



Effects of Aloe Vera Leaf Extract on Growth, Clinical Chemistry and Histology of Selected Organs in Turkey Poults Challenged with *Escherichia coli*

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ABSTRACT

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A 6-weeks feeding trial was carried out to elucidate the effect of different levels of aloe vera leaf extract on the performance and health of turkey poults challenged with *Escherichia coli*. Sixty (60) turkey poults were randomly grouped into five treatments which were further replicated three times in a completely randomized design. The first group of poults was without infection and fed on the basal diet alone, and served as the positive control (P-CON). Out of the rest four infected groups, one was fed the basal diet alone to serve as the negative control (N-CON), while the others were fed the basal diet with tetracycline (ANT), or aloe vera at 2-mL (ALV2) and 3-mL (ALV3) per litre of water. The response of turkey poults to the challenge were assessed in terms of their growth performance, histology, haematology and serum constituents. The growth parameters, such as survival, weight gain, feed conversion efficiency were significantly ($P < 0.05$) higher in poults given ALV3. Histological results of the organs (breast muscle, liver, spleen and ileum) showed normal morphological pattern for poults subjected to ALV2 while those kept on ALV3 showed normal for breast muscle, spleen and ileum but not for the liver. Similarly, birds raised on ANT showed normal organ morphology compared with the positive control. However, the birds challenged with *E. coli* without (N-CON) showed abnormal morphological pattern for all the organs investigated. There were also no adverse effects of aloe vera leaf extract on turkey poults health, as determined from the analysis of various haematological parameters and serum metabolites. The results indicated that Aloe vera leaf extract inclusion at 2mL (ALV2) in drinking water could successfully replace antibiotics in the rearing of turkey poults.

Key words: Aloe vera, Antibiotics, *E. coli*, Histology, Poults, Turkey.

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INTRODUCTION

Plant extracts have gained increasing interest as possible feed additives for animal productions (Windisch *et al.*, 2008). In recent years, phytogetic feed additives have attracted increasing interest as an alternative feeding strategy to replace antibiotic growth promoters. Phytobiotics (phytogetic additives) consist of substances derived from medicinal plants or spices which have positive effect on production and health of animals. Phytogetic additives influence improvement of consumption and conversion of food, digestibility and gain of broiler chickens (Ertas *et al.*, 2005). Mechanism of the action of these additives is not completely clear. Some plant extracts influence digestion and secretion of digestive enzymes, and, many also exhibit antibacterial, antiviral and antioxidant properties (Ertas *et al.*, 2005; Cross *et al.*, 2007).

Aloe vera (*Aloe barbadensis* Miller) is a species of succulent plant that probably originated in northern Africa. The species does not have any naturally occurring populations, although closely related aloes do occur in northern Africa. The species is frequently cited as being used in herbal medicines since the beginning of the first century AD. Extracts from aloe vera are widely used in the cosmetics and alternative medicine industries, being marketed as variously having rejuvenating, healing or soothing properties. The whole leaf of aloe vera contains over 200 compounds including aloesin, anthraquinones (aloin and aloemodin), acemannan, saponins, sterols, amino acids and vitamins (Grindlay *et al.*, 1986). Most of these compounds have various biological activities with potential health benefits, such as antibacterial, antiviral, wound healing, antioxidant, immunomodulatory, antineoplastic, antihypertensive and antidiabetic activities (Pandey *et al.*, 2010).

The biological and toxicological effects of aloe vera gel have been extensively studied. Gel formulations are used as functional food supplements, drugs and in cosmetics. Whole gel also has some therapeutic effects. Amongst its therapeutic properties, anti-inflammatory (Malterud *et al.*, 1993) immunostimulatory (Ramamoorthy and Tizard, 1998), and cell growth stimulatory (Rodriguez-Bigas, 1988) activities have been reported. Furthermore, activity against a variety of infectious agents has been attributed to Aloe vera; for instance, antibacterial (Ferro *et al.*, 2003), antiviral (Kahlon *et al.*, 1991) and anti fungal (Kawai *et al.*, 1998).

This study investigated the effects of levels of aloe vera leaf extract on the performance of *E. coli*-challenged Turkey poults.

MATERIALS AND METHODS

Collection of Aloe vera leaf extract

Fresh Aloe vera leaf was harvested within the Ilorin metropolis. The leaf was washed and the gel scraped into a clean flask. The extracted gel was mixed with water appropriately for the treatments.

Source of *E. coli*

Strains of *E. coli* were cultured at the Department of Microbiology, University of Ilorin, Nigeria. The plate was placed in an incubator set at 37°C to allow for growth for 24 hours. After the growth of the *E. coli* on the agar plate, samples were then inoculated into a test tube containing 10ml of Nutrient Broth that will aid the growth of the bacteria and again placed in an incubator at 37°C. After 24 hours the solution had become cloudy indicating growth of bacteria, the cloudy solution was then poured into a conical flask containing 200ml of the same broth. The birds were challenged orally with *E. coli* throughout the experiment at the rate of 1.23×10^8 cfu/ml (counted using serial dilution method). Birds were observed twice daily for 9 days post-challenge for clinical signs, and each individual was assigned daily clinical scores as follows: 0, normal; 0.5, slightly abnormal appearance, slow to move; 1, depression, reluctant to move; 2, inability to stand or reach food or water; and 3, dead. All of the birds were scored daily for 9 days post-challenge. Some birds that received a clinical score of 2 were euthanized by cervical dislocation. Chickens that were found dead or euthanized were necropsied immediately for various pathological lesions indicative of *E. coli*.

Management of experimental animals

A total of 60 six-weeks old turkey poults were used randomly assigned to five dietary treatments, each with 3 replicates and 4 birds each using a completely randomized design (CRD). The poults were fed a common basal feed *ad-libitum* (Table 1). The first group of poults was without infection and fed on the basal diet alone, and served as the positive control (P-CON). Out of the rest four infected groups, one was fed the basal diet alone to serve as the negative control (N-CON), while the others were fed the basal diet along with tetracycline (ANT), or aloe vera at 2-mL (ALV2) and 3-mL per litre of water. The birds were housed in a ventilated deep litter house.

Table 1. Composition of experimental diet[†] (%DM)

Ingredients	% Inclusion
Maize	35.0
Corn bran	6.0
Groundnut cake	25.0
Soyabean meal	24.0
Fishmeal	2.6
Bone meal	2.5
Oyster shell	1.5
Palm oil	3.0
Salt	0.2
Premix [‡]	0.2
Total	100.0

[†]Nutrient composition: CP, 24%; ME, 2700 kcal/kg

[‡]Premix supplied per kg of diet: vitamin A, 8×10^6 IU; vitamin D₃, 1500 IU

Data collection

The experiment was conducted for 6 weeks. Body weights of turkey poults were determined weekly. Feed consumption and weight gain were recorded and feed conversion ratio (food intake/weight gain) was calculated. Mortality was recorded as it occurred. At 42 days of age, two birds per replicate, chosen randomly were slaughtered and blood samples were collected into vials containing EDTA. The RBC and WBC counts were determined by a hemocytometer method using Natt-Herrick solution; hematocrit (HCT) and hemoglobin (Hb)

values were measured by microhematocrit and cyanmethemoglobin methods respectively (Kececi *et al.*, 1998). Also, blood samples were collected in non-heparinised tube at 42 days of age from 2 birds in each treatment was centrifuged to obtain serum. Individual serum samples were analyzed for total protein, total cholesterol, uric acid, creatine and glucose using commercial diagnostic kit (Pars Azmoon Company, Tehran, Iran).

The slaughtered birds were dressed, before the dissection of the carcass, the organs required for histology (breast muscle, ileum, spleen, and liver) were quickly dissected, and preserved in formalin solution. The tissues were trimmed, fixed in Bouin fixation for 24-h, embedded and sectioned at 5-6 μ with a microtome and stained with haematoxylin and eosin. The histological study was carried out according to the described method by King *et al.* (1980).

Statistical analysis

Data were subjected to analysis of variance using CRD according to Steel and Torrie (1980). Treatment means were subjected to Duncan Multiple range Test (Duncan, (1955) to test for differences.

RESULTS AND DISCUSSION

The effects of treatments on performance of turkey poult are summarized in Table 2. Feed intake and weight gain of poult in the N-CON group was significantly ($P < 0.05$) lower than those on the other treatments. However, weight gain of poult P-CON and ALV3 were similar ($P > 0.05$) and significantly ($P < 0.05$) higher than weight gain in other treatments. Increased feed intake in aloe vera gel groups can be attributed to changes in taste and stimulated appetite as reported by Windisch *et al.* (2008). Samarasinghe (2003) also argued that herbs can stimulate appetite and endogenous secretions which in turn improve performance. The poor performance of poult on negative control shows clearly the devastating effects *E. coli* could have on birds. Furthermore, improved performance of poult on ALV3 showed that it is more effective than antibiotics in treating *E. coli*. Antibiotics may control and limit the growth and colonization of a variety of pathogenic and nonpathogenic species of

Table 2. Effects of aloe vera leaf extract on performance of turkey poult challenged with *E. coli*

Treatment	Feed intake (g/day)	Weight gain (g/day)	Mortality (%)	Feed:Gain ratio
P-CON	63.12 ^a	17.57 ^{ab}	0.0 ^b	3.59
N-CON	35.72 ^c	11.14 ^c	8.3 ^a	3.21
ANT	49.97 ^b	16.57 ^{ab}	0.0 ^b	3.02
ALV2	47.14 ^b	15.49 ^b	0.0 ^b	3.05
ALV3	49.72 ^b	17.18 ^{ab}	0.0 ^b	2.89
SEM	2.56	1.22	4.56	1.80

^{abc}Means with different superscripts in a column differ significantly ($P < 0.05$)

bacteria in chick gut (Ferket, 2004). A more balanced biota population in the gastrointestinal tract of poultry could lead to a greater efficiency in digestibility and utilization of food, resulting in an enhanced growth and improved FCR (Bedford, 2000). In the same vein, Landy *et al.* (2011) reported an enhancement in body weight gain of broilers offered diets containing neem as alternative to antibiotics growth promoter. Furthermore, Aloe has been found to contain several beneficial ingredients, including vitamins, minerals, organic acids, and carbohydrates (Boudreau and Beland, 2006) that could influence body weight gain in Aloe-supplemented chickens.

The result of this experiment on the responses of different levels of Aloe vera leaf extract in turkey poualts has useful and valid information. The purpose of investigating breast muscle, liver, spleen and ileum is to serve as a tool to differentiate between normal and unhealthy animals at cellular levels. Treatments that contained aloe vera leaf extract ensured proper development and growth of these organs and tissues, especially in disease conditions.

From the results in Table 3, it was observed that breast muscle shows normal morphological pattern with a normal cellular appearance for all the treatments. This shows that the breast muscles for all the treatments were not affected by *E. coli*. The liver of birds given P-CON, ANT and ALV2 were normal with no abnormalities seen while birds on ALV3 and N-CON showed mild infiltration of the portal tract

Table 3. Effects of different levels of aloe vera leaf extract on organ histology of Turkey Poualts challenged with *E. coli*

Organs	Treatments	Descriptions
Breast muscle	P-CON	Section showed a normal morphological pattern of breast with a normal cellular appearance.
	N-CON	Section shows a normal morphological pattern of breast with a normal cellular appearance.
	ANT	Section showed a normal morphological pattern of muscle fibre.
	ALV2	Section showed a normal morphological pattern of breast with a normal cellular appearance.
	ALV3	Section shows a normal morphological pattern of breast with a normal cellular appearance.
Liver	P-CON	Section shows a normal pattern of the liver, no adverse reaction seen.
	N-CON	Section shows portal tract to be mildly infiltrated with lymphocytes, although morphological pattern is normal, no further abnormalities observed.
	ANT	Section shows a normal pattern of the liver, no abnormalities seen.
	ALV2	Section shows a normal pattern of the liver, no abnormalities seen.
	ALV3	Section shows portal tract to be mildly infiltrated with lymphocytes, although morphological pattern is normal, no further abnormalities observed.
Spleen	P-CON	Section shows a normal pattern of the spleen, no abnormalities seen.
	N-CON	Section shows an abnormal pattern of the spleen.
	ANT	-
	ALV2	Section shows a normal pattern of the spleen, no abnormalities seen.
	ALV3	Section shows a normal pattern of the spleen, no adverse reaction seen.
Ileum	P-CON	Intestine showing intact mucosa and muscle wall. No abnormalities observed
	N-CON	Intestine showing intact wall with mild focal loss of the overlying mucosa.
	ANT	Intestine showing intact mucosa and muscle wall. No abnormalities observed.
	ALV2	Intestine showing intact mucosa and muscle wall. No abnormalities observed.
	ALV3	Section shows a normal pattern of the ileum, no abnormalities observed.

of the liver with lymphocytes. This indicates that the use of antibiotics and other alternative antibiotic drugs discourage the growth of these pathogenic microbes (*E. coli*) from affecting the liver; this is line with the work of Klasing, (1988). The abnormalities associated with the high dose of aloe vera leaf extract may be associated with excess amount of aloin (Bottenberg *et al.*, 2007). The histology of spleen and the ileum of birds fed N-CON shows cellular aberrations such as splenal abnormal pattern and mild focal loss of ileal mucosa. This may be due to the heavy infestation of *E.coli* on the mucosa layer of these birds and however, the effects of ANT, ALV2 and ALV3 can be observed with normal spleen and ileum. This result is in line with that of Ravindran (2006) that alternative sources of antibiotics can prevent proliferation of pathogenic bacteria and modulation of indigenous bacteria so that the health, immune status and performance are improved. In this study, the birds challenged with *E. coli* without aloe vera (N-CON) showed abnormal morphological pattern for all the organs investigated.

The effect of experimental treatments on blood haematology in this study is summarized in Table 4. PCV and eosinophils did not differ markedly ($P > 0.05$) among treatments. RBC and hemoglobin concentration were significantly higher ($P < 0.05$) in birds receiving ANT in their drinking water. WBC count of N-CON groups was significantly higher ($P < 0.05$). However, WBC of birds in the other treatments was similar. Percentage of lymphocytes in turkey poultts receiving ALV2 was significantly different ($P < 0.05$) from the other groups.

Table 4. Haematological parameters of turkey poultts challenged with *E. coli* for eight weeks

Parameters	Treatments					SEM
	P-CON	N-CON	ANT	ALV2	ALV3	
PCV (%)	25.50	23.00	30.50	27.50	25.50	1.12
RBC ($\times 10^{12}/l$)	4.95 ^b	3.85 ^a	6.20 ^d	5.70 ^{cd}	5.10 ^{bc}	0.27
Hb (g/dl)	5.55 ^{ab}	4.50 ^a	6.90 ^b	5.15 ^{ab}	4.75 ^{ab}	0.35
WBC ($\times 10^9/l$)	5.35 ^a	9.55 ^b	5.65 ^a	4.70 ^a	5.55 ^a	0.65
Lymphocytes (%)	61.00 ^a	67.00 ^{ab}	61.50 ^a	85.50 ^c	75.00 ^b	3.22
Eosinophils (%)	2.50	1.50	4.00	1.50	0.50	1.70

^{abc}Means with different superscripts in a row differ significantly ($P < 0.05$)

Table 5. Serum biochemical constituents of turkey poultts challenged with *E. coli* for eight weeks

Parameters	Treatments					SEM
	P-CON	N-CON	ANT	ALV2	ALV3	
Uric acid (mg/dl)	0.0048	0.0052	0.0045	0.0043	0.0045	0.03
Creatinine (mg/dl)	0.91 ^{ab}	1.15 ^b	0.80 ^a	0.96 ^{ab}	1.03 ^{ab}	4.18
Glucose (mg/dl)	40.50	37.80	41.40	46.80	35.10	5.10
Cholesterol (mg/dl)	113.88 ^c	98.46 ^b	71.43 ^b	48.26 ^a	48.33 ^a	10.26
Total protein (g/l)	31.50	32.50	30.00	29.00	31.00	0.80

^{abc}Means with different superscripts in a row differ significantly ($P < 0.05$)

Changes in the physiological state often reflect alteration of haematological values. Blood indices are a fundamental tool used to monitor the effects of therapeutic, nutritional and environmental management in human and veterinary medicine (Toghyani *et al.*, 2011). A higher RBC count observed in turkey poult

s given aloe vera leaf extracts in their drinking water could imply that it may possess erythropoietic enhancing factors. These factors may also play such roles as immune system stimulation and in the function of organs related to blood cell formation such as thymus, spleen and bone marrow (Li *et al.*, 2002). Lymphocytes are responsible for immune responses. It has been showed that pathogens can stimulate activation and proliferation of T lymphocytes (Liu *et al.*, 2006).

The uric acid, glucose and protein were not significantly ($P>0.05$) different. However, serum creatinine and cholesterol concentrations were significantly different ($P<0.05$) among the treatments. Serum creatinine of bird on negative control was significantly higher compared to those on the other treatments that were similar. On the other hand the cholesterol concentration of birds given aloe vera gel was significantly reduced and highest in positive control birds. Creatinine is a waste product of creatine metabolism while uric acid is the end product of amino acid degradation in birds. Ninety-eight percent of the total body creatine is in skeletal muscle and 2% of this is converted each day to creatinine (Tillson, 2003). A measurement of creatinine in the blood may be used to determine renal function. Aberration in the serum creatinine and uric acid could be early pointer to depressed liver and kidney function (Tillson, 2003). The increase in serum uric acid and creatinine in this study could be due to breakdown of muscle protein of the kidney and a ruptured bladder.

The reduction of serum total cholesterol in ALV2 and ALV3 groups might be due to the reduction of synthetic enzyme activities. Similarly, cholesterol reduction was observed by Toghyani *et al.* (2011) when galic antibiotic growth promoter was included in broilers diets. However, Carrijo *et al.* (2005) did not report any alternation in serum levels of cholesterol and triacylglycerols after feeding broiler chicks with a diet containing up to 1% of garlic powder. Blood serum protein reflects the condition of an organism and the changes happening to it under influence of internal and external factors (Babalola *et al.*, 2009). Protein concentration did not vary statistically among treatments in our study. Therefore, to achieve good health conditions in turkey poult

s, it is recommended that Aloe vera leaf extract should be administered to turkey at 20 ml to a liter of water.

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