.2 g, cobalt 0.1 g, iodine 0.3 g, copper 10 g, zinc 50 g, iron 100 g, manganese 100 g.
the quinamide (Q) and quinamide + carbopol (QC) groups were statistically higher than the corresponding values for decoquinate (D) and the infected-untreated (IU) groups. Also, statistically significant differences were observed regarding body weight gain for Q and QC. Nevertheless, no difference was detected between the IU and D groups. On the other hand, chickens from the UU group had a similar final body weight gain as Q and QC groups. Groups Q and QC showed an increased mean body weight gain of 186 g and 254 g, respectively as compared to the D group.

Oocyst output reduction

Seven days after the experimental infection of litter, oocysts presence was confirmed in each pen of the infected groups. Starting from day 21 until the end of the study, the group that received quinamide + carbopol (QC) shed significantly (p < .05) less oocysts than infected groups treated with quinamide and infected birds that remained untreated. Uninfected and untreated (UU) animals did not shed oocysts. Oocyst excretion was similar in broilers that consumed decoquinate (D) and the combination of quinamide + carbopol (QC). These results are summarised in Table 3.

Mortality rate

One bird died due to coccidial infection on day 5 and two on day 16 in the IU group (initial n = 60), as confirmed by necropsy findings. Results reveal that both quinamide, quinamide + carbopol and decoquinate completely protected the medicated-infected groups against a challenge which killed 5% of the non-medicated-infected controls.

Lesion scoring

The severity of lesions was assessed by counting the number of times that a lesion score was observed in the different intestinal regions.

As far as lesions scores in the D group is concerned, the coccidial challenge induced first-degree lesion scores. Neither thickening of caecal walls nor caecal narrowing was observed. Nevertheless, the jejunal walls were erythematous and showed few petechial haemorrhages. In the Q group, the most frequent lesions did not reach a second-degree score, yet caecal walls were thickened. Moreover, mucus was observed in the intestinal walls. The QC group showed most of the first-degree lesions confined to the caecal region, where walls were thickened, and lumen size was reduced as compared to UU animals. Caeca of the QC birds were distended with soft, pasty faeces. On the other hand, caeca of IU animals were small, firm and walls were thickened, so lumen size was minimal or absent. As expected, UU birds showed no gross lesions (Table 4). Statistically significant differences in lesion scores frequencies were detected among all the groups (p = .001).

Table 2. Anticoccidial efficacy of three treatments based on broiler body weight gain (g) (Lmean ± SE, n = 60 per group).

| Group | Day | UU | IU | Q | QC | D |
|-------|-----|----|----|---|----|---|
| 1     | 420 ± 3.4 a | 410 ± 3.5 a | 410 ± 3.6 c | 420 ± 3.7 c | 420 ± 3.8 c |
| 7     | 240 ± 0.7 b,b | 247 ± 2.0 b | 237 ± 1.7 a | 247 ± 1.6 a | 242 ± 2.4 a,b |
| 14    | 355 ± 13.6 b,b | 356 ± 2.6 a | 377 ± 2.6 b | 391 ± 2.6 b | 344 ± 3.6 a |
| 21    | 713 ± 5.6 a | 709 ± 4.4 a | 756 ± 2.8 a | 720 ± 2.6 a | 694 ± 14.8 a |
| 28    | 1058 ± 18.4 a | 1083 ± 25.7 ab | 1164 ± 3.5 a | 1161 ± 3.0 b | 1076 ± 16.7 a |
| 35    | 1663 ± 2.3 a | 1613 ± 20.5 a | 1684 ± 3.8 a | 1730 ± 4.1 b | 1657 ± 23.0 a,b |
| 42    | 2147 ± 4.3 a | 1907 ± 24.8 a | 2078 ± 3.3 a | 2120 ± 4.9 b | 1969 ± 22.6 a |
| 49    | 2556 ± 48.6 a | 2216 ± 30.5 a | 2482 ± 3.3 a | 2550 ± 4.9 a | 2296 ± 21.9 a |

UU: uninfected-untreated control group; IU: infected-untreated control group; Q: quinamide 30 ppm; QC: quinamide 30 ppm, mixed with carbopol at 0.1% and D: decoquinate 30 ppm.

a–dDifferent letters within a row indicate statistically significant difference (p < .05).

Table 3. Quantification of oocysts per gram of litter (Lmean ± SE).

| Group | Day | UU | IU | Q | QC | D |
|-------|-----|----|----|---|----|---|
| 7     | 0 a | 1000 ± 108 b,c | 1250 ± 25 c | 1300 ± 184 b,c | 850 ± 23 c |
| 14    | 0 a | 5250 ± 209 b | 1000 ± 70 c | 800 ± 23 b | 1000 ± 47 b,c |
| 21    | 0 a | 4750 ± 348 b | 6000 ± 216 b | 1300 ± 47 b | 1150 ± 143 a,b |
| 28    | 0 a | 11000 ± 623 b | 9500 ± 209 b,c | 1500 ± 47 b | 2000 ± 187 a,b |
| 35    | 0 a | 15500 ± 418 b | 8000 ± 294 c | 1100 ± 47 b | 1200 ± 108 a,b |
| 42    | 0 a | 16000 ± 708 b | 8500 ± 117 d | 750 ± 84 b | 600 ± 81 c |
| 49    | 0 a | 15500 ± 294 b | 7750 ± 204 a,b | 400 ± 62 c | 150 ± 23 a |

UU: uninfected-untreated control group; IU: infected-untreated control group; Q: quinamide 30 ppm; QC: quinamide 30 ppm, mixed with carbopol at 0.1% and D: decoquinate 30 ppm.

a–dDifferent letters within a row differ significantly (p < .05).
Table 4. Most frequent intestinal lesion scores found in uninfected and Eimeria-infected broiler chickens treated or not with quinfamide, quinfamide + carbopol, or decoquinate.

| Group | Day 21 | Day 35 | Day 49 |
|-------|--------|--------|--------|
| UU    | 0 0 0  0 0 0 | 0 0 0  0 0 0 | 0 0 0  0 0 0 |
| IU    | 1 0 1  1 2 2 | 1 2 2  1 1 3 | 1 2 2  1 1 2 |
| Q     | 0 1 0  0 0 0 | 1 1 0  1 1 1 | 0 0 0  0 0 1 |
| QC    | 0 1 1  0 0 0 | 0 0 0  1 1 1 | 0 1 1  1 1 1 |
| D     | 0 0 0  1 1 1 | 0 0 0  1 1 1 | 0 0 0  1 1 1 |

UU: uninfected and untreated; IU: infected and untreated; Q: quinfamide 30 ppm; QC: quinfamide 30 ppm, mixed with carbopol at 0.1%; D: decoquinate 30 ppm.

Discussion

The primary strategy for controlling avian coccidiosis should focus on the prevention of the contamination of feed and water with sporulated Eimeria oocysts. The second line of defence is the prevention or treatment of subclinical or clinical coccidiosis by implementing control programmes that are based on anticoccidial drugs (Gotep et al. 2016). However, use of anticoccidial drugs that require multiple doses within a production cycle, are unsuitable for modern poultry production operations. Therefore, there is an imperative need for an alternative approach to control poultry coccidiosis (Gotep et al. 2016).

This study shows that quinfamide alone has limited anticoccidial activity, while the combination of quinfamide plus carbopol reduces, in a statistically significant manner, the oocyst per gram of litter shedding rate in broiler chickens. It is interesting to notice that carbopol increases activity of quinfamide against Eimeria. This effect may occur due to the mucoadhesive properties of this polymeric vehicle that may slow the gastrointestinal transit time, thus allowing a more prolonged contact of quinfamide with Eimeria spp. organisms (Paker & Neau 2009). A similar effect was observed for quinfamide in sheep, although the polymer used in that trial was chitosan, instead of carbopol (Aquino et al. 2013). Additionally, there were no evident adverse drug effects of quinfamide in chickens during this trial, as it occurs in humans (Padilla et al. 2002).

It has been demonstrated that poor gut health due to the disruption of the intestinal barrier, such as the one triggered by Eimeria, causes an undesirable impact in health and growth performance of broiler chickens (Chen et al. 2015). Inflammatory responses and gut disorders in broilers have economic consequences, such as a decrease in body weight gain and overall performance of the flock (Van Leeuwen et al. 2004; Shirzadi et al. 2010). In the current study, unaltered body weight gain, low mortality rate and low degree of lesions of the GI tract observed in treated birds, suggest that intestinal integrity was preserved, complying with previous reports (Van Leeuwen et al. 2004; Shirzadi et al. 2010; Chen et al. 2015). However, one interesting and unexpected result obtained, was the growth enhancement effects observed for Q and QC groups. Their body weight gain was similar to that of the UU group, in which chickens were likely to possess unaltered intestinal integrity. Part of this effect may be explained in terms of a reduction in oocyst shedding in the QC group, but not in the Q group, in which oocyst shedding was similar to that observed in group IU. Results demonstrated that the quinfamide-treated groups gained significantly more body weight than the D and the UU groups. Nevertheless, as no uninfected and quinfamide-treated group was included, it is not pertinent to assert that the growth enhancement effect may be attributed to quinfamide. Therefore, further studies are needed to provide enough evidence to define whether or not an increase in body weight gain is possible due only to quinfamide. Interestingly, decoquinate-treated animals gained significantly less weight than other treated groups (Q and QC). Further studies would be desirable to elucidate this outcome, considering as well that the mildest pathological findings were observed for the D group in the different intestinal regions. This is not completely atypical given that it has been shown lack of linearity when correlating the number of inoculated oocysts with the degree of lesions in the GI tract (Holdsworth et al. 2004).

These preliminary results should be ponder cautiously, as one of its major limitations was the low and non-uniform coccidial exposure, as demonstrated by low oocyst count estimation in litter samples and low-degree lesion scores. Yet, these results are in agreement with previous studies, which indicate that scarce uniformity of oocysts in floor-pen trials, result in build-up of immunity and not in clinical coccidiosis (Brewer & Kowalski 1970). Nevertheless, it is important to stand out that this study focussed on the anticoccidial properties of quinfamide, based on FDA guidelines and the statistically significant difference obtained in body weight gain between the Q and IU groups is a reliable indicator of the anticoccidial efficacy ($\alpha = 0.05$, two sided) (FDA 2012). The likelihood of this drug, typically used in human medicine, being licenced for veterinary use may prove challenging at best. Yet, this knowledge will hopefully motivate researchers to manipulate quinfamide to develop a compound with activity against Eimeria in broilers (FDA 2012).
Conclusions
This preliminary report describes that, without a clear-cut effect of quinamfide increasing feed intake, groups treated quinamfide and quinamfide-carbopol increased body weight gain and reduced both, oocyst output and intestinal lesion scores. Hence, utilisation of quinamfide, quinamfide-carbopol and/or chemical analogues as alternatives to control coccidiosis in broiler chickens, merit further research.

Disclosure statement
The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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