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RESEARCH ARTICLE

THE EFFICACY OF A PHYTO-SYNTHETIC DRUG OF COCCIDIA AS EVIDENCED FROM OOCYSTS COUNTS AND HISTOPATHOLOGY

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ABSTRACT

Cocccidiosisis a disease of economic importance in poultry industry causing morbidity and mortality and thereby leading to high annual economic loss to poultry farmers. In this study, graded concentrations of garlic powder (GP) was combined with some synthetic drugs and evaluated for their anticoccidial activities and the Histopathological effects of the various treatments in the caecum. Also, to know the concentration of garlic and amprolium that best mitigates the effects of the disease. Seventy day old broiler chickens were randomly assigned to seven groups of ten replicates (G1-G7). Groups 1 to 6 were orally inoculated with 6 x 103 sporulated oocysts of E. *tenella* on day 19 while Group 7 served as the negative control. G1 to G5 were treated with 12 mg GP +48 mg AMP, 24 mg GP + 48 mg AMP, 48 mg GP + 48 mg AMP, 48 mg AMP and 28 mg AMP + SUL respectively whereas G6 served as the positive control. The results of the seven days treatment showed the detrimental effects of E. *tenella* with respect to oocysts shedding and the positive effects of the tested drugs on birds. It also depicts the various degrees of damage to their intestines and the positive outcomes of the drugs. Feacal oocysts counts were significantly reduced in all the groups with the highest effect observed in G3 (48 mg GP + 48 mg AMP) and G5 (28 mg AMP + SUL). Histopathologically, the various levels of necrotic cells observed in the bird intestines also confirmed the level of damage caused by the parasite and the treatment effects of the drugs. G3 had the best intestinal architecture as compared with other treatments. In conclusion therefore, the best treatment was 48 mg of garlic powder in combination with 48 mg of amprolium which was comparable to the synergy of 28 mg of amprolium and sulphaquinoxaline.

INTRODUCTION

Coccidiosis is a major parasitic disease caused by Apicomplexan protozoa belonging to the subclass coccidian (Eimeridae: *Eimeria*) (Gararawa *et al*., 2011). There are various species of the intracellular protozoan parasites causing coccidiosis in poultry, each having a predilection site in the chicken digestive tract. The most common and pathogenic species affecting the poultry industry is *E. tenella* (Ayaz *et al.,* 2003), which results in 100 % morbidity and a high mortality due to extensive damage of the intestinal tract (Cook, 1988). Coccidiosis is known as the parasitic disease of poultry with the greatest economic impact worldwide (Allen and Fetterer, 2002) due to production losses and treatment costs or prevention (Shirley *et al.,* 2005). It is mainly controlled using chemical coccidiostats administered in feed (Shirley *et al.,* 2005), although the continuous use or misuse of these drugs have limited their efficiency in disease control due to development of resistance strains (Long, 1982; Ruff and Danforth, 1996). In the 1980s and early 1990s, sulphaquinoxaline, nitrofurans and amprolium were the commonly used drugs for the control of coccidiosis in poultry, Overtime, the parasites have developed resistance towards these drugs resulting in their inability to control the disease (Gararawa *et al.,* 2011). Furthermore, indiscriminate use of these anticoccidials have led to residual drugs in chicken products raising concerns about public health and food safety (Chapman, 1997; Orengo *et al.,* 2012).

Recently, researchers all over the world have tested plants for their anticoccidial activities and have found *Allium sativum* (garlic) to be effective in the control and prevention. Elbana *et al.* (2013) observed a significant decrease in oocyst counts in broiler chickens when infected with mixed sporulated *Eimeria* oocysts and treated with aqueous extract of *A. sativum* and *Aloe vera*. Similarly, El-Khtam *et. al.* (2014) observed a reduction in oocysts count in garlic supplemented group compared with turmeric supplemented group. This study aimed at studying the anticoccidial and histopathological effects of the combination of *A. sativum* and amprolium at different concentrations as compared with amprolium and the

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synergy of amprolium and sulphaquinoxaline and also to determine which concentration of the combination had the best treatment in broiler chickens that were experimentally infected with *E. tenella* sporulated oocysts.

MATERIALS AND METHODS

Plant collection and Preparation

Fresh bulbs of *A. sativum* were bought from a nearby vegetable market in Jos, Plateau State. They were peeled and shade-dried for one month. The dried garlic bulbs were then blended into powdery form with the aid of an electric blender (Binatone), sieved with a wire mesh and kept for use.

Experimental Drugs

Amprolium

Ancoban (Amprolium 20 %, Anglican Nutrition Products Company, UK) a commercially available anticoccidial drug for the routine treatment of avian coccidiosis due to *Eimeria* was purchased from a veterinary store in Jos metropolis. Amprolium acts by interfering with thiamine metabolism in the parasite. It was used to compare the anticoccidial effects garlic.

Amprolium and Sulphonamide

Prococ WDP (Amprolium 200 mg + Sulphaquinoxaline 200 mg + Vit K3 2 mg). Sulphaquinoxaline is a chemotherapeutic with bacteriostatic action against many gram-positive and gram negative-bacteria. It also poses a coccidiostatic activity against various *Eimeria* species in poultry.

Study Area

The research was carried out at the National Veterinary Research Institute (NVRI) Vom, Plateau State Nigeria where Birds were kept at the Large Animal House within the Institute and the Applied Entomology and Parasitology Unit of the Department of Zoology, University of Jos, Nigeria where analysis of data was conducted.

Experimental Animals

Apparently, healthy, unsexed day-old broiler chickens were obtained from a hatchery in Jos and brooded under standard conditions for nineteen days before the commencement of the study. They were kept under good hygienic conditions and drinking water was administered *ad libitum*. Chicks were fed on balanced commercial ration free from anticoccidials. Faeces were microscopically examined to ensure they were coccidia free prior experiment.

Experimental Design

A total of seventy day old Cobb-700 broiler chicks were randomly divided on day nineteen (after brooding) into battery cages and grouped into seven each of ten chicks (G1 to G7) and labeled accordingly. They were orally infected with 6 x 10^3 sporulated oocysts of *E. tenella* (0.2 ml) which was obtained from NVRI parasitology Laboratory. After about 90 % establishment of the infection (6 days pi) treatment was then commenced and lasted for seven days. Garlic powder was calculated as a percentage of amprolium in this study. Summarily, Group 1 was infected and treated with 12 mg GP+48 mg AMP; Group 2 was infected and treated with 24 mg GP + 48 mg AMP; Group 3 was infected and treated with 48 mg GP + 48 mg AMP; Group 4 was infected and treated with 48 mg of Amprolium alone; Group 5 was infected and treated with 28 mg of Amprolium + sulphaquinoxaline; Group 6 was infected but not treated (Positive control); Group 7 was neither infected nor treated (negative control); Birds were then monitored for clinical signs of coccidiosis.

Parasitological Examination (oocysts count)

Fresh faecal droppings were collected from the litter with the aid of clean gloved hand and was labeled with a permanent marker from day 3 post infection and repeated every 3 days until few or no oocysts was seen in stools. Evaluation of faeces for the Oocyts per gram (OPG) counts was performed using modified McMaster's counting technique according to the method described by Long and Joyner (1976).

Histopathological Examination

Seven days after treatment, tissue sample (intestines) of birds in all the groups were harvested from the birds immediately after sacrifice, fixed in 10 % buffered formal saline, embedded in paraffin wax, sectioned at 5 µ thickness, stained with haematoxylin and eosin (H & E) stain, cleared in xylene and mounted in a moutant (Akanbi and Taiwo, 2014; Drury and Wallington, 1976).

Statistical Analysis

The data obtained were statistically analysed by analysis of variance (ANOVA). Groups were compared using the least significant difference (LSD) at $p<0.05$ according to Petrie and Watson (2013).Data was computerized using SPSS version 20. In addition, the criteria used to measure the degree of coccidial infection and the efficacy of *Allium sativum* and amprolium were: The mean numbers of oocysts discharged in each group; Histopathological effects; Observation of clinical features of coccidiosis. This experiment lasted for fifty days

RESULTS

General Observations

Most of the infected chickens experienced decrease in weight, reduction of appetite, ruffled feathers and bloody diarrhea. All these were compared with their control counterparts although no mortality was recorded as a result of the parasitaemia prior treatment.

Effect of Treatments on Oocysts Count

It was observed as shown in Table 1 that on day 3 post infection, no oocyst was seen in all the groups. But from days 6 to 15 a constant increase in the number of oocysts shed was seen except for groups 3 and 4 that a slight drop in oocysts count was recorded on day 12. However, there was a significant decrease in oocysts output in all the treated groups from day 3 to day 9 post treatment. Group 1 birds shed 5 ± 2.3 x 10^2 oocysts which was significantly different from that of

Table 1. Comparative Efficacy of the combination of different Concentrations of *Allium satium* + Amprolium and Sulphaquinoxaline on *E. tenella* in broiler chickens

		Post Infection [Number of Oocysts Shed (x 10^2)]					Post Treatment		
Group	Treatment	Day 3	Day 6	Day 9	Day 12	Day 15	Day 3	Day 6	Day 9
1	25 % (GP + A)	-	12 ± 3.6^{ae}	21 ± 3.7^{ad}	23 ± 8.1^{a}	24 ± 3.6^{a}	5 ± 2.34^{cd}	14 ± 1.5^{ch}	6 ± 2.3^{i}
2	50 % (GP + A)	-	19 ± 2.9^{a}	16 ± 2.8^{cd}	22 ± 8.0^{ac}	27 ± 1.6^{a}	21 ± 1.0^{a}	13 ± 9.2^{dh}	4 ± 1.1^{i}
3	100 % (GP + A)	-	19 ± 1.2^{ag}	23 ± 3.6^{a}	18 ± 9.5^{bc}	27 ± 1.6^{a}	24 ± 3.2^{a}	11 ± 10.1^{eh}	4 ± 1.2^{i}
4	48 mg (Amp)	-	9 ± 2.6^{ah}	26 ± 1.4^{a}	23 ± 5.6^{a}	23 ± 2.7^{c}	24 ± 2.2^{a}	14 ± 12.4^{fh}	3 ± 1.1^{i}
5	28 mg(Amp + Sul)	-	14 ± 3.1^{e}	26 ± 1.2^{a}	24 ± 0.0^{a}	26 ± 1.5^{a}	20 ± 2.9^{a}	16 ± 9.3^{h}	9 ± 2.9^{i}
6	Control F(+ve control)	-	7 ± 0.0^{a}	25 ± 1.2^{a}	25 ± 0.0^{a}	27 ± 1.3^{a}	18 ± 2.3^{a}	25 ± 0.0^{a}	25 ± 1.5^{a}
7	Control G (-ve control)	-	-	-	-	-	-	-	-
	LSD(0.05)	NS	6.85	6.02	5.71	3.63	7.60	8.69	5.68

Legend: GP + A = *Allium sativum* +_Amprolium, (-) = no parasitic infection, +ve control (F) = infected not treated, -ve control (G) = not infected, not treated, NS = no significant difference where $P > 0.05$. Values are means ± SEM. Values with different superscripts along the same Column are significantly different ($P < 0.05$).Number of oocysts shed= x10²

Table 2. Efficacy of the Concentrations of *Allium sativum* and Amprolium in the mitigation of *E. tenella* in infected broiler chickens

		Post Infection [Number of Oocysts Shed (x 10^2)]					Post Treatment X10^2		
Group	Treatment	Day 3	Day 6	Day 9	Day 12	Day 15	Day 3	Day 6	Day 9
1	25 % (GP + A)	-	12 ± 3.6^{b}	21 ± 3.7^{a}	23 ± 8.1^{a}	24 ± 3.6^{ab}	5 ± 2.34^{c}	14 ± 1.5^{a}	6 ± 2.3^{a}
2	50 % (GP + A)	-	19 ± 2.9^{a}	16 ± 2.8^{ab}	22 ± 8.0^{a}	27 ± 1.6^{a}	21 ± 1.0^{ab}	13 ± 9.2^{a}	4 ± 1.1^{a}
3	100 % (GP + A)	-	19 ± 1.2^{a}	23 ± 3.6^{a}	18 ± 9.5^{b}	27 ± 1.6^{a}	24 ± 3.2^{a}	11 ± 10.1^{a}	4 ± 1.2^{a}
						NS	8.16	NS	NS

Legend**:** (GP + A) = *Allium sativum* + Amprolium, (-) = No parasitic infection, (AMP + SUL) = Amprolium + Sulphonamide. Values are means ± SEM.; NS= No significant difference where $P > 0.05$. Values with different superscripts along the same Column are significantly different ($P < 0.05$).

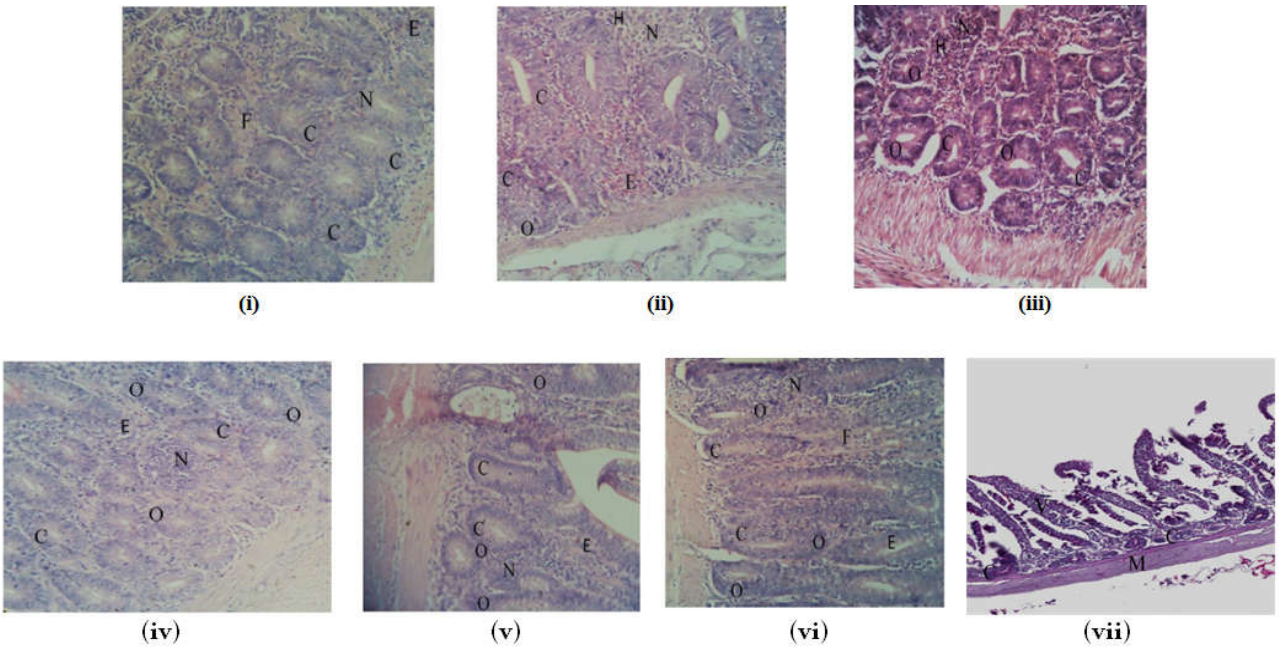

Figure 1. Photomicrograph of the intestine of chickens infected with *E. tenella* oocysts and treated with graded cocentrations of garlic powder incombination with amprolium, amprolium alone and the synergy of amprolium and sulphaquinoxaline with their controls. (i) G1 broiler chicken treated with 25 % (GP + A); Crypts of epithelial cells (C) moderately laden with varying developmental stages of protozoa parasites consistent with *Eimeria spp* and are necrotic (N). The submucosais expanded mainly by eosinophilic and heterophilic (H) polymorphs, with moderate macrophages and large quantity of organized fibrin (F). H & E stain x400. (ii) G2 broiler chicken treated with 50 % (GP + A); crypts of epithelial cells (C) are severely expanded and laden with varying developmental stages of protozoa parasite consistent with *Eimeria* species oocysts (O) and are moderately necrotic (N). The sub-mucosa and the inter-cryptic spaces are infiltrated mainly by moderate eosinophilic (E) and heterophilic (H) polymorphs. H & E stain x400. (iii) G3 broiler chicken treated with 100 % (GP + A); crypts of epithelial cells (C) are moderately expanded and laden with varying developmental stages of protozoa parasite consistent with *Eimeria* species oocysts (O) and are moderately necrotic (N). The sub-mucosa and the inter-cryptic spaces are infiltrated mainly by moderate lymphocytic mononuclear and heterophilic (H) cells. H & E stain x400. (iv) G4 broiler chicken treated with 48 mg (AMP); crypts of epithelial cells (C) are severely expanded and laden with varying developmental stages of protozoa parasite consistent with *Eimeria* species oocysts (O) and are severely necrotic (N). The sub-mucosa and the inter-cryptic spaces are infiltrated mainly by moderate eosinophilic (E) lymphocytic mononuclear and cells. H & E stain x400. (v) G5 broiler chicken treated with 28 mg (AMP + SUL); Villi epithelial cells (c) contain small amount of oocysts (O) of protozoa parasite consistent with *Eimeria* species and their sub-mucosa and lamina propria are moderately expanded by infiltrating Eosinophilic (E) and lymphocytic mononuclear inflammatory cells, fibrin and necrosis (N). H & E stain x400. (vi) G6 broiler chicken, Infected not treated; cryptic epithelial cells (C) are severely expanded and laden with oocysts of protozoa parasite consistent with *Eimeria* species and are severely necrotic (N). The sub-mucosa and lamina propria are moderately expanded by infiltrating lymphocytic (L) mononuclear inflammatory cells and fibrin (F). H & E stain. (vii) G7 broiler chicken, not infected not treated. Crypts epithelial cells (C). The intestine also shows villi (V) and muscular layer (M). Normal Intestine. H & E stain X100

birds in other groups. On day 9 post treatment, the least oocysts output was shed by birds in group 4; 3 ± 1.1 x 10^2 this was followed by groups 2 and 3; 4 ± 1.1 x 10^2 and 4 ± 1.2 x 10^2 respectively, then group 1 birds 6 ± 1.3 x 10^2. Group 6 (positive control) had the highest of 9 ± 2.9 x 10^2. No oocysts was shed by birds in group 7 (negative control) all through the experimental period. The most effective treatments at the end of the experiment with regards to oocyts shedding was in a decreasing order of Group 4; 48 mg (AMP), Group 3; 100 % (GP + A), Group 2; 50 % (GP + A) and Group 1; 25 % (GP + A).

Effects of Concentrations of *A. sativum* and Amprolium on broiler chickens

As indicated in Table two; few oocysts were counted on the day six, although there was no significant difference in the number of oocysts production among the three concentrations of *Allium sativum* and amprolium from day 3 to day 15 post infection indicating the same level of effects in the birds. On day 3 post treatment the lowest 5 ± 2.3 × 10^2 oocysts production was observed in group 1 broiler chickens, which was significantly different from the oocysts in the birds in groups 2 and 3. On day 9 post treatment, group 3 birds 100 % (GP + AMP) and group 2 chickens 50 % (GP + AMP) had the least faecal oocysts output among the three concentrations of garlic with amprolium used in the treatment of infected broiler chickens

Histological Findings

At the time of collection, all the birds had *Eimeria* oocyst except the group control (G7) "not infected not treated". The crypts of epithelial cells were laden with varying degrees of developmental stages of *Eimeria* species and necrosis is also very prominent in many cases, although this varied greatly among the infected groups. In the infected groups, the histopathological examination revealed increased inflammation of the intestinal villi characterized by lymphocytic infiltrates and heterophilic polymorphs indicating the presence of parasitic infection. Crypts of epithelial cells were also expanded in many cases due to parasitic actions. It was clearly observed that the treated groups showed some level of interventions compared to the positive control (G6) "infected but not treated which was severely laden with oocysts in the intestinal crypt and had severe expansion of the epithelial cell compared to other treated groups. Group 5 treated with 280 mg (AMP + SUL) had the least necrosis and the epithelial cells of the intestine was mildly expanded compared to the rest of the treatments this was followed by group 3 treated with 100 % (GP + A) which still maintained the shape of the crypt to a great extent, having moderate necrosis and less number of oocysts compared to Group 1; 25 % (GP + A), Group 2; 50 % (GP + A) and Group 4; 480 mg AMP.

DISCUSSION

Coccidiosis is an economic and health problem in the poultry industry which is capable of infecting any type of poultry in any facility in the world (McDougald and Reid, 1997). This was evidenced in this study by the high number of oocysts shed by all the infected birds from day 6 post infection which depicts greatly the level of harm *E. tenella* had on the broiler chickens. The detrimental effect was also seen in the birds with watery diarrhea seven day post infection. Oocysts count was zero in G7 'not infected not treated'. All the treatments used significantly reduced oocysts production when compared with the infected not treated group. However, groups that were treated with the combination of garlic and amprolium had a greater effect in the oocysts reduction although this was dependent on the concentration of garlic powder as the effect increased with increase concentration of GP. This confirms an observation by Khan *et. al.* (2012) who reported that the effects of garlic on the production of healthy broilers are different depending on variations, doses, processing and duration of feeding. Other researchers have obtained similar results when they treated coccidiosis with garlic and its extracts. For instance, Worku *et al.* (2012) reported that garlic extract contributes to the treatment of gastrointestinal infections in goat by reducing oocysts output and may improve performance in adult goats. Furthermore, Alnassan *et al.* (2015) studied the *in vitro* efficacy of allicin on chicken *E. tenella* sporozoites and observed about 99.9 % - 71.53 % inhibition using 180 mg/ml and 180 ng/ml dilution, respectively. Elbana *et al*. (2013) observed a significant decrease in faecal oocysts count in broiler chickens that were infected with mixed sporulated *Eimeria* oocysts and treated with aqueous extract of *Allium sativum* and *Aloe vera* alone or in combination. Similar result was obtained by El-Khtam *et al*. (2014) when they observed a reduction in total oocysts count in garlic supplemented group compared with turmeric supplemented group at different concentrations of 5 g/l and 10 g/l each in broilers infected with 10,000 sporulated oocysts of mixed *Eimeria* species in broiler chickens. Furthermore, Dkhil *et al*. (2011) reported a significant reduction of oocysts output in garlic treated mice infected with *E. papillata*. It can therefore be concluded that *A. sativum* posses anticoccidial activity which was comparable with that of anticoccidial drugs, amprolium and the synergy of amprolium and sulphaquinoxaline.

Conclusion

The severity of infection caused by *E. tenella* based on oocyts counts and histopathology was very high. Therefore coccidiosis caused by *E. tenella* has a destructive effect in the intestines and general well-being of broiler chickens and should be prevented. Due to the high nutritive value of garlic, the effect of this disease was greatly reduced with the highest effect observed in group 3: '48 mg of garlic powder combined with 48 mg of amprolium' and '24 mg of garlic powder combined with 48 mg of amprolium'. It can therefore be concluded that the combination of 100 % of garlic powder and 48mg of amprolium alleviated the effect of *E. tenella* by significantly reducing oocyst count to the bearest minimum and greatly improving the intestinal architecture of broiler chickens. The results are comparable with the synergy of amprolium and sulphaquinoxaline and should be used as an alternative therapy in the treatment of this disease. The photomicrographs of the intestines showed varying degrees of necrosis which was as a result of the parasitic infections by *Eimeria tenella* and evidently seen in the varying stages of their developmental stages. This is in sync with studies by Maskerem *et al.* (2013) who observed excessive tissue damage, hemorrhage, presence of clusters of large schizonts and merozoites in the caeca of birds infected with *E. tenella* and *E. brunette.* However, due to the treatment administered to

the birds, there was a moderate cryptic destruction in G3, G5 and G4. Obviously, G3 and G5 had the best architecture of epithelial crypts compared to G1 and G2. G6 had a severe destruction of its crypt due to lack of intervention. Evidently, G3 100 % (GP + AMP), had the best treatment which was followed by G5, that was treated with a synergy of amprolium and sulphaquinoxaline. These two therefore, had excellent effect in the reduction of *Eimeria tenella* in broiler chickens and can be attributed to the antiparasitic and antimicrobial effects of garlic.

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