COMPARATIVE ANTICOCCIDIAL EFFICACY OF TWO MADURAMICIN AND NICARBAZIN PREMIX (GROWMAX® AND PHAROSTATE®) ON EXPERIMENTALLY EIMERIA TENELLA INFECTED BROILER CHICKENS

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ABSTRACT

This study was carried out to evaluate the efficacy of maduramicin and nicarbazin combination (Growmax® and Pharostate®) in a dose of 3.75 mg maduramicin ammonium and 40 mg nicarbazin/kg feed in control of an experimentally 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 (Growmax®) and group (4) was infected and treated with (Pharostate®). 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 maduramicin and nicarbazin in feed for 7 days. This study was assessed by effect of maduramicin and nicarbazin 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 was also recorded. The results revealed that groups treated with maduramicin and nicarbazin (Growmax® and Pharostate®) showed great and significant improvement in (BW), (BWG) and (RGR) and also revealed the highest reduction in mortalities, lowered number of oocysts, indicating that maduramicin and nicarbazin (Growmax® and...
Pharostate®) is an effective anticoccidial drugs in treatment of *E. tenella* infection in chickens. Group provided with maduramicin and nicarbazin (Growmax® and Pharostate®) gave significant and satisfactory improvement in the assessment criteria when compared with infected non treated group and there were no significant changes between Growmax® and Pharostate® and both products can be used as interchangeable drug in veterinary medicine practice especially in poultry.

1. **INTRODUCTION**

Coccidiosis is one of the most important parasitic diseases of poultry causing death and impaired development rate in poultry industry (Lee et al., 2007). Conventional control of this problem depends mainly on anticoccidial drugs that are a significant cost to the industry. Moreover, development of resistance in most of the poultry parasites has endangered the economics of the poultry industry (Abbas et al., 2014).

Coccidiosis is a significant problem in the poultry industry throughout the world. It is responsible for 6–10% of all broiler mortalities. In subclinical coccidiosis, minor damage of the intestinal wall will lead to deteriorated performance. In such cases, the farm owners are complaining poor performance of the chicken. The diagnosis of coccidiosis is carried out promptly at the laboratory during routine parasitological examination. Under these conditions, broiler chickens are continually exposed to coccidial oocysts found in litter. Thus, at one time we could expect overlapping parasite cycles in which more than one parasitic stage will be present. Therefore, under these conditions, the anticoccidial efficacy will depend on the ability of drug to affect broad range of parasitic stages. This is typically occurring in subclinical coccidiosis, in which we expect different stages of a parasite at one time. In this study, the efficacy of amprolium and toltrazuril was investigated in cases of subclinical coccidiosis, in which the drug is given after the oocysts shedding started (Banfield et al., 1999).

Evaluation of any anticoccidial drug is based upon estimation of bird's performance criteria such as growth rate and feed conversion, and parasitological criteria including oocyst shedding and the presence of pathognomonic intestinal lesions (Chapman, 1997).

Maduramicin ammonium is an anticoccidial compound, produced by *Actinomadura yumaensis*, and belonging to the ionophores group. Its efficacy and safety have been repeatedly shown (Kantor and Schenkel, 1984; Berger et al, 1988).
Maduramicin, a potent polyether ionophore antibiotic isolated from the bacterium *Actinomadura yumaensis*, can inhibit the growth of the most continually occurring Eimeria species in poultry industry. (Additives and Authority, 2016; Additives and Feed, 2011; Labeda et al., 1985; Liu et al., 1983). It has been presented that maduramicin may form lipophilic complexes by combining ion (i.e. Na+ and K+) and interfere with the normal transportation of cation across cell membranes, increasing the osmotic pressure of coccidian cell, which causes the influx of excess water into cell thereby causing cell membrane disruption and cell death (Butaye et al., 2003; Kant et al., 2013). As an coccidiostat, maduramicin has been confirmed that possesses excellent preventive activity against various species of Eimeria when it is used as feed additive at the dose of 5 mg/kg (Folz et al., 1988; Logan et al., 1993). Although maduramicin has an outstanding anticoccidial activity, its toxic dose is very close to the recommended dosage. It has been demonstrated that 7.5 mg/kg of maduramicin in feed would decrease feed conversion of chickens and induce marked clinical symptoms, including anorexia, depression and diarrhea (Sivakumar et al., 2007).

Nicarbazin is a non-ionophoric synthetic complex composed of an equimolar amount of 4,4’-dinitrocarbanilide (DNC) and 2-hydroxy-4,6-dimethyl pyrimidine (HDP). It is authorized as a coccidiostat feed additive for individual use in chickens for fattening or in combination with the ionophore narasin, which has a monobasic carboxylic acid structure containing five cyclic ether rings (EFSA, 2008). Nicarbazin is well absorbed from the gastrointestinal tract and widely distributed in the body after oral administration. The components HDP and DNC have different pharmacokinetic characteristics; DNC is used as marker residue for evaluating food safety because it occurs at higher concentrations than HDP. DNC concentrates in the liver and kidneys, being excreted primarily in the faeces, while HDP is excreted in urine (FEEDAP, 2010; Goetting et al., 2011). Narasin is poorly absorbed in the gastrointestinal tract and rapidly metabolized, being largely excreted in the bile, leading to the excretion of a high proportion of the administered dose via the feces (EFSA, 2004).

The objective of this study was to investigate the efficacy of maduramicin and nicarbazin combination (Growmax® and Pharostate®) 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
Growmax®: It is a powder product administered via feed and manufactured by Zoetis, Australia.

Pharostate®: It is a powder product administered via feed and manufactured by Boston Company, Egypt. Each Kg from both products contains maduramicin ammonium 7.5 gm and nicarbazin 80 gm. This anticoccidial premix (Growmax® and Pharostate®) added to feed at a level of 500 g/tonne of broiler feed to obtain a concentration of 3.75 mg maduramicin ammonium and 40 mg nicarbazin/kg feed.

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 maduramicin and nicarbazin combination (Growmax®) and group (4) was infected and treated with maduramicin and nicarbazin combination (Pharostate®). 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 \textit{E. tenella} oocysts in the crop using a wide mouthed 1 ml pipette (Dalloul, 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:

\begin{equation}
\text{Relative growth Rate (RGR) = } 100 \times \frac{W_2 - W_1}{W_2 + W_1 / 2} \quad \text{(weight gain)}
\end{equation}

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. 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 $P < 0.05$ (Duncan, 1955).

3. RESULTS
The effect of maduramicin and nicarbazin combination (Growmax\textsuperscript{®} and Pharostate\textsuperscript{®}) 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 maduramicin and nicarbazin combinations** (Growmax® and Pharostate®) **in a dose of 3.75 mg maduramicin ammonium and 40 mg nicarbazin/kg feed on body weight (BW), body weight gain (BWG), relative growth rate (RGR) and mortalities in control, infected non treated and infected and treated groups (n=25).**

<table>
<thead>
<tr>
<th>Group</th>
<th>Non infected non treated</th>
<th>Infected non treated</th>
<th>Infected + Growmax®</th>
<th>Infected + Pharostate®</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean W₁ (gm)</td>
<td>307.52±25.98ᵃ</td>
<td>294.36±17.39ᵃ</td>
<td>298.31±15.04ᵇ</td>
<td>301.56±12.99ᵃ</td>
</tr>
<tr>
<td>Mean W₂ (gm)</td>
<td>2055.61±87.39ᵃ</td>
<td>1231.75±88.6ᶜ</td>
<td>1894.52±74.91ᵇ</td>
<td>1794.51±99.16ᵇ</td>
</tr>
<tr>
<td>W₂−W₁ (Weight gain)</td>
<td>1748.09ᵃ</td>
<td>937.39ᶜ</td>
<td>1596.21ᵇ</td>
<td>1492.95ᵇ</td>
</tr>
<tr>
<td>W₂+W₁/2</td>
<td>1181.56ᵃ</td>
<td>763.05ᶜ</td>
<td>1094.61ᵇ</td>
<td>1048.03ᵇ</td>
</tr>
<tr>
<td>RGR</td>
<td>147.94ᵃ</td>
<td>122.84ᵇ</td>
<td>145.58ᵃ</td>
<td>142.45ᵃ</td>
</tr>
<tr>
<td>No of dead birds/group</td>
<td>0/25ᶜ</td>
<td>16/25ᵃ</td>
<td>5/25ᵇ</td>
<td>6/25ᵇ</td>
</tr>
</tbody>
</table>

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

Maduramicin and nicarbazin combination (Growmax® and Pharostate®) induced significant reduction in the mean oocyst shedding when compared with infected non treated control and this was recorded in table (2).

**Table 2: Effect of treatment with Growmax® and Pharostate® in a dose of 3.75 mg maduramicin ammonium and 40 mg nicarbazin/kg feed on oocyst count (x10³ gm feces) from ⁵th to ¹⁰th day post infection in broiler chickens experimentally infected with Eimeria tenella (n=25).**

<table>
<thead>
<tr>
<th>Time</th>
<th>Control</th>
<th>Infected</th>
<th>Infected + Growmax®</th>
<th>Infected + Pharostate®</th>
</tr>
</thead>
<tbody>
<tr>
<td>⁵th pi</td>
<td>0</td>
<td>16</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>⁶th pi</td>
<td>0</td>
<td>115</td>
<td>72</td>
<td>74</td>
</tr>
<tr>
<td>⁷th pi</td>
<td>0</td>
<td>401</td>
<td>185</td>
<td>195</td>
</tr>
<tr>
<td>⁸th pi</td>
<td>0</td>
<td>119</td>
<td>65</td>
<td>63</td>
</tr>
<tr>
<td>⁹th pi</td>
<td>0</td>
<td>96</td>
<td>52</td>
<td>54</td>
</tr>
<tr>
<td>¹⁰th pi</td>
<td>0</td>
<td>43</td>
<td>18</td>
<td>17</td>
</tr>
<tr>
<td>Total oocyst count</td>
<td>0.0±0.0ᶜ</td>
<td>131.66 ± 56.41ᵃ</td>
<td>66.16±20.08ᵇ</td>
<td>68.16±27.58ᵇ</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.
When the anticoccidial (maduramicin) was withdrawn from the ration during the last 3 days of the study, feed conversion improved by this final compensatory growth (Radu et al., 1987).

Hirani et al., (2016) indicating better efficacy of Maduramicin after experimental Infection with with *E. tenella* in chickens. Overall result of body weight and body weight gain indicating better efficacy of maduramycin among treatment group. Salisch and Shakshouk, (1990) reported that broiler chickens infected with *Eimeria tenella* given Maduramicin at 5 ppm showed increased weight gain and feed conversion, when compared with narasin and monensin. The efficacy of maduramicin against *E. tenella, E. maxima, E. necatrix, E. brunetti and E. acervulina* in Hubbard - cross were studied by Folz et al., (1988) who reported that birds treated with maduramicin had significantly higher weight gain.

An overall result of feed efficiency and feed conversion ratio indicates better result by maduramicin which is completely in line with Azizi et al. (2010), Georgiva et al. (2010) reported better results with maduramicin in *E tenella* infected broilers by improving WG and FCR. da Costa et al., (2017) recorded that under conditions of coccidial challenge, nicarbazin significantly improved body weight gains in both temperature environments. Compared to standard temperature conditions, lower environmental temperatures exerted a positive effect on feed conversion rates of nicarbazin-fed broilers. As an anticoccidial, nicarbazin has earned an outstanding reputation for efficacy, resilience, and functional longevity over its many years of use (Mathis and McDougald, 1982; Chapman, 1994; Bafundo et al., 2008).

Administration of both drugs improved growth performance, gross lesion score and different microscopic lesions measured in this study, in comparison to challenged untreated group. No significant differences in the average weight gain and FCR were noted between unchallenged birds treated with either drug with the control group.

On conclusion, administration of maduramicin and nicarbazin combinations (Growmax® and Pharostate®) is very efficacious for the prevention and control of coccidiosis caused by *Eimeria tenella* in chickens.

**REFERENCES**

14. EFSA (European Food Safety Authority) Opinion of the scientific panel on additives and products or substances used in animal feed on the request from the commission on the


