COMPARATIVE ANTICOCIDIAL EFFICACY OF TWO ORAL CLOPIDOL PREMIX PRODUCTS (COYDEN 25%® AND CLOPI®) ON EXPERIMENTALLY EIMERIA TENELLA INFECTED BROILER CHICKENS

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ABSTRACT

This study was carried out to evaluate the efficacy of clopidol (Coyden 25%® and Clopi®) in control of experimental Eimeria tenella infection in broiler chickens. The trial was carried out on 100, day-old broiler chicks as they were divided into 4 main equal separate groups (25 chicks; each). Group (1) was non infected non treated (control positive), group (2) was infected non treated (control negative), group (3) was infected and treated with clopidol (Coyden 25%®) and group (4) was infected and treated with clopidol (Clopi®). Groups (2&3&4) were orally received 1ml of an inoculum containing 100,000 sporulated oocysts of Eimeria tenella/ chick at 14th days of age. Chickens of group (3&4) were infected and treated with clopidol in drinking water for 7 days. This study was assessed by effect of clopidol on the performance parameters including feed intake, body weight (BW), body weight gain (BWG), feed conversion ratio, feed efficiency ratio and also mortalities in infected and treated chickens. Also, oocyst count (shedding) in the dropping and lesion score was also recorded. The results revealed that groups treated with clopidol (Coyden 25%® and Clopi®) showed great and significant improvement in (BW), (BWG) and (RGR) and also revealed the highest reduction in mortalities, lowered number of oocysts and the lesion score, indicating that clopidol (Coyden 25%® and Clopi®) is an effective anticcocidial drugs in treatment of E. tenella infection in chickens. Groups provided with clopidol (Coyden 25%® and Clopi®) gave significant and
satisfactory improvement in the assessment criteria when compared with infected non treated group and there were no significant changes between Coyden 25%® and Clopi® and both products can be used as interchangeable drug in veterinary medicine practice especially in poultry.

1. INTRODUCTION
Amongst various parasitic infections, coccidiosis caused by obligate intracellular protozoan parasite of the genus Eimeria, is a major parasitic disease within the intensively reared poultry industry. Eimeria spp. are highly host-specific, with six species having economic impact on chicken (Arabkhazaeli et al., 2013). The annual worldwide cost is estimated at about $800 million (Allen and Fetterer, 2002). These estimates include the costs of prophylactic in-feed medication for broilers and broiler breeders, alternative treatments if medication fails and losses due to mortality, morbidity, impaired growth rate, temporary reduction of egg production in layers and poor feed conversion of chickens that survive outbreaks (Pirali-kheirabadi et al., 2008). It has been shown that coccidiosis also causes great economic losses in the poultry industry (Rahbari et al., 1995). For many years, prophylactic use of anticoccidial drugs has been the primary means of controlling chicken coccidiosis in broiler industry and has played a major role in the growth of this industry. Modern intensive poultry production is largely dependent upon chemoprophylaxis for the control of coccidiosis (Allen and Fetterer, 2002). The effective use of anticoccidial feed additives over the past 50 years has played a major role in the growth of the poultry industry and has allowed the increased availability of high quality, affordable poultry products to the consumer. These anticoccidials can be classified as (i) chemicals which have specific modes of action against parasite metabolism, such as amprolium, clopidol, decoquinate, halofuginone, or (ii) polyether ionophores such as monensin, lasalocid, salinomycin, narasin, and maduramycin, which act through general mechanisms of altering ion transport and disrupting osmotic balance (Mathis, 2001).

Clopidol, also known as meticlorpindol or clopindol, is a pyridinol with broad coccidiostatic activity against early development of Eimeria spp. by inhibiting mitochondrial energy production in sporozoites and trophozoites (Kant et al. 2013). A synergistic effect between meticlorpindol and 4-hydroxyquinolones has been described (Challey and Jeffers 1973; Jeffers and Challey, 1973). To achieve complete control, combination products with quinolones are marketed.
The objective of this study was to investigate the efficacy of clopidol (Coyden 25%® and Clopi®) on E. tenella experimentally infected broiler chickens.

2. MATERIALS AND METHODS

2.1. Chickens
A total of 100 one-day old unsexed Hubbard chicks with an average body weight of 45-50 gm were obtained from a private farm in Benha, Egypt. The rations were obtained from Cairo Company for poultry and rations, Egypt.

2.2. Drugs
Coyden 25%®: It is a feed additive premix, manufactured by Huvepharma, Bulgaria. Each 1 Kg contains clopidol 250 gm.

Clopi®: It is a feed additive premix, manufactured by Boston Company, Egypt. Each 1 Kg contains clopidol 250 gm.

2.3. Experimental design
The used 100, day-old Hubbard broiler chicks were kept on wire floor cages with daily examination of their dropping till the 14th day of life, where birds were randomly collected and divided into three equal separate groups (25 chicks each). Chicks of group (1) were kept as non infected and non treated control negative group. Chicks of group (2) were infected and non treated group. group (3) was infected and treated with clopidol (Coyden 25%®) and group (4) was infected and treated with clopidol (Clopi®). Treatment was performed for 7 days after appearance of symptoms.

2.4. Preparation of E. tenella sporulated oocysts
Oocysts of E. tenella were obtained from the parasitological laboratory of poultry diseases department, Faculty of Veterinary Medicine, Cairo University. The caeci of naturally infected chickens were separated by sieving and sedimentation techniques (Soulsby, 1978). The two caeci were emulsified in 2.5% potassium dichromate solution (in a ratio of one part of faecal sample to two parts of the solution), then filtrated and the filtrate was left for sedimentation. The sediment was taken and washed with distilled water several times. Finally, the washed oocysts were kept in 2.5% potassium dichromate solution at room temperature for sporulation.
2.5. Experimental infection

Each chick in the infected groups was orally inoculated at 14\textsuperscript{th} day of age with 1 ml solution containing about 100,000 sporulated E. tenella oocysts in the crop using a wide mouthed 1 ml pipette (Dalloul et al., 2003).

Birds of all groups were observed daily and mortalities were recorded as it occurred. Severe clinical signs (bloody dropping) were appeared at the 5\textsuperscript{th} day post infection (19\textsuperscript{th} day of age).

2.6. Growth performance parameters evaluation

2.6.1. Relative growth rate

Relative growth Rate (RGR) was calculated according to the following equation (Tawfik, 1991):

\[
\text{Relative growth Rate (RGR)} = 100 \times \frac{W_2 - W_1}{W_2 + W_1 / 2}
\]

Where \( W_1 \) = Mean initial weight of birds in each group just before infection (14\textsuperscript{th} day of age).

\( W_2 \) = Mean final weight at the end of the experiment.

2.6.2. Oocysts per gram of faeces (OPG)

Faeces were collected from five chicks in each group on days 5, 6, 7, 8, 9 and 10 after inoculation of sporulated oocysts for oocyst counts, which were expressed as oocyst/g faeces.

2.6.3. Lesion scoring

Three birds / group were sacrificed daily from 1\textsuperscript{st}, 3\textsuperscript{rd}, 4\textsuperscript{th}, 5\textsuperscript{th}, 7\textsuperscript{th} and 14\textsuperscript{th} day post infection (dpi) for detection of macroscopic caecal lesions score and scored on a scale of 0 to 4 by Lesion Scoring Technique as follows (Johnson and Reid, 1970)

0: No gross lesions.
+1: Very few scattered petechiae on the caecal wall; no thickening of the caecal walls; normal caecal contents present.
+2: Lesions more numerous with noticeable blood in the caecal contents; caecal wall is somewhat thickened; normal caecal contents present.
+3: Large amounts of blood or caecal cores present; caecal walls greatly thickened; little, if any, faecal contents in the caeca.
+4: Caecal wall greatly distended with blood or large caseous cores; faecal debris lacking or included in cores. Dead chicks were scored as +4.

2.6.4. Mortality percentage
Mortality percentage was recorded for each group of chickens.

2.7. Statistical analysis
Analysis of variance (ANOVA) using Duncan’s multiple range test for variables was computed for different parameters. Differences between group means were considered significant at P < 0.05.[14]

3. RESULTS
The effect of clopidol (Coyden 25%® and Clopi®) on the performance parameters including body weight gain (BWG) and the relative growth rate (RGR) and also mortalities in infected and treated chickens was recorded in table (1). Significant reduction in (BWG) and (RGR) was recorded in the infected non treated control positive chickens as compared with non-infected non treated control negative birds. Treated groups showed significant increase in (BW), improvement in (BWG) and (RGR) and reduced mortalities than infected non treated group.

Table 1: Efficacy of clopidol on body weight (BW), body weight gain (BWG), relative growth rate (RGR) and mortalities in control, infected non treated and infected and treated with clopidol (Coyden 25%® and Clopi®) groups (n=25 chicken for each group).

<table>
<thead>
<tr>
<th>Group</th>
<th>Non infected non treated</th>
<th>Infected non treated</th>
<th>Infected + Coyden 25%®</th>
<th>Infected + Clopi®</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean W1 (gm)</td>
<td>291.40±14.08 a</td>
<td>278.63±20.96 a</td>
<td>281.59±14.28 a</td>
<td>288.07±17.28 a</td>
</tr>
<tr>
<td>Mean W2 (gm)</td>
<td>1905.17±84.9 a</td>
<td>1027.09±74.1 c</td>
<td>1534.25±58.6 b</td>
<td>1567.28±82.05 b</td>
</tr>
<tr>
<td>W2-W1 (Weight gain)</td>
<td>1613.72 a</td>
<td>748.46 c</td>
<td>1252.66 b</td>
<td>1279.21 b</td>
</tr>
<tr>
<td>W2/W1 2</td>
<td>1098.31 a</td>
<td>652.86 c</td>
<td>907.92 b</td>
<td>927.67 b</td>
</tr>
<tr>
<td>RGR</td>
<td>146.92 a</td>
<td>114.64 b</td>
<td>137.97 a</td>
<td>137.89 a</td>
</tr>
<tr>
<td>No of dead birds/group</td>
<td>0/25 c</td>
<td>15/25 a</td>
<td>5/25 b</td>
<td>6/25 b</td>
</tr>
</tbody>
</table>

a, b, c Mean values having different letters in row differ significantly (P<0.05).

Clopidol (Coyden 25%® and Clopi®) induced significant reduction in the mean oocyst shedding and lesion score when compared with infected non treated control and this was recorded in table (2 & 3).
Table 2: Effect of treatment with clopidol in Coyden 25%® and Clopi® on oocyst count (x10³ gm feces) from 5th to 10th day post infection in broiler chickens experimentally infected with Eimeria tenella (n=25 chicken for each group).

<table>
<thead>
<tr>
<th>Time</th>
<th>Control</th>
<th>Infected</th>
<th>Infected + Coyden 25®</th>
<th>Infected + Clopi®</th>
</tr>
</thead>
<tbody>
<tr>
<td>5th pi</td>
<td>0</td>
<td>18</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>6th pi</td>
<td>0</td>
<td>119</td>
<td>78</td>
<td>79</td>
</tr>
<tr>
<td>7th pi</td>
<td>0</td>
<td>417</td>
<td>205</td>
<td>203</td>
</tr>
<tr>
<td>8th pi</td>
<td>0</td>
<td>127</td>
<td>74</td>
<td>78</td>
</tr>
<tr>
<td>9th pi</td>
<td>0</td>
<td>98</td>
<td>58</td>
<td>59</td>
</tr>
<tr>
<td>10th pi</td>
<td>0</td>
<td>56</td>
<td>22</td>
<td>23</td>
</tr>
<tr>
<td>Total oocyst count</td>
<td>0.0±0.0 c</td>
<td>139.16 ± 58.03 a</td>
<td>73.64±28.72 b</td>
<td>74.83±28.28 b</td>
</tr>
</tbody>
</table>

a, b, c Mean values having different letters in row differ significantly (P<0.05).

Table 3: Effect of treatment with clopidol in Coyden 25%® and Clopi® on lesion score (Mean ± S.E.) in broiler chickens experimentally infected with Eimeria tenella (n=25 chicken for each group).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Day</th>
<th>1st dpi</th>
<th>3rd dpi</th>
<th>4th dpi</th>
<th>5th dpi</th>
<th>7th dpi</th>
<th>14th dpi</th>
<th>X±SE dpi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>0.0±0.0 c</td>
<td>0.0±0.0 c</td>
<td>0.0±0.0 c</td>
<td>0.0±0.0 c</td>
<td>0.0±0.0 c</td>
<td>0.0±0.0 c</td>
<td>0.0±0.0 c</td>
</tr>
<tr>
<td>Infected</td>
<td></td>
<td>1.66±0.33 a</td>
<td>1.66±0.33 a</td>
<td>3.66±0.33 a</td>
<td>3.33±0.33 a</td>
<td>3.66±0.33 a</td>
<td>3.66±0.33 a</td>
<td>2.77±0.38 a</td>
</tr>
<tr>
<td>Infected+ Coyden 25%®</td>
<td></td>
<td>0.66±0.33 b</td>
<td>1.33±0.33 b</td>
<td>1.66±0.33 b</td>
<td>2.33±0.33 b</td>
<td>1.66±0.33 b</td>
<td>1.33±0.33 b</td>
<td>1.49±0.22 b</td>
</tr>
<tr>
<td>Infected+ Clopi®</td>
<td></td>
<td>0.66±0.33 b</td>
<td>1.33±0.33 b</td>
<td>1.66±0.33 b</td>
<td>2.33±0.33 b</td>
<td>1.66±0.33 b</td>
<td>1.66±0.33 b</td>
<td>1.55±0.23 b</td>
</tr>
</tbody>
</table>

a, b, c Mean values having different letters in row differ significantly (P<0.05).

4. DISCUSSION

Poultry industries engaged in broiler production have at one time or the other fed their broilers with ionophores or other types of anticoccidial drugs. Yet, intestinal coccidiosis, especially E. tenella has been a problem.

Coccidiosis is a widely occurring protozoan parasitic disease and is the most expensive disease facing the poultry industry. Coccidiosis causes a tremendous expense in terms of both prevention and loss of performance (Mathis, 2001). In the field of poultry coccidiosis, a sharp distinction is often made between chemical and ionophore anticoccidials. Chemical anticoccidials are typically characterized by a complete block of coccidial multiplication, which is in contrast to ionophore anticoccidials which always allow for a limited multiplication known as coccidial leakage (Vancraeynest et al., 2008). As both Diclazuril and
Clopidol are chemical anticoccidials, it is expected that they should be equally effective at blocking the multiplication of coccidia. This is in line with the findings of Pirali-kheirabadi et al. (2008) who reported that comparing anticoccidial effect of Salinomysin and Diclazuril indicated that Diclazuril was significantly more effective on oocyst shedding and was highly effective against combined infection of Eimeria spp. Peeters et al. (1994) reported that chemical drugs showed the highest activity against Eimeria, whereas ionophores were less efficacious. They also demonstrated that the highest activity against E. acervulina was obtained with Clopidol and the highest activity against E. maxima was obtained with Diclazuril.

Bird performance, particularly weight gain is widely used for assessing anticoccidial efficiency (Arabkhazaeli et al., 2013). In this study, supplementing diet with Clopidol and/or Diclazuril had positive effect on mean body weight, weight gain and FCR of infected groups only in the 4th week of the experiment. However, Clopidol and Diclazuril were effective in reducing oocyst shedding but, they did not have any significant effect on weight gain, mean body weight and FCR in the last 2 week of the experiment. In line with the results obtained in this study, Bahadoran et al. (2014) used two anticoccidial drugs, Clopidol and Amprolium + Ethopabate as protection in the diets of healthy chickens for 3 weeks and reported their adverse effects on the bird performance i.e. body weight and feed conversion rate.

Coccidiosis caused by parasitic Eimeria species remains one of the greatest burdens on the economics of production of poultry and poultry derived products. Approaches in control and management of coccidiosis are prophylactic feeding of anticoccidials, vaccination, and environmental management. Feed additive anticoccidials have been used for over 50 years. It is known that some anticoccidials reduce feed consumption, feed conversion and the absorption of specific nutrient from the intestine (Bell and Weaver, 2002) also, development of resistance in coccidian parasites against commonly used anticoccidials is a great problem (Lillehoj et al., 2010 and Williams, 2006). Neither of these anticoccidials is completely satisfactory, and new strategies are urgently required. Today, it seems to be more important than ever to find alternative coccidiosis control measures and develop new anticoccidials to provide for poultry producers high level of safety and efficacy. Alternative options include new anticoccidial vaccines, organic and herbal feed additives which should be studied more, as possible alternate for present synthetic anticoccidials.
Based on the results of this study, the reduction of WG, alteration of FI and subsequent alteration of FCR, in comparison to negative control, highlights the economic importance of the disease. Results of the sensitivity tests indicated that none of the field isolates was fully sensitive to the selected anticoccidials.

On conclusion, administration of clopidol (Coyden 25%® and Clopi®) is very efficacious for the prevention and control of experimental infection with Eimeria tenella in chickens.

REFERENCES
10. Lillehoj, H.S., Hong, Y., Kim, C. Quantitative genetic and functional genomics approaches to investigating parasite disease resistance and protective immune


