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Effect of *Aloe vera* Gel Extract on the Haematological Parameters in White Leghorn Chicks Vaccinated Against Infectious Bursal Disease Virus

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Infectious bursal disease virus (IBDV) causes an acute and highly contagious viral infection in poultry, which is highly immunosuppressive and increases susceptibility to various opportunistic pathogens. Various immunomodulators including herbs have gained wide attention in poultry production with their multiple health beneficial effects. The present study reports the effect of *Aloe vera* gel extract on the haematological parameters in IBDV vaccinated white leghorn chicks. Specific pathogen free (SPF) day old chicks (n=48) were divided into four groups viz., Group A (Control), B (*Aloe vera*), C (IBDV vaccinated), and D (IBDV vaccinated + *Aloe vera*). The 3% *Aloe vera* gel extract was given in water from day one to entire study period of 45 days. Blood samples were collected at weekly intervals to assess different haematological parameters like WBC, heterophils, lymphocytes, monocytes, RBC, Hb, PCV, MCV, MCH and MCHC. Group D chicks showed significantly (P<0.05) higher WBC and lymphocyte counts than other groups at all intervals tested. PCV, MCV and MCH did not differ significantly. The groups B, C and D showed higher (P<0.05) haematological values compared to control. The *Aloe vera* increased the leukocytes count in both vaccinated and unvaccinated chicks thereby stimulating non-specific immune response. This is the first report of the use of *Aloe vera* in relation to poultry vaccinated with IBDV, further studies could reflect its potential use as an additive in poultry ration to achieve better production and to boost immune status of poultry against infectious diseases.

Keywords: *Aloe vera*, hematological parameters, infectious bursal disease virus, vaccination, immunomodulation, white leghorn chicks.

Poultry industry is one of the fastest growing enterprises of agriculture sector and has captured large volume of meat and egg market all over the world. Poultry products are the most affordable class of animal protein available. However, this industry faces challenges in protection of occurrence of various diseases that seem to totally wipe out poultry population or severely affect their growth and production rate. Infectious bursal disease (IBD) is one such economically important disease due to its direct (mortality) and indirect effects (immuno suppression, leading to susceptibility to other microbial pathogens) in young chicken of age group 3-6 weeks old[1]. IBD is an acute and highly contagious viral disease which affects both broilers

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as well as layers caused by Infectious bursal disease virus (IBDV), a double stranded RNA virus belonging to the family *Birnaviridae*[2]. The IBD virus is a non-enveloped virion with icosahedral symmetry and a diameter ranging from 55-65 nm[3]. Two subtypes of IBDV occur namely, variant and classical forms. Three pathotypes of classical forms are attenuated, virulent and very virulent (vv). Two serotypes of IBDV (based on virulence) occur in chicken viz. virulent serotype 1 and avirulent serotype 2[4].

The vvIBDV strains causes mortality of about 50-60% in laying hens, 25-30% in broilers and 10-100% in susceptible leghorns[5] and causes depletion of B lymphocytes in bursal follicles, which results in immune-suppression[5]. Vaccination by live (based on classical virulent strains) and inactivated vaccines is used to protect or control this disease. Live vaccines are of three types viz. "mild", "intermediate" and "intermediate plus". Mild type vaccine cannot overcome the effect of maternally derived antibodies (MDA) against vvIBDV whereas the other two types are effective even in the presence of MDA but lead to certain degree of bursal atrophy thereby causing immunosuppression[6,7]. In order to overcome such untoward effects and to enhance the effectiveness of vaccine, various herbs and herbal preparations have been evaluated for their immunomodulatory activity and found to be effective[8,9,10,11]. The use of antibiotic growth promoter (AGP) in the poultry ration has been banned in the recent past because it leads to antibiotic resistance in animal and human body[12]. So, the focus is now shifted to find potential alternative to AGP by studying various additives like probiotics, prebiotics and herbs[13,14,15]. In this line, the *Aloe vera* is a semi-tropical plant that belongs to family *Asphodelaceae* and has been known since time immemorial for various health benefits[16]. It is a well accepted medicinal plant with proved wide range of properties like anti-bacterial, anti-viral, anti-inflammatory, anti-parasitic, anti-cancer, wound healing, anti-oxidants and also very good immunomodulating potential[17,18]. The *Aloe vera* has been explored for its therapeutic property against poultry diseases like Coccidiosis, Fowl Typhoid and Newcastle disease[19]. The gel of Aloe species contains certain immunomodulatory components such as aloctin A and acemannan[20,21]. Immunomodulatory potential of *Aloe vera* is mainly attributed to the increased leukocyte infiltration at the site of injury, activating macrophages to secrete nitric oxide, stimulating the production of IL-1 in turn boosting Th1/Th2 immune response and other cytokines[22]. Several studies have shown that it can stimulate the immune system of chickens as well[23,24]. Thus, the extract of *Aloe vera* can be explored to boost the health and immune system of birds vaccinated against infectious agents. Hence, the present study *Aloe vera* aimed to assess the changes in the haematological parameters and individual cell counts at different period of its administration in white leghorn chicks.

MATERIALS AND METHODS

Experimental chickens and housing

Specific pathogen free (SPF) eggs (n=55) for the present study were procured from M/S Venkateshwara Hatcheries Group Limited (VHL), Pune. These eggs were incubated and hatched in Hatchery Unit of ICAR-Central Avian Research Institute, Izatnagar. The hatched out White leghorn chicks (n=48) were housed under standard managemental conditions in the isolation sheds of Avian Disease Section, Division of Pathology, I.V.R.I, Izatnagar. The experiment was carried out as per the approved protocol of the Institute Animal Ethics Committee.

Source, extraction and purification of gel from *Aloe vera* plant

The *Aloe vera* plants were procured from local Nursery at Gandhi Puram, Izatnagar. The leaves were washed thoroughly with warm water, spines removed by trimming and the gel from leaves was separated by filleting the skin. Then, the separated gel was homogenised in homogenizer. The homogenized gel was purified by centrifugation at 10,000 rpm at 4 °C for 30 minutes[25] to separate the fibre and pure gel layer. The top pure and clear gel was collected without disturbing fibre content and stored at 4°C until further use.

Experimental design

Forty-eight SPF day-old Babcock chicks were randomly divided into four groups (n= 12 chicks/group). The chicks were reared under uniform managemental conditions for a period of 45 days. The chicks were divided as into four experimental groups: group A (control-only

drinking water), group B (*Aloe vera* treated only), group C (vaccinated with IBDV vaccine), and group D (IBDV vaccinated and *Aloe vera* treated). IBD Vaccine (Live intermediate plus strain) was administered through intra ocular route at second week of age and *Aloe vera* gel was administrated @ 3% through drinking water from day old to the entire study period (45 days).

Sample collection and analysis

Blood samples (~1.5 ml) were collected in EDTA anticoagulant from different groups of experimental chicks on 7, 14, 21 and 28 day post IBDV vaccination. The hematological parameters comprising of white blood corpuscles (WBC) count, red blood cells (RBC) count, total leukocyte (TLC) count (Lymphocytes, Monocytes, Heterophils), Hemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were analyzed using Automatic method (automatic cell counter) in Vet Hematology analyzer (Abacus junior, Radim, Italy) after putting the samples on electric mixer.

Statistical analysis

All the data obtained were analyzed using the statistical software SPSS®™ 20.0 (IBM, Corp. USA). Two-way analysis of variance (ANOVA) was used to determine the statistically significant differences in mean values between the groups. The means were subjected to Duncan Multiple range test and values with $p<0.05$ were considered significant.

RESULTS AND DISCUSSION

The results of hematological parameters at 7, 14, 21 and 28 day post IBDV vaccination for all the four experimental groups are summarized in Table. 1 and 2. The blood indices are a fundamental tool used widely to monitor the effects of nutrition, therapy and environment in veterinary medicine[26]. The groups (B, C, and D) showed significant ($P<0.05$) difference in WBC count on 7, 14, 21 and 28th day post vaccination (DPV). Especially, the group D (IBDV vaccinated plus *Aloe vera*) showed significantly higher (Pd"0.01) WBC count (21.55, 19.34, 26.79 and 28.38 X 10^3cell/µl) than the control group (16.99, 12.35, 13.29 and 12.21 X 10^3cell/µl) during the study period. The reason for higher WBC count in group B, C, and D might be due to the stimulation of immune response cells by both vaccine as well as by *Aloe vera* gel. Lymphocyte count was found to be highest in the group D (23.43 and 21.74 X 10^3cell/µl) on 21 and 28 DPV, respectively and it was significantly higher ($p<0.05$) than all other group. Although, the group B (16.84,12.64 X 10^3cells/µl) failed to show any significant difference with group A (15.06, 11.81 X 10^3/µl) on 7 and 21 DPV in terms of lymphocyte count, but it revealed its additive effects on hematological cells when combined with vaccine during entire study period. Our results are consistent with the findings of Valle et al.[27]who reported that inclusion of 2% *Aloe vera* gel in drinking water showed an increase in total WBC and lymphocyte counts in broilers vaccinated against Newcastle disease. Similarly, Darabighane et al.[28] observed that inclusion of *Aloe vera* gel in feed of broiler ration showed increased the WBC counts. In another study, the extract of *Aloe vera* gel @ 300 mg/ml administered intra-peritoneally in Swiss albino mice showed significantly ($P<0.01$) higher lymphoproliferative response than control group[29]. In addition, the turkey poults receiving 20 ml/l of *Aloe vera* gel have also been documented with significantly higher WBC and Lymphocytes counts than control group[30]. Recently, Darabighane et al.[31] reported that the boilers fed with either *Aloe vera* gel or peppermint @ 10g/kg or Vitamin E @ 100 mg/kg showed significantly ($p<0.05$) higher WBC count than control group.

Moreover, the group B showed significant higher monocyte count on 14 (0.93 X 10^3cell/µl) and 21 (1.17 X 10^3cell/µl) DPV than group A and C. The reason for higher monocyte count may be attributed to the presence of mannose polysaccharide (acemannan) which binds with mannose receptor on monocyte and stimulates production of various cytokines like IL-1, IL-6, IL-12 and TNF-±. These secreted cytokines further acts on the B and T lymphocytes to increase their growth and proliferation[32,33]. The acemannan compound also exhibits anti-tumor, anti-microbial and immunomodulatory effects as reported earlier[34].

As far as the heterophil counts are concerned, the group D showed 1.89, 3.24 and 5.85 X 10^3cell/µl on 14, 21 and 28 DPV, respectively, and group C showed 1.35, 1.89 and 1.45 X 10^3cell/

Table 1. Hematological changes in IBDV vaccinated and unvaccinated groups of chicks treated with *Aloe vera* gel extract

Parameters	Day 7	Day 14	Day 21	Day 28	Period mean	P-Value		
						Group	Period	G*P
RBC Count (x10^6/ µl)								
Control (A)	1.27^{bcde}±0.11	1.04^{ab}±0.14	0.86^{a}±0.01	1.13^{abc}±0.20	1.39^{A}	0.000	0.003	0.000
Aloe vera (B)	1.64^{fg}±0.08	1.52^{ef}±0.11	1.66^{fg}±0.03	1.87^{g}±0.12	1.37^{A}			
Vaccine (C)	1.49^{def}±0.10	1.49^{def}±0.00	1.07^{ab}±0.13	1.75^{fg}±0.11	1.33^{A}			
Vaccine+*Aloe vera* (D)	1.16^{abcd}±0.11	1.41^{cdef}±0.07	1.75^{fg}±0.08	1.65^{fg}±0.13	1.60^{B}			
Group Mean	1.08^{P}	1.67^{R}	1.45^{Q}	1.49^{Q}				
Haemoglobin (g%)								
Control (A)	8.83^{abc}±0.90	7.30^{a}±0.89	7.37^{a}±0.46	7.70^{a}±1.14	9.60^{A}	0.000	0.829	0.000
Aloe vera (B)	10.53^{bcd}±0.48	10.67^{cd}±0.78	11.90^{d}±0.19	10.83^{d}±0.23	9.88^{A}			
Vaccine (C)	11.03^{d}±0.36	10.53^{bcd}±0.34	8.73^{ab}±0.40	11.20^{d}±0.40	9.94^{A}			
Vaccine+*Aloe vera* (D)	8.00^{a}±0.57	11.03^{d}±0.33	11.77^{d}±0.11	10.03^{bcd}±0.88	9.94^{A}			
Group Mean	7.80^{P}	10.98^{Q}	10.38^{Q}	10.21^{Q}				
PCV (%)								
Control (A)	14.80^{cde}±1.04	11.17^{ab}±1.21	10.27^{a}±0.32	12.17^{abc}±1.84	15.67^{A}	0.000	0.002	0.000
Aloe vera (B)	17.27^{defgh}±0.13	15.59^{cdefg}±0.95	18.06^{efghi}±0.02	19.92^{hi}±1.56	14.29^{A}			
Vaccine (C)	16.78^{defgh}±1.56	15.38^{cdefg}±0.25	12.22^{abc}±0.87	18.62^{fghi}±1.35	15.39^{A}			
Vaccine+*Aloe vera* (D)	13.82^{bcd}±1.22	15.04^{cdef}±1.04	20.99^{i}±0.59	18.83^{ghi}±1.55	17.39^{B}			
Group Mean	12.10^{P}	17.71^{R}	15.75^{Q}	17.17^{QR}				
MCV (fl)								
Control (A)	117.33^{cde}±3.60	109.00^{ab}±2.53	119.00^{de}±1.90	109.67^{abc}±2.11	113.75^{C}	0.000	0.000	0.000
Aloe vera (B)	106.67^{ab}±4.85	102.67^{a}±1.05	103.00^{a}±0.00	105.67^{ab}±1.48	104.25^{A}			
Vaccine (C)	111.67^{bcd}±3.11	102.67^{a}±1.69	117.33^{cde}±5.48	105.67^{ab}±1.48	115.00^{C}			
Vaccine+*Aloe vera* (D)	119.33^{de}±0.42	102.67^{a}±1.12	120.67^{e}±2.11	113.67^{bcde}±1.28	108.67^{B}			
Group Mean	113.75^{R}	104.50^{P}	109.33^{Q}	114.08^{R}				
MCH (pg)								
Control (A)	69.03^{abcde}±1.65	71.23^{bcde}±0.86	85.60^{f}±4.11	69.87^{bcde}±1.73	69.82^{B}	0.003	0.000	0.000
Aloe vera (B)	64.33^{abc}±0.34	69.90^{bcde}±0.25	72.27^{cde}±2.49	58.90^{a}±3.04	72.77^{B}			
Vaccine (C)	76.27^{def}±6.78	70.40^{bcde}±2.15	85.00^{f}±5.69	64.87^{abc}±3.46	77.66^{C}			
Vaccine+*Aloe vera* (D)	69.63^{abcde}±1.37	79.53^{ef}±5.88	67.77^{abcd}±2.36	60.47^{ab}±1.33	63.53^{A}			
Group Mean	73.93^{Q}	66.35^{P}	74.13^{Q}	69.35^{PQ}				
MCHC (%)								
Control (A)	59.40^{abcd}±3.32	65.20^{bcdefg}±0.76	71.47^{efg}±2.36	63.53^{abcdef}±0.30	62.04^{AB}	0.020	0.000	0.000
Aloe vera (B)	60.97^{abcde}±2.30	68.20^{cdefg}±0.89	65.93^{bcdefg}±1.05	55.87^{ab}±3.71	69.36^{C}			
Vaccine (C)	69.43^{defg}±8.43	68.43^{cdefg}±1.03	72.00^{fg}±1.64	61.33^{abcdef}±3.59	66.26^{BC}			
Vaccine+*Aloe vera* (D)	58.37^{abc}±0.91	75.60^{g}±6.50	55.63^{ab}±0.86	53.17^{a}±0.66	58.48^{A}			
Group Mean	64.90^{PQ}	62.74^{P}	67.80^{Q}	60.69^{P}				

^{abcd} Means in row and columns bearing different superscripts differ significantly (P<0.05). ^{ABCD}Means in columns wise bearing different superscripts differ significantly (P<0.05).

^{PQRS}Means in row wise bearing different superscripts differ significantly (P<0.05).

Values (Mean+SE) RBC - Red Blood Cells; WBC- White Blood Cells; MCV - Mean Cell Volume; MCH - Mean Cell; Hb-Haemoglobin; MCHC - Mean Cell Haemoglobin Concentration; Hb – Haemoglobin, PCV – Packed Cell Volume

Table 2. TLC changes in IBDV vaccinated and unvaccinated groups of chicks treated with *Aloe vera* gel extract

Parameters	Day 7	Day 14	Day 21	Day 28	Period mean	P-Value		
						Group	Period	G*P
White Blood Cell Count (x10^3/µl)								
Control (A)	16.99^{bc}±1.01	12.35^{a}±1.12	13.29^{a}±0.86	12.21^{a}±1.03	19.68^{C}	0.000	0.000	0.000
Aloe vera (B)	19.26^{d}±0.18	16.63^{b}±0.00	15.26^{b}±0.07	16.35^{b}±0.14	16.69^{A}			
Vaccine (C)	20.91^{e}±0.21	18.43^{cd}±0.21	16.94^{bc}±0.58	16.50^{b}±0.21	18.07^{B}			
Vaccine+*Aloe vera* (D)	21.55^{e}±0.14	19.34^{d}±0.29	26.79^{f}±0.28	28.38^{g}±0.34	18.36^{B}			
Group Mean	13.71^{P}	16.88^{Q}	18.20^{R}	24.01^{S}				
Lymphocytes (x10^3/ µl)								
Control (A)	15.06^{b}±0.91	11.11^{a}±1.28	11.81^{a}±0.86	10.67^{a}±1.01	17.52^{B}	0.000	0.000	0.000
Aloe vera (B)	16.84^{bc}±0.04	15.07^{b}±0.13	12.64^{a}±0.39	15.16^{b}±0.00	14.81^{A}			
Vaccine (C)	18.48^{cd}±0.42	16.61^{bc}±0.53	14.96^{b}±0.79	14.69^{b}±0.30	15.71^{A}			
Vaccine+*Aloe vera* (D)	19.69^{d}±0.18	16.46^{bc}±0.54	23.43^{e}±0.38	21.74^{e}±1.13	15.57^{A}			
Group Mean	12.16^{P}	14.93^{Q}	16.19^{R}	20.33^{S}				
Monocytes (x10^3/ µl)								
Control (A)	0.39^{abcd}±0.19	0.21^{abc}±0.09	0.07^{a}±0.00	0.30^{abc}±0.15	0.45^{A}	0.001	0.201	0.000
Aloe vera (B)	0.81^{cde}±0.23	0.93^{de}±0.04	1.17^{e}±0.12	0.23^{abc}±0.09	0.65^{A}			
Vaccine (C)	0.50^{abcd}±0.25	0.48^{abcd}±0.24	0.09^{ab}±0.00	0.71^{bcde}±0.27	0.37^{A}			
Vaccine+*Aloe vera* (D)	0.11^{ab}±0.00	1.00^{de}±0.29	0.15^{ab}±0.00	0.78^{cde}±0.38	0.50^{A}			
Group Mean	0.24^{P}	0.78^{Q}	0.44^{P}	0.51^{P}				
Heterophils (x10^3/ µl)								
Control (A)	1.54^{a}±0.12	1.37^{a}±0.04	1.4 ^{a}±0.01	1.24^{a}±0.13	1.71^{A}	0.000	0.006	0.000
Aloe vera (B)	1.61^{a}±0.08	1.24^{a}±0.02	1.45^{a}±0.20	1.29^{a}±0.02	1.46^{A}			
Vaccine (C)	1.93^{a}±0.07	1.35^{a}±0.08	1.89^{a}±0.21	1.45^{a}±0.11	2.00^{AB}			
Vaccine+*Aloe vera* (D)	1.74^{a}±0.04	1.89^{b}±0.26	3.24^{b}±0.10	5.85^{c}±1.56	2.46^{B}			
Group Mean	1.39^{P}	1.40^{P}	1.65^{P}	3.18^{Q}				

^{abcd} Means in row and columns bearing different superscripts differ significantly (P<0.05).
^{ABCD}Means in columns wise bearing different superscripts differ significantly (P<0.05).
^{PQRS}Means in row wise bearing different superscripts differ significantly (P<0.05).
Values (Mean+SE) RBC - Red Blood Cells; WBC- White Blood Cells; MCV - Mean Cell Volume; MCH - Mean Cell; Hb-Haemoglobin; MCHC - Mean Cell Haemoglobin Concentration; Hb – Haemoglobin, PCV – Packed Cell Volume

µl on 14, 21 and 28 DPV, respectively, which are significantly higher than the group A. The increased heterophils count imparts its phagocytic activity against vaccine antigen. However, at 7 DPV the heterophil count did not differ significantly among the groups.

The RBC, Hb and PCV values were significantly higher in all the groups than the control group, which might be due the presence of erythropoietic factors in the extract of *Aloe vera* leaf[35]. Similarly, Mahdavi et al.[36] reported that the broiler fed with 1% *Aloe vera* gel powder in ration showed highest WBC, RBC and hemoglobin count. In another study, the broiler chickens fed with *Aloe vera* leaf juice @ 20 g/L in drinking water, daily for 42 days showed significantly ($P<0.05$) higher value of Hb concentration, PCV percentage and total leukocyte count than the control group[37]. Similarly, the broiler chicken fed with 7.5g/kg *Aloe vera* powder in ration revealed increased RBC count and Hb concentration[38]. The parameters like the MCV, MCH and MCHC measurements showed no significance difference (P d" 0.05) amongst the groups.

In the present study, the chicks fed with *Aloe vera* gel extract @ 3% in the drinking water did not show any adverse effects on their health and immune status rather it was found to stimulate the immune system as observed for the results for lymphocytes and other parameters/immune cells. This finding is consistent with that of Khan et al.[39] who reported that Fayoumi's chick fed with *Aloe vera* leaf @ 2.0% in ration did not show any sign of adverse effects on their health. In an earlier study by Pittman[40], the whole *Aloe vera* leaf was recommended for the control of immune deficiency disorders and Bolu et al.[30] stated that the extract would serve as a better alternative to antibiotic in poultry industry.

Seeing the importance of various herbs and their products gaining importance in animal and health production systems[41,42,15], the present findings with the use of *Aloe vera* with IBDV vaccinated chicks as a model, with addition to earlier beneficial reports of this herb[19,17,23,18,16,24] could pave way for further experimental and clinical trials with this valuable herb against virulent IBDV and other infectious pathogens of poultry as well as to be exploited as a promising immune-enhancer and health beneficial element.

In summary, the extract of *Aloe vera* gel increased the leukocytes count in IBDV vaccinated and unvaccinated white leghorn chicks thereby stimulating non-specific immune response without affecting their normal haematological parameters. It can be used as an additive in poultry ration to achieve better FCR and to boost health and immune status of poultry against IBDV, for which purpose further explorative studies carried out could reflect its beneficial potential to be use as an additive in poultry ration to achieve better production and health along with boosting the immune status of poultry birds against infectious diseases.

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Conflict of interest

Authors declares that no conflict of interest in this study

REFERENCES

1. Wang, M., Pan, Q., Lu, Z., Li, K., Gao, H., Qi, X., Gao, Y., Wang, X. An optimized, highly efficient, self-assembled, subvirus-like particle of infectious bursal disease virus (IBDV). *Vaccine.*, 2016. **24;34(30):** 3508-14.
2. Smith, J., Sadeyen, J. R., Butter, C., Kaiser, P., Burt, D.W. Analysis of the early immune response to infection by infectious bursal disease virus in chickens differing in their resistance to the disease. *J. virol.*, 2015; **89(5):** 2469-82.
3. Teshome, M., Admassu, T.F.B. Infectious Bursal Disease (gumboro disease) in chickens. *Brit. J. Poult. Sci.*, 2015; **4**: 22-8.
4. Morla, S., Deka, P. Kumar, S. Isolation of novel variants of infectious bursal disease virus from different outbreaks in Northeast. *India. Microb. Pathog.*, 2016; **93**: 131-6.
5. Ingrao, F., Rauw, F., Lambrecht, B., van den Berg, T. Infectious Bursal disease: a complex host pathogen interaction. *Dev. Comp. Immunol.*, 2013; **41**: 429-38.
6. Müller, H., Mundt, E., Eterradossi, N., Islam, M. R. Current status of vaccines against infectious bursal disease. *Avian Pathol.*, 2012; **41(2):** 133-9.
7. Wang, Y. S., Ouyang, W., Liu, X. J., He, K. W., Yu, S. Q., Zhang, H. B., Fan, H.J. & Lu, C. P. Virus-like particles of hepatitis B virus core

protein containing five mimotopes of infectious bursal disease virus (IBDV) protect chickens against IBDV. *Vaccine*., 2012; **30(12):** 2125-30.
8. Hung, C. M., Yeh, C. C., Chong, K. Y., Chen, H. L., Chen, J. Y., Kao, S. T., Yen, C. C., Yeh, M. H., Lin, M. S., Chen, C. M. Gingyo-san enhances immunity and potentiates infectious bursal disease vaccination. *Evid. based. Comp. Alt. Med.*, 2011.
9. Ma, A., Niu, X., Zhong, X. Effects of Echinacea Purpurea Extracts and Astragalus Extracts on Immunological Response to Infectious Bursal Disease Vaccination in Broilers. *Am. J. Trad. Chin. Vet. Med.*, 2013; **8(1):** 39-48
10. Saleem, M.H., Mehmood, K. Effect of a herbal supplement Livol on the growth performance and antibody response against infectious bursal disease virus in broiler chicks. *Pakistan J. Zool.*, 2013; **45(5):** 1387-1391.
11. Okonkwo, C., Oladele, O. A., Nwiyi, P. The Pattern of Immunomodulation of ImmuPlus on the Infectious Bursal Disease (IBD) Antibody of Vaccinated Broiler Chickens. *J. Vet. Adv.*, 2015; **1**(5): 808-13.
12. Yang, Y., Iji, P., Choct, M. Dietary modulation of gut microflora in broiler chickens: a review of the role of six kinds of alternatives to in-feed antibiotics. *World Poultry Sci. J.*, 2009; **65:** 97-114.
13. Verstegen, M.W., Williams, B.A. Alternatives to the use of antibiotics as growth promoters for monogastric animals. *Anim. biotechnol*., 2002; **13:** 113-27.
14. Dhama, K., Tiwari, R., Khan, R.U., Chakraborty, S., Gopi, M., Karthik, K., Saminathan, M., Desingu P.A., Sunkara, L.T. Growth promoters and novel feed additives improving poultry production and health, bioactive principles and beneficial applications: The trends and advances-A Review. *Int. J. Pharmacology*., 2014; **10(3):** 129-59.
15. Dhama, K., Latheef S.K., Saminathan M., Samad, H.A., Karthik K., Tiwari, T., Khan, R.U., Alagawany, M., Farag, M.R., Gazi Mahabubul Alam, Laudadio, V. Tufarelli, V.. Multiple beneficial applications and modes of action of herbs in poultry health and production – A review. *Int. J. Pharmacol*., 2015; **11(3):** 152-76.
16. Pandey, A., Singh, S. Aloe Vera: A Systematic Review of its Industrial and Ethno-Medicinal Efficacy. *Int. j. pharm. res. allied sci.*, 2016; **5(1):** 21-33.
17. Christaki, E.V., Florou-Paneri, P.C. *Aloe vera*: A plant for many uses. *J. Food Agric. Environ.*, 2010; **8**: 245-9.
18. Kargaran, M., Moradabadi, A. R., Arjomandzadegan, M., Hosseini, H., Habibi, G., Tayeboon, M., Karami, H., Akbari, A. Effects of the Aqueous Extract of *Aloe vera* on the Morphological and Physiological Properties of E. coli. *Iran. Red. Crescent. Med. J.*, 2016; e23896.
19. Mwale, M., Bhebhe, E., Chimonyo, M., Haliman, T. E. Use of herbal plants in poultry health management in the Mushagashe small-scale commercial farming area in Zimbabwe. *Int. J. Appl. Res. Vet. M*., 2005; **3:** 163-70.
20. Im, S. A., Lee, Y. R., Lee, Y. H., Lee, M. K., Park, Y. I., Lee, S., Kim, K., Lee, C. K. In vivo evidence of the immunomodulatory activity of orally administered *Aloe vera* gel. *Arch. Pharm. Res.*, 2010; **33(3):** 451-6.
21. Kumar, S., Tiku, A.B. Immunomodulatory potential of acemannan (polysaccharide from Aloe vera) against radiation induced mortality in Swiss albino mice. ***Food** Agr. Immunol.*, 2016; **27(1):** 72-86.
22. Gandhi, M., Shree, R. Immuno-modulatory Effect of Turmeric (Curcuma Longa) And *Aloe Vera* on Cultured Polymorphonuclear Cells (PMN) and Adherent Mononuclear Cells. *WebmedCentral ALTERNATIVE MEDICINE* 2016; **7(6):** WMC005113.
23. Ojiezeh, T. I., Ophori, E. A.. Haemogram and Serum Enzymes Activities of Newcastle Disease Virus Challenged Broiler Chickens Following Supplemental Treatment with *Aloe Vera* Extract. *J. Clin. Cell. Immunol*., 2015; **6:** 282.
24. Khaliq, K., Akhtar, M., Iqbal, Z., Hussain, I. *Aloe vera* Polysaccharides as Biological Response Modifiers in Chickens. *Int. j. agric. boil.*, 2016; **18(2):** 274-281.
25. Chandegara, V. K., Varshney, A. K. Effect of Centrifuge Speed on Gel Extraction from *Aloe vera* Leaves. *J. Food Process. Technol.*, 2014; **5**: 295.
26. Toghyani, M., Toghyani M, Gheisari, Abbasali G, Gholamreza E. Shahin. Evaluation of cinnamon and garlic as antibiotic growth promoter substitutions on performance, immune responses, serum biochemical and haematological parameters in broiler chicks. *Livest. Sci*., 2011; **138(1):**167-73.
27. Valle, P., Anunciado, S., Lapitan, R. Effects of *Aloe vera* on the white blood cell count and antibody titer of broiler chickens vaccinated against Newcastle disease. *Philipp. J. Vet. Med.*, 2005; **42:** 49-52.
28. Darabighane, B., Zarei, A., Shahneh, A.Z., 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**: e36.

29. Madan, J., Sharma, A., Inamdar, N., Rao, H., Singh, R. Immunomodulatory properties of *aloe vera* gel in mice. *Int. j. green pharm*., 2008; **2:** 152.
30. Bolu, S., Babalola, T., Elelu, N., Ahmed, R., Oyetunde, S., Ademola, P., Jiddah, S. 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 *Journal of Agricultural Research*., 2013; **2**: 223-9.
31. Darabighane, B., Mahdavi, A., Kasapidou, E. Comparison of the effect of Peppermint, *Aloe vera* and Vitamin-E supplementation on Broiler immune response. Scientific Papers, Series D. *Animal Science*., 2015; **58:** 107-10.
32. Zhang, L., and Tizard, I, R. Activation of a mouse macrophage cell line by acemannan: the major carbohydrate fraction from *Aloe vera* gel. Immunopharmacology, 1996; **35:** 119-28.
33. Ramamoorthy, L., Kemp, M.C., Tizard I.R. Acemannan, a beta-(1, 4)-acetylated mannan, induces nitric oxide production in macrophage cell line RAW 264.7. *Mol. pharmacol*., 1996; **50:** 878-84.
34. Choi, S., Chung, M. H. A review on the relationship between *Aloe vera* components and their biologic effects. *In Semin. Integr. Med.*, 2003; **1(1):** 53-62.
35. Li, M., Ciu, J. R., Ye, Y., Min, J. M., Zhang, L. H., Wang, K., Gares, M., Cros, J., Wright, M., Leung-Tack, J. Antitumor activity of Z-ajoene, a natural compound purified from garlic: antimitotic and microtubule-interaction properties. Carcinog., 2002; **23:** 573-9.
36. 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. *Brit. Poultry Sci*., 2012; **8:** 49-50.
37. Singh, J., Koley, K., Chandrakar, K., Pagrut, N. S. Effects of *Aloe vera* on dressing percentage and haemato-biochemidal parameters of broiler chickens. *Vet. World*, 2013; **6:** 803-6.
38. Naghi Shokri, A., Ghasemi, H. A., Taherpour, K. Evaluation of Aloe vera and synbiotic as antibiotic growth promoter substitutions on performance, gut morphology, immune responses and blood constitutes of broiler chickens. *Anim. Sci. J.*, 2016;
39. Khan, M. J. A., Khan, S.H., Naz, S., Gilani, S.S., Shafi, J., Hassan, F., Hassan, M. Anwar, M. Effect of Dietary Supplementation of *Aloe vera* Leaves on Growth Performance and Immunity of Fayoumi Chicks. *Pak. J. Nutr.*, 2014; **13(4):** 191-5.
40. Pittman, J.C. 1992. Immune enhancing effects of Aloe. Health conscious. **13:** 1-28.
41. Aghsaghali, A.M. Importance of medical herbs in animal feeding: A review. *Annals. Biol. Res.*, 2012; **3**(2): 918-23.
42. Mahima, Rahal, A., Deb, R., Latheef, S.K., Samad, H.A., Tiwari, R., Verma, A.K., Kumar, A. Dhama, K. Immunomodulatory and therapeutic potentials of herbal, traditional / indigenous and ethnoveterinary medicines. *Pak. J. Biol. Sci*., 2012; **15(16):** 754-74.