Influence of curcumin 
(Curcuma longa) as a natural 
anticoccidial alternative in 
adult rabbits: first results

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Abstract

Coccidiosis is one of the most common ailments in rabbits farming and is usually treated with drugs that can produce resistance; therefore, a natural alternative was sought. The objective of the current study was to evaluate the efficacy of the aqueous extract of curcumin (Curcuma longa) on the excretion of oocysts of Eimeria spp. in New Zealand white rabbits. Twenty-four eight-month-old rabbits were divided into four groups of six animals to be C. longa extract administered at 0 (Control), 10, 25 or 40 mg/kg body weight (BW). Rabbit weights were recorded and faeces samples were collected on d 0, 7, 14, 21, 28, 35 and 42. The McMaster technique was used for quantifying Eimeria spp. oocysts. Results were analysed using multivariate analysis of variance for repeated observations. Statistically significant differences (P<0.05) from d 28 were observed among the Control, the group of 25 mg/kg BW and that of 40 mg/kg BW. At d 42, statistically difference (P<0.05) among the Control group and the other three groups was observed. It could be concluded that C. longa decreased Eimeria spp. oocysts excretion efficiently at a dose of 40 mg/kg BW with 80.1, 63.7 and 64.9% for d 28, 35 and 42, respectively, with reducing concentration of eggs per gram of faeces with about 20.1, 15.6 and 17.8 for d 14, 21 and 35, respectively. However, further studies are needed to assess and confirm the antiparasitic activity of C. longa.

Introduction

Digestive diseases of rabbits are of great importance and can cause economic losses resulted from growth disturbances and rabbits death, in addition to decreased body weight (BW) and drug costs (García Segura et al., 2014; Szukic et al., 2014). It is generally accepted that intestinal damage following coccidiosis reduces nutrient absorption by the gut epithelium, thereby reducing BW and growth (Kim et al., 2013). Passalurus ambiguus, Encephalitozoon cuniculi, Cryptosporidium spp. and Eimeria spp. are the most prevalent endoparasites in rabbit farms worldwide; however, P. ambiguus and Eimeria spp. are the most prevalent endoparasites in Mexican rabbit farms (García Segura et al., 2014). Eimeria spp. is one of the leading causes of diarrhea, dehydration, listlessness, mortality, decreased BW and poor feed conversion from coccidiosis (Bhat et al., 1996).

Traditionally, in-feed anticoccidial drugs have proven highly effective in preventing morbidity associated with coccidiosis. Currently for treatment of coccidiosis, sulfonamides, salinomycin and robenidine are mainly used (Pakandi, 2009), which may become toxic to infants and pregnant females. Diclazuril has proven to effectively control rabbit coccidiosis (European Food Safety Authority, 2015). Nevertheless, the increase in the production of organic products is currently being observed in several countries due to consumer demands. On the other hand, parasites can potentially generate resistance for chemical anti-coccidial drugs due to bad practices in use. Furthermore, drugs and antibiotic residues in meat is potentially annoyance to consumer. By virtue of the above problems, it is important to find alternatives for the treatment of coccidiosis (Cedillo et al., 2014, 2015; Mejía Hernandez et al., 2014; Olmedo-Juárez et al., 2014); one such alternative is the use of curcumin. C. longa contains diferuloylmethane (curcumin), demethoxycurcumin, and bisdemethoxycurcumin as the main components (Arun Raj et al., 2014, 2015; Mejia Hernandez et al., 2014; Olmedo-Juárez et al., 2014); the pharmacological activities of turmeric are considerable dependent on the bioactivity of curcumin (Jayaprakasha et al., 2002). C. longa have been described for Plasmodium falciparum (Cui et al., 2007), Leishmania spp. (Koide et al., 2002), Giardia lamblia trophozoites (Shahiduzzaman et al., 2009) and Trypanosoma spp. (Nagajyothish et al., 2012). Khalfella et al. (2011) in vitro studied the effect of curcumin on Eimeria tenella, and found that there is a cytotoxic effect on the sporozoite through causing morphological changes. In addition, Pérez (2010) conducted an in vivo study to evaluate the antiparasitic effect of curcumin against coccidiosis in sheep, and reported 82.3% reduction in oocyst excretion at d 14 of treatment, and a reduction of 98.1% at d 21 of treatment compared to the
untreated group.

Therefore, the objective of the current study was to evaluate the in vivo antiparasitic efficacy of different doses of the aqueous extract of *C. longa* on the intestinal coccidiosis caused by *Eimeria* spp. in New Zealand white rabbits, with the hypothesis that the inclusion of *C. longa* causes a reduction in oocyst excretion.

**Materials and methods**

**Preparation of aqueous extract of *Curcuma longa***

About 500 g of macerated *C. longa* rhizome powder was weighed and mixed in 1 liter of bidistilled water and allowed to boil for 20 minutes. The mixture was allowed to stand for 24 h, then the supernatant was removed and introduced in a Buchi rotavapor at 60 °C under vacuum for sample drying, where 350 mg of aqueous extract were obtained. To determine the concentration of curcuminoiids, the UV-Vis method (Ultraviolet-visible spectroscopy) at 421 nm (Thermo Scientific spectrophotometer; Thermo Scientific, Inc.Waltham, MA, USA) was used according to Sharma et al. (2012). The obtained concentration of curcumin was 4.61 mg/100 mg of aqueous extract of *C. longa*.

**Animals and management**

Twenty-four NZW rabbits with eight-months-old and an average BW of 3 kg, from the Agricultural School, Autonomous University of Chapingo, Chapingo, State of Mexico, Mexico were used. The rabbits were housed in San Mateo Huxotla, Texcoco, State of Mexico, Mexico. Rabbits were naturally infected and belonged to a farm with a history of intestinal coccidiosis; with no experimental infection performing. Rabbits were kept in individual galvanized cages with 45 cm wide×30 cm long×30 cm high. Rabbits were fed on commercial diet (PURINA®, Lausanne, Switzerland) at 100 g daily. Diet contained 88% dry matter (DM), 90% organic matter (OM), 16% crude protein (CP), 3% crude fat, 17% crude fibre (CF), 42.5% of nitrogen-free extract, 1% calcium and 0.55% phosphorus. Water was offered *ad libitum*. For avoiding selection and decreasing feed waste, rabbits were offered pelleted feed at 07:00 and 17:00 h.

**Experimental design and sampling**

One-hundred g of a mixture containing an aqueous extract of 36.8 g of *C. longa* mixed with oatmeal and a non-nutritional apple flavoring additive were individually administered per os using a syringe. The untreated Control rabbits only received the mixture of oatmeal with the powdered artificial apple flavouring. The mixture was fed twice daily at 07:00 and 17:00 h. The composition of the mixture was: 91% DM, 98% OM, 13% CP, 6% crude fat and 12% CF. As rabbits were naturally infected, the stage of the life cycle of the parasite was uncertain at the time the first sample was taken. Therefore, we waited until all rabbits shed oocysts in faeces to start the experiment by speculating that the parasite had just finished the gametogony phase in most of the animals. As the life cycle is completed in 7 d (prepatent period), rabbits were sampled every 7 d to allow a life cycle to be completed. Thereafter, 7 d were skipped to check for efficacy after reinfection, considering that at least two life cycles of the parasites were completed by that time point. Therefore, the treatment of rabbits was administered from d 1 to 7, and allowed to rest in rabbits from d 8 to 14 and re-administered at d 15 to 21. Untreated Controls received the apple-flavored oatmeal mixture and the same commercial diet.

Before the beginning of the experiment (pretreatment), faeces samples from each rabbit were collected and examined by performing flotation technique quantitation of oocysts per gram faeces (OPG) with the technique of McMaster in order to confirm the natural infection of *Eimeria* spp. Based on the results of rabbits according to the load of OPG only be ordered, and randomly assigned to one of four treatments to make stockings for OPG similar groups (Kummuru et al., 2014). Oocyst counts ranged from 150 to 1100 OPG. Four groups of six rabbits each were formed where *C. longa* extract was administered at d 0 (Control), 10, 25 or 40 mg/kg BW. As explained previously, every 100 g of the *Curcuma/oatmeal/apple flavoring mixture contained: 36.8 g of the aqueous extract of *C. longa*; hence, for the 10 mg dose 27.1 mg of the mixture were used. The 25 mg dose included 67.9 mg of the mixture, whereas the 40 mg dose included 108.6 mg of the mixture. During the experiment, the weight of each rabbit was recorded and faeces as well as cecotrope samples were obtained and weighed on d 7, 14, 21, 28, 35 and 42 as the daily sampling procedure stresses the animals, which would have interfered with results and might have even caused mortality. A wire mesh was adapted at the cage floor to avoid urine to mix with faeces. Nevertheless, faeces did not fit into this mesh, so rabbits were in contact with their faeces. However, faeces from rabbits belonging to different experimental or Control groups did not have contact among them. Faeces samples were collected directly from the floor immediately after excretion and kept in plastic bags. Samples were kept refrigerated at -4°C until analysis and McMaster technique performing for quantifying *Eimeria* spp.

**Data statistical analysis**

Data were analyzed in a completely randomized design using a single factor-univariate analysis (ANOVA) with four levels of extract for each time. Multivariate analysis of variance for repeated measures was performed. Oocyst and eggs counts were natural log transformed, prior to analysis.

**Results**

**Oocyst quantification**

The multivariate analysis of variance showed a significant interaction (P<0.001) between time and treatment, which means that the number of OPG for each treatment did not exhibit homogeneity in behavior over time. As a consequence of the above result, a single factor-univariate analysis (ANOVA) with four levels for each time was performed. The Welch test was used because the variances of the groups were heterogeneous. Statistically differences (P<0.003) between groups from d 28 were found (Table 1). Dunnet T3 test was used for multiple comparisons (Figure 1). At d 28, statistically differences (P<0.05) in the log of average number of oocysts

| Levels of *Curcuma longa*, mg/kg BW | d 28 | d 35 | d 42 |
|----------------------------------|------|------|------|
|                                  | Mean | SE   | Mean | SE   | Mean | SE   |
| 0                                | 6.57 | 0.230| 6.50 | 0.242| 6.55 | 0.227|
| 10                               | 4.91 | 1.007| 4.91 | 0.991| 5.27 | 0.305|
| 25                               | 4.98 | 0.384| 4.80 | 0.252| 4.72 | 0.279|
| 40                               | 1.31 | 0.829| 2.36 | 0.879| 2.36 | 0.879|

BW, body weight. **Means in the same column with different superscripts significantly differ (P<0.05).
per gram of faeces in rabbits between the Control group and the groups receiving 25 mg/kg and 40 mg/kg BW of aqueous extract of *C. longa*, and between these two groups (Table 1). At d 35, decreased (P<0.05) numbers of oocysts were obtained with groups receiving 25 and 40 mg *C. longa* extract/kg BW compared to Control group (Table 1). At d 42, rabbits receiving *C. longa* extract at 10, 25 and 40 mg/kg BW had decreased (P<0.05) oocysts numbers compared to Control group (Table 1).

**Egg counts**

During the oocysts counting, analysis of *P. ambiguus* egg was performed. The multivariate analysis of variance showed a significant interaction between time and treatment (P<0.01) which means that the log average number of eggs per gram of faeces (HPG) for each treatment are not homogeneous behavior over time (Figure 2). As a consequence of the above result, univariate analysis of single factor with four levels for each time was performed. Welch test was used to analyze from d 0 to d 14. Statistically differences (P<0.002) were found up to d 14 and multiple comparisons were performed using the Dunnet T3 test. Statistically significant difference (P<0.05) was found between the Control group and the group receiving 40 mg/kg BW aqueous extract of *C. longa* (Table 2). At d 21, statistically significant differences (P<0.05) among the three groups (Control, 25 BW and 40 mg/kg BW aqueous extract of *C. longa*) were found, with lower values with the group receiving 25 mg/kg BW (Table 2). At d 28, statistically differences between the Control group with the groups receiving 25 and 40 mg/kg BW aqueous extract of *C. longa* were found. At d 35, statistically differences between the Control group and the group treated with 25 mg/kg BW aqueous extract of *C. longa* were found, with lowered value with the group receiving 25 mg/kg BW (Table 2). At d 42, no statistically differences among the three groups (Control, 25 and 40 mg/kg BW aqueous extract of *C. longa*) were found (Figure 2).

**Live weight, feed intake, faecal and cecotrope production**

During experiment and for all treatments, no significant difference in live weight was observed between rabbits at different days (Table 3). Significant differences (P<0.0001) were found in feed intake and faecal production between weeks 1 and 2 versus weeks 3 and 4, with a mean intake and faecal production of 98.32 and 40.88 g/d, respectively for the former two weeks and 162.15 and 78.67 g/d, respectively for weeks 3 and 4 (Table 4).

**Discussion**

The results indicate that the aqueous extract of *C. longa* at doses of 25 and 40 mg/kg BW decreased the parasite load of oocysts of *Eimeria* spp. with 24.2 and 80.1%, respectively compared with the Control group at d 28. These results are similar to those found by Pérez (2010), at a dose of 30 mg/kg BW; curcumin extract was able to reduce the parasite burden of *Eimeria* spp. in sheep. The dose of 10 mg/kg BW aqueous extract of *C. longa* decreased the burden of *Eimeria* spp. with 19.5% compared to the Control group at d 28. *C. longa* was reported to suppress the development of coccidiosis in chickens in diets at different concentrations (Allen et al., 1998). The reduction in sporozoite

![Figure 1. Concentration of oocysts in faeces of adult rabbits according to the groups and time (n=6 rabbits per treatment). A significant (P<0.001) difference was observed among groups from day 28 until the end of the experiment. OPG, oocysts in faeces.

![Figure 2. Concentration of eggs per gram of faeces of adult rabbits according to the groups and time (n=6 rabbits per treatment). A significant (P<0.001) difference was observed among groups from day 28 until the end of the experiment. HPG, number of eggs per gram of faeces.](image)

| Levels of Curcuma longa, mg/kg BW | d 14 Mean | SE | d 21 Mean | SE | d 35 Mean | SE |
|-----------------------------------|----------|----|----------|----|-----------|----|
| 0                                 | 5.57d    | 0.778 | 5.76a    | 0.139 | 5.84b     | 0.471 |
| 10                                | 2.62ab   | 0.829 | 0.00     | 0.000 | 0.00      | 0.000 |
| 25                                | 3.39abcd | 0.687 | 4.23a    | 0.139 | 3.80b     | 0.471 |
| 40                                | 4.45c    | 0.177 | 4.86d    | 0.152 | 4.86d     | 0.516 |

BW, body weight. "Means in the same column with different superscripts significantly differ (P<0.05).
Table 3. Live weight (kg) of adult New Zealand white rabbits treated with different levels of aqueous extract of Curcuma longa.

| Day | 0 mg/kg BW |          | 10 mg/kg BW |          | 25 mg/kg BW |          | 40 mg/kg BW |          |
|-----|------------|----------|-------------|----------|-------------|----------|-------------|----------|
|     | Mean       | SE       | Mean        | SE       | Mean        | SE       | Mean        | SE       |
| 0   | 3.06       | 0.10     | 3.06        | 0.06     | 3.06        | 0.06     | 3.08        | 0.06     |
| 7   | 3.13       | 0.12     | 3.10        | 0.08     | 3.07        | 0.03     | 3.15        | 0.06     |
| 14  | 3.20       | 0.11     | 3.16        | 0.08     | 3.12        | 0.04     | 3.24        | 0.06     |
| 21  | 3.21       | 0.12     | 3.21        | 0.08     | 3.16        | 0.04     | 3.27        | 0.06     |
| 28  | 3.26       | 0.12     | 3.30        | 0.10     | 3.22        | 0.05     | 3.20        | 0.09     |
| 35  | 3.33       | 0.11     | 3.33        | 0.10     | 3.28        | 0.05     | 2.81        | 0.57     |
| 42  | 3.35       | 0.11     | 3.35        | 0.09     | 3.31        | 0.04     | 2.85        | 0.57     |

BW, body weight.

Table 4. Mean weekly feed intake, faecal and cecotrope production.

| Week | Feed intake, g/d | Faecal production, g/d | Cecotrope production, g/d |
|------|------------------|------------------------|---------------------------|
| 1    | 97.78g           | 37.26g                 | -                         |
| 2    | 98.86g           | 44.89g                 | 40.71g                    |
| 3    | 161.74g          | 56.97g                 | -                         |
| 4    | 162.57g          | 100.37g                | 63.33g                    |

Different letters indicate a significant difference (P<0.0001).

Conclusions

It is important to note that the average weight of the animals was not affected by the application of aqueous extract of Curcuma longa as no statistically difference was found with respect to the average weight of the Control group, even though a significant difference was observed regarding faecal and cecotrope output, as well as a possible result of an increased feed intake. In vivo feeding of adult rabbits with an organic aqueous extract of Curcuma longa decreased the elimination of oocysts of Eimeria spp. more effectively at a dose of 40 mg/kg BW. The dose...
of 40 mg/kg DM reduced oocyst concentration in faeces with 80.1, 63.7 and 64.9% at d 28, 35 and 42, respectively, together with reducing the concentration of *P. Ambiguus* eggs per gram of faeces of about 20.1, 15.6 and 17.8 at d 14, 21 and 35, respectively. Further studies in rabbits and other species at different growth stages are suggested to assess and confirm the antiparasitic activity of *C. longa* spice.

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