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Evaluation of the coccidiocidal effects of *Vernonia amygdalina* and *Aloe vera* aqueous extracts on poultry in Bambili, North West, Cameroon

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Coccidiosis is a disease transmitted through ingestion of contaminated food or water with sporulated *Eimeria* oocysts. Bloody diarrhoea is the primary symptom. Farm poultry production systems are recently challenged with the concept of "clean, green, ethical" which promotes limited use of drugs, chemicals and hormones for poultry welfare. Substituting synthetic drugs with plant-based supplements such as *Vernonia amygdalina* and *Aloe vera* could ensure healthier food for humans. This study aimed at evaluating the use of these plant extracts in controlling chicken coccidiosis. Chicks were infected by inoculating oocysts in their water. Four chicks were placed in 24 separate cages, infection confirmed by observing for blood in faeces, followed by stool microscopy. Plant extracts were gotten by decoction and maceration extraction methods and administered at different concentrations (100, 200, 300 and 400 mg/L) to treat coccidiosis in 66 infected chicks. Chicks were weighed to check for weight gain and feed conversion ratio and growth performance. Different periods (4-9 days) were used to observe the effect of the extracts. Blood was collected from the chicks two weeks after inoculation of oocysts to determine whether these extracts could be beneficial to some haematological parameters. Concentration of 200 mg/L was the most effective dosage followed by 400 mg/L. The extracts showed some significant effects on the growth performance and on haematological parameters (Hb and platelets). Conclusively, *A. vera* and *V. amygdalina* are not only beneficial for improvement of chicken growth but also show some effects on haematological parameters and seem to be a good natural alternative for treatment of coccidiosis in chicken.

Key words: *Aloe vera*, Vernonia *amygdalina*, plant extract, coccidiocidal, chicken.

INTRODUCTION

Coccidiosis is an intestinal tract infection that is caused by single celled, eukaryotic, heterospecific organisms (protozoa) called coccidians. About 1800 *Eimeria* species infect the intestinal tract of different animals and birds

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(Haug et al., 2008) and infection with this parasite normally occurs through ingestion of contaminated feed or water contaminated with sporulated oocysts (Allen and Fetterer, 2012) and it is one of the most alarming problems for chicken, porcine and calf rearing industries (Pangasa et al., 2007a; Singla et al., 2018; Jaiswal et al., 2023). Parasitic diseases have remained a major problem limiting the expansion and profitability of developing agricultural countries (Mohammed and Zakaiya'a, 2015), where skilled husbandry inputs have not matched the rate of expansion and intensification of livestock holdings. Diarrhoea, which may become bloody in severe cases, is the primary symptom resulting from coccidiosis caused by the pathogenic *Eimeria* species, and other symptoms might include: dysentery, enteritis, emaciation, lower feed conversion rate, delayed sexual maturity, drooping wings, poor growth and low production with attendant high mortality and morbidity rates (Blake, 2015).

Most animals infected with coccidians are asymptomatic, but young or immunocompromised animals may suffer severe symptoms and even death. Coccidian parasites can infect a wide variety of animals, including humans, birds, and livestock, they are usually species-specific and re-infection by these species is possible. Coccidiosis is endemic in most of the tropical and subtropical regions where ecological and poor management conditions favour an all year-round development and propagation of casual agents.

Disease prevention and growth enhancement, feed intake and efficiency of feed are critical factors in modern animal production today (Kostadinvic and Levic, 2018). Farm poultry production systems are recently challenged with the concept of "clean, green, ethical" (CGE) animal production being promoted (Puvača et al., 2019). This CGE concept promotes limited use of drugs, chemicals and hormones with emphasis to reduce food production of the environment and poultry welfare. In the US, the use of antibiotics is strictly regulated by the US Food and Drugs Administration (FDA) while in Europe it is regulated by the European Agency for the Evaluation of Medicinal products (EMEA) (Kostadinovic et al., 2019). Recommendations from FDA, the World Health Organization (WHO) and the EMEA for veterinary medicine state that "whenever possible, synthetic drugs should be replaced with plant-based preparations in order to reduce the presence of synthetic drugs and their metabolites (residues) in final animal products".

One of the potential alternatives to synthetic drugs is the use of medicinal plant supplements or their essential oils because some have potent properties and complex bioactivity (Acimovic et al., 2019). Substituting synthetic drugs with plant based supplements could ensure healthier food for the human population, reduce reliance on synthetic drugs and thus reduce the development of pathogen resistance (Kostadinovic et al., 2019).

Medicinal herbs such as oregano, garlic, thyme, rosemary are currently the most frequently used phytoadditives in poultry nutrition (Pangasa et al., 2007b; Puvača, 2008; Stanaćev et al., 2010, 2011; Kostadinovic et al., 2011; Puvača et al., 2016).

The use of medicinal plant supplements and their extracts as feed additives have increased during the past decade due to their antibacterial (Lević et al., 2011; Oliveira et al., 2013), antioxidant (Botsoglou et al., 2002; 2004; Kostadinovic et al., 2010a; 2010b, 2011) and hypocholesterol activity (Srinivasan, 2004). Many other plant supplements have also been shown to improve growth and performance in poultry: such as, bitter leaf (*Vernonia amygdalina*) which contains bioactive compounds such as vernolide and vernodalol, which make up various phytochemicals including flavonoids, alkaloids and phenolics (Erasto et al., 2007). Similarly, it is reported to have antibacterial, antiviral, anti-inflammatory and anti-lipidaemia activity (Sani et al., 2012). *Aloe vera* is composed mostly of latex and gel which have physiologically active substances with biological effects, acting alone or indicating a synergistic effect (Boudreau and Beland, 2006).

This study was undertaken to assess the application of aqueous crude extracts of *Vernonia amygdalina* (bitter leaf) and *A. vera* in the prevention and treatment of coccidiosis in poultry. Bitter leaf and *A. vera* are plants which are readily available all year round and found in all areas of the North West region, Cameroon.

MATERIALS AND METHODS

Selection of poultry farms

A total 20 farms were randomly selected in Bambili based on the accessibility from different quarters around the locality. With the permission of the poultry owners, basic questions on coccidiosis, presence of infection in the poultry and how these farmers manage infection were asked. After a brief discussion with the farmers, access into the farms to collect stool samples was granted. Stool samples were collected in stool cups by using the spoons in the cup and picking up freshly passed out faeces. The activity was done by standing at the poultry site and observing the chicks excrete for a period of about one hour. Poultry farms with younger chicks were the preferred farms since infection is higher in young chicks. At least 10 samples (giving a total of 200 specimens) were collected from each farm depending on the size of the farm. Only the birds of farmers who welcomed the research team and gave answers to questions and were willing to participate in the study were enrolled. The birds of farmers who did not welcome the team were excluded from the study. In addition, birds that were already very sick in the poultry farms were not included in this study.

Oocysts conservation

Stool samples freshly collected from the various poultry farms were taken to the Biological Sciences Laboratory of the Faculty of Science, of the University of Bamenda and a simple microscopy test was done using a wet mount faecal test at 10X objective to

confirm presence of oocysts in the samples. Positive samples were confirmed by comparing oocysts using reference pictures (chats from the national institutes for health website). Positive samples were stored in 10 % potassium dichromate solution and at room temperature (25ºC) in the laboratory to be later used to inoculate the chicks.

Preparation of *V. amygdalina* and *A. vera* aqueous crude extracts

***V. amygdalina* (Bitter leaf) aqueous crude extract preparation**

V. amygdalina leaves were harvested from farms within Bambili locality, washed properly with clean water to remove any possible contaminants and were then taken to the Biological Sciences lab, air dried by placing the leaves in newspapers and allowed to dry for about 7 days (plant press method). The dry leaves were ground using a mortar and pestle and weighed using an electronic balance (Model: ALE-223, India).

Two different types of extraction methods were used: simple water extraction (maceration) and hot water extraction (decoction) (UNIDO, ICS, 2008).

In the maceration process, finely powdered bitter leaf (100 g) was placed in a plastic bottle-stoppered container containing water (1L) and allowed to stand at room temperature for a period of 3 days with frequent agitation until the soluble matter had dissolved. The mixture was then strained to remove large particles, the marc (the damp solid material) was pressed, and the combined liquids were filtered using Whatman filter 1 to remove any unwanted particles and the filtrate was stored at 4ºC in clean sterile bottles.

The decoction process was done by dissolving 20 g of the dry powder in 1L of distilled water. The whole solution was homogenized and boiled for 30 min. The starting ratio of crude extract to water was fixed, for example 1:4, v/v; the volume was then brought down to one-fourth its original volume by boiling (Gotep et al., 2016). The concentrated crude extract was passed in a square white nylon cloth and filtered twice on absorbent cotton and once on Whatman filter paper 1.

***A. vera* crude extract preparation**

A. vera leaves were harvested from farms within Bambili, washed thoroughly with distilled water and carefully and the spikes were cut out. The leaves comprise of green outer skin, yellow latex, and clear gel. The gel was carefully scrapped to avoid picking up the yellow latex. The gel was rinsed to remove some of the bitterness and also wash out any residual yellow latex. The gel was then ground using a laboratory mortar and pestle and was macerated using water so as to dilute the concentration of the *A. vera* gel.

Acquisition, maintenance and pre-screening of chicks

Seventy-two three-week-old vaccinated (against coccidiosis) chicks were obtained from a coccidiosis-free poultry confirmed by the poultry farmer selected at random in Bambili. The birds were observed for signs and symptoms such as bloody diarrhoea, dizziness and lack of appetite to check for possible coccidiosis infection. The chicks were randomly distributed in the 24 cages containing three chicks per treatment. The fowls were acclimatized for two weeks under ambient (fluctuating) conditions and with standard feeding including feed additives and treatments, which included the following:

1. EL-ROX growth booster composed of 0.4 g glucose oxidase and 79.6 g calcium carbonate which was mixed in 25 kg of food. It was given to boost the growth of the young fowls, enabling them to grow bigger with minimum quantity of feed intake.
2. AMIN TOTAL which is a multi-vitamin was added in their drinking water; 5 g of powder was dissolved in 15 L of water and administered for 3 days. This helped give the young birds their required vitamins and also served as an appetite remedy.
3. Deworming of the birds after administering vitamin; 5 g of piperazine (anthelmintics) powder was dissolved in 10 L of distilled water and administered for 1 day monthly.
4. HEPATURYL which is an all-round flush medication was also administered a day after deworming; 5 g of powder dissolved in 5 L of water and administered for 3 days. Hepaturyl helps flush all intestinal tract infections ensuring there was no intestinal tract infection in the birds before inoculation of *Eimeria* oocyst.

Day-to-day check-ups were done to check for any changes in the chicks such as: physical changes in chick as concerns growth and weight of chicks, possible signs and symptoms of infection, kind of faeces passed out by chicks, which was also followed by regular screening every 4 days based on pre-patent period of the disease (during acclimatization for 2 weeks). Feeders and drinkers were provided per cage and all sanitary measures were observed to avoid contamination of food and water.

Inoculation of oocysts

Infection of the chicks proceeded after acclimatization period, feed and water were provided as usual and the chicks were constantly monitored on a daily basis to check for any clinical changes. According to Gotep et al. (2016), 1 mL of oocyst suspension for the mixed species was put in 1 L of their drinking water daily. The fowls were continuously inoculated with oocysts for a period of 4 days. Based on the pre-patent period (4-7 days), screening was done on the 4th day and treatment began after the 7th day. Fowls were observed for macroscopic and microscopic changes:

1. Macroscopic changes observed were blood in faeces, dizziness in fowls, fowl isolation, change in feeding habit (high FCR) and loss of appetite, fowls looking listless, and cough, fowls looking dehydrated and even death.
2. Microscopic analysis was carried out on stool samples that looked greenish, reddish and also yellow foamy diarrhoea and presence of oocysts was confirmed.

When confirmed, treatment commenced using plant extracts at the various treatment levels and the birds were continuously monitored two times a day to take note of any the changes in the chicks that could indicate signs of infection.

Distribution of chicks in cages (test and control groups)

After infection was confirmed through microscopy and macroscopy, the chicks were randomly selected and placed in 24 groups of three chicks each. Groups of *A. vera* and *V. amygdalina and* their combination (50% *V. amygdalina* and 50% *A. vera*) for maceration and decoction extracts were separated based on different concentrations. The negative control group was given distilled water while the positive control group was given Amprolium which is a commonly used drug against chicken coccidiosis.

The different treatment groups included concentration of 100 mg/L for *A. vera* and *V. amygdalina maceration* and decoction preparations making a number of 4 groups for this concentration. For concentrations of 200, 300 and 400 mg/L: preparations for *A.*

vera, *V. amygdalina* and their combination for decoction and maceration extracts were separated making a total of 6 groups each for these concentrations.

A total of 22 treatment groups and 2 control groups were used and 3 chicks were placed in each cage resulting in 66 chicks used in the treatment groups and 6 chicks used in the control groups.

Assessment of chicken survival to oocysts after administration of treatment

Chicks were continuously monitored after oocysts inoculation to check for mortality rate caused by the *Eimeria* oocysts throughout the course of the study. Monitoring was done in the morning and evening to check for dead chicks in the various cages and number of deaths were recorded.

Assessment of bloody diarrhoea in chicken after administration of treatment

The effect of the plant extracts on the chicks during the seven days of treatment was observed. This was done by observing the intensity of bloody diarrhoea throughout the time of treatment particularly on days 2, 4 and 7. On these days, assessment was carried out for the presence of bloody diarrhoea: the approach of defined subjectivity in research was used to interpret the severity of the bloody diarrhoea; highly severe bloody diarrhoea (++++), severe bloody diarrhoea (+++), mild to moderate bloody diarrhoea (++), low bloody diarrhoea (+) and absence of bloody diarrhoea (-).

Growth performance

Average live weights were taken for the birds measured per cage from the 7th day after infection for 20 days; this was to examine the long-term effects of the plant extracts on coccidiosis in poultry. This was done by weighing the chicks in each cage and the total was divided by the number of chicks per cage for mean values. The live weights were used to calculate growth rate of the chicks. Growth parameters included: feed intake, body weight gain and feed conversion ratio.

Feed intake

It is the total amount of feed consumed by the chickens from the day of the administration of the plant extract to the last day.

$$\text{Average feed intake (g)} = \frac{\text{Quantity of feed given (g)} - \text{Quantity of leftover (g)}}{\text{Number of chicks}}$$

Body weight gain

Weight gain was observed on days 4, 6 and 9 using the weights of the chicks. Body weight gain (g) = Final body weight (g) – Initial body weight (g).

Feed conversion ratio

FCR was carried out for days 4, 6 and 9 of the treatment. Feed conversion was gotten as the ratio of feed intake to the body weight gain (g) (Unigwe et al., 2014).

$$\text{Feed Conversion Ratio (FCR)} = \frac{\text{Average feed intake (g)}}{\text{Average weight gain (g)}}$$

Assessment of the effects of treatment on haematological parameters in chicken

Blood samples were collected to check for the effect of blood properties after infection. This was done by collecting blood from the brachial (cutaneous ulnar or “wing”) vein as follows; the fowl was held carefully to restrain it from movements with one wing extended. Feathers found on the exposed area were removed to ease visibility. Cotton balls damped in 70% alcohol were used to clean the area and to increase visibility. The wing was supported by grasping the carpus. The vessel was held off by pressing down on the wing above the antebrachium. A needle (gauge size of 20) was inserted medially at a point just proximal to the elbow and the blood was collected into EDTA tubes. To avoid hematoma formation, pressure was applied on the site using a cotton ball for some minutes until blood had stopped flowing (Jaime, 2006). The blood samples collected were then transported in styrofoam boxes with ice packs to the University of Bamenda Health Center where full blood count test was carried out for haematological parameters such as red blood cells, haemoglobin, haematocrit, and platelets.

Data analysis

The data obtained was entered into Microsoft Excel, analysed using the statistical package for social sciences (SPSS) version 23. Mean values for different parameters were calculated and the values expressed as mean ± SEM. A Chi-Square (X^2) test was used to compare the effects of the two plants. Welch’s F-test ANOVA was used to determine the variability between group means and the variability of the observations within the means. The data was summarized and presented in the form of tables and charts. The level of significance was set at $p<0.05$.

RESULTS

General characteristics of the study population

A total of 72 chicks were used in this study and sixty-nine chicks were infected with *Eimeria* oocysts. After establishment of infection was confirmed through physical examination of the signs and symptoms (Figure 1) and through faecal microscopy, sixty-six chicks were treated with *V. amydalina* and *A. vera*, and three chicks were treated with a commercially manufactured pharmaceutical drug, Amprolium which was the positive control group (infected/treated group). A negative control (uninfected/untreated)

Effects of *V. amygdalina*, *A. vera* crude extracts and their combination on chicken survival

Out of the 66 chicks treated with plant supplements, four chicks died. The groups treated with *V. amydalina* crude extract had a survival rate of 91.7% with 22 chicks out of 24 surviving. The chicks had a survival rate of 88.9%, with 16 out of 18 chicks surviving when the crude extracts of the two plants were used. However, the groups’ treatment with *A. vera* showed 100% survival of chicken

Figure 1. Some signs and symptoms of *Eimeria* infection in chicks: (A) Chicks acting chilled and dizzy. (B) Bloody and watery diarrhoea in faeces.

Table 1. Chicken survival after oocysts inoculation and treatment with respect to crude plant extract, extraction method and concentration of extract.

Characteristics	Parameter	Number examined	Incidence (%)	Significance
Plant	*A. vera*	24	100.0 (24)	χ^2 =2.573, p = 0.276
	V. amygdalina	24	91.7 (22)	
	A. vera and *V. amygdalina*	18	88.9 (16)	
Extract	Decoction	33	93.9 (31)	χ^2 =0.000, p =1.000
	Maceration	33	93.9 (31)	
Concentration of extract (mg/l)	100	12	91.7 (11)	χ^2 =0.133, p =0.988
	200	18	94.4 (17)	
	300	18	94.4 (17)	
	400	18	94.17(17)	

after oocysts were inoculated as seen in Table 1.

Effects of crude extracts of *V. amygdalina*, *A. vera* and their combination on the level of blood in faeces

Decoction and maceration crude extracts were both effective in controlling the amount of blood in faeces in the chicks (χ^2 =0.086, p = 0.770). The prevalence of blood in faeces was highest with *A. vera* crude extract (95.8%), lower when the two plants were combined (94.3%), least in *V. amygdalina* crude extract (93.1%), which indicated that *V. amygdalina* crude extract was more effective in controlling blood in faeces. With respect to period (days), the plant crude extracts had more effect with time, indicating that on day 7 of treatment the degree of blood in faeces greatly dropped (86.4%) as compared to day 4 and 2 (98.5%) with a significant difference of χ^2 =12.208, p =0.002 (Table 2).

Effect of *V. amygdalina*, *A. vera* and their combination on weight gain (WG), feed conversion rate (FCR) and growth performance

The effect of V. amygdalina and A. vera on WG

The results on weight gain as seen on the Figures 2 and 3 shows some fluctuations in the weight of the chicks from day 4 to day 9 of treatment with *V. amygdalina, A. vera* and their combinations. Results for *V. amygdalina* crude extract shows that at a concentration of 100 mg/L,

Table 2. Presence of blood in faeces with respect to plants, method of extraction, concentration of extract and frequency of different periods.

Characteristics	Parameter	Number of stool samples examined	% Prevalence of blood in faeces (n)	Significance
Plant	*A. vera*	72	95.8 (69)	χ^2 =0.528, P = 0.768
	V. amygdalina	72	93.1(67)	
	A. vera and V. amygdalina	53	94.3(50)	
Extract	Decoction	99	93.9 (93)	χ^2 =0.086, P = 0.770
	Maceration	98	94.9 (93)	
Concentration of extract (mg/l)	100	36	86.1 (31)	χ^2 =7.904, P =0.048
	200	54	100.0 (54)	
	300	54	94.4 (51)	
	400	53	94.3 (50)	
Frequency different periods	Day 2	66	98.5 (65)	χ^2 =12.208, P =0.002
	Day 4	65	98.5 (64)	
	Day 7	66	86.4 (57)	

n= number treated.

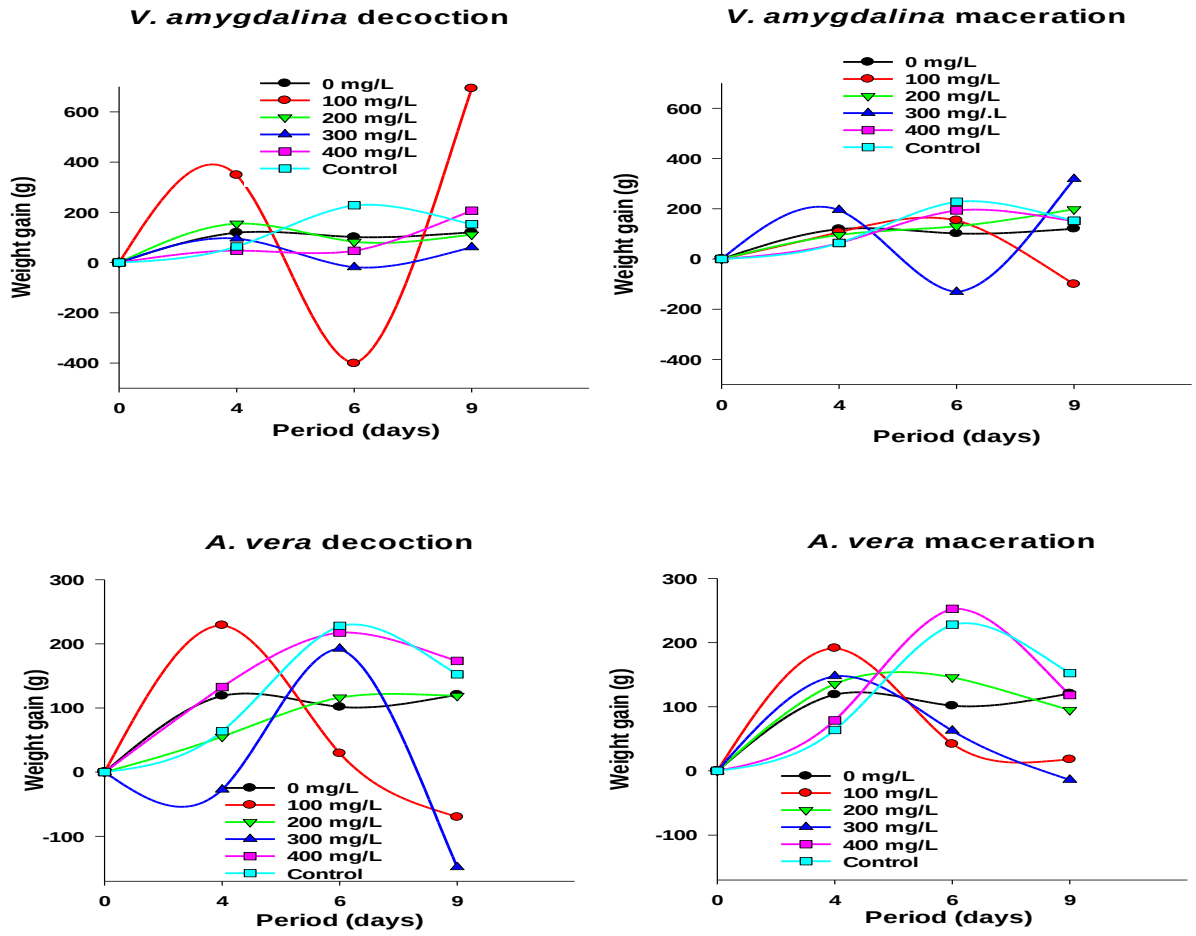

Figure 2. Effect of single plant crude extracts on weight gain.

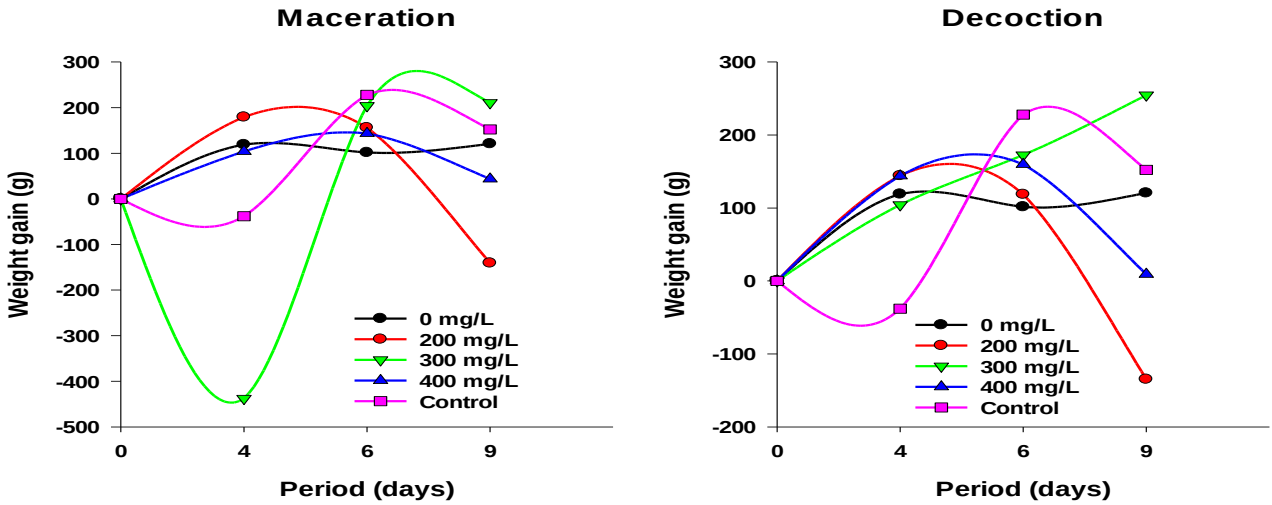

Figure 3. Effect of combined plant crude extracts on weight gain (WG).

there was a sharp drop in the weight of the chicks on day 6; however, there was an increase on day 9 for the decoction preparation. But results for 200 and 400 mg/L as well as the control/ treated group seemed to show similar effects on the weighs of the chicks. For the maceration extract, 100 mg/L showed a slow but steady increase from day 4 to 6 but a slight drop in weight from day 6 to day 9. At 300 mg/L, results indicated a drop from day 4 to 6 and a sharp increase in weight from day 6 to 9. Meanwhile at the other concentrations the results were seen to have similar trends to the control group (Figure 2).

With the administration of *A. vera* crude extract, decoction extraction method indicated at 100 mg/L a sharp increase in weight from the onset of treatment to day 4 and a sloppy decrease from day 4 to day 6 with a slight increase from day 6 to day 9. At 300 mg/L, weight increased steeply from day 4 to day 6 but decreased sharply from day 6 to day 9. At 400 mg/L, there's seen to be a steady increase in weight from day 4 to 6 and day 6 to day 9. Maceration extracts showed at 100 mg/L a steady increase from day 4 to 6 but a slow and steady decrease from days 6 to 9. At 200 mg/L, there was a steady increase from day 4 to day 9. At 400 mg/L, results indicated a sharp increase from day 4 to day 6 and a slight decrease from days 6 to 9 (Figure 2).

The combination of the two plant extracts showed that, at 300 mg/L for the maceration method there was a continuous drop in the weight of the chicks from the start of treatment to day 4, but a steep increase from day 4 to day 6. Also, at 200 mg/L, there was a gradual increase from the onset of treatment to day 6 and a sloppy decrease from day 6 to 9. For decoction extracts, results at 200 mg/L indicated a gradual increase from onset of treatment to day 6 but a steep decrease from day 6 to day 9 on the weights of the chicks. At 300 mg/L, there's a steady increase in the weight of the chicks from onset of treatment to day 9 (Figure 3).

The effect of V. amygdalina and A. vera on food conversion ratio (FCR)

With *V. amygdalina* maceration and decoction extraction methods, the results showed to have a slow but fluctuating increase in the FCR of the chicks as seen in the Figures 4 and 5. At 200 mg/L for maceration extraction methods, FCR levels dropped from day 4 to 6 but gradually increased from day 6 to 9. For the decoction extract, results at 200 mg/L showed a steady increase from onset of treatment to day 4 but sharply increase from day 6 to day 9 (Figure 4).

Results for *A. vera* showed at 200 mg/L steady FCR from onset of treatment to day 6 and a slight increase from day 6 to day 9. At 300 mg/L, there was a sloppy decrease in FCR from onset of treatment to day 6 and a steep increase from day 6 to day 9 for the maceration preparation. Results for decoction extract at 100 mg/L showed a gradual increase from onset of treatment to day 4, a steep increase from day 4 to day 6 and a sharp decrease from day 6 to day 9. At 300 mg/L, there is a gradual increase from onset of treatment to day 4 which slowly increased from day 4 to 6 and steadily from day 6 to day 9 (Figure 4).

Combining the 2 extracts showed a slight decrease from day 4 to 6, a sharp increase on day 6 to day in FCR at 200 mg/L for the decoction method, steady levels of FCR for 300 and 400 mg/L and the control group but a drop in FCR for the control uninfected group from day 6 to 9. Results for maceration extract showed a decrease in

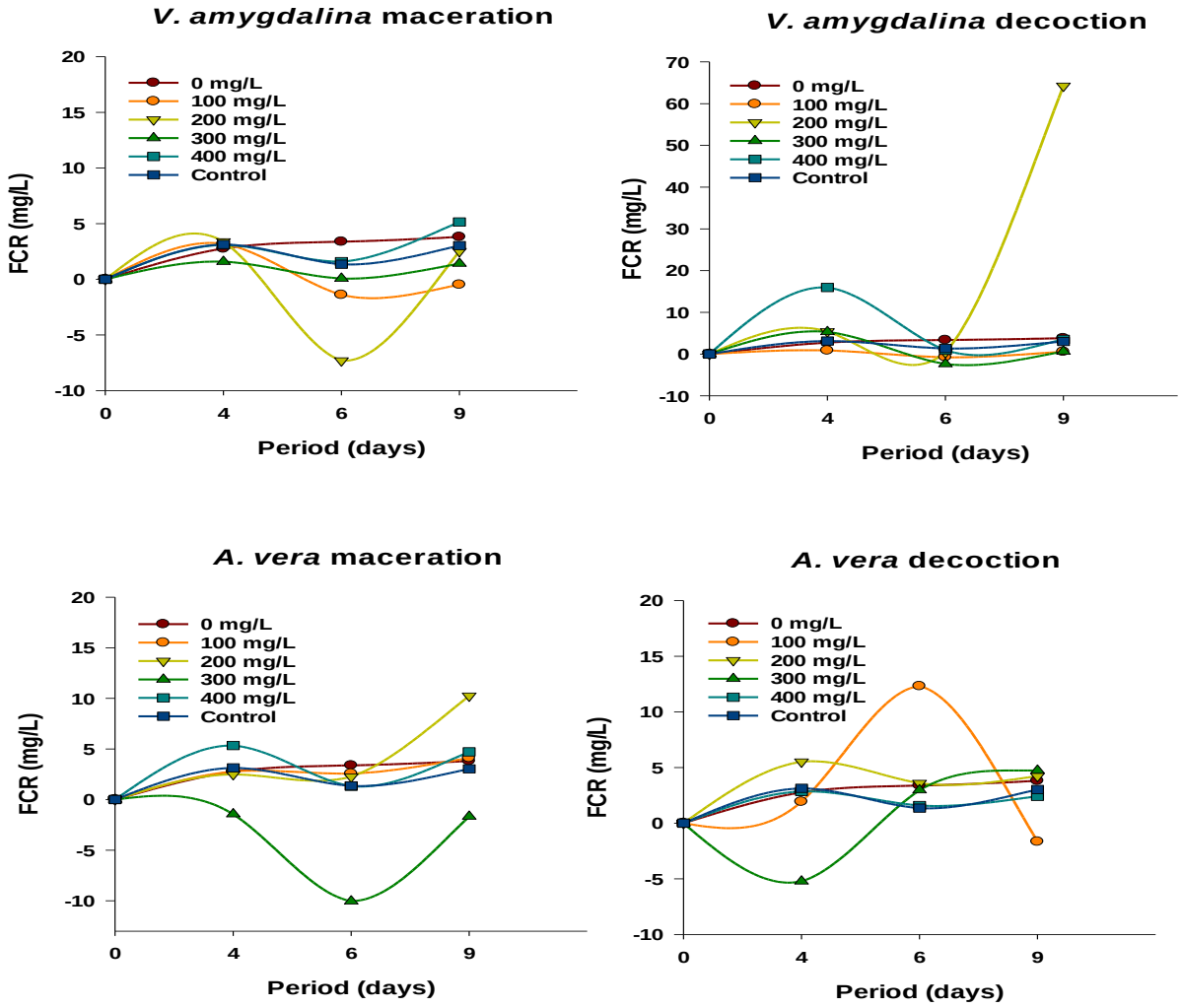

Figure 4. Influence of single plant crude extracts of *V. amygdalina* and *A. vera* on FCR.

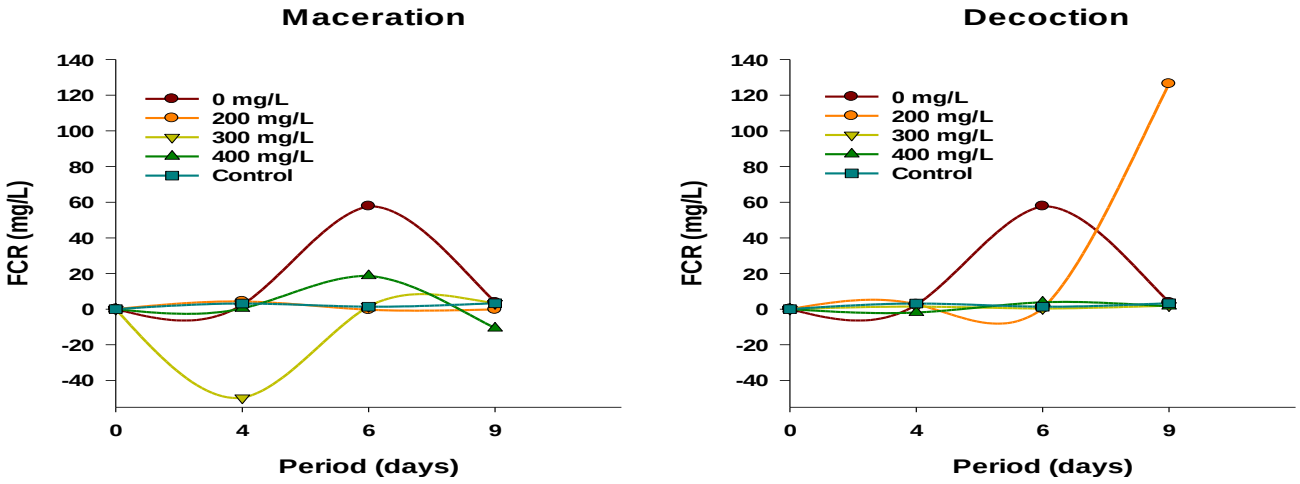

Figure 5. Influence of the combination of crude plant extracts of *V. amygdalina* and *A. vera* on FCR.

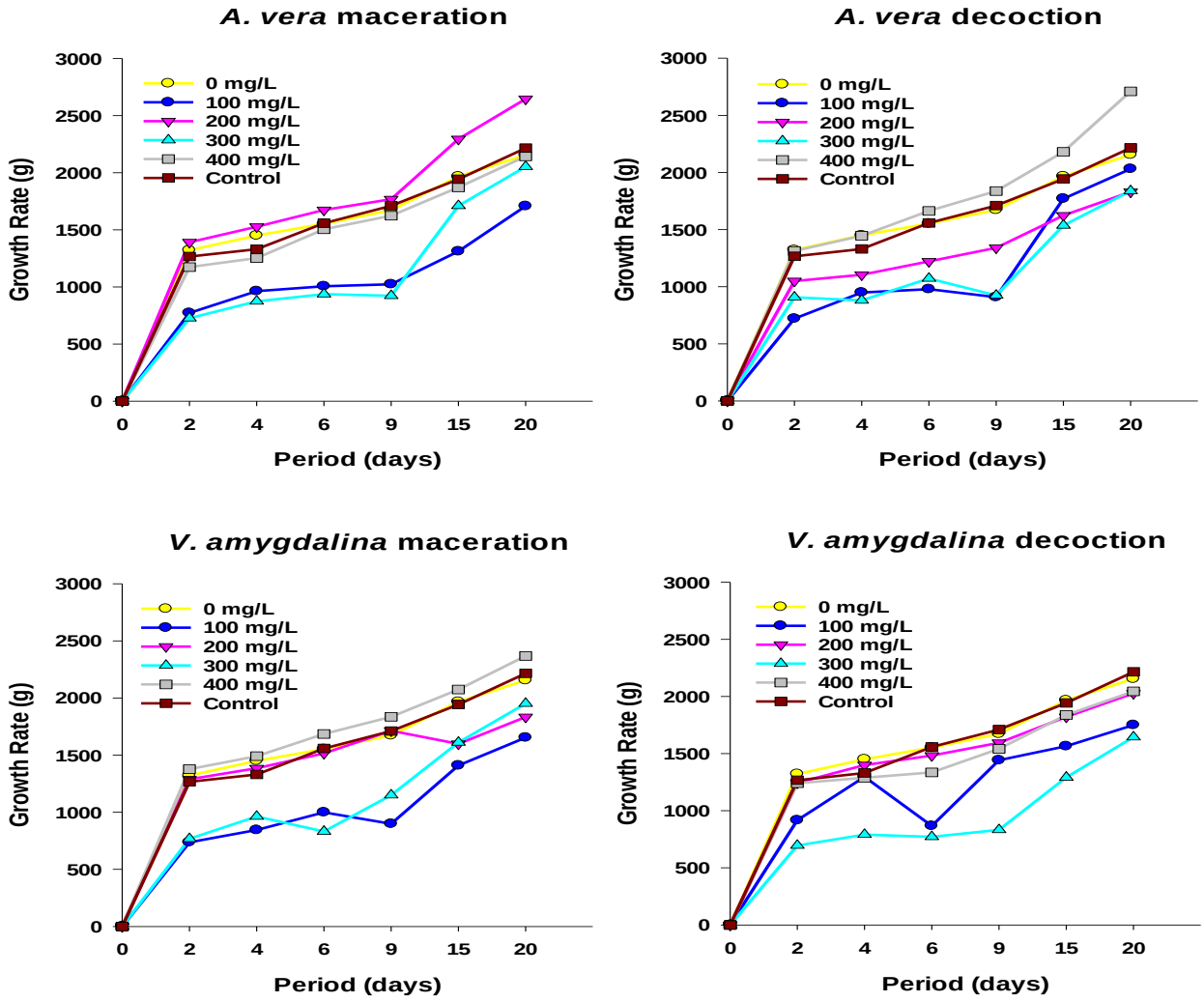

Figure 6. Effect of crude plant extracts on growth rate.

FCR from day 6 to 9 at 300 and 400 mg/L (Figure 5).

Effects of *V. amygdalina, A. vera* and their combination on growth performance of broiler birds with respect to period

The overall results for the growth rate of the chicks from the administration of *V. amygdalina, A. vera* and their combinations for a period of 20 days are shown on the Figures 6 and 7 below for the two extraction methods. These results show a steady increase in growth rate of the chicks regardless of the different treatment concentrations administered. Although some fluctuations were seen at 100 mg/L but the chicks showed steady increase in their growth as compared to the control treated and control untreated group. At a concentration of 400 mg/L, it was observed to have a higher effect on the growth of the chicks over the period of 20 days.

Effect of *V. amygdalina* on blood parameters two weeks post inoculation

There was no significant difference ($p > 0.05$) in RBC volume and HCT count within treated groups for decoction and maceration extracts when compared to the control group. However, there was a significant difference ($p < 0.05$) in HB concentration and PLT count when compared to the control group as described in Table 3.

Effect of *A. vera* on blood parameters two weeks post inoculation

There was no significant difference ($p > 0.05$) in RBC

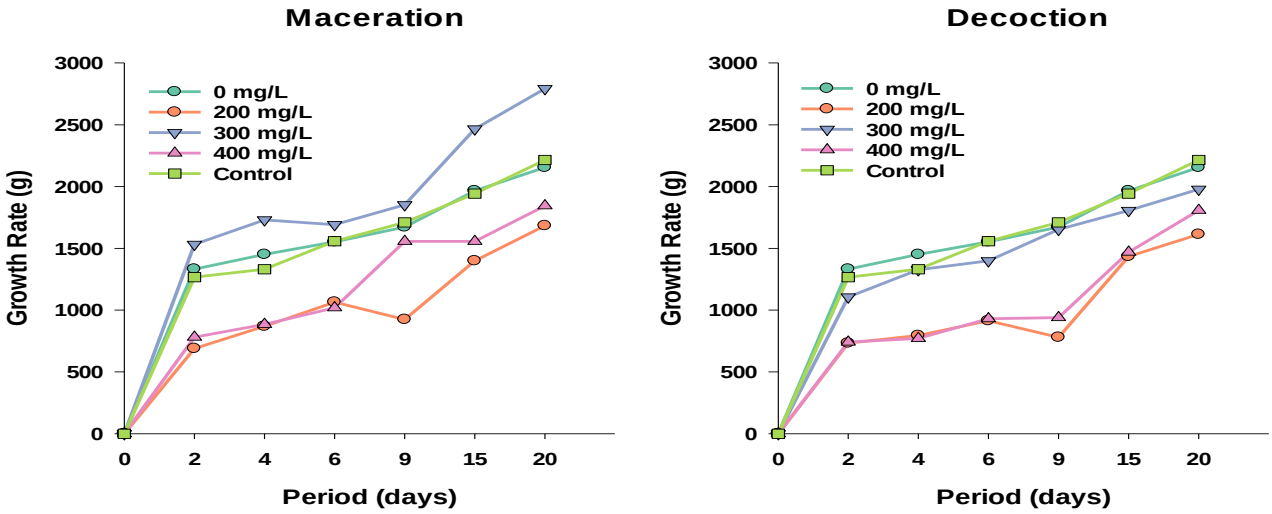

Figure 7. Effect of the combination of *V. amygdalina* and *A. vera* crude plant extracts on growth rate.

Table 3. Effect of *V. amygdalina* crude extracts on blood parameters two weeks post oocyst inoculation.

Concentration (mg/L)	Haematological parameters			
	RBC (*10^6 ul)	**HGB (g/dl)**	**HCT (%)**	**Platelet**(*10^3 ul)
Maceration				
0	2.39 ± 0.066^{a}	16.07 ± 0.03^{abc}	29.05 ± 0.10^{a}	32.33 ± 0.33^{a}
100	2.18 ± 0.12^{a}	10.33 ± 0.64^{c}	27.60 ± 2.08^{a}	11.00 ± 2.00^{b}
200	2.72 + 0.17^{a}	17.10 ± 1.10^{a}	30.18 ± 1.98^{a}	40.33 ± 9.33^{a}
300	2.41 ± 0.08^{a}	11.27 ± 0.27^{bc}	30.30 ± 0.50^{a}	8.00 ± 0.00^{b}
400	2.01 ± 0.33^{a}	13.70 ± 2.66^{abc}	26.63 ± 4.38^{a}	33.00 ± 0.58^{a}
Control	2.53 ± 0.18^{a}	16.13 ± 0.15^{ab}	30.55 ± 0.87^{a}	52.67 ± 4.63^{a}
$F_{(5;12)}$	1.95^{ns}	5.39 *	1.10^{ns}	15.70***
Decoction				
0	2.39 ± 0.06^{a}	16.07 ± 0.03^{a}	29.05 ± 0.10^{a}	32.33 ± 0.33^{abc}
100	2.44 ± 0.15^{a}	11.63 ± 0.47^{b}	31.27 ± 1.23^{a}	9.67 ± 0.67^{c}
200	2.46 ± 0.18^{a}	16.10 ± 0.15^{a}	29.28 ± 0.37^{a}	44.33 ± 7.17^{a}
300	2.25 ± 0.18^{a}	11.20 ± 0.13^{b}	28.67 ± 2.28^{a}	14.33 ± 1.76^{bc}
400	2.28 ± 0.19^{a}	15.27 ± 0.99^{a}	27.31 ± 1.97^{a}	40.00 ± 6.43^{a}
Control	2.53 ± 0.18^{a}	16.13 ± 0.15^{a}	30.55 ± 0.87^{a}	52.67 ± 4.63^{a}
$F_{(5;12)}$	0.45^{ns}	24.39***	1.03 ^{ns}	14.80***

Means with the same superscript in the same column (a, b, c) are not significantly different ($p > 0.05$), means with different superscript in the same column (a, b, c) are significantly different ($p < 0.05$). Values are expressed as mean ± SEM. RBC = red blood cells, HGB = haemoglobin, HCT = haematocrit value or packed cell volume. Control group was given amprolium at 240 mg/L which was the positive control group.

volume and HCT count within treated groups for decoction and maceration extracts when compared to the control group. However, there was a significant difference ($p < 0.05$) in HB concentration and PLT count when compared to the control group as described in Table 4.

Effect of the combination of *V. amydalina* and *A. vera* on blood parameters two weeks post inoculation

There was no significant difference ($p > 0.05$) in RBC volume and HCT count within treated groups for

Table 4. Effect of *A. vera* crude extracts on blood parameters two weeks post inoculation of oocysts.

Concentration (mg/L)	Haematological parameters			
	RBC (*10^6 ul)	**HGB (g/dl)**	**HCT (%)**	**Platelet** (*10^3 ul)
Maceration				
0	2.39 ± 0.06^{a}	16.07 ± 0.03^{a}	29.05 ± 0.10^{a}	32.33 ± 0.33^{ab}
100	2.26 ± 0.07^{a}	10.93 ± 0.38^{b}	29.47 ± 0.77^{a}	11.33 ± 2.03^{b}
200	2.27 ± 0.14^{a}	16.16 ± 0.37^{a}	28.64 ± 0.71^{a}	42.67 ± 7.22^{a}
300	2.26 ± 0.15^{a}	11.37 ± 0.47^{b}	31.07 ± 2.11^{a}	11.00 ± 2.30^{b}
400	2.46 ± 0.24^{a}	16.37 ± 0.27^{a}	30.30 ± 1.83^{a}	43.67 ± 9.14^{a}
Control	2.53 ± 0.18^{a}	16.13 ± 0.15^{a}	30.55 ± 0.87^{a}	52.67 ± 4.63^{a}
$F_{(5;12)}$	0.58^{ns}	67.61***	0.55^{ns}	11.13***
Decoction				
0	2.39 ± 0.06^{a}	16.07 ± 0.03^{a}	29.05 ± 0.10^{a}	32.33 ± 0.33^{b}
100	2.22 ± 0.07^{a}	10.73 ± 0.13^{a}	26.93 ± 1.97^{a}	8.67 ± 0.33^{c}
200	2.38 ± 0.11^{a}	15.65 ± 0.41^{a}	29.26 ± 0.43^{a}	43.00 ± 6.03^{ab}
300	2.24 ± 0.09^{a}	11.27 ± 0.18^{a}	30.26 ± 0.43^{a}	7.00 ± 1.16^{c}
400	1.92 ± 0.92^{a}	11.17 ± 5.34^{a}	19.79 ± 9.21^{a}	26.33 ± 4.67^{b}
Control	2.53 ± 0.18^{a}	16.13 ± 0.15^{a}	30.55 ± 0.87^{a}	52.67 ± 4.63^{a}
$F_{(5;12)}$	0.29^{ns}	1.51^{ns}	1.09^{ns}	24.69***

Means with the same superscript in the same column (a, b, c) are not significantly different ($p > 0.05$), means with different superscript in the same column (a, b, c) are significantly different ($p < 0.05$). Values are expressed as mean ± SEM. RBC = red blood cells, HGB = haemoglobin, HCT = haematocrit value or packed cell volume. Control group was given amprolium at 240 mg/L which was the positive control group.

decoction and maceration extracts when compared to the control group. However, there was a significant difference ($p < 0.05$) in HB concentration and PLT count when compared to the control group as described in Table 5.

DISCUSSION

Effects of *V. amygdalina*, *A. vera* and their combination on incidence of chicken survival

Chicken recovery from coccidiosis can take about 10-14 days. When coccidiosis is not detected early or parasitaemia is too high it could lead to death of the chicks; the severity of the infection is dependent on the number of *Eimeria* and *Eimeria* species that co-infect the birds (Abu-Akkada and Awad, 2012). According to Abdul et al. (2020) the mortality ratio can be 5% to 70% in young birds of 1 to 4 months old, and majority of deaths happen in 3 to 4 months. However, oocyst pathogenicity is negligible in adult birds since they develop a stronger immunity as they grow older. In this study, of the sixty- six chicks used, four chicks died which could have been as a result of high levels of oocyst infection. These findings agree with the findings of Yang et al. (2015) who observed an increase in the survival rate of broiler chicks when administered *Bidens pilosa* extracts as a coccidiocidal agent.

Effects of *V. amygdalina*, *A. vera* and the combination on the level of blood in faeces

One of the most visible clinical signs of chickens infected with coccidiosis is blood in diarrhoea (faeces) amongst many other clinical signs like weight loss, dizziness, dehydration, anorexia, fetid (McDougald and Fitz-Coy, 2008). This study showed high levels of bloody diarrhoea which reduced with administration of *V. amygdalina* and *A. vera* and the combination, also across a period of time. In this study, bloody diarrhoea was seen across all groups except the uninfected control group. The follow up period of 7 days showed a tendency of the efficacy of the plant extracts on oocyst load with time which could be because of the time needed for the chicks to absorb the plant supplement, distribute and breakdown or metabolize it. Results obtained on bloody diarrhoea showing a reduction on oocyst rates showed that all the plants were effective in reducing the initial oocyst loads of the parasite.

The active ingredients of *V. amygdalina* (quinines, vitamins A, B, C, E, B1) and *A. vera* (tannins) show antidiarrheal and antioxidant ability which empowered the intestinal microbiota and ultimately boosted the immunity

Table 5. Effect of the combination of *A. vera* and *V. amygdalina* crude extracts on blood parameters.

Concentration (mg/L)	Haematological parameters			
	RBC (*10^6 ul)	**HGB (g/l)**	**HCT (%)**	**Platelet** (*10^3 ul)
Maceration				
0	2.39 ± 0.06 ^{a}	16.07 ± 0.03 ^{a}	29.05 ± 0.03 ^{a}	32.33 ± 0.33 ^{b}
200	2.03 ± 0.00 ^{a}	9.60 ± 0.12 ^{b}	25.63 ± 0.43 ^{a}	9.33 ± 1.86 ^{c}
300	2.32 ± 0.25 ^{a}	14.9 ± 0.10 ^{a}	26.38 ± 0.31 ^{a}	28.67 ± 0.33 ^{b}
400	2.08 ± 0.18 ^{a}	10.87 ± 0.92 ^{b}	28.70 ± 2.50 ^{a}	10.00 ± 1.53 ^{c}
Control	2.53 ± 0.18 ^{a}	16.13 ± 0.15 ^{a}	30.55 ± 0.87 ^{a}	52.67 ± 4.63 ^{a}
$F_{(4;10)}$	1.76 ^{ns}	52.58 ***	2.79 ^{ns}	58.77 ***
Decoction				
0	2.39 ± 0.06 ^{a}	16.07 ± 0.03 ^{a}	29.05 ± 0.10 ^{a}	32.33 ± 0.33 ^{b}
200	2.25 ± 0.11 ^{b}	11.30 ± 0.15 ^{b}	30.37 ± 0.38 ^{a}	8.67 ± 1.20 ^{c}
300	3.25 ± 0.28 ^{a}	16.10 ± 0.95 ^{a}	28.00 ± 1.53 ^{a}	29.33 ± 1.20 ^{b}
400	2.19 ± 0.00 ^{b}	10.87 ± 0.07 ^{b}	27.83 ± 0.63 ^{a}	10.00 ± 1.00 ^{c}
Control	2.53 ± 0.18 ^{ab}	16.13 ± 0.15 ^{a}	30.55 ± 0.87 ^{a}	52.67 ± 4.63 ^{a}
$F_{(4;10)}$	7.39 *	39.40 ***	2.23 ^{ns}	64.70 ***

Means with the same superscript in the same column (a, b, c) are not significantly different ($p > 0.05$), means with different superscript in the same column (a, b, c) are significantly different ($p < 0.05$). Values are expressed as mean ± SEM. RBC = red blood cells, HGB = haemoglobin, HCT = haematocrit value or packed cell volume. Control group was given Amprolium at 240 mg/L which was the positive control group.

of the birds. This finding corroborate with those of Nghonjuyi et al. (2015) who demonstrated that vitamin A is essential for the integrity of chicken mucosal surfaces and enforces body resistance to coccidian and thus an increase in the levels of vitamin A, E and zinc would probably lead to a strong immune response to coccidiosis.

Effect of *V. amygdalina*, *A. vera* and their combination on weight gain (WG), feed conversion rate (FCR) and growth performance

The results on growth performance showed that a growth performance effect was exerted by the herbal supplements which were similar to the results gotten from the infected control group treated with amprolium and the uninfected control group. Abubakar and Mohammed (2019) reported that phytogenic food additives are often associated with the improvement of flavour and palatability of food. Thus *V. amygdalina* and *A. vera* extracts enhances production performance in birds as these plant crude extracts stimulate the digestive processes and improve gut health.

The results on FCR were not consistent but showed steady improvement with the course of treatment. These findings were similar to the findings of Abdul et al. (2020), who established the effects of neem crude extract in in enhancing the prophylactic levels in pigeons.

Weight gain was not consistent as the birds lost weight from the onset of treatment but slowly gained weight as treatment progressed. This could be as a result of the inclusion of different levels of *V. amygdalina and A. vera* in their meals and also the parasitaemia of the birds. The effect of *Vernonia amagydalina* on the WG was seen to be higher at a concentration of 200 and 400 mg/ L which showed that a higher dosage of *V. amygdalina* might have increased the absorption of nutrients in the chicken's digestive tract thus leading to the weight gain of the chicks. This study showed non-consistence in the weight gain of the poultry. This corroborates with the findings of Stanacev et al. (2011) who showed a non-consistency in the weight gain of the infected- untreated group and a gradual but significant weight gain in both the infected- untreated group and the infected- treated group in reference to amprolium.

The combination of the two plant extracts was found to have a higher significance on the improvement of the growth performance of the birds. Increase in WG and FCR observed in the combination of the two plant extracts could be as a result of increasing fibre content of the diet which may have impaired nutrient digestibility and absorption (Ige et al., 2006; Onu, 2010).

This could be as a result of similar bioactive ingredients (tannins) in both plants which improved growth rate. The combination of the two plants on growth performance showed synergistic effects on the chicks as the growth rate was higher when the two plants were combined as compared to the effect of the individual plants.

Effect of *V. amygdalina, A. vera* and their combination of *V. amygdalina* and *A. vera* extracts on blood parameters two weeks post oocyst inoculation

Blood parameters represent a means of assessing clinical and nutritional health status of animals in feeding trials and the haematological variables most commonly include PCV (haematocrit), RBC, HB, MCHC, MCV and the clotting factor. The results observed for the HCT and RBC did not show any significant difference with the different treated groups but the observed difference in HGB and platelet count is indicative of the erythropoietic inducing ability of the plants and also their combined effects which is beneficial since the parasite in the epithelia of the intestines of the chicks causes bloody diarrhoea and consequently anaemia. At 200 mg/L, *V. amygdalina* maceration extract appeared to be higher for both decoction and maceration extracts although higher platelet count was recorded in decoction extract at that concentration in reference to Amprolium.

A. vera concentrations of 200 and 400 mg/L for the maceration extract increased with reference to Amprolium but no significant difference was observed when administered with the decoction extract which could be that *A. vera* lost some of its properties when boiled for a long period; although platelet values were higher at a concentration of 400 mg/L than at 200 mg/L.

The combination of the plants showed an increase in RBC with the decoction extract in reference to Amprolium and the uninfected group at 300 mg/L showing the synergestic effects of the plants when combined. Also, at 300 mg/L, the HGB and platelet count were seen to be higher compared to other concentrations.

Haemoglobin and RBC levels increased in the treated birds. These findings are in accordance with Gotep et al. (2016) who also established an increase in RBC and HGB in infected chicken infected with *Eimeria* with some plant extracts. The iron properties in these plants explains the significant increase in HGB levels and RBC levels which is beneficial since *Eimeria* parasite in the epithelia of the intestines causes bloody diarrhoea and consequently anaemia.

According to Hamman et al. (2016), *V. amygdalina* has been reported to contain tannins, glycosides, cellulose, minerals like; potassium, calcium, phosphorus, iron, sulphur, manganese, sodium and copper: some of which not only enhance recovery of wound and tumour and also enhance healing of the renal structure and renal functioning, but also enhances body blood system. *A. vera* contains tannins which enhances recovery of wound and tumour and also enhance healing of the renal structure and renal functioning, but also enhances body blood system through the production of erythropoietin.

A significant decrease in platelet count in blood was observed at a concentration of 100 mg/l as compared to the control group which could be an indication of the probable ability of the extract to inhibit the actions of platelet activating factor especially when administered at a longer duration, hence reducing the blood clotting potentials.

Conclusion

Coccidiosis remains a major health problem in the production of chicken. This study aimed at evaluating the efficacy of *V. amygdalina* and *A. vera* in controlling chicken coccidiosis and the effects of the plant supplements on growth, parasitological and haematological parameters of broiler chickens infected with *Eimeria* species. *V. amygdalina* and *A. vera* have proven to be of significant importance in fighting coccidiosis in chicken as shown in the reduction of bloody diarrhoea and survival rate of the chicks. *V. amygdalina* showed variations in the growth performance of the chicks for both the macerartion and decoction preparations and *A. vera* showed a sharp and steady decrease in the weight of the birds for both the decoction and maceration preparations at concentrations of 100 and 300 mg/L. *V. amygdalina* and *A. vera* significantly improved haematological parameters in chicks such as HB and PLT count of chicks. Therefore, *V. amygdalina* and *A. vera* could be used as a natural alternative to synthetic chemicals to control coccidiosis and improve chicken health.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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