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Effect of supplemental *Aloe vera* gel and amla fruit extract in drinking water on growth performance, immune response, haematological profiles and gut microbial load of broiler chicken

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ABSTRACT

The use of herbs and spices has gained increasing interest as feed additives and possible alternatives to antibiotics in poultry production. The effects of using different levels of aloe vera and amla extract on growth performance, selected blood parameters, intestinal microflora, and immune response of broiler chickens were investigated in this study. 144 commercial day-old Cobb 500 broiler chicks were randomly allotted to six treatment groups with three replicates of eight chicks each. The treatments were T_1 (Control), T_2 (Aloe vera, 5ml/L), T_3 (Aloe vera, 10ml/L), T_4 (Amla 5ml/L), T_5 (Amla 10ml/L), T_6 (Antibiotic 0.2g/L). The experimental treatments were administered through drinking water. All the experimental birds were provided standard husbandry conditions like light, space, ventilation and relative humidity. The result showed that the average body weight of the birds at the end of the 28 days was higher ($p<0.05$) in all treated groups than the control. Feed conversion ratio for both the supplemented groups together with antibiotic group was significantly improved than the control group. However, Feed intake was non-significant ($p>0.05$) among the treatment groups. All haematological parameters (RBC, WBC, HCT and Hb) did not show any significant ($p>0.05$) difference among the treatments and the values were found to be within the normal ranges. Aloe vera gel group exhibited significantly improved antibody titre ($P<0.05$) against NDV compared to broilers in the control and other groups. Furthermore, supplementation of aloe vera and amla significantly ($P<0.05$) decreased gut Escherichia coli and Salmonella spp. then the control, but did not affect the total bacterial count. Thus, the findings showed that aloe vera and amla improved performance indices and gut microflora in broiler chicks. It also induced the immune response in the studied population. Therefore, aloe vera and amla extract may potentially be used as an antibiotic alternative in poultry production.

Key Words: *Antibiotic, Feed additive, Herbs, Poultry production and Productive performance*

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I. Introduction

Antibiotics have been used for more than half a century in poultry feed for improving performance, reducing some pathogenic microorganisms and increasing some useful microorganisms in the intestinal tract of these birds (Gibson and Fuller, 2000). However, antibiotics in the animal feed used as growth promoters have been banned recently due to potential development of antibiotic resistant human pathogenic bacteria (Patterson and Burkholder, 2003). It is of interest to investigate potential alternatives with the removal of antibiotic growth promoters from poultry diets to maintain good growth performance and good intestinal microbial populations, particularly to control the growth of harmful bacteria. Numerous additives are used or proposed as a means to reduce or eliminate pathogens or improve growth and FCR (Joerger, 2002). Probiotics (Awad et al., 2006), prebiotics (Biggs and Parsons, 2007), organic acids (Gunal et al., 2006), enzymes (Viveros et al., 1994) and herbal extracts (Sakine et al., 2006) used extensively in poultry feed in different countries of the world. Aloe vera contains over 200 various compounds including aloesin, anthraquinones (aloin and aloe emodin), acemannan, saponins, sterols, amino acids and vitamins (Grindlay et al., 1986). Most of the compounds have various biological functions with potential health benefits, such as antibacterial, antiviral, wound healing, antioxidant, immunomodulatory, antineoplastic, antihypertensive and anti-diabetic properties (Pandey et al., 2010).

Phyllanthus emblica (syn. *Emblica officinalis*), the Indian gooseberry or amla, which is a deciduous tree of the Phyllanthaceae family and is known for its edible fruit. Medical studies conducted on amla fruit suggest that it has anti-viral properties (Koul et al., 2010) and also acts as an anti-bacterial and anti-fungal agent (Linda, 1994). Amla powder contains 5.05 to 6.78% moisture, 0.23 to 0.59% fat and minerals like calcium 79.6mg, phosphorous 12.38mg and iron 88.03mg/100g (Poonam et al., 2009). Amla is one of the richest sources of Vit-C and contains 700mg (Saini et al., 2008). It has antioxidant properties and a high density of tannins (Wei et al., 2011). The fruit also contains other polyphenols: flavonoids, kaempferol, ellagic acid and gallic acid (Rehman et al., 2007). Animals fed on amla powder showed a better ability for uptake and killing of bacteria due to the presence of tannins that stimulates phagocytic cells (SaiRam et al., 2001).

Various studies suggest that herbs, spices and various plant extracts have appetizing and digestion-stimulating properties and antimicrobial effects which ultimately help the overall performance of poultry. However, very little work has been done to study the efficacy of aloe vera and amla for improving the growth performance of broilers. Hence, the present study was undertaken to evaluate the effects of aloe vera and amla on performance, immunity, blood parameters and gut microbial loads of broiler chickens. Various medicinal plants show many bioactivities such as antimicrobial activities, immune enhancement, and stress reduction. The use of these plants as dietary supplements may positively affect poultry health and production. Aloe vera and amla are the two of them which may be included in the poultry diet as feed additives for utilizing their benefits. Thus, the present research program was undertaken to evaluate their effects on the productive performance of broiler chickens as well as to investigate the effectiveness of aloe vera and amla as a growth promoter.

II. Materials and Methods

Site of the experiment and duration of work

The research project was conducted at the poultry farm of Sher-e-Bangla Agricultural University. The trial had been conducted for 28 days (From 01.08.2015 to 28.08.2015).

Experimental design

One hundred forty four (144) day old Cobb 500 broiler chicks were purchased from the local market and divided into 6 experimental groups with 3 replications of 8 chicks each. The treatments were T_1 (Control), T_2 (Aloe vera, 5ml/L), T_3 (Aloe vera, 10ml/L), T_4 (Amla 5ml/L), T_5 (Amla 10ml/L) and T_6 (Antibiotic 0.2g/L). The experimental treatments were administered through drinking water. At day time, drinking water was provided according to treatment. But only freshwater was provided in all groups at every night.

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Ration for broiler

Broiler starter and broiler finisher diet were fed *ad libitum* to birds from 1-14 and 15-28 days of age; respectively. The diet was subjected to proximate analysis as per (AOAC, 1995). The ingredients composition of broiler starter and finisher diets are presented in Table 01.

Table 01. Ingredients composition of broiler diet

Ingredients (%)	Starter (1-14 days)	Finisher (15-28 days)
Maize	51.5	56
Soybean meal	31	26
Rice polish	7	6
Soybean oil	2.5	3
Meat and bone meal	5.25	6.25
Lime stone	1.2	1.2
Dicalcium phosphate	0.4	0.4
Lysine	0.2	0.2
DL- methionine	0.18	0.16
Group super (GS) premix	0.25	0.25
Common salt	0.25	0.25
Choline chloride	0.05	0.05
Toxin binder	0.2	0.2
Coccidiostat	0.05	0.05
Total	100 Kg	100 Kg

Management

The birds were housed in a well-ventilated deep litter house. Each group was kept in a separate brooding place of similar specifications. Each experimental unit of the chicks was separated by using a fence. The temperature was measured during brooding period using digital thermometers. All the six groups of chickens were kept under similar management conditions like space, litter material, brooding, light, ventilation and relative humidity. The balanced feed was supplied *ad-libitum* all the time. The birds were vaccinated against ND and Gumboro disease according to the standard vaccination schedule.

Data collection

The experiment was conducted for 4 weeks. Birds were weighed at the beginning of the experiment and subsequently, on a weekly basis. Feed consumption and weight gain were recorded and feed conversion ratio (feed intake/weight gain) was calculated. Mortality was recorded daily.

Immunological study

Blood samples were collected (one bird per replicate) at the end of four weeks and serum was separated. The antibody titre in the serum was detected by haemagglutination test (Alexander, 1998). Antibody titer against NDV was reported as $\log_2$.

Haematological parameters

At 28 days of age, two birds per replicate were randomly chosen, then slaughtered and blood samples were collected into well-labeled sterilized bottles containing ethylenediaminetetraacetic acid (EDTA) as anti-coagulant. The blood samples were collected via the wing veins using sterile needles and syringes. The samples were investigated for the following haematological parameters – red blood cell count (RBC), white blood cell (WBC), hemoglobin (Hb) and hematocrit (HCT) (Kececi et al., 1998).

Faecal sample collection and examination

At the end of the study period, one bird from each replicate was randomly chosen and fresh faecal samples (approximately 2 g/bird) were collected with swab sticks and dropped inside sterile sample bottles for determination of microbial load. About 1g of gut content from each bird was diluted with 9 ml of sterilized physiological saline solution and thoroughly mixed and a serial dilution up to 10^{-9} was done. From each dilution, 1ml of aliquot was spread on the appropriate selective agar plates and incubated at 37°C for 24 hours. Plate count agar, salmonella shigella agar (SSA), Eosin methylene blue agar were used as the media for the total bacterial count, salmonella and *E. coli* count; respectively.

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After incubation, the colonies were counted and expressed as the numbers of colony forming units (cfu) per gram of gut content (Quinn et al., 1992).

Statistical analysis

The performance data were presented as mean ± standard deviation. The data collected were analyzed by analysis of variance technique using Completely Randomized Design. The differences in means of the treatments were compared by Duncan's Multiple Range Test. Values of $p< 0.05$ were considered to be significant.

III. Results and Discussion

Growth performance

The chemical composition of experimental rations is presented in Table 02. The crude protein content of starter and finisher rations ranged between 20.19 to 22.32% and metabolizable energy (ME) contents between 3027 to 3169 kcal/kg. Initial body weight and final body weight with FCR and mortality% in different treatment groups are presented in Table 03. The initial body weight of chicks on different treatments was non-significant ($p>0.05$). The average bodyweight of the birds at the end of the 4th week was higher ($p<0.05$) in all treated groups than the control. The result indicates that, as a growth promoter, both Aloe vera and amla improved growth and FCR similar to antibiotic growth promoter. This result supports the observation made by Patel et al. (2016), Mmereol (2011) and Mehala and Moorthy (2008). The improvement in body weight gain might be due to the presence of antimicrobial, antioxidant and antistress properties in herbal preparations which is similar with the findings of Maini et al. (2007), Patil et al. (2012), Kumari et al. (2012), Patil et al. (2014), Sujatha et al. (2010) and Kumar et al. (2013). Feed conversion ratio for both the supplemented groups and antibiotic group was significantly improved than the control group ($p<0.05$). Average feed intake was non-significant ($p>0.05$) among the treatment groups (Table 03). Dietary supplementation of both aloe vera and amla at levels respectively (5 ml and 10 ml) did not show any adverse effect on feed intake in broilers. The mortality was 4.17% in both T_1 and T_4 groups (Table 03). The data indicated that the percentage of mortality was within the normal limit (below 5%).

Table 02. Chemical composition of basal diet used during experimental feeding

Attributes	Starter (1-14days)	Finisher (15-28days)
Dry Matter, (%)	10.07	10.42
Crude Protein, (%)	22.32	20.19
Ether extract, (%)	2.84	3.67
Crude fiber, (%)	4.86	4.97
Total ash, (%)	5.35	5.14
ME (kcal/kg)	3027.3	3169.8

Table 03. Growth performance of broiler

Treatment	Initial BW (g)	Final BW (at 28d) (g)	Av. BWG (g)	Av. FI (g)	FCR	% Mortality
T_1 (Control)	44.16±0.06	1274.44±18.97^b	1230.28±18.91^b	2082.53±25.50	1.69±0.01^a	4.17
T_2 (Aloe vera, 5ml/L)	44.13±0.08	1323.13±18.63^a	1279.00±18.56^a	2101.00±27.84	1.64±0.00^b	0
T_3 (Aloe vera, 10ml/L)	44.13±0.05	1319.50±21.69^a	1275.37±21.65^a	2111.68±33.02	1.66±0.01^b	0
T_4 (Amla 5ml/L)	44.12±0.04	1333.69±25.73^a	1289.58±25.70^a	2114.33±43.10	1.64±0.00^b	4.17
T_5 (Amla 10ml/L)	44.15±0,04	1336.54±26.78^a	1292.39±26.74^a	2127.67±36.47	1.65±0.01^b	0
T_6 (Antibiotic 0.2g/L)	44.15±0.07	1317.84±33.03^a	1273.69±33.00^a	2097.67±37.81	1.65±0.02^b	0
Level of Sig.	NS	*	*	NS	*	

Data were presented as mean ± standard deviation, $^{a, b}$ Means bearing different superscripts in a column differ significantly ($p<0.05$). NS= Non significant;*= Significant

Haematological profiles

Changes in the physiological condition often reflect the alteration of blood composition. Therefore, haematological characteristics of livestock have been observed as factors determining the response of livestock to the diet they are fed (Madubuike et al., 2006). The consequence of treatments on blood

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profiles in this study is summarized in Table 04. All haematological parameters (RBC, WBC, HCT and Hb) measured did not show any significant ($p>0.05$) difference across treatments. Values of haematological parameters measured fall within the normal ranges (Mitruka et al., 1977). This implies that inclusion of amla and aloe vera as a dietary supplement does not have adverse effects on haematological parameters. The results are similar to the findings of Mekala (2014) and Mmereol (2011) who reported that there was no significant difference in haematological parameters in aloe vera supplemented broilers compared to control. These results indicate that the supplementation of amla and aloe vera in the diet is considered safe for broilers. Aloe vera gel and amla contain thiamine, riboflavin, folic acid and other essential amino acids which may trigger the erythropoietic system to produce red cells. These constituents would play a role in the immune system stimulation and in the function of organs which related to blood cell formation such as thymus, spleen and bone marrow (Li et al., 2002).

Table 04. Blood profiles of broiler

Treatment	RBC ($\times 10^6$/mm^3)	WBC ($\times 10^3$/mm^3)	HCT (%)	Hb (g/dl)
T_1 (Control)	2.19±0.24	138.70±12.08	30.63±2.84	10.12±0.40
T_2 (Aloe vera, 5ml/L)	2.33±0.24	131.20±11.26	29.98±2.22	9.88±0.36
T_3 (Aloe vera, 10ml/L)	2.18±0.35	126.72±10.17	31.59±2.06	10.21±0.50
T_4 (Amla 5ml/L)	2.03±0.22	141.29±11.08	30.86±1.93	10.03±0.70
T_5 (Amla 10ml/L)	2.29±0.22	130.24±10.92	30.74±2.08	9.97±0.34
T_6 (Antibiotic 0.2g/L)	2.26±0.27	136.28±13.55	31.16±2.34	10.15±0.44
Level of Significance	NS	NS	NS	NS

Data were presented as mean ± standard deviation. NS= Non-significant

Immunity

In the present study, broilers on the aloe vera gel group exhibited significantly improved antibody titer ($p<0.05$) against NDV compared to broilers in the control and other groups. In this experiment, a significant increase in the titre value against Newcastle disease was observed only when aloe vera was included at10 ml in drinking water but not in 5 ml inclusion. Numerically improved that antibody titre against NDV was found in all treated groups as compared to control (Table 05). The findings may be attributed to the fact that active component of herbs (Aloe vera and Amla) may improve digestion and stimulate the immune function in broiler. The findings are very much consistent with the report of Darabighane et al. (2012), Valle-Paraso et al. (2005), Fallah (2014) and Reddy et al. (2012). They reported that dietary inclusion of aloe vera and amla showed significant increases in antibody titre against Newcastle disease virus (NDV).

Table 05. Immunological response against Newcastle at 28 days of age (log_2)

	T_1 (Control)	T_2 (Aloe vera, 5ml/L)	T_3 (Aloe vera, 0ml/L)	T_4 (Amla 5ml/L)	T_5 (Amla 10ml/L)	T_6 (Antibiotic 0.2g/L)	Level of Sig.
Antibody titre against Newcastle	$3.80±0.39^a$	$4.50±0.24^a$	$5.14±0.25^b$	$4.32±0.18^a$	$4.20±0.20^a$	$4.54±0.23^a$	*

Data were presented as mean ± standard deviation. ^{a,b} Means bearing different superscripts in a row differ significantly ($p<0.05$). *= Significant

Gut health

The sequel of dietary treatments on gut microbial load (*Escherichia coli* and *Salmonella*) of broilers receiving different dietary treatments are shown in Table 06. The results revealed that supplementation of aloe vera and amla significantly ($p<0.05$) decreased *Escherichia coli* and *Salmonella population* than the control, but had no effect on total bacterial count ($p>0.05$). However, there were numerical variations among the treatments. The result of the present study is almost similar to the findings of (Amaechi and Iheanetu 2014). They revealed that microflora of the faecal samples was significantly influenced by the dietary inclusion of aloe vera and enramycin in the broiler's diets. Generally, aloe vera and amla are made of different constituents which possess antibacterial, antifungal and antiviral properties with a direct or indirect effect on gut microflora population.

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Table 06. Gut microbial load (log_{10}cfu/g) of broilers at four weeks of age

Treatment	Total bacterial count	E. coil	Salmonella
T_1 (Control)	5.39±0.16	5.07±0.14[a]	3.98±0.12[a]
T_2 (Aloe vera, 5ml/L)	5.36±0.15	4.37±0.07[b]	2.51±0.18[c]
T_3 (Aloe vera, 10ml/L)	5.30±0.20	4.25±0.17[b]	2.59±0.13[c]
T_4 (Amla 5ml/L)	5.48±0.20	4.34±0.11[b]	3.30±0.10[b]
T_5 (Amla 10ml/L)	5.43±0.10	4.20±0.12[b]	3.19±0.09[b]
T_6 (Antibiotic 0.2g/L)	5.40±0.19	4.28±0.11[b]	2.43±0.12[c]
Level of significance	NS	*	*

Data were presented as mean ± standard deviation. [a, b, c] Means bearing different superscripts in a column differ significantly ($p<0.05$). NS= Non significant; *= Significant

IV. Conclusion

The findings represent that aloe vera and amla enhanced performance indices and gut microflora in broiler chicks. It also persuades the immune response against Newcastle disease. Though aloe vera and amla supplementation had shown positive response in the present study, further investigation is needed at different dietary inclusion levels to get more information about its efficacy. Also, cost effective study needs to be performed as well. However, it is concluded that aloe vera and amla extract may potentially be used as alternatives to the antibiotic in poultry production.

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Conflict of interest statement

The authors have no conflict of interest to declare.

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