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Immunomodulatory and Protective Effects of a Polyherbal Formulation (Immon) against Infectious Anemia Virus Infection in Broiler

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ABSTRACT
The present investigation was undertaken to assess the role of a polyherbal immunomodulator with additional elements (Immon) against the *Chicken infectious anemia virus* (CIAV) infection. A total of 60 broiler chicks (day old age) were divided into three groups (n = 20) and vaccinated against Newcastle Disease (ND). The group I chicks were kept as healthy control while group II and III chicks were infected with 1 mL CIAV ($10^{4.5}$ $TCID_{50}$/0.1 mL) per chicken intramuscularly. Group III chicks were supplemented with Immon (1 mL/10 birds in the drinking water) for 21 days. Subsequently, chicks of all three groups were monitored for hematological (Hb, PCV, TEC, TLC and DLC) and biochemical parameters (AST, ALT, ALP and uric acid) along with ND antibody titers, organ: body weight ratios, and mean live body weight at 7th, 14th, 21st, 28th and 35th day of the experiment. At 7-24 days of CIAV infection, the group II birds showed a significantly lower count of erythroid and myeloid cells; increase in enzyme activities and uric acid; decline in mean live body weight and organ: body weight ratios of lymphoid organs and decline in ND antibody titers. However, at these day intervals the CIAV immunosuppression was less severe in Immon supplemented chicks which showed significantly ($p<0.05$) higher values of all the test parameters as compared to virus control group II chicks. Thus, the present findings support that Immon is an effective immunomodulating agent in CIAV affected birds, reduces pathogenicity of the virus, ameliorate the depressed immune responses and protects the virus induced adverse effects on growth performances.

Key words: *Chicken infectious anemia virus*, immunomodulator, polyherbal supplement (Immon), haematology, biochemical enzymes, broiler

INTRODUCTION

Chicken infectious anemia virus (CIAV), belonging to *Gyrovirus* genus of family Circoviridae, causes acute disease (chicken infectious anaemia, CIA) in young chicks of up to 4 weeks of age with clinical stage developing after an incubation period of 10-14 days (McNulty, 1991; Todd *et al*., 2000; Schat, 2003). CIAV has been reported worldwide and is

considered as an economically important viral disease of poultry (Dhama *et al*., 2008; Schat, 2009; Bhatt *et al*., 2011). The CIA is manifested by inconsistent pathognomic symptoms of depression, paleness, weakness, anorexia, ruffled feathers and stunted growth (McNulty, 1991; Hagood *et al*., 2000). Anemia, the only specific sign with a peak at 14-16 Days Post Infection (DPI) is noticeable on the non-feathered areas with marked pallor that may extend to the internal organs (Pope, 1991). CIAV causes suppression of differentiation and proliferation of haemopoietic precursor cells, leading to anemia and panleukopenia. Repopulation of the bone marrow with proerythroblasts and promyelocytes and recovery of haematopoietic activity (erythropoiesis) and lymphocyte repopulation occur during recovery phase (Taniguchi *et al*., 1982; Liu *et al*., 1997; Wani *et al*., 2015). The virus plays a key role in the etiology of several multifactorial diseases like haemorrhagic syndrome, haemorrhagic anemia syndrome, infectious/aplastic anaemia, anaemia-dermatitis syndrome, gangrenous dermatitis and blue wing diseases (Pope, 1991; Toro *et al*., 2000; Hagood *et al*., 2000). Certain notable characteristics such as vertical transmission, detection in Specific Pathogen Free (SPF) eggs, its highly contagious, hardy and ubiquitous nature and the potential for inducing marked immunosuppression have attracted the global poultry production systems towards the potential threat of CIAV infection in poultry production (Todd, 2000; Dhama *et al*., 2008; Basaraddi *et al*., 2013). As with other viral infections there is no specific therapeutic approach for the treatment of CIA. Vaccination strategies inclusive of live-attenuated and inactivated vaccines are available but have limitations; DNA vaccine has been developed recently but need to be applied yet (Dhama *et al*., 2008; Sawant *et al*., 2015; Zhang *et al*., 2015). Birds can be provided with haematinics and immunostimulants so as to check anaemia and boost the immune system (Bhatt *et al*., 2013; Latheef *et al*., 2013). Therefore, the present investigation was carried out to study the immunomodulatory and protective effects of a polyherbal preparation with additional nutrients named 'Immon' in CIAV infected chicks.

MATERIALS AND METHODS

Virus: *Chicken infectious anaemia virus* (CIAV), maintained in the Avian Disease Section, Division of Pathology, Indian Veterinary Research Institute (IVRI), Izatnagar, UP (India) was propagated in MDCC-MSB1 cells and used as the challenge virus for inducing infection. Virus at a titer of $10^{4.5}$ $TCID_{50}$/0.1 mL was used to infect the broiler.

Immunomodulator: An immunomodulator 'Immon' (Regen Biocorps, Vadodara, Gujrat, India) which is a polyherbal formulation (*Asparagus adscendens, Moringa oleifera* and *Picrorhiza kurroa*) with micro and macro nutrients like amino acids, minerals, vitamins (E and C), nucleotides, spirulina, β-glucans and probiotics was used in the present study.

Experimental chicks: During the study, 60 day old age broiler chicks were maintained under ideal conditions and given normal basal ration and water *ad libitum*. All the experimental procedures on the chicks were carried out according to the recommendations and approval of the Institute Animal Ethics Committee (IAEC) under the guidelines set forth by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Experimental design: Sixty day-old broiler chicks were divided into three equal groups with 20 birds in each group (Group I-III). The chicks were vaccinated against Newcastle Disease (ND) by live attenuated 'F' strain (NDV-F) and Georgia strain of Infectious Bursal Disease (IBD) on 1st and 12th day, respectively following manufacturer's instructions. The chicks of group I were given normal basal ration and plain drinking water, and served as healthy negative control. The chicks of group II and III were infected intramuscularly with 1 mL of CIAV infected MDCC-MSB1 cell culture fluid ($10^{4.5}$ $TCID_{50}$ 0.1 mL^{-1}) per bird. The chicks of group II were given normal basal ration and plain drinking water, and served as virus positive control. The chicks of group III in addition were supplemented with Immon each 1 mL/10 birds in drinking water for 21 days.

Pooled blood samples of 5 mL were collected randomly from 3 birds from each group on 7th, 14th, 21st, 28th and 35th day of the experiment. The blood was divided into two parts: one part was taken in heparinised vials (10-20 IU heparin mL) for hematological estimations and the second part was taken in sterilized vials for serum separation for studying biochemical and immunological parameters. The mean live body weight and organ: body weight ratios of lymphoid organs (thymus, bursa of Fabricius, spleen and liver) were studied in experimental chicks of all the groups at regular intervals on 7th, 14th, 21st, 28th and 35th day of the experiment after scarification of birds. On these interval days, three birds from each group were sacrificed to calculate body: weight and ratio.

Hematological parameters: Hematological parameters, viz., Hemoglobin (Hb), Packed Cell Volume (PCV) (Jain, 1986), Total Erythrocyte Count (TEC), Total Leukocyte Count (TLC) (Natt and Herrick, 1952) and Differential Leukocyte Count (DLC) (Lucas and Jamroz, 1974) were estimated with pooled blood samples (5 mL from 3 birds) from each group on 7th, 14th, 21st, 28th and 35th day of the experiment as per standard procedures.

Biochemical parameters: Biochemical parameters, viz., Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and uric acid (UA) were estimated with serum samples from 3 birds from each group on 7th, 14th, 21st, 28th and 35th day of experiments using reagent kits (IFCC Kinetic Method, Erba, Mannheim Ltd., Germany; Uricase PAP Method, Span Diagnostics Ltd., Surat, Gujarat, India).

ND HI titer: Haemagglutination Inhibition (HI) test was performed on the serum samples obtained from 3 birds from each group on 7th, 14th, 21st, 28th and 35th day of experiment as described by Allan and Gough (1974) to evaluate the

humoral immune response to NDV vaccine. The HI-titre of the serum was calculated as the reciprocal of the highest dilution of the test sera showing complete inhibition of haemagglutination of chicken RBCs. The mean HI titre (log_2) for different experimental groups was calculated for comparison.

Mean live body weight: To determine the ameliorative effect of immunomodulator 'Immon' on growth performance of the CIAV infected chicks, the average live body weight of 5 chicks each of three groups was recorded on day 0, 7th, 14th, 21st, 28th and 35th of the study for comparison.

Organ: Body (O: B) weight ratio: Organ: Body weight ratios of the lymphoid organs (thymus, bursa of Fabricius, spleen and liver) were calculated for the 5 chicks each of three groups recorded on day 0, 7th, 14th, 21st, 28th and 35th of the study to know the protective effect of 'Immon' on overall body growth and growth of various lymphoid organs of the virus infected birds for comparison. This ratio was calculated by dividing respective organ weight with body weight (g) and multiplying with 100.

Statistical analysis: All the values obtained in the study were analysed statistically and are represented as Mean±SE. The data was analyzed by analysis of variance (ANOVA) (Snedecor and Cochran, 2004) by using SPSS (2007) version 16.

RESULTS AND DISCUSSION

Hematological parameters: All the estimated hematological parameters of CIAV challenged and unchallenged groups are summarized in Table 1. A significant ($p<0.05$) decline in the Hb, PCV and TEC was observed in the CIAV challenged group II from day 7 onwards which was lowest at day 21 and then again started increasing when compared to the healthy group I. In the myeloid lineages also, a significant ($p<0.05$) decline in the TLC, percent lymphocyte count and percent eosinophil and basophil count was observed in all CIAV infected birds of group II from day 7 onwards and maximum decrease was observed on day 21. These observations are in agreement to those reported in earlier studies related to experimental CIAV infection (Dhama, 2002; Schat, 2003; Bhatt *et al*., 2013). The reduction in the Hb, PCV, TEC and TLC levels in all the CIAV infected chicks might be due to the destructive effect of CIAV on erythroid and myeloid tissues of the bone marrow where it causes suppression of differentiation and proliferation of haemopoietic precursor cells. This drastically affects erythropoiesis and myelopoiesis leading to anaemia and panleukopenia (Schat, 2003; Pope, 1991; Bhatt *et al*., 2013). The chicks of group III supplemented with Immon showed significantly ($p<0.05$) higher values of hematological parameters as compared to group II (virus positive control). Herbal and other ingredients of 'Immon' possess immunostimulant properties which can be a reason to counteract panleukopenia and immunosuppression during CIAV infection (Chae *et al*., 2006; Rajapakse *et al*., 2010; Dhama *et al*., 2011; Hussain *et al*., 2013).

Biochemical parameters: Biochemical parameters of all the three groups were estimated and are summarized in Table 1. A significant ($p<0.05$) elevation in the activities of ALT, AST and ALP enzymes, and uric acid in the CIAV inoculated group II birds was found which can be associated with the damage to the organs like liver, kidneys and muscles of CIAV infected chicks (McNulty, 1991; Dhama *et al*., 2008). It has been well documented that AST, ALT and ALP are found in the liver and the ALP is found in the kidney also (Kaneko *et al*., 1997). CIAV has a widespread distribution in the body resulting in damage and focal necrosis of liver, kidney and spleen (Dhama *et al*., 2008). Hemorrhages in the proventricular mucosa and subcutaneous tissues and muscular hemorrhages within the wing tips are sometimes associated with severe anaemia (Yuasa and Imai, 1986; Engstrom *et al*., 1988; Pope, 1991). Ingredients of 'Immon' with immunostimulant properties might have limited the immunosuppression during CIAV infection and thus the immunomodulator treatment was found to significantly reduce the effect of CIAV induced increase in the value of biochemical enzymes (ALT, AST and ALP) and uric acid (Rajapakse *et al*., 2010; Dhama *et al*., 2011; Hussain *et al*., 2013).

ND HI titer: The humoral immune response (HIR) to NDV-F vaccination in chicks of the three experimental groups was measured by haemagglutination (HI) test at weekly intervals, i.e., day 7, 14, 21, 28 and 35. Mean HI antibody titers in the sera of all three groups obtained at different days post vaccination expressed as HI titer (log_2) are presented in Table 2. No significant difference was observed in the mean HI antibody titers of all the three groups on 7th day of the experiment. The group II challenged with CIAV showed a significant ($p<0.05$) decline in the mean HI antibody titer on 14th, 21st, 28th and 35th day of the investigation. The group III chicks supplemented with 'Immon' showed significantly higher values of HI titers as compared to group II CIAV challenged birds only. The destructive effect of CIAV is due to suppression of the population of both helper ($CD4^+$) and cytotoxic ($CD8^+$) T-lymphocytes in the thymus as suggested by Hu *et al*. (1993) and Adair (2000), which could be the reason for poor immune response in CIAV inoculated chicks. Amongst both CIAV inoculated groups II and III, the group supplemented with 'Immon' showed significantly higher HI antibody titers as compared to the non-supplemented group. Herbal supplementation has been reported to have ameliorative effects against immunosuppression induced by CIAV (Bhatt *et al*., 2013; Latheef *et al*., 2013).

Mean live body weight: The mean body weights recorded in the healthy control group I, CIAV challenged virus control Group II and 'Immon' treated group III of chicks at different post-infection intervals are presented in Table 3. The chicks of healthy group I showed maximum weight gain as compared to

Table 1: Hematological and biochemical parameters of healthy, CIAV challenged and 'Immon' treated groups of chicks

		Day of observation				
Parameters	Groups	7th day	14th day	21st day	28th day	35th day
Hb (g L^{-1})	I	116.67±0.18^{A}	118.00±0.12^{A}	120.67±0.07^{A}	121.33±0.07^{A}	118.67±0.18^{A}
	II	100.33±0.30^{Ca}	86.33±0.12^{Cb}	52.67±0.29^{Cc}	88.33±0.18^{Cb}	102.67±0.29^{Ba}
	III	109.33±0.24^{Ba}	102.67±0.29^{Bb}	92.67±0.54^{Bc}	109.33±0.47^{Ba}	111.33±0.44^{Ba}
PCV (L L^{-1})	I	0.34±0.01^{A}	0.34±0.01^{A}	0.35±0.01^{A}	0.35±0.01^{A}	0.34±0.01^{A}
	II	0.28±0.00^{Ca}	0.26±0.00^{Cb}	0.18±0.01^{Cc}	0.25±0.00^{Cb}	0.29±0.01^{Ba}
	III	0.31±0.00^{Ba}	0.30±0.01^{Ba}	0.29±0.01^{Bb}	0.31±0.01^{Ba}	0.32±0.00^{Aa}
TEC ($\times 10^{12}$ L^{-1})	I	3.29±0.06^{A}	3.37±0.02^{A}	3.39±0.01^{A}	3.40±0.01^{A}	3.39±0.12^{A}
	II	2.97±0.12^{Ba}	2.43±0.06^{Bb}	1.52±0.07^{Cc}	2.60±0.03^{Cb}	2.99±0.10^{Ba}
	III	3.10±.0.05^{ABa}	2.82±0.09^{Bb}	2.60±0.12^{Bc}	3.21±0.21^{Ba}	3.35±0.13^{Aa}
TLC ($\times 10^{9}$ L^{-1})	I	20.60±0.42^{A}	21.20±0.70^{A}	21.53±0.37^{A}	21.50±0.32^{A}	21.50±0.32^{A}
	II	18.47±0.40^{Ba}	17.00±0.20^{Cb}	14.67±0.57^{Cc}	17.33±0.29^{Cab}	17.97±0.12^{Bab}
	III	19.43±0.09^{Ba}	19.60±0.12^{Ba}	18.27±0.18^{Bb}	19.87±0.48^{Ba}	20.07±0.37^{Ba}
PLC (%)	I	60.67±0.58^{A}	59.00±0.58^{A}	59.34±0.67^{A}	59.00±0.58^{A}	59.00±0.58^{A}
	II	52.67±0.58^{Ba}	49.67±0.88^{Cb}	48.33±0.33^{Cb}	49.67±0.88^{Cb}	50.00±0.58^{Cb}
	III	56.33±0.33^{Ba}	55.67±0.67^{Ba}	54.33±0.33^{Ba}	54.67±0.88^{Ba}	57.67±0.88^{Ba}
PHC (%)	I	33.00±0.58^{B}	34.67±0.88^{B}	32.67±0.34^{B}	33.00±1.00^{B}	32.67±0.88^{B}
	II	40.00±0.58^{Ac}	43.33±0.33^{Ab}	47.33±0.33^{Aa}	44.33±0.88^{Ab}	43.67±0.67^{Ab}
	III	36.67±0.33^{Aa}	37.33±0.67^{Ba}	39.33±0.88^{Ba}	38.33±0.88^{Ba}	35.33±0.89^{Ba}
PMC (%)	I	3.67±0.34	4.00±0.58	4.67±0.33^{A}	4.33±0.34^{A}	4.67±0.58^{A}
	II	4.33±0.34	4.33±0.88	2.67±0.33^{B}	3.67±0.33^{B}	3.67±0.58^{A}
	III	4.33±0.58	4.33±0.67	3.67±0.33^{AB}	4.33±0.33^{A}	4.00±0.00^{A}
P (B+E) (%)	I	2.67±0.33	2.33±0.33	3.67±0.33^{A}	3.67±0.33^{A}	3.67±0.33
	II	3.00±0.58	2.67±0.33	1.67±0.33^{B}	2.33±0.33^{B}	2.67±0.33
	III	3.33±0.33	3.33±0.33	2.67±0.67^{AB}	2.67±0.33^{AB}	3.00±0.00
AST (IU L^{-1})	I	172.50±3.00^{C}	174.80±2.30^{C}	174.33±1.96^{C}	174.33±1.42^{C}	175.30±1.85^{B}
	II	228.10±5.13^{Ac}	291.83±3.77^{Ab}	322.00±4.44^{Aa}	296.50±2.84^{Ab}	190.87±4.12^{Ad}
	III	197.70±4.85^{Bc}	224.77±2.11^{Bb}	290.83±3.00^{Ba}	221.43±2.19^{Bb}	181.47±3.27^{Bd}
ALT (IU L^{-1})	I	33.35±0.43^{C}	33.51±0.34^{C}	33.58±0.31^{C}	33.62±0.32^{C}	33.50±0.33^{B}
	II	47.64±0.63^{Ac}	60.03±0.66^{Ab}	71.06±1.08^{Aa}	59.47±0.32^{Ab}	36.23±0.62^{Ad}
	III	42.06±1.05^{Bc}	46.97±0.88^{Bb}	60.03±0.66^{Ba}	48.22±0.89^{Bb}	35.83±0.49^{Bd}
ALP (IU L^{-1})	I	485.33±4.15^{C}	487.00±3.33^{C}	489.40±2.46^{C}	492.23±1.52^{C}	491.07±2.69^{B}
	II	527.33±4.87^{Ac}	571.83±3.65^{Ab}	606.83±3.17^{Aa}	561.50±2.77^{Ab}	502.33±1.30^{Ad}
	III	513.50±2.18^{Bc}	529.67±3.94^{Bb}	571.83±3.66^{Ba}	533.73±2.64^{Bb}	494.83±2.05^{Bd}
UA (mg dL^{-1})	I	5.89±0.13^{C}	5.92±0.06^{C}	5.94±0.08^{C}	5.93±0.03^{C}	6.01±0.04^{B}
	II	7.08±0.06^{Ad}	7.91±0.09^{Ac}	8.98±0.06^{Aa}	8.37±0.06^{Ab}	6.29±0.05^{Ae}
	III	6.37±0.04^{Bd}	7.18±0.03^{Bb}	7.77±0.05^{Ba}	7.02±0.05^{Bc}	6.18±0.08^{Be}

Values (Mean±SE) having atleast one common superscript (Capital letters in columns and small letters in rows) does not differ significantly ($p<0.05$) for a parameter

Table 2: ND HI titer (log_2) in healthy, CIAV challenged and 'Immon' treated groups of chicks

	Days of observation				
Groups	7th day	14th day	21st day	28th day	35th day
I	1.67±0.33^{Ad}	9.67±0.33^{Aa}	8.67±0.33^{Aab}	7.67±0.33^{Abc}	6.67±0.33^{Ac}
II	1.00±0.58^{Ad}	6.33±0.33^{Ca}	5.00±0.58^{Bab}	4.67±0.33^{Bbc}	3.33±0.33^{Bc}
III	1.33±0.33^{Ac}	7.67±0.33^{Ba}	7.33±0.33^{Aa}	6.67±0.33^{Aab}	5.67±0.33^{Ab}

Values (Mean±SE) having atleast one common superscript (Capital letters in columns and small letters in rows) does not differ significantly ($p<0.05$) for a parameter

Table 3: Mean live body weight (g) of healthy, CIAV challenged and 'Immon' treated groups of chicks

	Days of observation					
Groups	0day	7th day	14th day	21st day	28th day	35th day
I	39.80±0.51^{Ae}	84.40±4.48^{Ae}	153.20±6.51^{Ad}	256.32±9.77^{Ac}	366.75±9.01^{Ab}	730.00±19.64^{Aa}
II	39.70±0.51^{Ae}	66.85±2.13^{Ce}	121.20±4.97^{Cd}	194.38±8.75^{Cc}	252.50±5.32^{Cb}	443.00±18.83^{Ca}
III	39.90±0.60^{Ae}	77.60±2.40^{Be}	139.15±4.66^{Bd}	234.78±7.31^{Bc}	327.00±6.63^{Bb}	597.00±15.78^{Ba}

Values (Mean±SE) having atleast one common superscript (Capital letters in columns and small letters in rows) does not differ significantly ($p<0.05$) for a parameter

CIAV infected groups II and III. The CIAV affected group II showed minimum weight gain as compared to the immunomodulator supplemented group III which had significantly ($p<0.05$) higher weight gains as compared to virus positive group II on all the experimental days. The mean live body weight of the chicks of group II challenged with CIAV showed a significant ($p<0.05$) decline as compared to the control group I which is in accordance with the findings of

Table 4: Organ:body weight ratios of all lymphoid organs in healthy, CIAV challenged and 'Immon' treated groups of chicks

Organs	Groups	Days of observation				
		7th day	14th day	21st day	28th day	35th day
Thymus	I	0.21 ± 0.01^{A}	0.21 ± 0.01^{A}	0.20 ± 0.00^{A}	0.20 ± 0.00^{A}	0.19 ± 0.01^{A}
	II	0.13 ± 0.01^{Ba}	0.10 ± 0.00^{Bb}	0.09 ± 0.00^{Cb}	0.09 ± 0.01^{Cb}	0.11 ± 0.01^{Cb}
	III	0.15 ± 0.00^{Bab}	0.14 ± 0.01^{Bc}	0.13 ± 0.01^{Bbc}	0.14 ± 0.01^{Bbc}	0.15 ± 0.01^{Ba}
Bursa	I	0.18 ± 0.01^{A}	0.17 ± 0.01^{A}	0.17 ± 0.01^{A}	0.18 ± 0.00^{A}	0.18 ± 0.00^{A}
	II	0.12 ± 0.00^{Bb}	0.10 ± 0.00^{Bc}	0.09 ± 0.01^{Bc}	0.10 ± 0.01^{Bc}	0.14 ± 0.01^{Ba}
	III	0.14 ± 0.00^{Bb}	0.13 ± 0.00^{Bb}	0.14 ± 0.01^{Ab}	0.17 ± 0.00^{Aa}	0.17 ± 0.01^{Aa}
Spleen	I	0.18 ± 0.00^{A}	0.18 ± 0.01^{A}	0.18 ± 0.01^{A}	0.17 ± 0.00^{A}	0.19 ± 0.00^{A}
	II	0.12 ± 0.00^{Ba}	0.11 ± 0.01^{Ba}	0.11 ± 0.01^{Ba}	0.12 ± 0.01^{Ca}	0.12 ± 0.01^{Ba}
	III	0.12 ± 0.00^{Bb}	0.12 ± 0.01^{Bb}	0.12 ± 0.01^{Bb}	0.14 ± 0.00^{BCb}	0.15 ± 0.00^{Ba}
Liver	I	3.66 ± 0.03^{B}	3.64 ± 0.07^{B}	3.63 ± 0.07^{C}	3.68 ± 0.06^{B}	3.69 ± 0.06^{B}
	II	3.92 ± 0.03^{Ac}	4.13 ± 0.04^{Ab}	5.02 ± 0.10^{Aa}	4.28 ± 0.03^{Ab}	3.90 ± 0.04^{Ac}
	III	3.73 ± 0.02^{Bc}	4.03 ± 0.03^{Bb}	4.27 ± 0.05^{Ba}	4.12 ± 0.07^{Ba}	3.63 ± 0.03^{Bc}

Values (Mean±SE) having atleast one common superscript (Capital letters in columns and small letters in rows) does not differ significantly ($p<0.05$) for a parameter

Dhama (2002). The chicks of the group III supplemented with 'Immon' showed a higher body weight gain as compared to the group II CIAV challenged chicks which may be due to growth stimulation with presence of β-glucan, yeast and spirulina (Chae *et al*., 2006; Rajapakse *et al*., 2010; Mariey *et al*., 2012).

Organ: Body (O: B) weight ratios: The mean percent ratios of all the lymphoid organs, viz., thymus, bursa, spleen and liver were calculated in the healthy group I, CIAV infected group II and 'Immon' treated group III of chicks at different post-infection intervals of the investigation and are summarized in Table 4. The group II challenged with CIAV showed a significant ($p<0.05$) decline in their mean percent ratios of thymus, bursa and spleen to body weight on 7th, 14th, 21st, 28th and 35th day of the investigation with the maximum decline on 21st day. The group challenged with CIAV showed a significant ($p<0.05$) elevation in their mean percent ratio of liver to body weight as compared to control group I on all the days of investigation. These findings are in accordance with the CIAV experimental infection results documented by Dhama (2002) in day old chicks. The 'Immon' supplemented group exhibited minimal alterations in mean percent ratios of lymphoid organs among both the CIAV inoculated group II and III and had significantly ($p<0.05$) improved ratios for lymphoid organs. The results of the organ: body weight ratios in the present investigation connote that CIAV causes atrophy of the thymus, bursa and spleen, and increases the size of liver. Goryo *et al.* (1987) also observed thymus and bursal atrophy with liver swelling in 50% of CIAV affected birds. Sommer and Cardona (2003) observed declined relative bursal weight and thymic weight in CIAV affected birds as compared to the control birds. Dhama (2002) and Vachhani (2005) also reported similar findings in accordance with the present observations. The 'Immon' supplemented group exhibited minimal alterations in mean percent ratios of lymphoid organs among all the CIAV inoculated chicks which can be attributed to the presence of amino acids, nucleotides and probiotics in the immunomodulatory agent used (Rajapakse *et al*., 2010; Hussain *et al*., 2013; Dhama *et al*., 2014).

Immunomodulatory, growth performance enhancing and protective effects of various components of 'Immon' viz., Picrorhiza kurroa (Sharma *et al*., 1994; Hussain *et al*., 2013), Moringa oleifera (Sudha *et al*., 2010; Dougnon *et al*., 2011), β-glucan (Estrada *et al*., 1997; Chae *et al*., 2006; Rajapakse *et al*., 2010), Spirulina (Qureshi *et al*., 1994; Khan *et al*., 2005; Mariey *et al*., 2012), vitamin E (Konjufca *et al*., 2004), probiotics (Dhama *et al*., 2011), amino acids, minerals, vitamin C and nucleotides have been reported in animals and poultry by several workers (Mahima *et al*., 2012; Dhama *et al*., 2014). Based on these findings, 'Immon' was selected for the present study to explore its potential regarding immunomodulatory and ameliorative/protective effects in CIAV infected birds. CIAV infection in chicken markedly depresses the immune system and reduces the size of lymphoid organs with reduction in overall growth performance, and in this situation the results of the present trial indicate that 'Immon' could serve as a potential immunomodulatory and protective polyherbal formulation with additive nutritional elements to ameliorate CIAV infection in broiler chicks.

CONCLUSION

On the basis of above findings, it can be concluded that "Immon" can be used as an immunomodulator to reduce the pathogenicity of CIAV and to alleviate the depressed immune response of CIAV affected chicks. Its supplementation can increase the growth performance of the virus affected birds which could be utilized to minimize economic losses being suffered by poultry producers due to CIAV infection. Hence, it is suggested that immunomodulators need to be supplemented to the young chicks during early ages to minimize the pathological effects and immunosuppressive potential of CIAV and to safeguard their growth performances against this economically important pathogen of broiler.

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REFERENCES

Adair, B.M., 2000. Immunopathogenesis of chicken anemia virus infection. Dev. Comp. Immunol., 24: 247-255.

Allan, W.H. and R.E. Gough, 1974. A standard haemagglutination inhibition test for Newcastle disease. (1). A comparison of macro and micro methods. Vet. Record, 95: 120-123.

Basaraddi, M.S., K. Dhama, M.Y. Wani, P.M. Sawant and R. Tiwari *et al*., 2013. Downregulation in cytokines profiles and immunopathological changes in chicks infected with chicken infectious anaemia virus. Afr. J. Microbiol. Res., 7: 2464-2474.

Bhatt, P., S.K. Shukla, M. Mahendran, K. Dhama, M.M. Chawak and J.M. Kataria, 2011. Prevalence of Chicken Infectious Anaemia Virus (CIAV) in commercial poultry flocks of Northern India: A serological survey. Transbound. Emerg. Dis., 58: 458-460.

Bhatt, P., S.K. Shukla, M.Y. Wani, R. Tiwari and K. Dhama, 2013. Amelioration of chicken infectious anaemia virus induced immunosuppression by immunomodulator and haematinic supplementation in chicks. Veterinarski Arhiv, 83: 639-652.

Chae, B.J., J.D. Lohakare, W.K. Moon, S.L. Lee, Y.H. Park and T.W. Hahn, 2006. Effects of supplementation of β-glucan on the growth performance and immunity in broilers. Res. Vet. Sci., 80: 291-298.

Dhama, K., 2002. Pathogenecity and immunosuppressive effects of Chicken Infectious Anaemia Virus (CIAV) in chicks and evaluation of diagnostic tests for its detection. Ph.D. Thesis, Deemed University, Indian Veterinary Research Institute (IVRI), Izatnagar, (U.P), India.

Dhama, K., M. Mahendran, R. Somvanshi and M.M. Chawak, 2008. Chicken infectious anaemia virus: An immunosuppressive pathogen of poultry: A review. Indian J. Vet. Pathol., 32: 158-167.

Dhama, K., V. Verma, P.M. Sawant, R. Tiwari, R.K. Vaid and R.S. Chauhan, 2011. Applications of probiotics in poultry: Enhancing immunity and beneficial effects on production performances and health: A review. J. Immunol. Immunopathol., 13: 1-19.

Dhama, K., R. Tiwari, R.U. Khan, S. Chakraborty and M. Gopi *et al*., 2014. Growth promoters and novel feed additives improving poultry production and health, bioactive principles and beneficial applications: The trends and advances: A review. Int. J. Pharmacol., 10: 129-159.

Dougnon, J.T., A.P. Edorh, H.S. Bankole, M. Kpodekon and J. Gbenou, 2011. Efficiency of ethanol extract of Moringa oleifera lam leaves for the treatment of Staphylococcus aureus infections in chicks. J. Med. Plants Res., 5: 6704-6708.

Engstrom, B.E., O. Fossum and M. Luthman, 1988. Blue wing disease of chickens: Experimental infection with a Swedish isolate of chicken anaemia agent and an avian reovirus. Avian Pathol., 17: 33-50.

Estrada, A., C.H. Yun, A. Van Kessel, B. Li, S. Hauta and B. Laarveld, 1997. Immunomodulatory activities of oat β-Glucan in vitro and in vivo. Microbiol. Immunol., 41: 991-998.

Goryo, M., Y. Shibata, T. Suwa, T. Umemura and C. Itakura, 1987. Outbreak of anemia associated with chicken anemia agent in young chicks. Jap. J. Vet. Sci., 49: 867-873.

Hagood, L.T., T.F. Kelly, J.C. Wright and F.J. Hoerr, 2000. Evaluation of chicken infectious anemia virus and associated risk factors with disease and production losses in broilers. Avian Dis., 44: 803-808.

Hu, L.B., B. Lucio and K.A. Schat, 1993. Depletion of $CD4^+$ and $CD8^+$ T lymphocyte subpopulations by CIA-1, a chicken infectious anemia virus. Avian Dis., 37: 492-500.

Hussain, A., W. Shadma, A. Maksood and S.H. Ansari, 2013. Protective effects of Picrorhiza kurroa on cyclophosphamide-induced immunosuppression in mice. Pharmacogn. Res., 5: 30-35.

Jain, N.C., 1986. Schalm's Veterinary Haematology. 5th Edn., Lea and Febiger, Philadelphia, pp: 1221.

Kaneko, J.J., J.W. Harvey and M.L. Bruss, 1997. Clinical Biochemistry of Domestic Animals. 5th Edn., Academic Press, San Diego, CA, USA., Pages: 376.

Khan, M., J.C. Shobha, I.K. Mohan, M.U.R. Naidu and C. Sundaram *et al*., 2005. Protective effect of Spirulina against doxorubicin-induced cardiotoxicity. Phytother. Res., 19: 1030-1037.

Konjufca, V.K., W.G. Bottje, T.K. Bersi and G.F. Erf, 2004. Influence of dietary vitamin E on phagocytic functions of macrophages in broilers. Poult. Sci., 83: 1530-1534.

Latheef, S.K., K. Dhama, M.Y. Wani, H.A. Samad, R. Tiwari and S.D. Singh, 2013. Ameliorative effects of Withania somnifera, Azadirachta indica, Tinospora cordifolia and E care SE herbal preparations on chicken infectious anaemia virus induced haematological changes in chicks and their live body weights. South Asian J. Exp. Biol., 3: 172-182.

Liu, Z.G., Z.Y. Zhou, Y.B. Xu, X.R. Wang, S.M. Zheng and L.P. Yang, 1997. Haematopathological changes in chicks with chicken infectious anaemia. Acta Vet. Zootech. Sin., 28: 434-443.

Lucas, D.M. and C. Jamroz, 1974. Atlas of Avian Haematology. Oxford and IBH Publishing Co., New Delhi, Pages: 228.

Mahima, A. Rahal, R. Deb, S.K. Latheef and H.A. Samad *et al*., 2012. Immunomodulatory and therapeutic potentials of herbal, traditional/indigenous and ethnoveterinary medicines. Pak. J. Biol. Sci., 15: 754-774.

Mariey, Y.A., H.R. Samak and M.A. Ibrahem, 2012. Effect of using *Spirulina platensis* algae as a feed additive for poultry diets. Egypt. Poult. Sci., 32: 201-215.

McNulty, M.S., 1991. Chicken anaemia agent: A review. Avian Pathol., 20: 187-203.

Natt, P.M. and C.A. Herrik, 1952. A new blood diluent for counting the erythrocytes and leucocytes of chicken. Poult. Sci., 31: 735-738.

Pope, C.R., 1991. Chicken anaemia agent. Vet. Immunol. Immunopathol., 30: 51-65.

Qureshi, M.A., D. Garlich, M.T. Kidd and R.A. Ali, 1994. Immune enhancement potential of Spirulina platensis in chickens. Poult. Sci., 73: 46-46.

Rajapakse, J.R., M.D. Buddhika, M. Nagataki, H. Nomura, Y. Watanabe, Y. Ikeue and T. Agatsuma, 2010. Effect of sophy β-glucan on immunity and growth performance in broiler chicken. J. Vet. Med. Sci., 72: 1629-1632.

SPSS., 2007. Statistical Packages for Social Sciences. Version 16, SPSS Inc., Linois, USA.

Sawant, P.M., K. Dhama, D.B. Rawool, M.Y. Wani, R. Tiwari, S.D. Singh and R.K. Singh, 2015. Development of a DNA vaccine for chicken infectious anemia and its immunogenicity studies using high mobility group box 1 protein as a novel immunoadjuvant indicated induction of promising protective immune responses. Vaccine, 33: 333-340.

Schat, K.A., 2003. Chicken Infectious Anemia. In: Diseases of Poultry, Saif, Y.M., H.J. Barnes, J.R. Glisson, A.M. Fadly, L.R. McDougald and D.E. Swayne (Eds.). 11th Edn., Iowa State University Press, Ames, IA., pp: 182.

Schat, K.A., 2009. Chicken infectious anaemia. Curr. Top. Microbiol. Immunol., 331: 151-183.

Sharma, M.L., C.S. Rao and P.L. Duda, 1994. Immunostimulatory activity of Picrorhiza kurroa leaf extract. J. Ethnopharmacol., 41: 185-192.

Snedecor, G.W. and W.G. Cochran, 2004. Statistical Methods. 8th Edn., Oxford and IBH Publications, New Delhi, India.

Sommer, F. and C. Cardona, 2003. Chicken anemia virus in broilers: Dynamics of the infection in two commercial broiler flocks. Avian Dis., 47: 1466-1473.

Sudha, P., S.M. Asdaq, S.S. Dhamingi and G.K. Chandrakala, 2010. Immunomodulatory activity of methanolic leaf extract of *Moringa oleifera* in animals. Indian J. Physiol. Pharmacol., 54: 133-140.

Taniguchi, T., N. Yuasa, M. Maeda and T. Horiuchi, 1982. Haematopathological changes in dead and moribund chicks induced by chicken anaemia agent. Natl. Inst. Anim. Health Q. (Japan), 22: 61-69.

Todd, D., 2000. Circoviruses: Immunosuppressive threats to avian species: A review. Avian Pathol., 29: 373-394.

Todd, D., M.S. McNulty, A. Mankertz, P.D., J.L. Dale and J.W. Randles *et al*., 2000. Family Circoviridae. In: Virus Taxonomy: Classification and Nomenclature of Viruses: Seventh Report of the International Committee on Taxonomy of Viruses, Murphy, F.A., D.H.L. Carstens, E.B. Carstens, M.K. Estes and S.M. Lemon *et al*. (Eds.). Academic Press, New York, pp: 299-303.

Toro, H., C. Gonzalez, L. Cerda, M. Hess, E. Reyes and C. Geisse, 2000. Chicken anemia virus and fowl adenoviruses: Association to induce the inclusion body hepatitis/hydropericardium syndrome. Avian Dis., 44: 51-58.

Vachhani, K.V., 2005. Etio-immuno-pathological studies on chicken infectious Anemia- gangrenous dermatitis syndrome in pullets. M.V.Sc. Thesis, Anand Agricultural University, Anand, Gujarat, India.

Wani, M.Y., K. Dhama, R. Tiwari, R. Barathidasan, Y.S. Malik, S.D. Singh and R.K Singh, 2015. Immunosuppressive effects of chicken infectious anaemia virus on T lymphocyte populations using flow cytometry and hematological parameters during experimental subclinical infection in chicks. Adv. Anim. Vet. Sci., 3: 143-150.

Yuasa, N. and K. Imai, 1986. Pathogenicity and antigenicity of eleven isolates of Chicken Anaemia Agent (CAA). Avian Pathol., 15: 639-645.

Zhang, X., B. Wu, Y. Liu, W. Chen, Z. Dai, Y. Bi and Q. Xie, 2015. Assessing the efficacy of an inactivated chicken anemia virus vaccine. Vaccine, 33: 1916-1922.