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Phyto-Synthetic Combination as Great Enhancers of Haematological Parameters: A Case Study in Poultry

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Abstract

This present study was conducted to evaluate the effect of graded concentrations of *Allium sativum* (garlic) powder (GP) in combination with amprolium (AMP) on oocysts counts and haematological parameters of coccidian in experimentally induced Cobb 700 broilers. They were compared with commercial drugs, amprolium and sulphaquinoxaline (SUL). Seventy day-old broiler chicks were used for this research. Broiler chickens were randomly divided into seven groups of ten replicates and five treatments (T1-T5) were administered to five groups while the last two groups served as positive and negative controls. On day nineteen, sixty birds were orally inoculated with 6×10^3 sporulated oocysts of *Eimeria tenella* and treatment commenced on day 16 Post Infection (PI). T1 to T3 were treated with 12 mg (GP)+48 mg (AMP), 24 mg (GP)+48 mg (AMP) and 48 mg (GP)+48 mg (AMP) respectively. T4 and T5 received 48 mg (AMP) and 28 mg (AMP+SUL) respectively while T6 and T7 were the positive (Infected not treated) and negative controls (not infected not treated). Results showed that infection of broiler chickens with 6 x 10^3 sporulated oocysts of *E tenella* coccidian oocysts had deleterious effects on the birds, caused faecal oocysts shedding, significant reduction of; red blood cells (RBC), haemoglobin concentration (HB) and packed cell volume (PCV). Infection also resulted to a significant increase in heterophils (HEU), lymphocyte counts (LYM) and mean corpuscular volume (MCV). Treatment commenced when 90% of infection was established (day 16 PI) and lasted for seven days. Faecal oocysts counts significantly reduced in all the treated groups with the highest effect observed in groups T3, T4 and T5; while the untreated group T6 had the highest oocyst output and T7 did not shed oocysts all through the experimental period. In the haematological indices, PCV was highest in T1 and T3 while RBC was highest in T3. HB was highest in T1. T3 and T4 had the least HEU values, T2 and T1 had the lowest LYM values while T1, T2 and T4 had the lowest MCV values post treatments. It was concluded that since the addition of *A. sativum* to amprolium significantly reduced oocysts shedding and improved haematological indices of broiler chickens, it could be used as a substitute for pure chemical drugs in the treatment of chicken coccidiosis.

Keywords: *Allium sativum*; Amprolium; Amprolium; Sulphaquinoxaline; Poultry; Haematological parameters

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Introduction

Coccidiosis has been reported to be an endemic parasitic disease of poultry causing huge damage and economic losses to poultry farmers all over the world [1]. Commercial poultry farming is constantly expanding due to its contribution in the provision of affordable and high quality proteins [2,3]. However, this sector is seriously challenged with enteric diseases like coccidiosis which is hindering its progress [4,5]. Poultry farmers over time mainly rely on the prophylactic and therapeutic use of chemicals for the

control of avian coccidiosis. However, frequent and indiscriminate uses of anticoccidial drugs have led to development of drug resistance in Eimerian species [6], rendering commercial drugs ineffective. Thus new drugs must therefore be synthesized in order to keep pace with the evolution of drug resistance strains. It is therefore a prompt necessity to evaluate alternative materials such as herbs or plant materials prepared for the control of this disease in poultry especially in rural poultry holdings where varieties of constraints prevent farmers from readily accessing medications for their birds due to its expensiveness, resistance nature and distance from commercial veterinary products. Sulphonamides have been added to amprolium in the control of *Eimeria* and other related mycotoxins. Following this basic knowledge therefore, garlic (*Allium sativum*) which is known to contain 33 sulphur compounds among which are sulphanimide, sulfonamide an antibacterial will be used. Garlic has been known to have antimicrobial, antiparasitic, antihelminthic and anticarcinogenic properties [7]. Apart from the cost effectiveness of garlic, it is also very much available and can be consumed in large quantity without causing harm to animals. This research therefore is aimed at evaluating the anticoccidial strength of garlic powder in combination with amprolium in the treatment of *E. tenella* experimentally infected chickens on oocysts counts and haematological parameters.

Materials and Methods

Location/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 research was conceived and analysis of data was conducted.

Collection and processing of *Allium sativum* powder

Mature bulbs of *A. sativum* were purchased from a vegetable market in Jos-North local government area of Plateau State, following the onset of dry season. They were peeled and air dried in a shade to minimize the effects of ultra-violet rays from the sun on the active ingredients of garlic. Drying lasted for two months, was blended to almost a fine powder and sieved with wire mesh.

Experimental birds

Seventy day old Cobb 700 unsexed broiler chickens were obtained from a hatchery in Jos metropolis and they were brooded under standard conditions for nineteen days before the commencement of the study. They were fed standard pelletized broiler starter feed for the first four weeks and followed with a finisher until the end of the experiment. Adequate light and water was given ad libitum. For the first 2 weeks, the birds were vaccinated against infectious Bursal disease and Newcastle disease following the conventional vaccination schedule for broilers. The vaccines were obtained from NVRI, Vom and were administered via their drinking water after 12 hours of water starvation. Birds were then moved into individual battery cages for the commencement of study.

Innoculation of birds with coccidia

The sporulated oocysts of *Eimeria tenella* inoculum used was 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. T1-T6 received same number of oocysts while T7 was given distilled water all through the experimental period.

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). 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 and application of drugs

A total of seventy birds were randomly divided into seven groups of ten replicates, groups T1 to T7. The amount of garlic used for treatment was calculated as a percentage of the amprolium. Thus: T1 represents infected and treated with 48 mg of amprolium and 12 mg of garlic powder (25% of garlic); T2 represents infected and treated with 48 mg of amprolium and 24 mg of garlic powder (50% of garlic); T3 represents infected and treated with 48 mg of amprolium and 48 mg of garlic powder (100% of garlic); T4 represents infected and treated with 48 mg of amprolium only. T5 represents infected and treated with 28 mg each of amprolium and sulphaquinoxaline. T6 represents infected and not treated (positive control). T7 represents non infected and non-treated (negative control). Treatment commenced on day sixteen PI at the establishment of about 90% level of parasitaemia which was confirmed by clinical signs and oocysts counts. The dose of amprolium and sulphaquinoxaline given was according to manufacturer's prescription. Drugs were weighed using PB153 Mettler Toledo weighing scale and dissolved in 0.4 ml of distilled water for each bird and was given orally. Treatment lasted for seven days.

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 Long and Joyner [8]. Results were recorded as the number of opg shed by each bird.

Blood collection from birds

Collection of blood samples from three birds in each group was done six days post infection (PI) and seven days post treatment (PT). About 2 ml of blood was randomly collected from three birds in each group to evaluate blood parameters such as red blood cell counts (RBC), packed cell volume (PCV), heterophil (NEU), lymphocyte (LYM) and haemoglobin concentration (HBC). The blood collection was done via the wing vein with the aid of needle and syringe and immediately transferred into a sterile tube containing the anticoagulant, ethylenediaminetetraacetic acid (EDTA) and labeled accordingly. Samples were taken to the NVRI Heamatology Laboratory and analysed using standard laboratory techniques. Particular attentions were given to the staining methods for heterophils and lymphocytes.

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 [9]. Data was computerized using SPSS version 20.

Results

General observations

It was generally observed that most of the infected chickens experienced decrease in weight gain, reduction of appetite, paleness of their combs, ruffled feathers and bloody stools were also seen. These features were absent in T7 were all the birds remained healthy and did not shed oocysts in their faeces. No mortality was recorded in all the groups until the end of the experiment.

Evaluation of parasiteamia

Fresh feacal samples of the broilers were collected every three days PI and no parasite was seen after the first collection. In **Table 1**, it was observed that there was a constant increase in the number of oocysts shed from day 6 to day 15 PI in all the infected groups except in groups T3 and T5 where there was a slight reduction in the trend on day 12 PI. On day 3 PT, a slight reduction in oocyst output was recorded in all the groups except in T1 where occysts was greatly reduced from 24 ± 3.6 × 10^2 to 5 ± 2.34 × 10^2 and marked significant difference from other groups. At the end of the experiment, T4 had the least oocyts count of 3 ± 1.1 × 10^2 which was immediately followed by T2 and T3 with 4 ± 1.1 × 10^2 and 4 ± 1.2 × 10^2 respectively. Positive control group T6 had the highest oocysts output of 25 ± 1.5 × 10^2 while negative control group T7 still maintained a state of no oocysts at the end of the experiment. **Table 2** shows a comparison of the treatments with garlic powder and amprolium at different concentrations. No significant difference was observed in all the treatments although the effect of the concentrations of GP and AMP was highest in T2 and T3. T1 had the highest oocyts output at the end of the experiment.

Haematological evaluation

The comparative effect of different treatments among the groups of broilers with respect to their blood parameters are indicated in **Table 3**. A significant difference was observed between all haematological parameters among the different groups of birds except for lymphocytes and mean corpuscular volume, post infection. The highest percentage of PCV was recorded in group T5; (28 mg AMP+SUL) post infection while the least (50% GP+A), was observed in group T2. Group C (100% GP+A) and group T4 (480 mg AMP) had same significant level. The red blood cell count (RBC) recorded was lowest (1.46 ± 0.04) in group T1, which was significantly different from the rest of the treatments post infection. No significant difference was observed among the various treatment groups with respect to the haemoglobin concentration except for the controls where the positive control had the least and the negative control had the highest haemoglobin concentrations post infection. The highest 19 ± 0.04 percentage of heterophil (NEU) was recorded in group T3: 100% (GP+A), while the least 11 ± 0.31 and 11 ± 0.32 values were observed in 25% (GP+A) and 50% (GP+A) respectively. There were significant differences observed across all haematological parameters except HB concentrations post treatment. The highest (30 ± 0.86) percentage of PCV among all the treatments was observed in group T3: 100% (GP+A), while the least 24 ± 0.66 was observed in group T2 post treatment. In the red blood cell (RBC) post treatment, a significant difference was observed along the treatments with group T3 having the highest 4.78 ± 0.13 count 100% (GP+A) while the least 1.65 ± 0.04 was observed in group T2, 50% (GP+A). Observations in heterophil counts showed a significant difference along the treatments. The least percentage 5.30 ± 0.15 was observed in group T3: 100% (GP+A) and group T4: 480 mg (AMP) 5.80 ± 0.17. As observed in the Lymphocyte counts, the highest 47 ± 1.36 was recorded in group T3: 100% (GP+A) while the least 26 ± 0.75 was recorded in group T1: 25% (GP+A) and group T2: 50% (GP+A) 23 ± 0.67. The MCV value recorded the highest 139 ± 4.01 in 50% (GP+A), while the least 63 ± 81 was in group T3: 100% (GP+A).

Discussion

This study reveals that the combination of garlic powder of graded concentrations with amprolium induced anticoccidial effect against *E. tenella* oocysts and the results were comparable to pure chemical drugs that were used, that is, amprolium alone and the synergy of amprolium and sulphaquinoxaline. The effect was dependent on concentration as it increased with increasing concentration of garlic powder. The group treated with 48 m Ggp+48 mg AMP and 24 mg GP+48 mg AMP had the least oocysts output at the end of the experiment. Similar dose related responses in faecal oocysts counts and mortality in coccidian infections of broiler chickens has been reported by other researchers. Biu [10] reported dose dependent reduction in faecal oocysts from 57,000 to 5800 o/g for 200 mg/kg, 81,000 to 22,000 o/g for 1,600 mg/kg, 78,100 to 1,300 o/g for 800 mg/kg and 74,100 to 2,000 o/g for 1600 mg/kg neem (*Azadirachta indica*) aqueous extract at 4 days post treatment while El-Khatam [11] reported dose dependent faecal oocyts counts on broilers infected with *Eimeria* species and treated with turmeric (*Curcumalonga)* or garlic at 10 g/l or 5 g/l of each agent. The result obtained was comparable with the oocyst reduction observed in birds treated with control drugs; amprolium and

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

		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
T1	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}
T2	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}
T3	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}
T4	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}
T5	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}
T6	Control (+ve)	-	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}
T7	Control (-ve)	-	-	-	-	-	-	-	-
	LSD(0.05)	NS	6.85	6.02	5.71	3.63	7.60	8.69	5.68

GP+A=*Allium sativum*+Amprolium, (-)=no parasitic infection, +ve control=infected not treated, -ve control=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)

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 (× 10^2)]					Post Treatment × 10^2		
Group	Treatment	Day 3	Day 6	Day 9	Day 12	Day 15	Day 3	Day 6	Day 9
T1	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}
T2	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}
T3	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

(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)

Table 3 Comparative Effect of the combination of *Allium sativum*+Amprolium, Amprolium and Sulphonamide on Haematological parameters of Broiler Chickens.

		Post Infection (Values)						Post Treatment (Values)					
Group	Treatment	PCV (%)	RBC (cells/L)	HB (g/L)	HEU	LYM	MCV	PCV	RBC	HB	NEU	LYM	MCV
T1	25 % (GP +A)	21.10 ± 0.6^{a}	1.46 ± 0.04^{c}	9.80 ± 0.28^{ch}	11 ± 0.31^{ci}	89 ± 4.40	132 ± 6.6	31 ± 0.89^{a}	2.80 ± 0.08^{ci}	15.24 ± 0.43	7.30 ± 0.21^{bg}	26 ± 0.75^{ci}	11 ± 3.20^{bh}
T2	50%(GP+A)	18.88 ± 0.5^{be}	1.60 ± 0.04^{ag}	9.80 ± 0.28^{dh}	11 ± 0.32^{di}	89 ± 4.40	128 ± 6.4	23 ± 0.66^{cf}	1.65 ± 0.04^{dh}	11.28 ± 5.45	7.50 ± 0.22^{cg}	23 ± 0.67^{di}	9 ± 4.01^{cg}
T3	100%(GP+A)	20.4 ± 0.5^{ae}	1.96 ± 0.10^{dfg}	12.60 ± 0.36^{ei}	19 ± 0.54^{eh}	81 ± 2.31	133 ± 6.5	30 ± 0.86^{a}	4.78 ± 0.13^{e}	7.96 ± 0.23	5.30 ± 0.15^{dh}	47 ± 1.36^{e}	63 ± 1.81^{d}
T4	48mg(AMP)	20.1 ± 0.5^{ade}	1.75 ± 0.09^{ah}	11.60 ± 0.33^{f}	15 ± 0.43^{f}	85 ± 2.4	136 ± 6.8	27 ± 0.77^{d}	37 ± 0.06^{f}	10.17 ± 0.33	5.80 ± 0.17^{efhi}	41 ± 1.18^{fj}	14 ± 1.81^{eh}
T5	28MG(AMP+Sul)	23.77 ± 0.8^{cd}	1.80 ± 0.09^{eh}	12.10 ± 0.34^{gi}	13 ± 0.37^{g}	86 ± 2.4	133 ± 6.5	24 ± 0.69^{ef}	3.00 ± 0.08^{gi}	11.52 ± 0.33	6.20 ± 0.18^{ai}	38 ± 1.09^{j}	80 ± 2.30^{f}
T6	Control(+ve)	21.10 ± 0.6^{a}	1.65 ± 0.04^{a}	8.80 ± 0.44^{a}	17 ± 0.49^{a}	82 ± 2.36	131 ± 6.55	30 ± 0.86^{a}	3.32 ± 0.09^{a}	14.08 ± 0.40	6.00 ± 0.17^{a}	30 ± 0.87^{a}	90 ± 2.59^{a}
T7	Control(-ve)	22.06 ± 0.6^{ad}	1.95 ± 0.08^{bf}	13.60 ± 0.25^{b}	20 ± 0.57^{bh}	90 ± 2.59	139 ± 6.95	23 ± 0.66^{bf}	1.75 ± 0.05^{bh}	14.64 ± 0.40	6.20 ± 0.18^{af}	38 ± 1.09^{bh}	31 ± 3.78^{ag}
	LSD (0.05)	1.78	0.15	0.99	1.36	NS	NS	2.37	0.26	NS	0.56	3.12	9.40

Positive control (+ve)= Infected not treated, Negative Control (-ve)= Not infected not treated, (GP+AMP)= *Allium sativum*+Amprolium, AMP=Amprolium, (AMP+Sul)=Amprolium+Sulphonamide, PCV=Packed Cell Volume, RBC=Red Blood Cell counts, HB- Heamoglobin concentration, HEU=Heterophils, LYM=Lymphocytes, MCV=Mean Corpuscular Volume, NS=No Significant difference where P>0.05

sulpaquinoxaline and thus it can be concluded that garlic powder posses anticocidial agent. Similar reports abound in literatures, for instance, Elbana [12] 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. Similarly, El-Khtam [11] 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 [13] reported a significant reduction of oocysts output in garlic treated mice infected with *E. papillata*. With respect to haematological analysis, it was observed that there was a significant reduction in the packed

cell volume, haemoglobin concentration and red blood cell counts post infection. An increase in the neutrophils, lymphocyte counts and mean corpuscular volume was also recorded among the infected groups of birds. Coccidiosis is associated with clinical signs of bloody diarrhea and anaemia in chickens [14-16]. These parameters changed significantly post treatment and increase in the red blood cell, heamoglobin concentration and packed cell volume was recorded. There was also a reduction in heterophils, lymphocytes count and mean corpuscular volume following treatments. PCV and RBC were highest in group T3: 100% (GP+AMP) post treatment. These results agree with those of Witlock [17], who observed a significant decrease in red blood cells, Haemoglobin concentration and Packed Cell Volume of *E. tenella* infected chickens and suggested that the decline may be due to the severe bleeding and tissue damage in the mucosa of duodenum originated from invasion of *E. tenella*. Furthermore, Patra [18] recorded an increase in the infiltration of heterophils when they infected broiler chickens with *E. tenella*. They observed heterophils infiltration increases immediately after infection as a first defense line followed by Eosinophils concentration as a response to parasitic infection. Ogbe [19] also observed a slight drop in PCV in the broilers infected with the Houghton strain of *E. tenella* on the seventh day post infection and attributed it to the virulent nature of *E. tenella* in chickens, their results confirmed the effectiveness of both amprolium and *Ganoderma lucidium* extract against the species. Anaemia caused by *E. tenella* was characterizsed by the decrease in number of red blood cells and decreased PCV. This effect was ameliorated by 100% (GP+AMP), the highest values of PCV and RBC were recorded after treatment in group T3 broiler chickens. The increase in lymphocyte count may be attributed to the effect of the inflammations of the caeca and intestine.

Conclusion

The administration of the combination of *Allium sativum* powder in different concentrations was able to emeliorate coccidian infection by reduction in oocyts output. This effect was greatest in 48 mg GP+48 mg AMP and 24 mg GP+48 mg AMP which was comparable to that observed in control drug 48 mg of amprolium. Due to the high nutritive value of garlic added to the synthetitic drugs, haematological indices; PCV, HB and RBC were able to improve in all the treated groups. RBC value of group T3 birds was comparable to those that were treated with the synergy of 28 mg of sulphaquinosalin and amprolium. Finally, these suggests that garlic powder can be used in combination with amprolium in the treatment of coccidiosis caused by *Eimeria tenella* at a high dose of 48 mg GP+48 mg Amp. Based on this research, it can be concluded that the combination of garlic powder and amprolium is coccidiocidal.

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