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Effect of dietary supplementation of Aloe vera and *Lactobacillus acidophilus* on hematological, biochemical and immune parameters of broiler birds

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Abstract
An experiment was conducted at the College of Veterinary Science and Animal Husbandry, Selesih, Aizawl, Mizoram to assess the effect of dietary supplementation of Aloe vera and Probiotic (*Lactobacillus acidophilus*) on haemato-biochemical parameters and antibody titre of broiler birds. Two hundred day old (Ven Cobb) broiler chicks were randomly distributed into five treatments groups. Dietary groups consisted of Group-1 birds were fed basal diet (control); groups 2 supplemented with BMD (Bacitracin methylene disalicylate) @500g/ton of feed (AB); Group-3: with 0.5% Aloe vera powder (ALV); Group-4 with *Lactobacillus acidophilus* (LAB); Group-5: with 0.5%Aloe vera powder plus *Lactobacillus acidophilus* (ALVLB) respectively. The hematological parameters namely Hb, PCV, WBC, RBC, MCV, MCH, MCHC and biochemical parameters viz. glucose, globulin, albumin, albumin/globulin, HDLC, LDLC, Cholesterol, Triglyceride were not affected. The Ferric Reducing Antioxidant Power value for both blood serum and meat increased significantly. Antibody titre against Newcastle disease virus was not affected at day 28 and day 35.

Keywords: Broiler, aloe vera, probiotic, *Lactobacillus acidophilus*, haemato-biochemical parameter

Introduction
The basic objectives of modern broiler farming are faster growth, high feed conversion efficiency and livability. The economics of production is very important criteria for broiler production where feed is the major important factor affecting the productive performance and economics of broiler production, next to genetic potential. To ensure more net return and to minimize high expenditure on feed are the main challenges, for which many research strategies have been practiced such as introducing feed supplements and feed additives. Antibiotic feed additive as growth promoters have long been supplemented to poultry feed to improve the general performance and prevent some specific intestinal pathology (Hassan *et al*., 2010) [1]. Antibiotic removal has led to problems in poultry performance and a rise in incidence of certain poultry diseases (Dibner and Richard, 2005) [2]. Such situation has compelled the researcher to use alternatives to antibiotic growth promoter like organic acids, enzymes, probiotics, prebiotic, herbs, essential oil and immunostimulants as feed additives in poultry (Jackson *et al*., 2004) [3]. Among the alternatives phytobiotics and probiotics have the potential to improve the production performance of poultry.

Probiotics are live microbial feed supplements which beneficially affect the host animal by improving its intestinal microbial balance, feed conversion efficiency, weight gain and reduce mortality or a live microbial feed that is beneficial to health (Patterson and Burkholder, 2003) [4].

Phytobiotics are another alternate to antibiotic growth promoter. A well-known herb that has received particular attention from researchers is Aloe vera (*Aloe barbadensis*), is a tropical or a sub tropical plant with turgid lanced shaped leaves with jagged edges and sharp points (Qiao *et al.*, 2013) [5]. Aloe vera gel contains acemannan, which has been identified as the primary polysaccharide (Hamman 2008) [6]. It can affect the humoral immune response and cellular immunity (Zhang *et al.*, 2011) [7].

Materials and Methods

Location & Climate

This experiment was conducted at Instructional Livestock Farm Complex, Department of Animal Nutrition, College of Veterinary Sciences & Animal Husbandry, Selesih, Aizawl, Mizoram during the early summer months (Feb-April) during which environmental temperature was in the range of 18°C to 25° C. Permission for using the animals for the experiment was duly taken from Institutional Animal Ethics committee (IAEC) constituted as per the Article No. 13 of the CPCSEA rules laid down by Government of India.

Experimental birds, experiment design & diet

Two hundred one day old broiler chicks were randomly distributed into five treatments groups with five replicates (n=5) per group. Each replicate contained 8 chicks five dietary groups consisted of: 1) basal diet of as per BIS standard, 2007 without any supplementation (control); 2) basal diet with BMD (Bacitracin methylene disalicylate) 0.5g/kg of feed (AB); 3) basal diet with 0.5% Aloe vera powder (ALV); 4) basal diet with *Lactobacillus acidophilus* (LAB) and 5) basal diet with 0.5% Aloe vera powder plus *Lactobacillus acidophilus* (ALVLB). The chicks were reared group wise under the similar management condition and health care. The birds were vaccinated against Ranikhet and Infectious Bursal Disease at the 7th and 14th day respectively. The chickens were house in floor pen on fresh rice husk and saw dust. Plastic wire nest up was used to separate the different pens. Three types of standard broiler diets have been prepared i.e. broiler pre-starter (1-7 days of age), broiler starter (8-21 days of age) and broiler finisher (22-42 days of age) as per BIS (2007) specification.

Procurement of Aloe Vera powder

The natural pure Aloe vera powder was purchased from local market.

Preparation of probiotic culture (*Lactobacillus acidophilus*)

The probiotic product (*Lactobacillus acidophilus)* was procured from National Dairy Research Institute, Karnal, India. From the stock culture, a loop full of *Lactobacillus acidophilus* was transferred aseptically to glycerol solution for maintaining the micro-organism culture by incubating for 24 hrs. at 37^0C. The bacterial culture was kept in deep freeze. Then by using MRS agar the bacterial colony was grown, which was suspended in PBS solution and concentration was checked in Mac-Ferlend. So, Minimum concentration of *Lactobacillus acidophilus* was maintained 10^6/ g of feed.

Aloe vera powder and culture of *L. acidophilus* were added to basal diet and mixed thoroughly to obtain different treatment diets.

Haemato-biochemical characteristics of broilers

(i) Collection of blood sample

Blood were collected from ten experimental birds of each group i.e. two broiler birds from each replicate on 14, 21, 28 and 42 days for experiment period.

Blood samples were collected from the wing vein on inner side of the elbow joint of the birds. A 2 ml Tuberculin syringe fitted with a sterile needle was carefully inserted into the vein after manual ligation and about 2 ml of blood were withdrawn and quickly added to Vacutainer containing ethylene diaminetetraacetic acid (EDTA). The sample bottle was shaken gently to mix up the blood with the EDTA to prevent clotting. For estimation of blood bichemical parameter, another 2 ml of blood was taken in a vacutainer having clott activator and the serum was separated by centrifugation at 4000 rpm for 5 minutes and stored at -20 °C until further analysis.

1. Estimation of haematological parameter

Estimation of Haematological parameters like Haemoglobin, PCV, RBC, WBC, MCV, MCH and MCHC were done with the help Automated Haematology Cell Counter (Model: MS4e) by following standard procedures as per the manufacturers protocol.

2. Estimation of blood- biochemical parameter

Blood-Biochemical parameter has been estimated by the use of commercially available diagnostic kits

3. Antioxidant profile

Antioxidant Profile of blood serum was done by using Ferric Reducing Antioxidant Power (FRAP) Assay method (Benzie and strain, 1996) [8]

4. Immune response

Vaccination against Newcastle disease (NDV) was done on 4th day and 20th day. Antibody titre will be measured by indirect ELISA using commercial kit following the manufacturer's instructions on 28th day and 35th day aged groups. The plates will be read by ELISA plate reader at required wavelength suggested by the manufacturer. Following protocol was followed for performing the HI test. Two milliliter of blood was collected from the wing vein on inner side of the elbow joint of the birds and put in sample bottles without anti-coagulant and the serum was separated. The sample was decomplemented by heating at 56^0C for 30min and Haemagglutination inhibition test (HI) as per the OIE protocol OIE (2012).

Statistical analysis

The data was analyzed by one way analysis of variance (ANOVA) using SPSS (1997) by completely randomized design. The test was employed for identifying the significant differences amongst the different treatments probability values less than 0.05 is considered to be statistically significant and the values $P<0.01$ was declared as trend.

1. Haematological Parameter: Changes in physiological state generally reflect alteration of hematological values. Therefore, blood indices are a fundamental tool used to monitor the effect of therapeutic, nutritional and environmental management in veterinary medicine.

1.1 RBC

Average values of RBC in blood of different in blood of different groups of different groups of experimental birds during all the collection period as a whole were 2.71±0.13, 2.49±0.07, 2.47±0.06, 2.53±0.07, 2.66±0.07 71 (×10^6/µl) for Control, AB, ALV, LAB, and ALVLB respectively for the whole collection period and there was no statistically significance ($p>0.05$) difference among the groups for all the collection period as a whole. In contrast to our finding, (Mahdavi *et al*., 2012) [9] observed that red blood cell count was highest in broiler group supplemented with 1%Aloe vera than compared to control.

1.2 WBC

Average values of WBC in blood of different in blood of

different groups of different groups of experimental birds during all the collection period as a whole were 19.47±1.00, 18.41±0.17, 18.49±0.42, 19.76±0.84, 20.33±0.71 ($\times 10^3$/µl) for Control, AB, ALV, LAB, and ALVLB respectively for the whole collection period and there was no statistically significance ($p>0.05$) difference among the groups for all the collection period as a whole.

There was a significant ($P<0.05$) increase in total WBC count along with absolute differential count of monocytes, lymphocytes and heterophils. Valle paraso *et al*. (2005) [10], Darabighane *et al*. (2011) [11] reported an increase in total white blood cell count of broilers as a result of adding *Aloe vera* gel to broiler feeds. In another study that used *Aloe vera* gel powder in broiler feeds, a significant increase was observed in total white blood cell count, red blood cell count, and hemoglobin in groups treated with *Aloe vera* gel powder compared to the control group, with the 1% *Aloe vera* gel powder group showing the highest hemoglobin, red blood cell, and white blood cell count (Mahdavi *et al*., 2012) [9].

Table 1(a): Effect of Aloe vera and probiotic (*L. acidophilus*) supplementation on, RBC ($\times 10^6$/ µl), WBC ($\times 10^3$/ µl), PCV (%) and MCV (fL) changes in experimental birds

	Treatment					
Attributes	**Group-1 (Control)**	**Group-2 (AB)**	**Group-3 (ALV)**	**Group-4 (LAB)**	**Group-5 (ALVLB)**	**P value**
RBC						
d 28	2.33±0.68	2.29±0.12	2.29±0.12	2.41±0.66	2.30±0.54	0.34^{NS}
d 35	2.60±0.25	2.63±0.09	2.59±0.03	2.75±0.07	2.77±0.09	0.82^{NS}
d 42	2.36±0.25	2.46±0.14	2.47±0.15	2.38±0.11	2.78±0.05	0.28^{NS}
Average± SE	2.41±0.13	2.49±0.07	2.47±0.06	2.53±0.07	2.66±0.07	0.22^{NS}
WBC						
d 28	$21.30±0.19^{c}$	$18.95±0.14^{b}$	$18.88±0.44^{b}$	$16.46±0.03^{a}$	$17.13±0.28^{a}$	<0.01
d 35	21.52±0.89	18.68±0.24	19.33±0.83	21.56±0.92	21.61±1.18	0.06^{NS}
d 42	$16.32±1.75^{a}$	$17.82±0.17^{abc}$	$17.41±0.37^{ab}$	$19.93±1.48^{bc}$	$20.97±0.69^{c}$	0.041^{*}
Average± SE	19.47±1.00	18.41±0.17	18.49±0.42	19.76±0.84	20.33±0.71	0.24^{NS}
PCV						
d 28	33.16±1.58	29.4±0.46	29.3±0.75	30.53±0.61	29.53±0.59	0.057^{NS}
d 35	35.94±2.10	32.94±0.36	36.56±0.62	38.0±1.96	38.58±0.87	0.07^{NS}
d 42	$20.44±0.90^{a}$	$24.18±1.14^{ab}$	$26.4±2.37^{b}$	$27.6±1.94^{bc}$	$32.06±0.40^{c}$	<0.01
Average± SE	29.33±2.23	28.75±1.19	30.97±1.58	32.27±1.67	33.98±1.14	0.15^{NS}
MCV						
d 28	$131.53±0.48^{b}$	$124.07±1.27^{a}$	$134.40±2.77^{b}$	$125.33±0.66^{a}$	$134.43±2.66^{b}$	0.006^{*}
d 35	135.18±6.89	130.08±2.94	137.62±2.63	143.72±1.25	138.66±1.01	0.157^{NS}
d 42	111.84±2.83	111.11±3.20	110.52±2.95	105.32±1.21	104.12±1.11	0.111^{NS}
Average± SE	125.36±4.10	121.41±2.90	126.45±3.97	124.7±4.90	124.37±4.70	0.936^{NS}

NS= Non significant,[abc] means with different superscript in the same row differ significantly; AB=Antibiotic, ALV= Aloe vera; LAB=*L. acidophilus*; ALVLB=*Aloe vera* & *L. acidophilus*,* means (P<0.05)

1.3 PCV

The present findings was in agreement with Bolu *et al*. (2013) [12] observed that there were no effects of *Aloe vera* gel on turkey poults health, as determined from various haematological parameters and serum metabolites. Mmereole (2011) [13] while studying the effect of the dietary inclusion of 1% aloe vera and terramycin antibiotic growth promoters in broilers reported that aloe vera supplementation had significantly (P<0.05) better effect on haematological parameters compared to antibiotic supplemented group. However, these parameters are statistically equal to the control group.

1.4 Mean corpuscular volume

The present observation was comparable with the findings of Singh *et al.* (2013) [14] who reported that supplementation of Aloe vera (@20g/l) in broiler diet have no significant difference in MCV value amongst all the groups. Mmereole (2011) [13] reported increase MCV values in *Aloe vera* treated group as compared to antibiotic supplemented group.

1.5 Haemoglobin

The present observation of numerically higher hemoglobin concentration in agreement with Cetin *et al.* (2005) [15] observed that the probiotic supplementation caused statistically significant increase in the haemoglobin concentration of Turkeys. The present observation was not in agreement with the finding Singh *et al.* (2013) [14] showed significantly higher value of Haemoglobin (Hb) concentration, in chicks supplemented with Aloe vera juice @ 20g/l in drinking water as compared to control and drug control groups. Tariq *et al*. (2014) [16] reported that Hb concentration was not affected by Aloe vera & clove supplementation in Japenese quails.

1.6 Mean Corpuscular Haemoglobin

On statistical observation it was observed that there was no significance ($p<0.05$) difference in MCH values of blood among the various treatments.

In contrast Yadav *et al.* (2017) [17] reported that significant increase in haematological values for Hb, PCV, TEC & TLC was observed in aloe vera powder (0.5%) and aloe vera juice (0.2% in drink) supplemented broiler bird compared with the control group. Mmereol (2011) [13] reported increase MCH values in *Aloe vera* treated group as compared to antibiotic supplemented group.

1.7 Mean corpuscular haemoglobin concentration

On statistical observation it was observed that there was no significance ($p<0.05$) difference in the MCH of blood among the various groups of experimental birds.

The present observation was comparable with the findings of Singh *et al.*, (2013) [14] who reported that supplementation of Aloe vera (@20g/l) in broiler diet have no significant difference in MCHC value amongst all the groups. Mmereol

(2011) [13] reported increase MCHC values in *Aloe vera* treated group as compared to antibiotic supplemented group.

Table 1(b): Effect of aloe vera and probiotic (*L. acidophilus*) supplementation on Haemoglobin (g/dl), MCH (pg), MCHC (g/dl) changes in experimental birds

Attributes	Treatment					P value
	Group-1 (Control)	Group-2 (AB)	Group-3 (ALV)	Group-4 (LAB)	Group-5 (ALVLB)	
Hb						
d 28	9.46±0.32	9.43±0.43	9.40±0.43	10.13±0.76	10.16±0.63	0.732^{NS}
d 35	10.18±0.52	9.44±0.14	9.40±0.12	10.44±0.18	9.82±0.26	0.07^{NS}
d 42	9.54±0.42	10.02±0.55	9.72±0.05	10.30±0.03	10.28±0.09	0.468^{NS}
Average± SE	9.76±0.42	9.66±0.23	9.52±0.10	10.31±0.21	10.07±0.17	0.055^{NS}
MCH						
d 28	$35.7±0.66^{a}$	$39.43±0.56^{bc}$	$36.93±2.01^{b}$	$36.26±0.37^{ab}$	$41.43±0.61^{c}$	0.014^{*}
d 35	$38.40±0.52^{b}$	$36.04±0.53^{a}$	$36.08±0.38^{a}$	$36.72±0.27^{a}$	$36.44±0.69^{a}$	0.020^{*}
d 42	42.90±1.54	46.66±2.63	47.22±1.69	42.74±0.98	41.88±1.32	0.129^{NS}
Average± SE	39.51±1.02	40.90±1.67	40.56±1.67	38.93±1.69	39.93±0.92	0.821^{NS}
MCHC						
d 28	$27.46±0.35^{a}$	$30.10±0.25^{b}$	$28.70±0.83^{ab}$	$27.36±0.53^{a}$	$29.46±0.63^{b}$	0.02^{*}
d 35	$28.54±1.16^{b}$	$27.44±0.63^{ab}$	$25.96±0.23^{a}$	$26.56±0.76^{ab}$	$25.36±0.12^{a}$	0.03^{*}
d 42	$31.68±1.98^{a}$	$33.48±1.61^{a}$	$39.50±4.03^{ab}$	$43.08±2.73^{b}$	$32.68±1.48^{a}$	<0.01
Average± SE	29.50±0.97	30.37±0.98	31.80±2.30	33.10±2.49	29.12±1.07	0.44^{NS}

NS=Non significant, ^{abc} means with different superscript in the same row differ significantly; AB=Antibiotic, ALV= Aloe vera; LAB=*L. acidophilus*; ALVLB=*Aloe vera & L. acidophilus*,* means (P<0.05)

2. Blood Biochemical parameter

2.1 Glucose

On statistical observation it was observed that there was no significance (p>0.05) difference in the Glucose of blood among the various groups of experimental birds. The present observation was comparable with the observation of Pourakbari *et al*. (2016) [18] conducted experiment with broiler bird fed diet supplemented with different levels of probiotic and found blood glucose was higher. There was a highly significant (*P*<0.05) decrease in serum glucose level after intra-peritoneal administration of bitter principle of *Aloe vera* rabbits. Choudhary *et al.* (2014) [19] found that the use of Aloe vera gel powder significantly reduced blood glucose, lipid profile and blood pressure.

2.2 Total Protein

On statistical analysis, there was significant increased (p<0.05) in blood total protein level in the treatment groups supplementation with AB, ALV, LAB & ALVLB compared to control.

The present observation was not in agreement with those of Dimcho *et al*., (2005) [20] who found that probiotic supplementation did not affect the total proteins concentrations of chickens.

2.3 Albumin

This observation revealed that the Albumin level in the blood was highest in LAB (3.14±0.34) and lowest in Control (2.13±0.09).On statistical analysis there was significant differences (p<0.05) between the treatment. Similar observation was also reported by Pourakbari *et al*. (2016) [14] who conducted experiment with broiler bird fed diet supplemented with different levels of probiotic and found albumin tended to be higher in the supplemented treatments. On the contrary, Alkhalf *et al*. (2010) [21] who reported the serum concentrations of albumin were not affected by any of the three levels of probiotic supplementation. On contrary Singh *et al*. (2013) [14] found no significant difference (p>0.05) in the Albumin value among the group treated with Aloe vera (@20g/l) and the other groups. This may be attributed to the fact that no detrimental effect due to Aloe vera and standard antibiotic in the diet.

2.4 Globulin

On statistical analysis, there was no significant increased (p>0.05) in blood globulin level among the treatment groups. The present observation was similar to Singh *et al.*, (2013) [14] found no significant difference (p>0.05) in the globulin concentration in serum among the treatments.

2.5. Albumin/Globulin

On statistical analysis, there was no significant difference (p>0.05) in blood globulin level among the treatment groups. The present observation was similar to Singh *et al.* (2013) [14] who found no significant difference (p>0.05) in the Albumin/Globulin value among the group treated with Aloe vera (@20g/l) and the other groups. Tariq *et al.* (2014) [16] reported that serum protein, albumin, globulin and ratio were not affected by aloe vera and clove supplementation in Japanese Quails.

2.6. Triglyceride

Triglyceride level in serum of broiler was not affected (P>0.05) by the various treatments. Mansoub (2010) [22] reported that there was decrease (P<0.05) in triglyceride concentration in serum of broiler fed diet supplemented with *L. acidophilus* and *L. casei* in comparison to control. The present finding are not comparable with the observation of Arun *et al*. (2006) [23] who found that serum triglycerides were reduced significantly by dietary supplementation of probiotic containing *L. sporogene* at 100 mg per kg diet. Corcoran *et al.* (2005) [24] observed that *Lactobacillus* spp. decreased the gall bladder acids during digestion of lipid and this resulted in reduction in the ability of feed digestion and consequently lower triglyceride level in blood.

2.7 Cholesterol

Blood Cholesterol level was not affected (P>0.05) by different dietary treatment. Similar results were reported by Arun *et al*. (2006) [23] who found that serum total cholesterol and triglycerides were reduced significantly by dietary supplementation of probiotic containing *L. sporogene* at 100 mg per kg diet. It was speculated that *Lactobacillus acidophillus* reduces the cholesterol in the blood by deconjugating bile salts in the intestine, thereby preventing

them from acting as precursors in cholesterol synthesis (Abdulrahim *et al*., 1996) [25]. Alkhalf *et al.* (2010) [21] demonstrated that probiotic microorganisms inhibit hydroxymethyl- glutaryl-coenzyme A; an enzyme involved in the cholesterol synthesis pathway thereby decrease cholesterol synthesis. On the other hand, probiotics de-conjugate the bile salts and thus may interfere with the absorption mechanism of cholesterol in the gut (Li *et al.,* 2007) [26].

2.8 LDL-Cholesterol

On statistical analysis, there was no significant increased (p>0.05) in blood LDLC level among the treatment groups. The present study was similar Pourakbari *et al*. (2016) [18] who observed a significant reduction in LDL and improvement in HDL levels of the blood in the treatment consisting of probiotic.

2.9. HDL-Cholesterol

On statistical analysis, there was no significant increased (p>0.05) in blood HDLC level among the treatment groups. Shokryazdan *et al.* (2017) [27] reported that supplementation of *Lactobacillus salivarium* significantly (P<0.05) reduced LDL-Cholesterol in serum in broiler but not HDL-Cholesterol in serum was not affected by probiotic supplementation. Similar results of reduced LDL- Cholesterol but not HDL-Cholesterol were also reported by Kalavathy *et al.* (2003) [28]. Panda *et al.* (2006) [29] observed similar finding of reduced LDL-Cholesterol in broiler supplemented with *L. sporogens* at 100 and 200 mg/kg diet. On the contrary, Ashayarizadeh *et al.* (2011) [30] did not find significant difference in serum LDL and HDL-Cholesterol concentration of chicken fed the commercial probiotic Primalac, in compared to control group.

Table 2(a): Effect of aloe vera and probiotic (*L. acidophilus*) supplementation on serum Glucose (mg/dl), Total protein (g/dl), Albumin (g/dl) and Globulin (g/dl) in broiler chickens

Attributes	Treatment					P value
	Group-1 (Control)	Group-2 (AB)	Group-3 (ALV)	Group-4 (LAB)	Group-5 (ALVLB)	
Glucose						
d 28	250.57±23.77	228.81±13.41	232.68±8.43	234.85±5.18	260.09±7.72	0.45^{NS}
d 35	$209.87±9.17^{a}$	$237.27±19.57^{ab}$	$257.54±12.29^{b}$	$269.16±7.22^{b}$	$253.80±14.72^{b}$	0.044^{*}
d 42	$269.14±12.98^{bc}$	$229.42±5.06^{a}$	$246.57±7.43^{abc}$	$242.57±12.62^{ab}$	$278.30±13.26^{c}$	0.027^{*}
Average± SE	242.06±10.59	232.23±7.78	247.58±6.05	251.01±6.78	264.68±7.92	0.07^{NS}
Total Protein						
d 28	4.63±0.32	5.16±0.49	4.50±0.17	5.26±0.24	4.70±0.40	0.463^{NS}
d 35	$4.66±0.15^{a}$	$5.78±0.22^{ab}$	$6.62±0.74^{b}$	$5.46±0.38^{ab}$	$6.36±0.21^{b}$	0.021^{*}
d 42	$5.58±0.36^{a}$	$6.60±0.30^{b}$	$6.36±0.14^{ab}$	$7.46±0.32^{c}$	$5.76±0.21^{ab}$	<0.01
Average± SE	$5.07±0.20^{a}$	$5.95±0.23^{b}$	$6.03±0.36^{b}$	$6.18±0.34^{b}$	$5.74±0.22^{a}$	0.041^{*}
Albumin						
d 28	$1.79±0.08^{a}$	$1.99±0.04^{b}$	$1.67±0.03^{a}$	$1.99±0.05^{b}$	$2.31±0.05^{c}$	<0.01
d 35	$2.31±0.12^{a}$	$2.22±0.13^{a}$	$2.64±0.13^{ab}$	$2.58±0.23^{a}$	$3.13±0.18^{b}$	<0.01
d 42	$2.16±0.17^{a}$	$3.76±0.21^{bc}$	$3.59±0.18^{b}$	$4.40±0.42^{c}$	$2.49±0.11^{a}$	<0.01
Average± SE	$2.13±0.09^{a}$	$2.76±0.24^{ab}$	$2.78±0.22^{ab}$	$3.14±0.34^{b}$	$2.69±0.12^{ab}$	0.047^{*}
Globulin						
d 28	2.83±0.28	3.17±0.46	2.82±0.20	3.27±0.19	2.39±0.42	0.41^{NS}
d 35	2.34±0.21	3.55±0.13	3.97±0.65	2.87±0.47	3.22±0.17	0.06^{NS}
d 42	3.41±0.49	2.84±0.45	2.76±0.19	3.05±0.10	3.27±0.30	0.63^{NS}
Average± SE	2.87±0.24	3.19±0.21	3.24±0.29	3.03±0.18	3.05±0.18	0.799^{NS}
Albumin/Globulin						
d 28	0.64±0.06	0.66±0.11	0.60±0.05	0.61±0.02	1.03±0.17	0.06^{NS}
d 35	1.02±0.11	0.62±0.03	0.73±0.10	1.05±0.27	0.98±0.08	0.19^{NS}
d 42	0.77±0.25	1.59±0.44	1.34±0.15	1.46±0.27	0.80±0.11	0.11^{NS}
Average± SE	0.84±0.11	1.00±0.20	0.93±0.11	1.11±0.15	0.92±0.06	0.73^{NS}

NS=Non significant, ^{abc} means with different superscript in the same row differ significantly; AB=Antibiotic, ALV= Aloe vera; LAB=*L. acidophilus*; ALVLB=*Aloe vera & L. acidophilus*,* means (P<0.05)

Table 2(b): Effect of Aloe vera and probiotic (*L. acidophilus*) supplementation on Triglyceride (mg/dl), Cholesterol (mg/dl), LDL (mg/dl), HDL (mg/dl) in broiler chicken

Attributes	Treatment					P value
	Group-1 (Control)	Group-2 (AB)	Group-3 (ALV)	Group-4 (LAB)	Group-5 (ALVLB)	
Triglyceride						
d 28	65.66±5.20	82.50±3.81	88.33±10.13	90.00±8.03	82.50±9.46	0.260^{NS}
d 35	102.00±2.15	93.50±5.45	95.50±2.89	97.0±2.15	95.0±2.23	0.421^{NS}
d 42	91.20±3.52	95.5±3.39	100.48±6.91	93.90±3.17	86.10±6.93	0.390^{NS}
Average± SE	89.46±4.38	91.73±2.83	95.76±3.59	94.19±2.22	88.69±3.53	0.537^{NS}
Total Cholesterol						
d 28	94.85±6.21	96.84±1.44	94.64±3.84	93.71±2.22	94.69±.50	0.977^{NS}
d 35	93.24±3.91	99.31±0.35	96.18±3.16	92.73±2.29	97.94±2.01	0.347^{NS}
d 42	95.84±2.48	98.36±6.07	93.79±1.80	100.05±3.98	99.96±3.17	0.726^{NS}
Average± SE	94.61±2.08	98.37±2.21	94.91±1.53	95.77±1.96	97.97±1.46	0.493^{NS}
LDL						
d 28	39.76±6.21	40.80±0.66	42.26±3.61	33.01±2.29	35.03±2.09	0.345^{NS}
d 35	35.08±1.25	29.37±3.23	36.72±2.13	33.16±3.77	41.50±3.28	0.083^{NS}
d 42	45.54±2.47	41.05±2.87	36.39±3.66	36.74±3.41	42.26±1.75	0.180^{NS}

Average± SE	40.18±2.05	36.50±2.25	37.87±1.81	34.50±1.95	40.30±1.62	0.185^{NS}
HDL						
d 28	48.79±3.44	54.59±4.12	47.56±2.59	51.98±0.32	60.26±1.64	0.055^{NS}
d 35	46.96±1.92^{a}	59.99±3.59^{b}	54.48±3.11^{ab}	58.53±2.97^{b}	58.69±3.61^{b}	0.046*
d 42	53.06±1.25	51.25±2.51	55.24±3.58	56.21±3.18	49.02±2.05	0.335^{NS}
Average± SE	49.73±1.32	55.38±2.08	53.17±1.98	56.12±1.71	55.33±2.10	0.115^{NS}

NS=Non significant, ^{abc} means with different superscript in the same row differ significantly; AB=Antibiotic, ALV= Aloe vera; LAB=*L. acidophilus*; ALVLB=*Aloe vera & L. acidophilus*

3. Antioxidant Profile

The average FRAP value of blood serum in different groups of experimental birds during different collection period have been presented in table 4.10. On statistical analysis, there was significant increased (p<0.01) in blood FRAP value among the treatment groups.

Jakubcova *et al.* (2014) [31] explained that antioxidant activity is a marker of total amount of antioxidants in a given sample. It is a value, which is used to evaluate the ability of organism to uptake free radicals, protect against their creation or to change them into less reactive forms. Lowered antioxidant activity leads to oxidative stress, which is related to higher rate of impairment of organism (disease, impaired productive). Farahi (2012) [32] studied the effect of dietary supplementation of *Melissa Officinalis* and *Aloe Vera* on hematological traits, lipid oxidation of carcass and performance in Rainbow Trout (*Oncorhynchus Mykiss*). They found Aloe herbs could be protective against Lipid peroxidation in fish meat during chilling storage (4°C, 7 days).

Table 3: Effect of aloe vera and probiotic (*L. acidophilus*) supplementation on FRAP (mmole Fe^{+2}/l) value in blood serum and meat (mmole Fe^{+2}/kg)

	Group-1 (Control)	Group-2 (AB)	Group-3 (ALV)	Group-4 (LAB)	Group-5 (ALVLB)	P value
FRAP OF Blood Serum						
d 28	0.473±0.01	0.51±0.01	0.50±0.01	0.53±0.02	0.47±0.02	0.22^{NS}
d 35	0.41±0.02^{a}	0.49±0.003^{bc}	0.47±0.01^{ab}	0.56±0.02^{d}	0.54±0.02^{cd}	<0.01
d 42	0.47±0.02	0.44±0.01	0.50±0.01	0.53±0.02	0.48±0.03	0.08^{NS}
Average± SE	0.44±0.01^{a}	0.47±0.009^{ab}	0.49±0.01^{b}	0.54±0.01^{c}	0.50±0.01^{bc}	<0.01
Frap of Meat Sample						
d 42	0.23±0.005^{a}	0.23±0.002^{a}	0.25±0.00^{b}	0.28±0.014^{c}	0.28±0.005^{c}	<0.01

NS=Non significant, ^{abc} means with different superscript in the same row differ significantly; AB=Antibiotic, ALV= Aloe vera; LAB=*L. acidophilus*; ALVLB=*Aloe vera & L. acidophilus*,* means (P<0.05)

4. Immune status

The findings are similar with Valle- Paraso *et al.* (2005) [10] stated that oral supplementation of Aloe vera showed increase in mean antibody titres to NDV in broiler chickens. The exact mechanism of immunomodulation by probiotics has not been explained but probiotic may stimulate different subsets of immune system cells (Dallout *et al.* (2003) [33]. Antibody titre against the common poultry diseases Newcastle Disease, Infectious Bronchitis and Infectious Bursal Disease was increased by the use of probiotic product Primalac (Landy and Kavyani 2013) [34]. It is also reported that bioactive peptide release by the lactic acid bacteria during fermentation could contribute to immunomodulation effect (Leblanc *et al*., 2004) [35] and interaction between host cells and pathogen may lead to modulation of + cell mediated or B cell mediated immune response (Haghighi *et al.,* 2006) [36]. Similar findings were reported by Besharatian *et al.* (2012) [37] did not observe a significant difference in weight of lymphoid organs, but reported a weight gain in spleen and bursa. Such relative increase in the weight of lymphoid organs as a result of adding *Aloe vera* to feed or drinking water suggests immune (humoral and cellular) system readiness against antigens.

Table 4: Effect of Aloe vera and Probiotic (*L. acidophilus*) supplementation on wt. of lymphoid organ and antibody titre (log_{10}) against NDV in experimental birds

Attributes	Group-1 (Control)	Group-2 (AB)	Group-3 (ALV)	Group-4 (LAB)	Group-5 (ALVLB)	P value
d 28	2.33±0.33	2.66±0.33	3.33±0.33	3.33±0.33	3.33±0.33	0.17^{NS}
d 35	3.33±0.33	3.33±0.33	3.66±0.33	4.33±0.33	3.66±0.33	0.27^{NS}
lymphoid organ weight						
Spleen	1.91±0.08	1.86±0.03	1.92±0.07	1.86±0.02	1.84±0.03	0.782^{NS}
Spleen %	0.106±0.005	0.09±0.006	0.101±0.007	0.09±0.004	0.09±0.006	0.789^{NS}
Liver	31.66±1.66	31.66±1.66	33.33±8.81	41.66±6.00	30.00±5.77	0.609^{NS}
Liver %	1.76±0.08	1.65±0.04	1.72±0.38	2.16±0.25	1.53±0.21	0.41^{NS}
Bursa	0.99±0.12	0.86±0.02	1.28±0.11	1.03±0.02	1.09±0.05	0.052^{NS}
Bursa %	0.05±0.008	0.046±0.003	0.066±0.003	0.053±0.003	0.056±0.006	0.23^{NS}

NS=Non significant, AB=Antibiotic, ALV= Aloe vera; LAB=*L. acidophilus*; ALVLB=*Aloe vera & L. acidophilus*

Aloe vera, as an additive to broiler chicken feed, has great potentials for improving growth performance, carcass characteristics, haemato-biochemical parameters, intestinal health, immune system response. Therefore, more studies are required to determine effective dosage and form of use.

Conclusion

Considering the overall performance of broiler birds in terms of feed intake, body weight gain, feed conversion efficiency and digestibility of nutrients it can be concluded that, *Aloe vera* and *Lactobacillus acidophilus* have the potential to be used as alternative to antibiotic growth promoters.

References

1. Hassan M, Kjos M, Nes I, Diep D, Lotfipour F. Natural antimicrobial peptides from bacteria: characteristics and potential applications to fight against antibiotic resistance. J Appl. Microb. 2010; 113(4):723-736.
2. Dibner J, Richards D. Antibiotic growth promoters in agriculture: history and mode of action. Poult. Sci. 2005; 84:634-643.
3. Jackson ME, Geronian K, Knox A, McNab J, McCartney E. A dose-response study with the feed enzyme β-mannanase in broilers provided with corn-soybean meal based diets in the absence of antibiotic growth promoters. Poult. Sci. 2004; 83:1992-1996.
4. Patterson JA, Burkholder KM. Application of prebiotics and probiotics in poultry production. Poult. Sci. 2003; 82:627-631.
5. Qiao J, Li HH, Zheng J, Feng ZY, Wang W. Dietary supplementation with Aloe vera polysaccharide enhances the growth performance and immune function of weaned piglets. J Anim. Feed Sci. 2013; 22:329-334.
6. Hamman JH. Composition and applications of *Aloe vera* leaf gel. *Molecules* 2008; 13(8):1599-1616.
7. Zhang H, Du X, Chen X, Zhao B, Lv Y, Liu H *et al*. Astragalus polysaccharides enhance the humoral and cellular immune responses of hepatitis B surface antigen vaccination through inhibiting the expression of transforming growth factor β and the frequency of regulatory T cells. FEMS. Immunol. Med. Microbiol. 2011; 63:228-235.
8. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: the FRAP assay. Anal. Biochem. 1996; 239:70-76.
9. Mahdavi A, Alemi F, Ghazvinian K, Ghaderi M, Darabighane B. Study of effects of different levels of *Aloe vera* gel powder on antibody titre against sheep red blood cells and other blood parameters in broilers. Bri. Poult. Abstracts 2012; 8:49-50.
10. Valle-Paraso M, Vidamo P, Anunciado R, Lapitan A. Effects of *Aloe vera* (*Aloe barbadensis*) on the white blood cell count and antibody titre of broiler chickens vaccinated against Newcastle disease. Philipp. J Vet. Med. 2005; 42:49-52.
11. Darabighane B, Zarei A, Zareshahneh A, Mahdavi A. Effects of different levels of Aloe vera gel as an alternative to antibiotic on performance and ileum morphology in broilers. Ital. J Anim. Sci. 2011; 10:189-194.
12. Bolu SA, Babalola TO, Elelu N, Ahmed RN, Oyetunde SA, Ademola PF *et al*. Effect of supplemental *Aloe vera* gel in drinking water on some performance histology, hematology, serum constituents and growth of turkey poults challenged with Escherichia coli. Wudpecker J Agric. Res. 2013; 2(8):223-229.
13. Mmereol FUC. Evaluation of the Dietary Inclusion of Aloe vera as an Alternative to Antibiotic Growth Promoter in Broiler Production. Pakistan J Nutri. 2011; 10:1-5.
14. Singh J, Koley KM, Chandrakar K, Pagrut NS. Effects of aloe vera on dressing percentage and haemato-bio chemidal parameters of broiler chickens. Vet. World 2013; 6(10):803-806.
15. Cetin N, Gu¨ c lu¨ BK, Cetin E. The effects of probiotic and mannan oligosaccharide on some haematological and immunological parameters in Turkeys. J of Vet Medicine Series A – Physiology Pathology Clin. Med. 2005; 52:263-267.
16. Tariq H, Rao PV, Mondal BC, Malla BA. Effect of Aloe vera and clove supplementation on immune status, haematological and serum biochemical parameters in Japanese quails. Indian J Anim. Nutri. 2014; 31(3):293-296.
17. Yadav DC. *Aloe vera* as a feed additive in broiler chicken production: A Review. Intr. J Vety. Sci. Anim. Husb. 2017; 2(5):30-38.
18. Pourakbari M, Seidavi A, Asadpour L, Martinez A. Probiotic level effects on growth performance, carcass traits, blood parameters, cecal microbiota, and immune response of broilers. Anaisda Academia Brasileira de Ciências, 2016. doi: org/10.1590/0001-3765201620150071.
19. Choudhary M, Kochhar A, Sangha J. Hypoglycemic and hypolipidemic effect of Aloe vera in non-insulin dependent diabetics. J Food Sci. Tech. 2014; 51:90-96.
20. Dimcho, Djouvinov, Svetlana, Boicheva, Tsvetomira, Simeonova *et al*. Effect of feeding Lactina_ probiotic on performance, some blood parameters and caecal microflora of mule ducklings. Trakia J Sci. 2005; 3:22-28.
21. Alkhalf A, Alhaj M, Al-homidan I. Influence of probiotic supplementation on blood parameters and growth performance in broiler chickens. Saudi J Bio. Sci. 2010; 17:219-225.
22. Mansoub NH. Effect of Probiotic Bacteria Utilization on Serum Cholesterol and Triglycrides Contents and Performance of Broiler Chickens. *Global Veterinarian* 2010; 5(3):184-186.
23. Arun K, Panda RR, Savaram V, Mantena VLN, Raju S, Sharma R. Dietary supplementation of Lactobacillus Sporogenes on performance and serum biochemico-lipid profile of broiler chickens. J of Poul. Sci. 2006; 43:235-240.
24. Corcoran BM, Stanton C, Fitzgerald GF, Ross RP. Survival of probiotic lactobacilli in acidic environments is enhanced in the presence of metabolizable sugars. App. Environ. Microbio. 2005; 71(6):3060-3067.
25. Abdulrahim SM, Haddadin MSY, Hashlamoun EAR, Robinson RK. The influence of Lactobacillus acidophilus and bacitracin on layer performance of chickens and cholesterol content of plasma and egg yolk. Bri. Poult. Sci., 1996, 341-346.
26. Li XJ, XS Piao, SW Kim, P Liu, L Wang *et al*. Effects of chito-oligosaccharide supplementation on performance, nutrient digestibility and serum composition in broiler chickens. Poult. Sci. 2007; 86:1107-1114.
27. Shokryazdan P, Faseleh Jahromi M, Liang JB, Ramasamy K, Sieo CC, Ho YW. Effects of a *Lactobacillus salivarius* mixture on performance, intestinal health and serum lipids of broiler chickens. PLoS ONE 2017; 12(5):e0175959. https://doi.org/10.1371/journal.pone.017595
28. Kalavathy R, Abdullah N, Jalaludin S, Ho YW. Effects of Lactobacillus cultures on growth performance, abdominal fat deposition, serum lipids and weight of organs of broiler chickens. Brit. Poult. Sci. 2003; 44(1):139-144.
29. Panda AK, Rao SVR, Raju MVLN, Sharma SR. Dietary supplementation of *Lactobacillus sporogenes* on performance and serum biochemico-lipid profile of broiler chickens. Poult. Sci. 2006; 43:235-240.

30. Ashayerizadeh A, Dabiri N, Mirzadeh KH, Ghorbani MR. Effect of dietary supplementation of probiotic and prebiotic on growth indices and serum biochemical parameters of broiler chickens. Journal of Cell and Animal Biology. 2011; 5(8):152-156.
31. Jackubcova Z, Mares P, Jurikova T, Mlcek J, Balla S, Kalhotka L *et al*. Influence of garlic extract on antioxidant status of chicken. Potravinarstvo. 2014; 8(1):315-320.
32. Farahi A, Kasiri M, Sudagar M, Soleimani iraei M, Zorriehzahra SMJ. Effect of dietary supplementation of *Melissa officinalis* and carcass and performance in rainbow trout (*Oncorhynchus Mykiss*). J Anim. and Feed Res. 2012; 1:01-05.
33. Dallout RA, Lillehoj HS, Shellem TA, Doerr JA. Enhanced mucosal immunity against Eimeria acervulina in broilers fed a Lactobacillus-based probiotic. Poult. Sci. 2003; 82:62-66.
34. Landy N, Kavyani A. Effects of using a multi-strain probiotic on performance, immune responses and caecal micro flora composition in broiler chickens reared under cyclic heat stress condition. Ir. J Appl. Anim. Sci. 2013; 3(4):703-708.
35. Leblanc J, Fliss I, Matar C. Induction of a humoral immune response following an Escherichia coli O157:H7 infection with an immunomodulatory peptidic fraction derived from Lactobacillus helveticus-fermented milk. Clin. Diag. Lab. Immuno. 2004; 11:1171-1181.
36. Haghighi HR, Gong J, Gyles CL, Hayes MA, Zhou H, Sanei B, Chambers JR *et al.* Probiotics stimulate production of natural antibodies in chickens. Clin. Vaccine Immunol. 2006; 13:975-980.
37. Besharatian M, Arshami J, Valizade R, Tahmasebi A, Bahari KR. Effects of *Aloe vera* leaf powder and extract on immune response in broilers. Proc. 5th Iranian Congress on Animal Science, Isfahan, Iran. 2012, 366-370.