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An Evaluation of the Anticoccidial Potency of Some Commonly Used Anticoccidial Drugs in Broiler Industry in Abia State

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Abstract: Avian coccidiosis is a common parasitic disease of broiler chickens caused by single protozoan parasite of the genus Eimeria that infects the intestinal tract and is transmitted among the birds via ingestion of infective oocysts during feeding. 150 day old broiler chicks were used for this study. They were randomly grouped into 6 groups (A – F), weighed and introduced to the new litter containing contaminated litter from a previous coccidial outbreak at the farm. Each group of birds contained 25 birds. Groups A, B, C, D and E were placed on deep litter while group F was kept on a suspended wire mesh. Groups A, B, C and D were medicated from day 6 post infection with different anticoccidial agents respectively. Group E was infected non – medicated while group F was non – infected/non-medicated. PCV was taken across the groups at 14, 21 and 28. Also weight gains were taken across the groups at days 1, 7, 14, 21, 28, 35, 42, 49 and 56. Parameters such as mortality, postmortem, activity score and facial score were observed. Flotation technique was used for coprological analysis. The study lasted for 56 days. Groups B, C and D showed reduced efficacy. Group A was most efficacious in comparison with group F and the rest of the groups as the major parameters used revealed statistical difference in favour of group A. The data obtained from this study were summarized and represented by means. There was significant difference ($P<0.5$) in the treatment groups. The results seen from this study has shown that coccidiosis intervention should start early in broiler production and Amprolium 250® is the best recommended drug from this study.

Key words: Coccidosis · Broilers · Amprolium · Abia

INTRODUCTION

Poultry industry, a subsector of the livestock sector, outnumbers all other forms of livestock sector in developing countries and is the fastest way of obtaining animal protein within a short period because of its short generation interval [1-4].

Coccidiosis is one of the most detrimental and lethal management diseases of poultry (One of the most significant poultry diseases is coccidiosis [5] and it is said to cost commercial poultry producers a loss of at least 1.5 billion US$ every year globally [6-8]. In broilers, control of coccidiosis is not limited to the prevention of clinical disease and mortality, since mild and subclinical infections are also important as even minor intestinal lesions can interfere with growth and feed efficiency and therefore profitability. In addition to management measurements (Litter condition, flock density); the disease has largely been controlled by addition of anticoccidial drugs to the feed [9]. The worldwide intensive use of anticoccidial drugs to prevent coccidiosis, has inevitably led to the development of resistance to all anticoccidial drugs as long term exposure to any drug will result in loss of sensitivity

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[10-12]. The World Health Organization Scientific Group [14] defined resistance in broad terms as 'the ability of a parasite strain to survive and / or to multiply despite the administration and absorption of a drug given in doses equal to or higher than those usually recommended but within the limits of tolerance of the subject'. Generally, drug resistance in coccidia can be complete, in which case increasing doses up the maximum tolerated by the host is ineffective (i.e. diclazuril, nicarbazin). In contrast, relative resistance to anticoccidial drugs is characterized by the fact that increasing doses tolerated by the host still will show efficacy (i.e. ionophores).

This study has been conducted to investigate the efficacy of some of the most commonly used anticoccidials in Abia state and to evaluate some usual anticoccidial efficacy indices used in literature for measuring resistance.

MATERIALS AND METHODS

Location and Time of Experiment: The experiment was carried out in Michael Okpara University of Agriculture, Umudike. Abia State. Southeast of Nigeria. The study was conducted between May and July, 2014. The experiment had a course of 56 days.

Study Population: 150 broiler chicks, purchased at day old from a commercial hatchery were used for the study. They were kept in a deep litter standard poultry houses. The stocking density was approximately 13 birds per m^3.

Sampling Procedures and Sampling Size: The birds were divided randomly into 6 groups and weighed at day old by a restricted randomized procedure (RRP) that initially equalized their weights. Thereafter, they were allocated 25 birds per pen. The birds were reared at a standard condition and given feed and water *ad libitum*. They were routinely vaccinated against Newcastle and Infectious bursal diseases.

Control 1 was 25 birds and were housed on a suspended wire meshed cage. The birds were kept in accordance with the recommendations in the Guide for Care and Use of Laboratory Animals [14].

Parasites and Sample Collection: Natural infection with *Eimeria* was allowed to occur by providing all the necessary conditions needed for the coccidian infection. This was aimed at providing the condition (moisture and warmth) necessary for the sporulation of the oocysts, as we are in the tropical area where warmth is always available. Thus, three conditions necessary for sporulation, i.e. warmth, moisture and oxygen were provided [15, 16].

Drugs Used: The drugs that were used were Embazine forte® (Sulphaquinoxaline 188mg/l and diaveridine 19.6mg/l). Amprolium (Amprolium 250WSP®,). Dicoxin plus® (Amprolium HCl 17,5g and Sulphaquinoxaline 17,5g) and Pantacox® (Sulphamerazine sodium 200mg, sulphaquinoxaline sodium, pyrimethamine sodium, furaltadone Hcl.

Infection of Birds: The birds were introduced to the litter from day old following arrival. This was done to mimick a field condition. Clinical signs were recorded. The dead birds were examined for any post mortem lesions. Intestinal scrapings and faecal floation were conducted.

Drug Administration: Embazine forte® was obtained and administered in drinking water for 3-1-2 days according to manufacturer's instructions. Amprolium (Amprolium 250WSP®) was administered in water for a period of 5 days as prescribed by the manufacturer. Dicoxin plus® and Sulphaquinoxaline 17, 5g, Vitamin A and K) was administered in water for a period of 5 days. Pantacox® was administered in water for a period of 3 consecutive days.

Experimental Design: The birds were randomly divided into 6 groups (A, B, C, D, E and F) from day one each group containing 25 birds. Of these, birds of five groups (i.e. group A-E) were kept on deep litter containing moist infected/contaminated litter. Group F (Control 1) birds were kept on suspended wire meshed cage free of wood shaving and this group was the non-infected non medicated group. Group E (Control 2) infected non-medicated

At day 6 post infection (PI) birds in group A were medicated with amprolium while birds of groups B, C and D received Dicoxin plus® (Amproliuum HCl and Sulphaquinoxaline), Pantacox® (Sulphamerazine sodium 200mg, Vitamin K3 5mg, Vitamin A 15,000iu), Embazin forte® (Diaveridine and Sulphaquinoxaline) respectively.

Evaluation of Drug Efficacy: Mortality was recorded each day during the experiment. All birds that died in the course of the study were examined for intestinal lesions due to coccidiosis [17]. Ten birds were randomly picked from each group and weighed at days 1, 7, 14, 21, 28, however, when the infected non – medicated group reduced by mortality to 6, 6 birds from across the groups were weighed at 35, 42, 49 and 56. The body weight gains

were recorded, calculated and expressed in grams, average and percentages. Activity scores were recorded with 5 being normal (Alert) activity, 4-slight deviation of normal activity, 3- showing weakness, depression, somnolence and 2 and 1 representing moribund state. Also the colour and consistency of faeces was recorded from day 5 post infection. Blood samples (0.6-1.3 ml) were collected by jugular vein puncture from 3 chickens randomly selected from each group in all groups (A-F) at days 8, 17, 24 of age of the chickens. The packed cell volume was determined by microhaematocrit method of Campbell and Coles [18].

Statistical Analysis: The data (On weight gains, consistency and colour of faeces, packed cell volume and activity score) obtained from the study were summarized and represented as means=SEM. Statistical analysis of the treatment groups were made using statistical package for social sciences (SPSS). The results were made from the analysis.

RESULTS

The result shows that different mortalities were recorded across the different treatment groups from day 6 to day 27 of the experiment. Mortalities were observed from group A – E before and in the course of treatment. Mortality before treatment was recorded as follows; groups A – 2; B – 1; C – 2; D – 2; E – 1 and F – 0. In the course of treatment and after treatment mortality across the groups were; Groups A – 0; B – 10; C – 10, D – 8, E – 18 and F – 0 birds There was no mortality in group F (Control 1) throughout the course of the study. Amprolium treated birds (Group A) recorded only 2 mortalities which were prior to the commencement of medication. Mortality was highest in infected non – medicated birds (Group E), followed by Pantacox treated birds (Group C), Dicoxin plus treated birds (Group B) and Embazine forte treated birds (Group D) in that order. Mortality was not recorded in non – infected – non – medicated birds (Group F). At day 6, mortality reached up to 5 in group E as shown in Figure 1. Necropsy revealed gross lesions typical of coccidiosis (Ballooning of the large intestines and petechial haemorrhages of the serosal surface of the large intestine, thickening of the wall of the caeca with a blood filled lumen), Anosa *et al*. [14]. There was no mortality in group F (Control 1) throughout the course of the study. Amprolium treated birds (Group A) recorded only 2 mortalities which were prior to the commencement of medication.

The result shows that different weight gains were recorded across the different groups from day 1 to day 56 of the experiment. From week 5 to week 8 there was accelerated growth among the groups (Figure 2). However, this accelerated growth was most prominent in group F and least in group E.

Non – infected non – medicated birds (Group F) had the highest average weight gain followed by amprolium treated birds (Group A); Embazine forte treated birds (Group D); Pantacox treated birds (Group C); Dicoxin plus treated birds (Group B) and infected non – medicated birds (Group E) in that order. Group E had the least weight gain among the groups, thus the group averaged the least weight among the groups at any given time. At day 56 of age, the mean body weight gain of group E birds (1841.67g) was significantly lower than the group F (2825g) birds. The mean weight gains of group B (1841.67g), group C (1891.67g) and group D (1966.67g) were significantly lower than Amprolium group (2825g) birds (Figure 2).

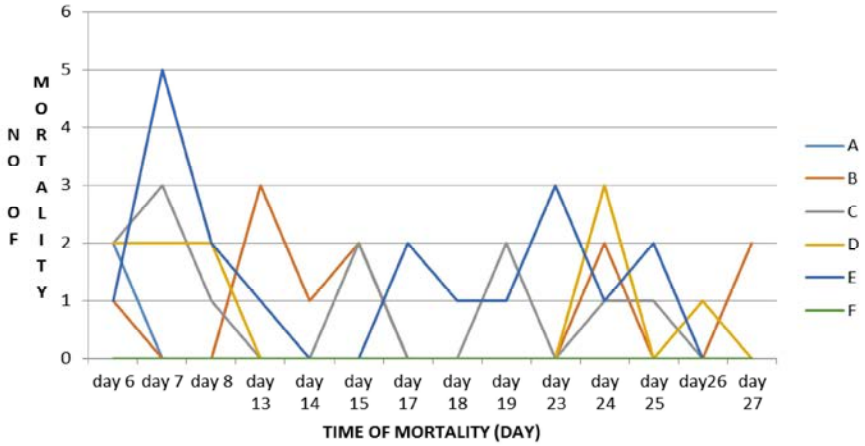

Fig. 1: Line graph of mortality across different groups of birds

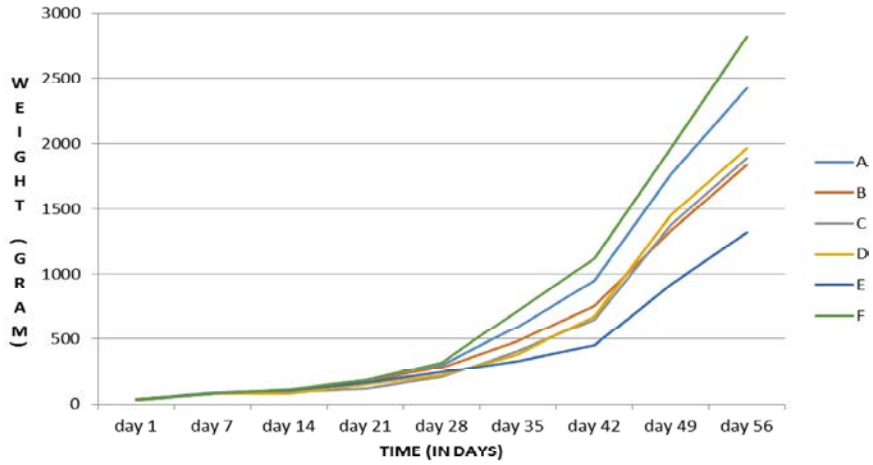

Fig. 2: Line graph of average weight gain per week of the different treatment

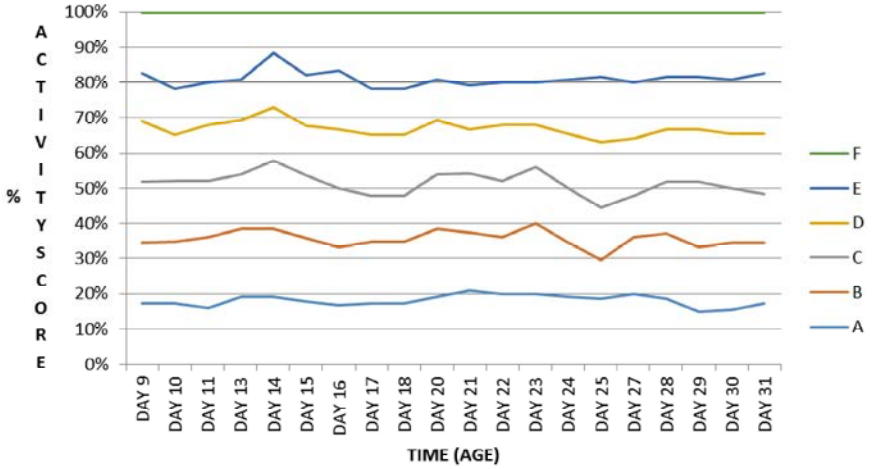

Fig. 3: Line graphs of activity score across different bird groups.

Table 1: Packed Cell Volume of the Treatment Groups Within the Period of Infection

DAYS	GROUP A	GROUP B	GROUP C	GROUP D	GROUP E	GROUP F
14	24.67±2.0817^{ab}	25.33±5.6862^{ab}	21.83±5.0083^{b}	24.50±4.3301^{ab}	20.33±1.5275^{b}	29.33±1.5275^{a}
21	26.67±1.5275^{b}	24.17±1.0408^{b}	20.33±1.5275^{c}	20.67±2.5166^{c}	20.00±1.7321^{c}	31.33±1.15470^{a}
28	25.67±2.0817^{b}	24.67±1.1547^{b}	22.67±1.1547^{b}	25.33±5.1316^{b}	24.00±3.0000^{b}	32.67±4.1633^{a}

Different superscripts within a column indicate significant difference between the means, abcd. $p<0.05$.

The result shows that different packed cell volume (PCV) were recorded across the treatment groups from day 14 to day 28 of the experiment. The PCV was highest in the non – infected – non – medicated birds (Group F) whereas the PCV across groups A-E was not clearly separated (Table 1)

The activity score across different treatment groups were recorded from day 9 to 31 of the experiment and the result shows that non – infected – non – medicated birds (Group F/control 1) was the most active, followed by Pantacox treated birds (Group D), Embazine forte treated birds (Group C), Dicoxin plus treated birds (Group B) and amprolium group in that order as seen in figure 4. Group E had the lowest mean activity score of 3.65 while groups A, B, C and D had much higher scores of 4.65, 4.50, 4.05, 4.00 respectively (Figure 3). There were no significant difference ($P<0.05$) between groups A, B and F. However, groups A, B and F had significant difference to groups C, D and E. group C had significant difference to group E but not to group D. Group D was not difference in significance to group E. Group E (Control 2) had the highest mean brown faeces of 1.45 while groups A, B, C and D had much lower scores of 0.50, 0.50, 0.60 and 0.95 respectively (Figure 3).

Plates: Coccidia Oocyst: The results of intestinal scrapings (of mortalities) at PM was taken from different sites of the intestine from groups A – E. Microscopic examination shows that the oocysts had varied sizes and the oocysts size are species dependent thus, revealed that the coccidiosis was due to mixed *Eimeria* species infection. Also, the different locations from which the scrapings was collected confirmed that the coccidiosis was a consequence of mixed *Eimeria* species infection, this is because different species of *Eimeria* are known to affect a particular location of the intestine. This is in line with what the earlier workers reported [3, 15, 16] who said that field outbreak of coccidiosis are usually a mixed infection.

The result of gross PM lesion revealed that the coccidiosis was due to mixed *Eimeria* species infection, with caecal coccidiosis predominating as it is seen in all the birds examined at PM (Plate A - D).

DISCUSSION AND CONCLUSSION

Chicken coccidiosis is a persistent problem of universal importance in poultry production [17]. Evaluation of some contemporary drugs in the treatment of coccidiosis in broiler was studied. These drugs were selected from the commonest ones in present day coccidial intervention. They included those that have coccidiocidal activity like amprolium; Dicoxin plus (A combination of amprolium and sulphaquinoxaline); Embazine fort which is about the most frequently used; and those with coccidiostatic action like pantacox which is sulphonamide based. The parameters used in this evaluation include mortality, weight gain, packed cell volume (PCV), activity score and presence of brown faecal materials (Faecal score).

In this study, mortalities were observed from group A-E before and in the course of treatment and PM examination done. From the result shown Figure 1, the ability of amprolium to prevent mortality in the medicated group of birds shows that the drug was more efficacious in the control of coccidiosis when compared to Dicoxin plus® (Amprolium HCl and sulphaquinoxaline), Pantacox®(sulphamerazine sodium, sulphaquinoxaline sodium, pyrimethamine sodium, furaltadone HCl) and Embazine forte®(sulphaquinoxaline, Diaveridine) which showed reduced efficacy; these results were better than seen in group E (Control 2). This shows that coccidia organisms had varied resistance across groups B, C and D. There was significant difference ($P<0.05$) across the groups; Group E had the highest mean mortality followed by group C>B>D>F (Figure 1). This agrees with the findings of some authors [3, 11, 14, 16]. Mortality is the primary criterion of measuring efficacy and ratios of mean weight gains and faecal scores are used as indicators of morbidity [19]. Mortality, despite treatment, is a very good indicator of resistance problems in coccidal cases on the field [20]. The first sign of coccidiosis was sudden onset of mortalities. This agrees with [21]. Mortalities observed at day 6 of age in this study has shown that there is a shift in age of affected chicks. This is contrary to earlier reports by some workers [21] reported that disease typically occurs at 3 – 6 weeks of age but may occur in older birds [22] in the same vein reported that caecal coccidiosis rarely affect chicks below two weeks. Mortality stopped at day 27 of the experiment, implying that the disease is self-limiting which agrees with finding of earlier workers [16].

From the results above amprolium was more efficacious in improving mean weight gains when compared to the non-medicated/infected group E (control 2) and non-medicated/non – infected group F (Control 1). In addition, amprolium was also more efficacious than Decoxin plus® (Group B), Pantacox® (Group C) and Embazine fort® (Group D) in improving weight gains. This does not agree with those of Anosa *et al*. [14] who showed that birds treated with Embazine fort® had a higher mean weight than birds treated with amprolium but no significant difference statistically. This could be due to the fact that with increased use of a particular anticoccidial, over time, the coccidia organism has a tendency of developing resistance [4, 12, 14]. This is true of this study because the coccidia organisms used to infect birds in this study was gotten from previous outbreak in the farm where the study was conducted. This farm has the history of preferring Embazin Fort® to other anticoccidials [23] has established that a reduced efficacy of an anticoccidial drug always has an adverse effect on weight gain and or on the feed conversion. These factors play a decisive part in practice, since they determine the economic success of the operation [20]. Comparing the results of mortalities and weight gain in this study, mortality as well as reduced weight gain due to coccidiosis should be the most reliable sign of resistance to anticoccidials. This is contrasted with Bedrnik [23] who reported that ‘sthe occurrence of deaths due to coccidiosis is the most reliable sign of resistance to anticoccidials. This is important because almost almost 70% of the estimated cost (€2.3 billion of coccidiosis annually in Sweden) is due to subclinical coccidiosis, by impact on weight gain and feed conversion rate [24].

The result of the mean PCV (Table 1) shows that the disease resulted to anaemia. Anaemia might have been due to intestinal destruction, resulting to blood loss, a consistent finding in *Eimeria* species infection in chickens [17].

At day 28 of age, the mean PCV of group A (25.67), B (24.67), C (22.67), D (25.33) and E (24.00) were significantly lower ($P<0.05$) than that of group F (32.67%) at 5% but had no significant difference across groups A – E. The result equally showed no significant difference across the groups, this is probably due to recovering mechanism of the body at this time. At day 21 of age, there was no significant difference ($P<0.05$) in PCV between the groups A and B and no significant difference ($P<0.05$) in PCV between groups C, D and E but groups A and B had significant difference to groups C, D and E. This therefore implies that PCV may not be a good indicator in efficacy monitoring of coccidials especially in the field where chickens are maintained more often than not in the same litter or environment during and after treatment. Nonetheless, the result of this study shows that there was a positive correlation between high PCV and weight gain. This is because group F birds maintained high PCV at day 14 (29.33%), day 21 (31.33) and day 28 (32.67). The result of brown faeces or faecal score in Figure 4 shows that Amprolium and Dicoxin plus® were most efficacious in reducing the deviation of the appearance of droppings from normal, followed by pantacox® and embazine fort® in that order. This is in agreement with previous studies on the anticoccidial activity of amprolium [16].

The result of the activity score in Figure 4 shows that with development of resistance, activity score may not show variance with infected non – medicated and birds medicated with drugs to which the organism is resistant to. This result implies that when any anticoccidial is used in the treatment of coccidiosis and the activity score of the birds shows no improvement, it is most probably an indication for coccidia resistance to the drug. Hence, the drug should be changed. This is in agreement with Douglas *et al.* [26] who defined disease as abnormality of structure or function with an identifiable pathological or clinicopathology basis and with a recognized syndrome.

The outcome of this evaluation has highlighted the severe economic loss emanating from coccidial infection in broiler chicken. This was evident as the final average weight of the non-infected non treated birds doubled that of the infected non-treated birds. Therefore, every step should be taken to prevent its occurrence if profitability is to be maximized. Also, it is recommended that coccidial intervention be introduced early as chicks are now coming down with coccidiosis at a very early age. Although Embazine fort®, Pantacox® and Dicoxin plus® were efficacious, Amprolium 250® was superior and is thus recommended for therapy of coccidiosis in broiler chicken. Shuttle or rotation program (The use of different coccidial drugs at different growth stage or use of different coccidial drugs for different batches of birds respectively) [17] should be adopted to avoid the risk of developing resistance by coccidia organisms. Embazine fort® produced reduced efficacy in this study when compared to that it produced in Anosa *et al*. [14]. This is perhaps due to the history of use of Embazine fort® in this farm. Pantacox®, a coccidiostat showed the poorest performance among the treatment groups as the group recorded the second highest mortality and a lower weight gain. Therefore, with the exception of breeder stock, Pantacox may not be appropriate for use in broiler chickens due to its coccidiostatic action which enhances immunity development against coccidiosis over time [27]. This may not be of use in the broiler industry as these birds are early maturing and are not kept for long on farms. Also, they are meat producing and the withdrawal time should be considered.

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