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Studies on the Effect of the Combination of Boerhavia Diffusa and Costus Afer Leaf extracts on the Haematological Parameters of Broiler Chickens

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Abstract

Ethanol extracts of *Boerhavia diffusa* and *Costus afer* and their combination were tested for their effects on haematological parameters of broiler chicken and possible synergistic effect due to their combination. The parameters were Red Blood Cell count, White Blood Cell count, Platelet count, Blood Glucose Packed Cell Volume, Haemoglobin content and Electrolyte concentration. At the end of the analysis period of four weeks, it was found that *B. diffusa* extract and the combination of the two extracts increased red blood cell count significantly above the control and those fed with *C. afer* extract. However, *C. afer* extract increased the white blood cell count from 10.1 to 13.1 x 10^{12}, *B. diffusa* extract from 10.7 to 11.8 x 10^{12} and the combination from 11.5 to 12 x 10^{12}. The control value remained constant. All the extracts, including the combination increased platelet count significantly above the control. Blood glucose was most significantly reduced by the combination of the two extracts, while other samples including the control showed constant values. The packed cell volume (PCV) and haemoglobin content showed a drop in value in all samples except those fed a combination of the two extracts, which increased PCV value from 32-33% and increased haemoglobin values from 10.7 to 11g/dl. Electrolyte concentration at the end of research period ranged from 97.8 to 104.5 for chloride, 3.6 to 4.3 for potassium ion and 138.4 to 142.6 for sodium ion.

Keywords: Broilers, Blood, Costus Afer, Boerhavia Diffusa, Haemoglobin, Packed Cell Volume, Feed Additive, Growth-Promoting Effect

Introduction

The increasing demand for organically grown poultry in the International and local markets is due to the discoveries of adverse effect of synthetic feed additives and feed stuff (Eisa and Sohar, 2003). These adverse effects include cancer, hormonal imbalance and even poor culinary value

of the product (Ocak et al., 2008). This is because synthetic feed additives are often bio-accumulative and toxic in small amounts (Ocak et al., 2008; Barreto et al., 2008). Because these feed additives are used to boost growth, immune function and increase feed conversion ratio, it is necessary to discover plant materials that can effectively serve as feed additives (Biu et al., 2009). Nature provides many plants that have since antiquity been proven to boost immune function, resulting in higher resistance to diseases and infections, stimulate the appetite, resulting in higher feed conversion ratio and as such higher growth rate and cure diseases to which the birds are routinely exposed. These plants include *Zingiber officinale, Afromomum melegueta, Cnidoscolus acontifolius, Boerhavia diffusa* and *Costus afer (*Biu et al., 2009; Durrani et al., 2008; Awasth and Verma, 2006; Anaga et al., 2004; Nworgu et al., 2007). They have been shown to contain bioactive active components, phytochemicals and hormone precursors in addition to being protein and energy sources (Nworgu et al., 2007; Ansari et al., 2010). Studies have shown that vegeterians live longer and are less susceptible to infectious diseases due to higher immunity (Iwu, 1993). However, many of these plants, though abundant even in the wild are grossly under-utilized as just one plant can contain many bioactive components and act as a diuretic, an appetizer, alkaline phosphatase stimulator, anti-bacterial, anti-fungal and anti-viral agent (Al-Kassae, 2009).

Boerhavia diffusa is a herbaceous member of the family Nyctaginaceae. Widely distributed in the tropics, it is found in the Phillipines, the USA, Brazil and all over Africa (Ansari et al., 2008). It also occurs abundantly as a weed in India. It is a perennial creeping weed with a stout and fusiform root and a woody root stock. The stem is prostrate, cylindrical often purple in colour and hairy especially at the nodes. It has been shown to contain a large number of bioactive compounds such as vitamins, flavonoids, alkaloids, steroids, triterpenoids, lipids, lignins, carbohydrates, proteins and glycoproteins. It also contains large quantities of potassium nitrate (Joy et al, 1998; Awasth and Verma, 2006).

B. diffusa has found several applications in traditional medicine. It is, for example, used in the treatment of dyspepsia, jaundice, enlargement of spleen, abdominal pain and tumors (Awasth and Verma, 2006). It is also used to treat convulsions and is also a mild laxative and febrifuge. Its root extracts have been shown to possess anti-hepatotoxic properties and has been used in the treatment of liver disorders. It also possesses diuretic, anti-inflammatory, antifibrinolytic and antibacterial properties (Iwu, 1993). Also, the aqueous solution of its roots also contains a basic protein known as systemic resistance-inducing protein. This protein has been shown to induce strong systemic resistance in several susceptible plants against commonly occurring viruses such as Tobacco Mosaic Virus, Water Melon Mosaic Virus (Awasth and Verma, 2006).

Costus afer Ker Gawl is a member of the Zingiberaceae family. It is a tall (up to 4m) perennial herbaceous, unbranched creeping plant commonly found in West Africa including Nigeria Ghana and Cameroun. It is commonly known as ‘ginger lily’ or ‘bush cane’, ‘okpete’ in Igbo land, ‘Kakizawa’ in Hausa, ‘tete-egun’ in Yoruba and ‘mberitem’ in Efik (Anaga et al., 2004).

Costus afer has been shown to contain phytochemicals such as steroidal saponins, sapogenins, oxalates, furans, furan derivatives and starches. It also contains lanosterol, tigonenin, diogenin, sapogenin and costugenin (Anyasor et al., 2010). It is used traditionally to treat tachycardia via inflorescence infusion, its stem is mashed and is used to treat stomach upsets, cough, sore throat (Iwu, 1993). Its leaf sap is used as eye drops for eye infections and nose drops to treat headache and vertigo. It is also used to treat oedema and fever. Its stem sap is applied to treat urethral

discharges, venereal diseases, jaundice and to prevent miscarriage (Iwu, 1993). An infusion of the aerial parts is also used to treat hypertension while the powedered stems are used as enema to expel worms and treat haemorrhoids (Anaga et al., 2004). The whole boiled root has been shown to reduce carageenan-induced oedema in rat paw while checking diarrhea caused by arachidonic acid and castor oil (Anyasor et al., 2010). It has also been shown to ameliorate all signs associated with adjuvant induced polyarthritis in rats.

Based on the reported nutritive and phytochemical properties of the both plants, they were investigated for their growth promoting effect and possible improvement of haematological parameters of broiler chicks.

Materials and Methods

Sample Collection

B. diffusa and C. afer plants were obtained from the Botanical Garden, Abia State Polytechnic, Aba and identified in the Department of Biology/Microbiology, Abia State Polytechnic, Aba. The leaves were then washed with water to remove sand and other impurities. They were then dried in a laboratory oven at 65^{o}C for 1 hour until crispy (while retaining their greenish colour). The leaves were then milled to a powdery consistency. Extracts were then prepared via soxhlet extraction using ethanol as solvent after which the ethanol was evaporated and the dry extract stored.

Seventy-five day old chicks were purchased from a commercial hatchery. The chicks were, for the first two weeks prior to the commencement of the experiments, fed with starters' mash. Subsequently, they were then fed with finishers' mash till the end of the experiment. The chicks were then divided into three main groups (Those fed *B. diffusa* extract, those fed *C. afer* and those fed a combination of *B. diffusa* and *C. afer* extracts) and from each group randomly assigned to five different concentrations of leaf extract inclusions (0%, 1%, 2%, 5% and 10% w/v) into drinking water. The inclusions for the combination were prepared by dividing the inclusion level into two and collecting one-half from each of the ethanol extracts of each plant. For example, 1% inclusion (*B. diffusa+C. afer*) was prepared by adding 0.5% from *B. diffusa* and 0.5% from *C. afer*. 0% inclusion level served as the control. Five birds were used per inclusion level.

Collection of Blood Samples

Every week, three broilers from each treatment (inclusion level) were randomly selected and weighed. 2ml of blood was then collected from the wing vein via sterile syringes and stored in sterile bottles treated with ethylene diamine tetra acetic acid (EDTA).

The following analyses were carried out weekly

1. Blood glucose
2. Red Blood Cell Count
3. White Blood Cell Count
4. Platelet count
5. Haemoglobin concentration
6. Packed Cell Volume (%)
7. Electrolyte concentration (K^+, Na^+, Cl^-)

Results

Figs. 1 to 7 illustrate the effect of the combination of *B. diffusa* extract and *C. afer* extract on Red Blood Cell (RBC) Count, White Blood Cell (WBC) Count, Platelet Count, Blood Glucose, Packed Cell Volume (%PCV), Haemoglobin levels and Serum Electrolyte levels respectively.

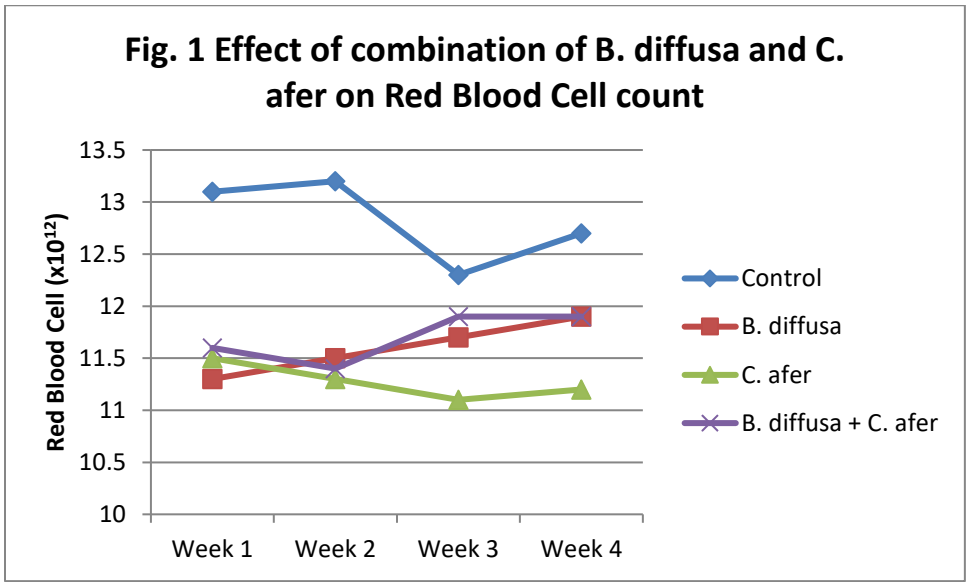

Fig. 1: Effect of combination of *B. diffusa* and *C. afer* extracts on Red Blood Cell Count

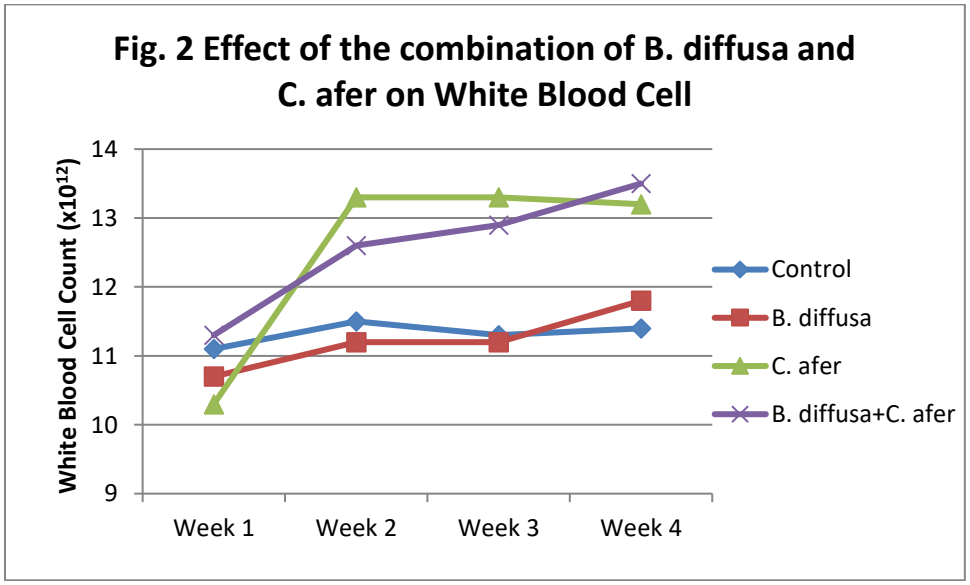

Fig. 2: Effect of the combination of *B. diffusa* and *C. afer* on White Blood Cell Count

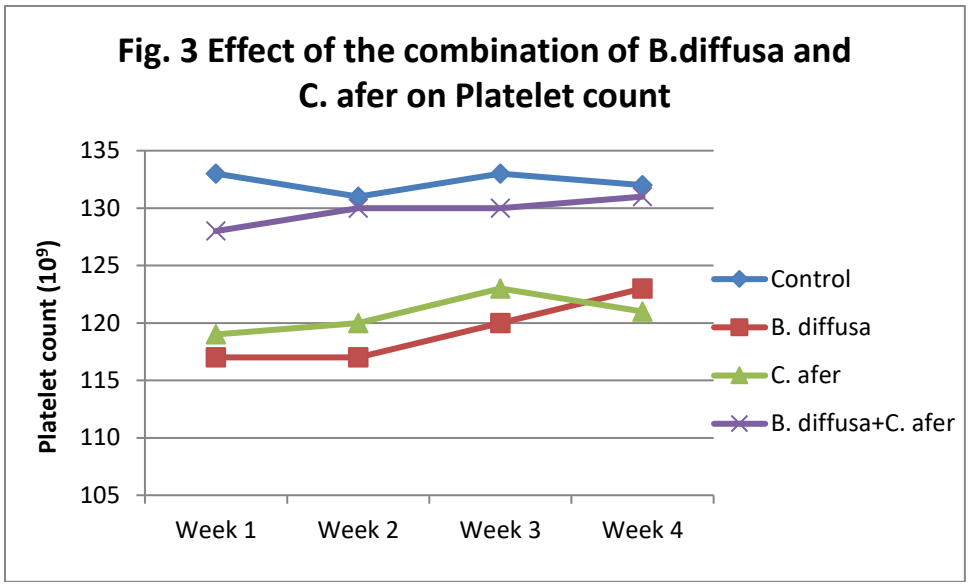

Fig. 3: Effect of the combination of *B. diffusa* and *C. afer* on platelet count

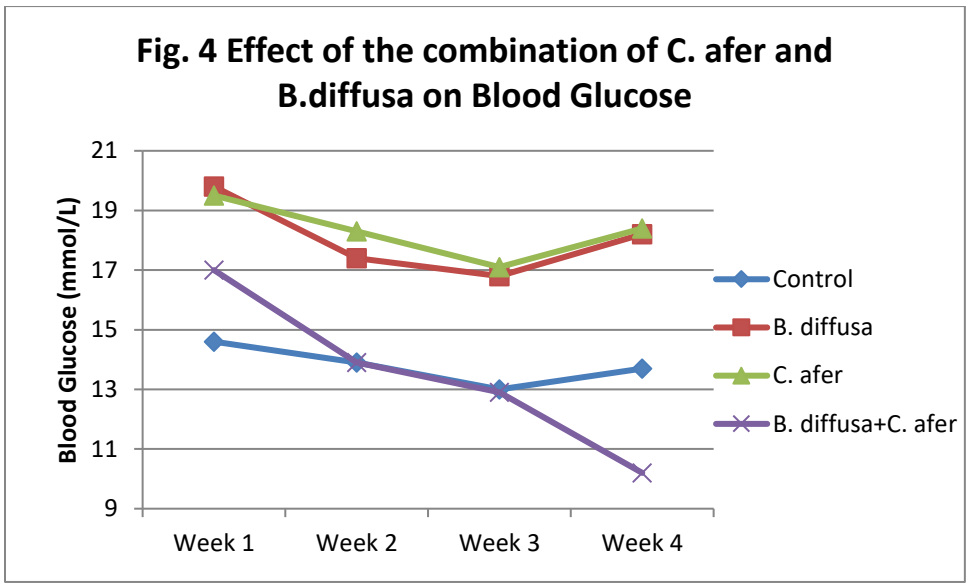

Fig. 4: Effect of the combination of *C. afer* and *B. diffusa* on blood glucose

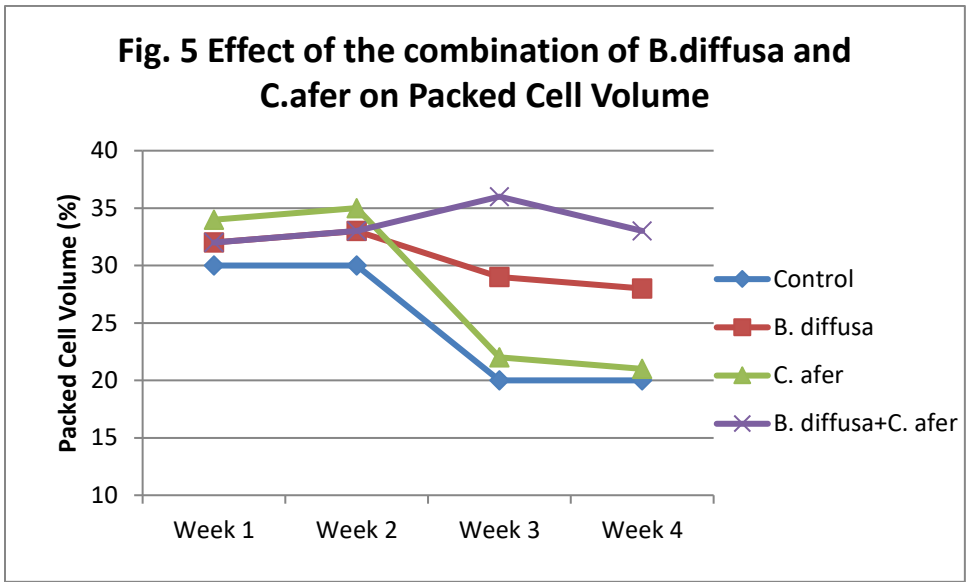

Fig. 5: Effect of *B. diffusa* and *C. afer* extracts on Packed Cell Volume

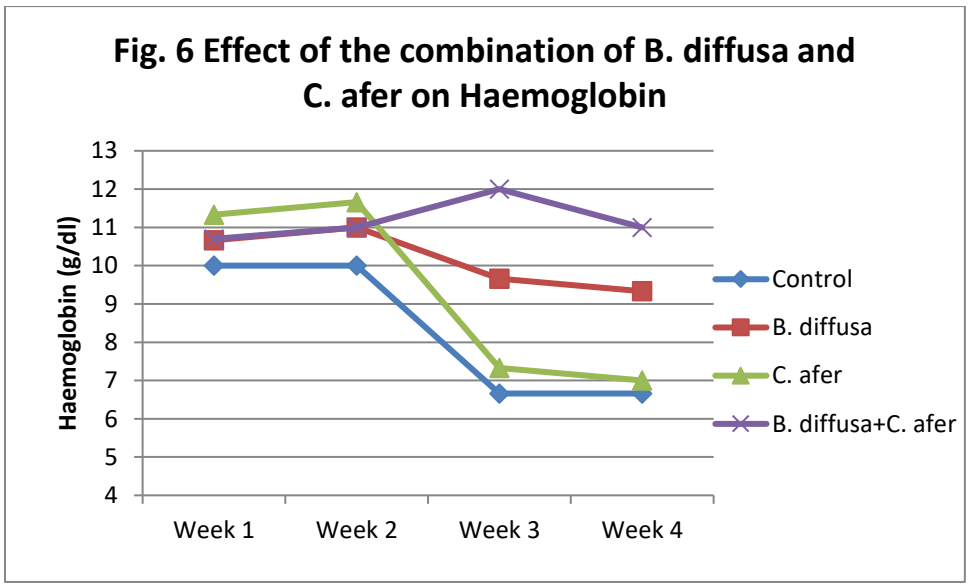

Fig. 6: Effect of *B. diffusa* and *C. afer* extracts on Haemoglobin content of blood

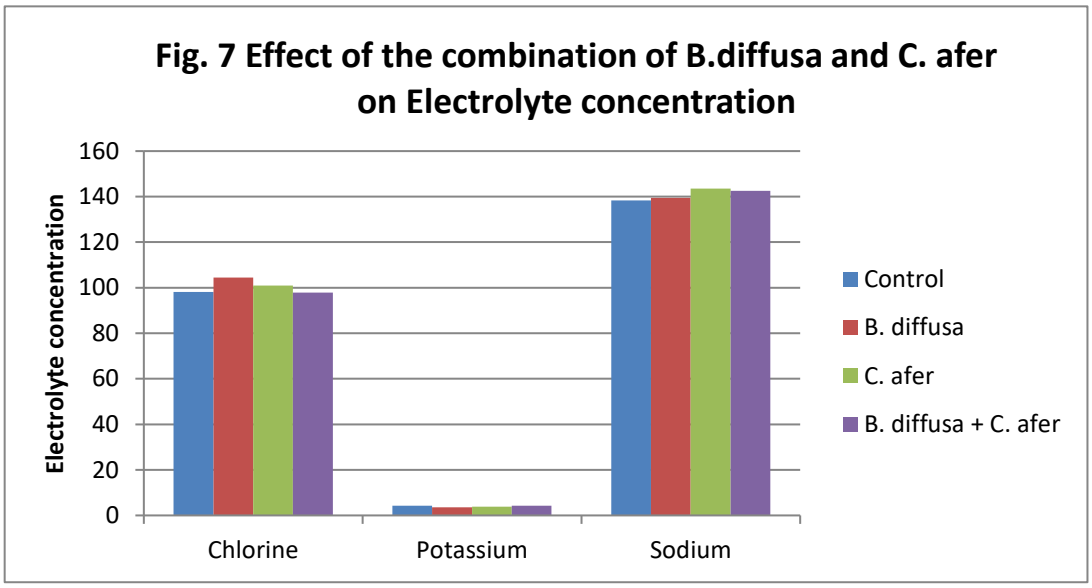

Fig. 7: Effect of the combination of *B. diffusa* and *C. afer* on Blood Electrolyte Concentration (after four weeks)

Discussion

Fig. 1 shows that the red blood cell count of the control broilers and those treated with *C. afer* dropped during the research period. Significant increase in red blood cell count, however, was observed in broilers on *B. diffusa* treatment and those treated with the combination of the two extracts.

Fig. 2 shows that there was a slight increase in the population of white blood cells in the control. However, this increase is more dramatic in birds fed with inclusions of the two extracts and their combination as they showed a significant increase in white blood cell count. Also, samples treated with *C. afer* showed a good increase in white blood cell count. White blood cells also known as phagocytes are a major defense system against the attack of germs and infections.

Fig. 3 shows the effect of the extracts and their combination on platelet count of the test broilers. While the control values remained steady, other samples showed varying levels of increase. Samples treated with *B. diffusa* showed the best rate, followed by samples treated with a combination of the two extracts. In the fourth week of the study platelet counts of samples treated with *C. afer* showed a small retracement from their previous values.

Fig. 4 shows the effect of extracts on blood glucose. All samples except the control, which remained steady, showed reduction in blood glucose levels. Samples treated with *B. diffusa* and those treated with *C. afer* showed blood glucose reduction of about (1.8mmol/L) during the test period. However, the most significant drop in blood glucose levels was shown in samples fed a combination of the two extracts. This can be attributed to a synergy of the two anti-diabetic agents resulting in a sharper drop in blood glucose.

Fig. 5 shows the effect of the two extracts and their combination on Packed Cell Volume of the experimental broiler chickens. Control samples and those treated with *C. afer* and *B. diffusa* showed a drastic reduction in PCV between week 2 and 3. However, samples treated with a

combination of the two extracts showed an improvement in PCV in the same period of time. This is indicative of synergistic interaction between the two extracts in vivo.

Fig. 6 shows the effect of *B. diffisa* and *C. afer* on haemoglobin content of blood. Akin to fig. 5, the graph shows an obvious drop in haemoglobin in the control, samples treated with *B. diffusa* and those treated with *C. afer*. However, broilers served a combination of the two extracts showed an increase in the period instead of a decrease in haemoglobin values. This is a positive indicator of synergistic action on haemoglobin production by the two plants. Samples treated with *B. diffusa* had a slower rate of haemoglobin reduction than those treated with *C. afer*.

End-of–research results for blood electrolytes are shown in fig. 7. They show little difference in electrolyte concentration (Cl^-, K^+ and Na^+). Chlorine content was slightly higher in birds treated with *B. diffusa*, followed very closely by those with *C. afer*. Control samples showed were higher than samples treated with a combination of the two plant extracts used which had the lowest value. Further research is necessary to reveal whether this is indicative of synergistic action as the two plant extracts alone raised the chlorine content of the broilers. There was no significant difference in potassium ion concentrations in tested birds. Sodium content was highest in samples treated with *C. afer* followed very closely by samples treated with a combination of *B. diffusa* and *C. afer*. Broilers treated with *B. diffusa* had a slightly higher sodium ion concentration than the control samples which had the lowest sodium concentration.

Conclusion

In summary, the results showed that *B. diffusa* extract gave the highest rate of increase in the red blood cell count and platelet count of test broilers. While all extracts reduced blood glucose, the combination of the two extracts showed a synergistic interaction in the rate of reduction of blood glucose as a more rapid drop was observed. The synergy between the two extracts was also evident in Packed Cell Volume (PCV) and haemoglobin levels as it significantly improved these values while other samples suffered a drop. There was no significant difference in the end-of-research electrolyte concentration with respect to extracts used.

This research clearly establishes that it is advantageous to the poultry farmer to incorporate plant extracts especially those used in this experiment into feed as they can improve the haematological parameters of the animal. Since blood is essentially the livewire of an animal, it can be said that improvement in haematological parameters will translate into more resistant (as white blood cells are responsible for attacking germs), stronger and larger birds, which will in turn enrich the poultry farmer.

More research, however, is necessary to determine and compare the growth promoting effect of *B. diffusa*, *C. afer* and the feasibility of replacing ccommercial growth promoters with these plant extracts.

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