

Haemogram and Serum Enzymes Activities of Newcastle Disease Virus Challenged Broiler Chickens Following Supplemental Treatment with *Aloe Vera* Extract

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Abstract

Aloe products have been promoted for use in constipation, coughs, diabetes, arthritis, immune-system deficiencies and many other conditions, but the clinical efficiency in chickens is unknown. Therefore, the study was designed to determine the haemogram and serum enzymes activities of Newcastle Disease Virus (NDV) challenged broilers following supplementation of *Aloe vera*. One hundred and forty day-old broilers were grouped into seven 20 broilers/group, were treated with different concentrations of the extract for 30 days. Both vaccinated and unvaccinated groups were challenged with 0.2 saline suspension of 10⁶ ELD₅₀ intradermal inoculation of NDV challenged strain up to the 30th day. Increase in serum protein, aspartate transaminase, creatinine, urea and gamma globulin levels among challenged group was concentration dependent (50 mg>100 mg>150 mg) but not statistically significant (P>0.05). Lymphocytosis among supplemented and challenged group was concentration dependent (50 mg>100 mg>150 mg) and statistically significant (P<0.05). Control group lymphocyte count was within normal range (55-70%). Heterophil and lymphocyte (H/L) ratio was lower in supplemented and challenged group. Oral intake of *Aloe vera* modulated leucocytes proliferation of the broilers and enhanced the cell differentiation in favour of the lymphocyte. Serum enzymes activities in broilers challenged with NDV following supplementation with *A. vera* juice were positively influenced, and modulated the excessive leakage of protein, globulin, creatinine and alkaline phosphatase in infected birds. This extract may therefore be seen as a good immunomodulator.

Keywords: *Aloe vera*; Enzymes; Haemogram; Newcastle disease virus; Supplementation

Introduction

It has been recommended that most diet be augmented with supplemental antioxidants and vitamins. Some of the best studied and most readily available supplements are beta carotene, selenium, vitamin C, vitamin E and vitamin A. Populations that have diets high in beta carotene have a lower incidence of certain forms of cancer [1].

Historical use of various *Aloe* species by humans has been well documented, though the species of *Aloe* used and their clinical effectiveness remain not fully understood [2]. Of the 300 species of *Aloe*, only few have been used traditionally as herbal medicine. Some species, in particular *Aloe vera* is used in alternative medicines and as a first aid material in some homes. Both the translucent inner pulp and the resinous yellow exudates from wounding the *Aloe* plant are used externally to relieve skin discomforts [3]. It has been reported to facilitate wound healing [4]. Systematic reviews of randomized and controlled clinical trials have provided no evidence that *Aloe vera* has a strong medicinal effect [5,6].

Bioactive compounds such as alkaloids, flavonoids, steroids, terpenoids glycosides, carbohydrates and tannins were reported to be present in the leaves of *Aloe vera* [7]. Davis and Robson further showed that *Aloe vera* has been traditionally used as antibacterial, anti-fungal and antiviral agents, immune enhancement, wound

healing, anti-inflammatory properties, constipation and for the female reproductive system [8,9]. *Aloe* products for internal use have been promoted for constipation, coughs, wounds, ulcers, diabetes, cancer, headaches, arthritis, immune-system deficiencies, and many other conditions. However, the only substantiated internal use is as a laxative [10]. There have been some studies in animal models which indicate that extracts of *Aloe* plant may have a significant anti-hyperglycemic effect, and may be useful in treating Type II diabetes. These studies have not been confirmed in humans [11].

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Materials and Methods

Laboratory animals (broiler chickens)

The experimental animals comprised of one hundred and forty (140) day-old broilers were obtained from Obasanjo Hatchery, Oluyole Estate, Ibadan, Oyo State for the study.

Inocula

A vial of lyophilized challenged strain of Newcastle disease virus (NDV) was obtained from Regional Laboratory for Avian Influenza and Tran boundary Animal Diseases, National Veterinary Research Institute, Vom, Plateau state, Nigeria. The vial was transported under cold chain and standard bio-safety practice to Owo, Ondo State. A saline suspension of 10^6 ELD₅₀ was prepared by taken up the vial in 1.5 ml of sterile diluents (physiological saline), then take 1 ml of the reconstituted virus to add 99.0 ml sterile normal saline.

Aloe vera (AV) extract

Samples of the succulent leaves of *Aloe* plant were harvested and washed with distilled water. Samples deposited in Biological Science Department, Achievers University, Owo, were identified and authenticated. *Aloe vera* juice was prepared following the method described by Wu et al. with slight modification [12]. The modification included the following steps: Freshly harvested leaves of the plant were washed, drained and cut open. The inner pulp was scrapped into a clean beaker and warmed at 50°C for 30 min until the viscous light-yellow pulp became less viscous. The extract was filtered with a muslin cloth. The AV extract was evaporated to dryness at 37°C using a Speed Vac (Model 7811001, Labconco, USA). The recovered extract was weighed and formulated in distilled water to give the required dose.

Treatment of broilers

Throughout the entire study the broilers were fed with feeds compounded carefully to meet 23% crude protein (CP) and 3200Kcal metabolizable energy (ME) for broiler starter and 20% CP and 3000Kcal for broiler finisher. The level of mycotoxins in feed were maintained relatively low throughout the experiment, the quality and quantities of groundnut cake (GNC), soya bean and rice bran included in the feeds were the same for both starter and finisher mash. The percentage CP in feed and ingredients were determined by the biurette method [13] while the metabolizable energy was determined by the Bomb calorimeter method [14]. The broilers were housed in battery cages 0.31 m²/bird as recommended by Mustafa et al. [15]. All experimental protocols complied with NIH guidelines (NRC, 1985), as approved by the ethical and research committee, Achievers University, Owo. All the birds received necessary medication and vaccination exempting NDV vaccine with the exception of the NDV vaccinated control group. The birds were divided into 7 groups of 20 birds each; they were allowed to acclimatize for 16 days and were fed with standard broilers feed and water *ad libitum*. Group I were not supplemented with *Aloe vera* extract i.e 0 mg, group II were supplemented with 50 mg, group III were given 100 mg, group IV were given 150 mg, group V were given 100mg but not challenged with NDV (NSC), group VI were not supplemented and not challenged with NDV (NSNC) and group VII were vaccinated, not supplemented and not challenged with NDV (VNSNC). Each treatment group was supplemented for 30 days. Birds were then challenged with intramuscular administration of inoculums bearing 0.2 ml of 10^6 ELD₅₀ (50 percent Embryo Lethal Dose) of saline suspension of NDV on the 30th day, and were examined for clinical signs and symptoms (though not reported in this article).

Haemogram

Haematological studies of blood samples from broiler chickens were carried out at the Haematology Laboratory of Federal Medical

Centre, Owo, Ondo State, using manual method to ensure accuracy Pack Cell Volume (PCV) was determined by the method described by [16]. Haemoglobin (Hb) concentration was measured by cyanmethaemoglobin method described by Lewis et al. [17]. Leukocyte was estimated from blood smears, the film was stained using Leishman's method as described by Lewis et al. [17], the total WBC and differential count on on hemacytometer as described by [18].

Serum biochemistry

The chickens blood collected into Lithium heparinised bottles were centrifuged and the plasma separated. The plasma of each bird was analyzed for levels of uric acid, Total Protein (TP), Albumin (Ab), Globulin (G), Creatinin (CRK), urea, γ -Glutamyltransaminase (γ GT), Alkaline Phosphate (AKP), Alanine Aminotransaminase (ALT) and Aspartate Aminotransaminase (AST) using method described by [13]. All the parameters were measured as a batch from each plasma sample using biochemical assay kits of Randox Lab. Ltd (UK) and Biochemistry Analyser (Spectrumlab 755S, England).

Statistical analysis

Results presented are means \pm SEM of 5 values of individual birds per supplementation group. Data obtained from this study were statistically analyzed using one way analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) using SPSS 17.0. Significant differences between the treatment means were determined at 95% confidence level.

Results

Serum biochemistry of *Aloe vera* supplemented broilers

Serum biochemistry of broiler chickens challenged with NDV after supplementation with *Aloe vera* is shown in Table 1 below. Total Protein (TP) (g/l) level was elevated in supplemented but challenged (SC): 50 mg, 100 mg and 150 mg group and control group (NSNC, SNC and VNSNC. The increase in serum protein level among challenged group was concentration dependent (50 mg>100 mg>150 mg) but not statistically significant ($P>0.05$.) when compared with not supplemented but challenged (NSC) control group (26.06 ± 0.1). Ab (g/l) level was elevated in SC and control group but low in NSC (0.32 ± 0.02). The increase in serum Ab level of SC group was concentration dependent (50 mg>100 mg>150 mg) and the difference was statistically significant ($P<0.05$). Among SC group the increase in serum globulin level was not concentration dependent (50 mg<100 mg<150 mg) and not statistically significant ($P>0.05$). The increase in serum AKP level was not concentration dependent (50 mg<100 mg<150 mg) and not statistically significant ($P>0.05$). γ GT (iu/l) of broiler chickens was elevated in SC group but low in NSC and control group, increase γ GT serum level among SC was concentration dependent (50 mg>100 mg>150 mg) and statistically significant at $P<0.05$, NSC recorded low serum γ GT level (1.16 ± 0.2).

AST (iu/l) was observed to be elevated among SC and control group but low in NSC (41.00 ± 0.1), and there was no statistical significant difference in AST level of the NSNC and SNC ($P>0.05$). Urea level among SC group was concentration dependent (50mg>100mg>150mg) but not statistically significant ($P<0.05$), urea level in NSC group was high (2.54 ± 0.1). Serum analysis for uric acid (mmol/l) was observed to be low in the entire group (SC, NSC and control groups), the level of uric acid among SC group was not

concentration dependent (50 mg<100 mg<150 mg) and not statistically significant (P<0.05), uric acid among NSC group was low (0.21 ± 0.03). CRK (mmol/l) level was elevated in SC and control group but low in NSC group (10.41 ± 0.04). The increase in SC group was concentration dependent (50 mg>100 mg>150 mg) and the difference was statistically significant (P<0.05). Among control group, VNSNC recorded higher serum CRK (50.34 ± 0.3), followed by NSNC (37.50 ± 0.2) and least in SNC (32.38 ± 0.4) the differences among control group was statistically significant at P<0.05.

The haemogram of *A. vera* supplemented broiler chickens after NDV challenge and control group is shown on Table 2 below. The total white blood cell count (1 × 10³/μl) was significantly low in SC group but slightly increased among control group but within the reference range (5.0-11.0), the reduction among SC group was not concentration dependent (150 mg>100 mg>50 mg) and the difference was statistically significant at P<0.05. Among control group SNC had higher count (21.0 ± 0.1) followed by NSNC (9.4 ± 0.1) and least in VNSNC (6.6 ± 0.0).

Parameter	0mg (NSC)	50mg	100mg	150mg	NSNC	SNC	VNSNC
TP (g/l)	26.04 ± 0.0	40.48 ± 0.5	41.02 ± 0.0	41.65 ± 0.7	45.30 ± 0.3	50.54 ± 0.6	45.48 ± 0.5
Ab (g/l)	0.31 ± 0.01	2.41 ± 0.39	3.07 ± 0.07	4.97 ± 0.01	0.92 ± 0.02	8.55 ± 0.05	6.96 ± 0.01
Globulin (g/L)	25.73 ± 0.01	38.07 ± 0.01	37.95 ± 0.01	36.68 ± 0.01	44.38 ± 0.01	42.00 ± 0.01	38.52 ± 0.01
AKP (iu/l)	60.72 ± 5.0	122.12 ± 4.0	94.00 ± 1.0	69.00 ± 2.0	93.47 ± 3.0	324.29 ± 24.0	98.28 ± 2.0
YGT (iu/l)	1.16 ± 0.2	6.94 ± 0.2	10.53 ± 0.0	16.20 ± 0.2	2.93 ± 0.0	1.74 ± 0.0	2.32 ± 0.3
AST (iu/l)	41.00 ± 0.1	51.67 ± 0.1	71.08 ± 0.1	80.00 ± 10.0	45.40 ± 0.4	48.00 ± 1.0	64.08 ± 0.1
Urea (mmol/l)	2.54 ± 0.1	2.01 ± 0.0	2.45 ± 0.5	3.34 ± 0.3	2.24 ± 0.2	2.83 ± 0.1	2.07 ± 0.1
Uric acid (mmol/l)	0.21 ± 0.03	0.37 ± 0.10	0.25 ± 0.03	0.14 ± 0.01	0.25 ± 0.02	0.25 ± 0.03	0.26 ± 0.01
CRK (μmol/l)	10.41 ± 0.4	44.20 ± 0.2	54.00 ± 4.0	62.40 ± 0.4	37.50 ± 0.2	32.38 ± 0.4	50.34 ± 0.3

AKP: Alkaline Phosphatase; TP: Total Protein; Ab: Albumin; γGT: Gamma Glutamyltransaminase; AST: Aspartate Transaminase; CRK: Creatinine Kinase; NSC (0 mg): Not Supplemented but Challenged; NSNC: Not Supplemented Not Challenged; NSC: Not Supplemented but Challenged; VNSNC: NDV Vaccinated, Not Supplemented Not Challenged.

Table 1: Serum enzymes activities of NDV challenged broilers following Aloe vera supplementation and control groups. P<0.05 (significant at 0.05), Mean ± SEM of 5 values per supplementation group.

Treatment groups (mg) >	0mg	50mg	100mg	150mg	NSNC	SNC	VNSNC
Total WBC (10 ³ /μL)	4.0 ± 0.2	5.4 ± 0.2	1.8 ± 0.1	2.6 ± 0.4	9.4 ± 0.1	21.0 ± 0.1	6.6 ± 0.0
Heterophil (%)	22	17	15	14	24	31	27
Lymphocyte (%)	70	74	80	81	65	62	69
H/L ratio	0.31	0.23	0.19	0.17	0.37	0.5	0.39
Monocytes (%)	5	6	2	2	4	4	1
Basophil (%)	2	3	2	3	3	3	3
Eosinophil (%)	1	0	1	0	1	0	0
Hb (g/dl)	9.7	10.2	9.8	9.4	10.1	9.8	11.1
PCV (%)	29	30	29	28	30	29	33
Plasma cell	-	+	+	+	-	-	-

NSNC: Not Supplemented Not Challenged; SNC: Supplemented but Not Challenged; NSC: Not Supplemented but Challenged; VNSNC: NDV Vaccinated Not Supplemented Not Challenged

Table 2: Haematological parameters of NDV challenged broilers following Aloe vera supplementation and control groups. P<0.05 (significant at 0.05), Mean ± SEM of 5 values per supplementation group.

Heterophils (%) was low among SC group while the control group was slightly elevated but the count was within the normal range (20-35), the difference in SC was concentration dependent (150

mg>100 mg>50 mg) but was not statistically significant at P>0.05. Lymphocytosis was observed in SC group and it was concentration

dependent and statistically significant at $P < 0.05$. Among the control group, lymphocyte (%) count was within the normal range (55-70).

Heterophil and lymphocyte (H/L) ratio was raised among control group and lower in the SC group. Monocytosis was also observed in 50mg, NSC (0mg), NSNC and SNC groups, and the increase was statistically significant at $P \leq 0.05$. There was no significant difference in the monocytes count in 150mg, 100mg and VNSNC at $P > 0.05$.

Basophils (%) and Eosinophils (%) count among birds supplemented with *A. vera* were not statistically significant at $P < 0.05$. Packed Cell Volume (%) recorded in both SC and control groups were within the normal range (28-42) and was statistically significant at $P < 0.05$. Haemoglobin (Hb) was observed to be within the normal range (9.0 g/dl) in the entire group and there was no significant difference. Plasma cells were also observed on blood film of challenged group except NSC group and completely absence in control group.

Discussion

The quality of blood obtained from supplemented and challenged group especially on the haematology and blood chemistry assays indicated that the liver enzymes aspartate aminotransaminase (AST) and γ -glutamyltransaminase (γ GT) were significantly elevated among supplemented and challenged group, an observation indicative of tissues damage [13]. Creatinine kinase (CRK) was also observed to be elevated in supplemented group which also indicate some sort of muscle damage, CRK has value in distinguishing muscle from liver damage [19], though, CRK is very insensitive and is a relatively poor diagnostic test in birds. However, Transaminases are the most commonly used indicators of cellular necrosis and increase in serum concentration may indicate liver malfunction [20]. They occupy a central position in amino acid metabolism; the elevation observed in this study could have a consequential effect on the amino acid metabolism in the birds, it may indicate some sort of injury to the organs like liver, heart, kidney, and brain, such injury may cause leakage of enzymes from damaged organs to the blood stream, the concentration of these enzymes predict the severity of damage.

The significant low values of AST, γ GT, AKP and Ab in the affected broilers indicate adverse health condition. The role of liver in the detoxification and regulating of body temperature cannot be over emphasized. AST, γ GT and AKP are enzymes used in the assessment of liver functions. AST and ALT are been used to determine hepatocellular integrity, the lower value observed in AST activity in the NSC group may be an indication that viral challenge alone has no effect on hepatocellular integrity and for the same reason values of AKP and γ GT observed in NSC group brings up the thought that NDV challenge has no adverse effect on the biliary system. TP, Albumin and globulin, AKP, γ GT and AST level was observed to be high in SC group and when compared with those of the control groups is in agreement with the report of [21], who stated that serum AKP could be influenced by physiological status (Pregnancy) and disease of animals. It has also been noted that lower AKP level could be linked to dietary zinc deficiencies [19]. Similar trend was observed by Alletor and Futuga [22] on the activities of AKP in blood of rats injected with legume anti nutrients. It appears that both viral challenged and *Aloe vera* supplementation has a synergistic effect on the synthetic capability of the liver, similarly, the production of γ GT, AKP and AST by the liver or other organs. A phenomenon in which both Albumin and enzymes are elevated in plasma may not be unconnected to the effect of the supplementation on the liver alone; it may also be due to

other organs that produce AST, AKP and γ GT such as the heart, bone marrow and pancreases. More so, The increase in AKP observed in SNC group as against the NSNC group shows that AV has a positive effect on the synthetic function of the liver while it has an adverse effect on the AKP producing tissues. The fact that it did not do the same to γ GT and AST could be due to the fact that AV may have no adverse effect on the liver in the absence of NDV challenge.

Our finding suggest that oral intake of *Aloe vera* modulated leucocytes proliferation of the supplemented broilers and enhanced the cell differentiation in favour of the lymphocyte as shown in the blood smear. An increasing practice among ornithologists is the use of blood smears to assess immune function of birds by counting the numbers and proportions of white blood cells (leukocytes) on blood smears, a leukocyte profile (or differential) can be obtained for the individual, giving insight into its immune function at the time of sampling [23]. Our finding shows that there was a significant reduction in total leucocytes count among the challenged broiler chickens and the effect of which was more on supplemented birds, which indicate that the extracts administered to the individual broilers impacted their immunity by regulating the total leucocytes count. This finding is consistent with Davis et al., who showed that stressful events such as disease could induce a significant decrease in white blood cell count in peripheral blood of birds [23]. Research with poultry has also shown that overall white blood cell numbers decrease within 1 h after stressful events [24]. It is probable that the significant lower leukocyte count (leucopenia) of birds on *A. vera* supplement is an indication that the birds were immunologically challenged. The reduced number of total WBC (leucocytosis) and increase in lymphocytes (lymphocytosis) in SC group is a characteristic of viral infection [25]. The reduced leukocyte counts of birds could also be a physiological adjustment presented against negative antigenic effect associated with the viral challenge.

The total white blood cell recorded in the present study was slightly below the values reported by [26] for broiler finishers fed control diets. Differential leucocytes counts were used as indicators of stress response and sensitive biomarkers crucial to immune functions [27]. It has however been reported that bacterial and viral diseases affect the number of white corpuscles and the ratio between the different types of white corpuscles and the percentages of the various types in healthy animals vary slightly but are greatly modified in sick animals [28,29]. Since corticosterone (stress hormone) level increase in wild birds within minutes of capture in response to stress [30], the corticosterone hormone released in response to stress may indirectly act to reduce the number of leucocytes in the peripheral blood stream in exotic disease condition of NDV infection. However, further research into avian immune systems would be needful to verify this.

The H/L ratio has been increasingly used by ornithologists to monitor immune function, as it appears to increase with disease [23], injury [31], and urbanization [32]. The relationship between increase in H/L ratio and lower immunoglobulin level is more difficult to interpret. It might be possible that birds with higher in H/L ratios are more responsive to the challenge because of circulating number of lymphocyte and their involvement in antibody production-an essential factor in humoral immunity, than those with lower H/L ratio, which ought to translate to healthier living by counteract the exotic effects of the challenge. The decrease in H/L ratio in the present study is at variance with the report of Davis et al. [23]; this phenomenon may be peculiar to Newcastle disease virus infection.

The haemogram of test birds slightly varied with supplementation, it has been documented that diets affect blood profile of animal [33-36]. The haemoglobin concentration (Hb) and packed cell volume (PCV) recorded in in this study was within the Hb (9.10 g/dl) and PCV (30.90%) of the control group of finisher broilers administered with oral *Telferia occidentalis* leaf extract as reported by Alabi et al. [26] which indicate that AV extracts affected the haemotopioses of the birds.

Plasma cells in the blood films which ordinarily should be absent on peripheral blood smear but may be seen with bacteria or viral infections, drug and other allergic responses, immunization and systemic lupus erythromatosus and in patients with multiple myeloma. The finding of plasma cells on the blood smears of broiler chickens supplemented with AV of different concentrations and challenged with NDV suggest that there was active viral infection. Plasma cell production increases as activated B-cells differentiate [37]. Surge in plasma cell production is followed by gradual decrease and sustained levels of circulating antibodies. For instance, plasma cells will likely secrete IgG3 antibodies, if they matured in the presence of the cytokine-interferon- γ . Since B cell maturation also involves somatic hypermutation (a process completed before differentiation into a plasma cell), these antibodies frequently have a very high affinity for their antigen. Plasma cells can only produce a single kind of antibody in a single class of immunoglobulin. In other words, every B cell is specific to a single antigen, but each cell can produce several thousand matching antibodies per second [38].

Conclusion

In NDV infection, H/L ration was seen to decrease And *A. vera* extract affected haematopioses of the poultry birds in favour of lymphocytes. This information should be added to the list of other vegetables/herbs tonics that enhances blood formation. Serum enzymes activities in broiler chickens challenged with NDV following supplementation with *A. vera* juice were influenced by the extract, especially modulating the excessive leakage of protein, globulin, creatinine and alkaline phosphatase enzymes in affected birds.

Conflict of Interest

The authors do not have a direct financial relationship with the commercial identity mentioned in this paper.

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