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Development of a Potent Anti-coccidial Drug: A Phyto-Synthetic Approach

Adulugba IA[1], Goselle ON[1], Ajayi OO[1] and Tanko JT[2]

1 Applied Entomology and Parasitology Unit, Department of Zoology, University of Jos, Nigeria
2 National Veterinary Research Institute Vom, Plateau State, Nigeria

Corresponding author:
Goselle N

obeto247@yahoo.com

Applied Entomology and Parasitology Unit, Department of Zoology, University of Jos, Nigeria.

Tel: 2348038500285

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Abstract

Coccidiosis is a well-known parasitic disease causing major economic problem in the poultry industry and responsible for several deaths of poultry and loses to poultry farmers all over the world. Despite several progresses aimed at the mitigation of this disease through formulation of synthetic drugs and phyto-medicine, the scourge of the disease is still recorded in different farms and at an uncontrollable state. Although different mode of its transmission has been recorded and lots of potent drugs formulated, identifying the specific survival strategy of the parasites and the quest to develop a portent drug that could completely kill the parasites still remains an enigma. Here, we report an extraordinary case where we conducted a study through the addition of garlic to synthetic drugs to test the efficacy of the anti-coccidial effect of graded concentrations of garlic powder (GP) in combination with amprolium on faecal oocysts counts of coccidia and measured weight gain in broiler chickens post treatment. To achieve our set goal, seventy day old birds were randomly assigned to seven groups (A-G) of 10 replicates each. Sixty of them were orally inoculated with 6×10^3 sporulated oocysts of *Eimeria tenella* on day 19 while the remaining 10 as negative control. Groups A-C were treated with 12 mg GP+48 mg AMP, 24 mg GP+48 mg AMP and 48 mg GP+48 mg AMP respectively, while Groups D and E received 48 mg of amprolium alone and 28 mg of the synergy of amprolium and sulpha quinoxaline respectively, with Group F serving as the positive control whereas Group G as negative control. Via intense monitoring of the experiment, we were able to understand that inoculation of broiler chickens with 6×10^3 sporulated oocysts of *Eimeria* significantly reduced growth rate and triggered the shedding of oocysts in their faeces. A closer look at the understanding of the unfolded results after seven days of treatment, indicated that faecal oocysts count reduced significantly in all the treated birds with the highest effect in groups treated with 48 mg GP+48 mg AMP, 24 mg GP+48 mg AMP and 28 mg (AMP+SUL) while the untreated group had the highest oocysts output with a significant difference established ($P<0.05$) between treatments. With regards to weight gain, a significant increased in all the treated groups was recorded with the highest effect observed in groups treated with 24 mg GP+48 mg AMP and 28 mg AMP+SUL. In conclusion, the combination of 48 mg of garlic powder and 48 mg of amprolium had a similar anti-coccidial effect when compared with the synergy of 28 mg of amprolium and sulphaquinoxaline in the treatment of *Eimeria tenella* in chickens and can therefore be used as an alternative therapy.

Keywords: Chickens; Coccidia; *Eimeria tenella*; Garlic; Amprolium; Sulphaquinoxaline weight gain

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Introduction

Coccidiosis is a poultry disease caused by a protozoan parasite of the genus *Eimeria* comprising of nine species which includes: *Eimeria tenella, E. necatrix, E. maxima, E. acervulina, E. mitis, E. brunette, E. nagani, E. mivati and E. praecox* [1] and of these species, three (*E. tenella, E. maxima* and *E. acervulina*) have been reported to be mostly common [2]. The effect of Coccidiosis in chickens have been reported to cause poor growth rate and poor feed efficiency in broilers, leading to high mortality, decreased productivity, and high medical cost [3]. The severity of this disease has also been characterized by excretion of faecal oocysts and the presence of intestinal lesions [4]. Coccidiosis is transmitted through hard thick-walled sporulated oocyst which is able to survive for a long time in the poultry litter, soil particles and water and is usually resistant to disinfectants. Although the disease is mainly controlled by good hygiene practice and the use of chemotherapeutic agents or chemical coccidiocidal agents [5] frequent use or misuse of these drugs has led to drug resistance [6-8] and this has remain an endemic problem especially in the use of chemical drugs and ionophore compounds [9-12]. Due to the outrageous development of drug resistance to coccidiostats, elevated cost of synthetic vaccination and increasing consumer demand for natural food products, plant based alternatives is now considered as alternatives over chemicals for coccidian control in poultry farming [13] With the revelations from many reports showing the efficacy of *Allium sativum* (garlic) in the treatments and prevention of wide range of diseases all over the world in both humans and animals, Garlic is now cultivated the world over and it is considered one of the most essential and useful herbs used for medicinal purposes. It has been described to exhibit broad antimicrobial activity against bacteria, fungi and virus [14-16], anti-tumour activity [17] as well as anti-thrombotic, anti-arthritic, hypolipidemic and hypoglycemic activities [18,19]. It has also been discovered to be effective against various parasites such as amoeba [20], Leishmania [21], Trypanosoma [22] and cryptosporidium [23]. Experimental evidenced by Wahba [23] Dkhil [24] showed that treatment of mice with garlic extract significantly decreased oocysts expulsion, while Toula and Al-Rawi [25] showed that Garlic possess anti-coccidial activity in rabbits whereas El-khatam [26] established that garlic powder was effective against coccidiosis in broilers. The use of several drugs alone or in combination with other drugs has proven to be effective over the mitigation of coccidiosis, although pockets of resistance still occur especially after prolong use [27]. With the discovery of sulphonamides as a potent compound in the control of *Eimeria* over six decades ago, its use as anti-coccidial drugs have continued to generate great interest. This prompted the inclusion of Sulphonamides to amprolium in the control of Eimeria and other related mycotoxins. Following this basic knowledge, and blessed from the benefit of hindsight that garlic contain 33 sulphur compounds among which are sulphonamides; the need to increased the contents of sulphanamides to the synergy of Amprolium and sulphaquinoxaline or alternatively to a lone dose of amprolium especially in countries where the synergistic drugs are unavailable or costly was therefore necessary if a complete mitigation of coccidial is to be achieved. We therefore used graded concentrations of garlic powder in combination with amprolium in the treatment of *Eimeria tenella* in experimentally infected birds and compared with pure chemical drugs in this research.

Materials and Methods

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.

Collection of *Allium sativum*

The mature bulbs of *Allium sativum* were purchased from the popular vegetable market in Faringada, Jos-North local government area of Plateau state in November 2015 at the onset of dry season.

Processing of *Allium sativum*

The bulbs were peeled and shade dried to minimize the effect of ultra-violet rays from the sun on the active ingredients of the plant material. The drying lasted from December to February. They were then blended using an electric blender (Binatone) into powdery form and sieved with a fine wire mesh.

Experimental animals

A total of 70 healthy day old broiler chickens of both sexes were obtained from a hatchery in Jos, Nigeria and brooded under standard conditions for nineteen days before the commencement of the study. The broilers were fed standard pelletised Commercial broiler starter feed (Vital feed, Grand Cereals, Nigeria Plc., Jos, Nigeria) and water offered *ad libitum*. Birds were then moved into individual battery cages with sufficient lighting and heat. They were vaccinated against Infectious bursal diseases (IBD) and Newcastle disease virus (IBD and La Sota vaccines) on days 7 and 14, using NVRI, Vom, vaccines. The vaccines were then administered in their drinking water after 12 hours of water starvation. Faeces were microscopically examined to ensure coccidia-free birds prior inoculation.

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 reputable veterinary store in Jos metropolis. Amprolium acts by interfering with thiamine metabolism in the parasite. It was used to compare the anticoccidial effects of plant material.

Amprolium and sulphonamide: Prococ WDP (Amprolium 200 mg+Sulphaquinoxaline 200 mg+Vit K3 2 mg) was also purchased at same store. Sulphaquinoxaline is a chemotherapeutic with bacteriostatic action against many gram-negative and gram-positive bacteria. It also has a coccidiostatic activity against various *Eimeria* species that infect chickens

Experimental design

The seventy purchased birds were randomly divided into seven groups (A-G) of ten replicates. The amount of garlic used for treatment was calculated as a percentage of the amprolium. Thus: Group A represents infected and treated with 48 mg of amprolium and 12 mg of garlic powder (25% of garlic). Group B represents infected and treated with 48 mg of amprolium and 24 mg of garlic powder (50% of garlic). Group C represents infected and treated with 48 mg of amprolium and 48 mg of garlic powder (100% of garlic). Group D represents infected and treated with 48 mg of amprolium only. Group E represents infected and treated with 28 mg each of amprolium and sulphaquinoxaline. Group F represents infected and not treated (positive control) while Group G represents non-infected and non-treated (negative control).

Weighing of birds

Weight of each bird was recorded and repeated after every three days until the end of the experiment. This was achieved with the aid of Camry Weighing Scale.

Inoculation of experimental animals with coccidia

The sporulated oocysts of *Eimeria tenella* inoculum used were obtained from the Parasitology Laboratory of NVRI, Vom. On day 19, each bird was orally inoculated with 6×10^3 sporulated oocysts (0.2 ml) as a single gavage. Groups A-F received same number of oocysts while group G was given distilled water all through the experimental period.

Parasitological examination (Oocysts Count)

Evaluation of faeces for the oocyst per gram (opg) counts was carried out in the Parasitology Laboratory of NVRI Vom. Fresh faecal samples were collected from each bird and was examined for the presence of *Eimeria* oocysts on day 3 post infection and subsequently examined after every 3 days. The mean number of oocysts per gram faeces for each bird was counted using the McMaster counting technique according to the method described by [28]. Results were recorded as the number of opg shed by each bird. In addition, the criteria used to measure the degree of coccidian infection and the efficacy of *Allium sativum* and amprolium were: The mean numbers of oocysts discharged in each group; Weight gain or loss across the groups; Observation of clinical features of coccidiosis. The experiment lasted for fifty days.

Statistical analysis

The data obtained were statistically analyzed by analysis of variance (ANOVA). Groups were compared using the least significant difference (LSD) at $P<0.05$ as recommended by Petrie and Watson [29]. Data was computerized using SPSS version 20.

Results

General observation

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

Parasitaemia evaluation

From **Table 1** on day 3 post infection, no oocyst was observed in all the groups. But from days 6 to 15 a constant increase in the number of oocysts shed was observed except for groups C and D where 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 A birds shed $5 \pm 2.3 \times 10^2$ oocysts which was significantly different from that of birds in other groups. On day 9 post treatment, the least oocysts output was shed by birds in group D ($3 \pm 1.1 \times 10^2$) this was followed by groups B and C ($4 \pm 1.1 \times 10^2$ and $4 \pm 1.2 \times 10^2$ respectively), then group A birds $6 \pm 1.3 \times 10^2$. As anticipated, Group F (positive control) had the highest of $9 \pm 2.9 \times 10^2$ but no oocysts were shedded by birds in group G (negative control) all through the experimental period. From the observed results, the most effective treatments at the end of the experiment with regards to oocyts shedding was in a decreasing order of Group D 48 mg (AMP), 100% (GP+A), 50% (GP+A) and 25% (GP+A) which were most sensitive to coccidian reduction in broilers at the same significant level.

Effect of treatments on weights of broiler chickens

From day 6 to 12 Post Infection (PI), a significantly similar mean weight gain was observed across all the treatment groups compared with their controls; negative control group G had the highest while positive control group F had the least. No significant difference was observed in birds in groups A, B and C on day 15 PI. It was observed 3 days Post Treatment (PT) that weight gains in all the treated groups were similar except for group C where the weights gain was lower. From days 6 to 9 PT, groups A, B, D and E had similar weight gain while group C had the least among the treatments. When compared with the controls, group F ("infected not treated") had the least weight gain while group G "not infected not treated" had the highest weight gain at the end of the experiment.

Discussion

This study reveals that the combination of garlic powder of various concentrations with amprolium, amprolium and sulphonamides as well as amprolium alone induced anti-coccidial effect against *Eimeria tenella.* The effect was dependent on concentration as it increased with increasing concentration of garlic powder. Garlic by its high concentration exerted the greatest percentage reduction in oocysts production and a slight increase in the weight gain of infected broilers **(Table 2).** This work also showed the detrimental effect of coccidiosis on clinical signs of birds ranging from weight gain reduction, intestinal necrosis leading to reduction in appetite, decreased activity due to depression, and reduction in the concentration of red blood cells. The detrimental effect also showed in the bloody diarrhea that was observed seven days post infection. The number of oocysts recorded in all the groups post treatment significantly reduced compared to the infected non-treated control group F which recorded the highest

Table 1 Comparative Efficacy of the combination of different Concentrations of *Allium satium*+Amprolium and Sulphaquinoxaline on *E. tenella* in broiler chickens 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= × 10^2.

		Post Infection (Number of Oocysts shed (× 10^2)					Post Treatment		
Group	Treatment	Day 3	Day 6	Day 9	Day 12	Day 15	Day 3	Day 6	Day 9
A	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}
B	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}
C	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}
D	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}
E	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^{j}
F	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}
G	Control G (-ve control)	-	-	-	-	-	-	-	-
	LSD (0.05)	NS	6.85	6.02	5.71	3.63	7.60	8.69	5.68

Table 2 Effect of the Combination of Concentrations of *Allium sativum*+Amprolium, Amprolium, and Sulphonamide on the Weights of Broiler Chickens GP+A=*Allium sativum*+Amprolium, AMP=Amprolium, (AMP+Sul)=Amprolium+Sulphonamide, + ve Control(F)=Infected not treated, -ve Control (G)=not infected not treated, (-) No Parasitic infection, NS=No Significant difference where P>0.05; Values are means ± SEM. Values with different superscripts along the same column are significantly different.

		Post Infection Weight (g)					Post Treatment Weight (g)		
Group	Treatment	Day 3	Day 6	Day 9	Day 12	Day 15	Day 3	Day 6	Day 9
A	25%(GP+ A)	0.46 ± 0.02^{a}	0.55 ± 0.02^{bg}	0.62 ± 0.02^{bgh}	0.71 ± 0.02	0.83 ± 0.03^{cg}	1.03 ± 0.02^{cgh}	1.54 ± 0.02^{ch}	2.38 ± 0.02^{a}
B	50%(GP +A)	0.44 ± 0.01^{a}	0.53 ± 0.01^{cg}	0.64 ± 0.02^{chi}	0.70 ± 0.02	0.83 ± 0.02^{cg}	1.06 ± 0.02^{dg}	1.72 ± 0.02^{dgh}	2.46 ± 0.01^{ae}
C	100%(GP+ A)	0.43 ± 0.00^{a}	0.51 ± 0.03^{adg}	0.62 ± 0.03^{dgi}	0.71 ± 0.03	0.81 ± 0.02^{d}	0.89 ± 0.02^{ah}	1.15 ± 0.02^{a}	2.04 ± 0.02^{c}
D	480 mg (AMP)	0.41 ± 0.02^{a}	0.50 ± 0.03^{aeg}	0.61 ± 0.02^{egi}	0.71 ± 0.02	0.70 ± 0.03^{e}	1.13 ± 0.03^{eg}	1.86 ± 0.02^{eg}	2.41 ± 0.02^{a}
E	280 mg(AMP+Sul)	0.44 ± 0.01^{a}	0.51 ± 0.02^{afg}	0.62 ± 0.02^{fgi}	0.70 ± 0.03	0.91 ± 0.03^{f}	1.11 ± 0.04^{fg}	1.86 ± 0.04^{fg}	2.59 ± 0.05^{ae}
F	+ve Control (F)	0.44 ± 0.01^{a}	0.47 ± 0.01^{a}	0.54 ± 0.01^{a}	0.64 ± 0.01	0.93 ± 0.02^{a}	0.82 ± 0.02^{a}	0.94 ± 0.09^{a}	1.35 ± 0.07^{d}
G	-ve Control G)	0.51 ± 0.00^{b}	0.43 ± 0.01^{ad}	0.58 ± 0.01^{ag}	0.76 ± 0.03	0.92 ± 0.02^{b}	1.16 ± 0.03^{bg}	1.91 ± 0.04^{bg}	2.72 ± 0.04^{be}
	LSD (0.05)	0.04	0.06	0.06	NS	0.07	0.17	0.24	0.31

oocysts count. No significant difference was recorded in the oocysts output post treatment among the groups. The oocysts count was zero in uninfected group. Large number of oocysts was produced in all the infected groups. However, addition of *Allium sativum* of various concentrations with amprolium to the drinking water of infected chickens significantly reduced the total oocysts count. From this result it is concluded that *Allium sativum* possess anti-coccidial activity which was comparable with that of anti-coccidial drugs amprolium and the synergy of amprolium and sulphaquinoxaline. Previous researchers have found *Allium sativum* to possess a high anti-coccidial effect on avian species. For instance, Elbana [30] 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-Khatam. [26] 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. [24] reported a significant reduction of oocysts output in garlic treated mice infected with *E. papillata*. It is well known that coccidial infection reduces the growth rate of birds [31-33] but could be altered on treatment. The findings from our study revealed an increase in the body weight gain of infected and treated birds as compared to those infected and non-treated positive control in group F, but almost comparable to the negative control of group G which had the lowest and highest body weight gain post treatments. Although the highest weight gain was recorded in Group B birds, 50% (GP+AMP) which was comparable with the treatment in group E, 280 mg (AMP+SUL) on day 9 post treatment. This result is in sync with other researchers who have reported an increase in the body weight gain of chicken after they were treated with *Allium sativum*. For instance, Pourali [34], reported a significant increase in average daily weight gain of broiler chickens infected with 7.5 × 10^2 mixed oocysts of *Eimeria* species infected birds, fed with the basal diet supplemented with 29% garlic powder; and also similar to the results obtained by Elbana [30] who observed an improvement in growth performance and feed efficiency of broiler chickens supplemented with aqueous extract of *Allium sativum* and *Aloe vera* either in combination or alone after infecting them with mixed *Eimeria* species. But our obtained results is a per with the findings of Ashayeriadeh [35] who demonstrated that garlic powder added to broiler feed had no effect on body weight gain and those of Mohebbifar and Torki [36] who reported that feeding garlic at 2 g/kg did not affect feed intake, feed conversion rate and weight gain in Ross broilers. Such a wide range of results may be attributed to the dose, duration and processing of the medicinal plants as well as the experimental conditions. It can therefore be concluded that 24 mg of garlic powder with 48 mg of amprolium significantly increased the body weight gain of broiler chickens 9 days post treatments.

Conclusion

The severity of this disease based on oocysts counts and weight gain was very high. Therefore, coccidiosis caused by *E. tenella* has a destructive effect on broiler chickens. The results of this study suggests that the combination of 48 mg of garlic powder when combined with 48 mg of amprolium had a similar effect as compared to the synergy of amprolium and sulphaquinoxaline when dissolved in their drinking water in the reduction of oocysts count. Similarly, 24 mg of garlic powder when combined with 48 mg of amprolium also exerted similar effects on weight gain of chickens as the synergy of sulphaquinoxaline and amprolium in broilers chickens. Conclusively, the addition of garlic to chemical drugs (phyto-synthetic) has been shown to have great effect in the control of coccidians in chickens.

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