Research Article

Immunomodulatory and Therapeutic Prospective of a Protein Supplement with Vitamins and Selenium (Multimune) against Chicken Infectious Anaemia in Broiler Chicks

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Abstract | Chicken infectious anaemia virus (CIAV) is a well-established cause of generalized lymphoid atrophy accumulating severe immunosuppression subsequent to infection. In this study, we assessed the role of a protein supplement with vitamins and selenium (Multimune) against the CIAV infection in broiler chicks. Experiment was performed on 60 day-old broiler chicks grouped into 3 groups of 20 birds in each and all the birds were vaccinated against Newcastle Disease (ND) and infectious bursal disease (IBD). The chicks of group I served as healthy control and other 2 group chicks (group II and III) were exposed to CIAV ($10^{4.5}$ $TCID_{50}$/0.1 ml). The Multimune was fed for 3 weeks to chicks of group III @1 gm / 10 birds. Successively, all the birds were examined for ND antibody titres, various haematological and biochemical parameters, organ: body weight ratios, and mean live body weight at weekly interval until 35th day of the study. The chicks of group II showed significant decline in the count of erythroid and myeloid cells, mean live body weight and organ: body weight ratios of lymphoid organs, and ND antibody titres, whereas upturn was observed in enzyme activities and uric acid values. In contrast, the immunosuppression was less severe in (group III in comparison to group II chicks. The haematological and biochemical parameters, mean live body weight and organ body weight ratios were significantly ($P<0.05$) higher in Multimune supplemented chicks. Present study highlights the use of Multimune as an effective immunomodulating agent in immune suppressed CIAV affected birds with reduced virus pathogenicity, enhanced immune responses and safeguards the virus prompted ill effects on bird's growth performances.

Keywords | Chicken infectious anemia virus, Immunosuppression, Immunomodulator, Multimune, Chicken

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INTRODUCTION

Chicken infectious anemia virus (CIAV), belongs to the genus *Gyrovirus* under the family *Circoviridae* (Todd et al., 2000). It causes acute anemic condition (chicken infectious anaemia, CIA) in young chicks below 4-5 weeks of age. CIA is a worldwide problem and acclaimed as the most economically important viral diseases of poultry (McNulty, 1991; McIlroy et al., 1992; Bulow and Schat, 1997; Farkas et al., 1998; Rosenberger and Cloud, 1998; Dhama et al., 2008; Schat, 2009; Bhatt et al., 2011; Wani et al., 2013). The birds exhibit symptoms of depression, se-

vere anaemia, paleness, weakness, anorexia, ruffled feathers, poor weight gain, aplasia of the bone marrow, lymphoid atrophy, subcutaneous and muscular haemorrhages with increased case fatality (McNulty, 1991; Bulow and Schat, 1997; Hagood et al., 2000). The prominent sign of anaemia, prevails during the end of 2nd weeks post infection (PI) and manifest mainly the non-feathered areas that may extend to internal organs (Pope, 1991). There is marked damage of haematopoietic and lymphopoietic tissues viz. stem cells in bone marrow and precursor T-lymphocytes in thymus. The bursa, spleen and other lymphoid organs are also depleted of lymphoid cells though less severely (Goryo et al., 1989; Dhama, 2002). Repopulation of the bone marrow with proerythroblasts and promyelocytes, and recovery of haematopoietic activity (erythropoiesis) and lymphocyte repopulation occur during convalescent phase (Taniguchi et al., 1982; Liu et al., 1997). CIAV is a potent immunosuppressive agent for very young unprotected chicks thereby increasing their susceptibility to secondary infections (Van Den Berg, 1996; Adair, 2000; De Herdt et al., 2001). The CIAV infection makes birds prone to several other diseases like haemorrhagic syndrome, haemorrhagic anaemia syndrome, infectious/aplastic anaemia, anaemia-dermatitis syndrome, gangrenous dermatitis and blue wing diseases (Pope, 1991; Hagood et al., 2000; Toro et al., 2000). CIAV infections have been associated with a number of vaccination failures, vaccination reactions or aggravation of the residual pathogenicity of attenuated vaccine viruses viz. Newcastle disease virus (NDV), Marek's disease virus (MDV), infectious laryngotracheitis virus (ILTV) and fowl pox virus (FPV) (Box et al., 1988; Otaki et al., 1988; Rosenberger and Cloud, 1989; De Boer et al., 1994; Zheng and Liu, 1996; Liu et al., 2001; Dhama, 2002). The notable characteristics such as vertical transmission, highly contagious, hardy and ubiquitous nature, and the potential for inducing marked immunosuppression have attracted the global scientific community towards the potential threat of CIAV infection in poultry industry (Todd, 2000; Dhama et al., 2008; Basaraddi et al., 2013). Alike other viral infections there is no specific treatment option for CIA and vaccination strategies of live-attenuated and inactivated vaccines are available with some limitations, also advanced vaccines like DNA vaccine and others have been developed but need to be applied yet (McNulty, 1991; Schat, 2003; Dhama et al., 2008; Sawant et al., 2015; Zhang et al., 2015). In such a case there is a need to evaluate other effective therapeutic regimens to subside the massive immunosuppression produced by the CIA virus. As an alternative approach birds can be supplemented with haematinics and immunostimulants to arrest anaemic stage and enhance the immune system (Bhatt et al., 2013; Latheef et al., 2013). With this aim, the present study was carried out to assess the immunomodulatory and therapeutic potential of a protein supplement containing essential vitamins and selenium (Multimune) in CIAV infected chicks.

MATERIALS AND METHODS

Virus

The cell culture (MDCC-MSB1 cells) adapted chicken infectious anaemia virus (CIAV), maintained in the Avian Disease Section, Division of Pathology, Indian Veterinary Research Institute (IVRI), Izatnagar, UP (India) was used to infect the chicks in the present study.

Immunomodulator (Multimune)

Multimune (Interface Pharmaceuticals, New Delhi, India), a protein supplement containing essential vitamins and selenium, was used as an immunomodulator in the study.

Experimental Chicks

Sixty day-old age broiler chicks were procured from Instructional poultry farm of the university. Chicks were reared in deep litter system under hygienic conditions and given normal basal ration and water *ad libitum*. The experiment on chicks was performed following 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). Prior to experiment all birds were checked for presence of CIAV by using IDEXX FlockChek CAV test kit (IDEXX Laboratories, USA) and were found free of infection.

Experimental Design

Sixty day-old broiler chicks were distributed in three equal groups (Group I-III). Chicks of all three groups were vaccinated against Newcastle disease (ND) by live attenuated 'F' strain (NDV-F) and Georgia strain of infectious bursal disease (IBD), respectively as per manufacturer's instructions. Group I chicks served as healthy control while chicks of group II and III were inoculated with 1 ml of CIAV infected cell culture supernatant ($10^{4.5}$ $TCID_{50}$/0.1 ml) per bird intramuscularly. Group II chicks served as virus positive control and group III chicks were supplemented with Multimune at the dose of 6.0 gm / 60 birds in drinking water for 21 days.

Sampling for haematology and biochemical parameters was based on pooled blood samples (5 ml) collected from 3 birds of each group weekly up to 35th day of experiment (i.e. 7th, 14th, 21st, 28th and 35th day). One part of aseptically collected blood was separated in heparinised vials (10-20 IU heparin / ml) for haematological estimations and another part for serum analysis (biochemical and immunological studies). The mean live body weight was studied in experimental chicks of all the groups at regular intervals at weekly intervals up to 35th day of experiment after scarification of birds. At weekly intervals, three birds from each group were sacrificed to calculate ratio of lymphoid organs (thymus, bursa of Fabricius, spleen and liver) to body weight.

Haematological Parameters

Haemoglobin (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, 1961) were estimated with pooled blood samples (5 ml from 3 birds) from each group at weekly intervals up to 35th day (i.e. 7th, 14th, 21st, 28th and 35th) of experiment.

Biochemical Parameters

Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and uric acid (UA) were estimated with sera samples from 3 birds from each group at weekly intervals up to 35th day of experiment using reagent kits (IFCC Kinetic Method, Erba, Mannheim Ltd., Germany; Uricase PAP Method, Span Diagnostics Ltd., Surat, Gujarat, India).

ND HI Titer

For determining the antibody titer against ND vaccination, Haemagglutination inhibition (HI) test was carried out on the sera samples obtained from 3 birds from each group on 7th, 14th, 21st, 28th and 35th day of experiment following method of Allan and Gough (1974). The mean HI titre (log_2) for different experimental groups was calculated for comparison.

Mean Live Body Weight

To assess the immunomodulatory effect of 'Multimune' on growth performance of the CIAV infected chicks (group II and group III), the average live body weight of 5 chicks each from the three groups was recorded at weekly interval (day 0, 7th, 14th, 21st, 28th and 35th) of the study for comparison.

Organ: Body (O: B) Weight Ratio

To assess the protective effect of 'Multimune' on lymphoid organs of the virus infected birds, the Organ: body weight ratios of the thymus, bursa of Fabricius, spleen and liver were calculated from the 5 chicks each, from all three groups. Assessment was done on day 0, 7th, 14th, 21st, 28th and 35th of the study for comparison. This ratio was calculated by dividing respective organ weight with body weight (gm) and multiplying with 100.

Statistical Analysis

The statistical analysis was performed by analysis of variance (two way ANOVA) (Snedecor and Cochran, 2004) by using SPSS (2007) version 16. All the values obtained in the study are represented as mean ± SE.

RESULTS AND DISCUSSION

Haematological Parameters

As shown in Table 1 a significant (P<0.05) decrease in the values of Hb, PCV and TEC was noticed in the chicks of CIAV infected group (group II) from 7th day PI where lowest values were seen at 21 day PI in comparison to the chicks of healthy group (group I). The normal value of Hb concentration of healthy group (116-121 g/L) was reduced to 52.67±0.29 in CIAV infected chicks on 21st day, and the improvement in Hb concentration was recorded in the immunomodulator treated (70.00±0.20 g/L) chicks (group III) on 21st day PI. The normal value of PCV was found to be 0.34-0.35 (v/v) in healthy chicks. CIAV infection was found to decrease normal PCV values on 7, 14, 21 and 28 DPI. Similar observations were recorded in the myeloid lineages with significant (P<0.05) decline in the TLC, percent lymphocyte count and percent eosinophil and basophil count (PE+BC) in group II birds from 7th day PI and lowest count was seen on 21 DPI. Immunomodulator treatment was found to significantly reduce the effect of CIAV induced reduction in PCV. Similar to the values of Hb and PCV, TEC also showed a significant reduction in CIAV challenged groups with maximum reduction on 21st day (1.52×10^{12}/L), whereas the Multimune supplemented group showed significantly (P<0.05) improved values of TEC (2.09×10^{12}/L) on the 21st day. The TLC value of healthy group was found to be 20-22×10^9/L, and CIAV infection was found to be reduced on days 7 (18.47±0.40), 14 (17.00±0.20), 21 (14.67±0.57), 28 (17.33±0.29) and 35 (17.97±0.12) PI, respectively. But immunomodulator group showed significantly (P<0.05) better values of TLC on day 7 (19.23±0.14), 14 (19.03±0.05), 21 (17.60±0.35), 28 (19.00±0.29) and 35 (19.43±0.27) PI, respectively. Similarly, the mean PLC and PE+BC values were significantly decreased while the PHC values showed an increasing trend in all the CIAV inoculated chicks. Our results are in agreement with earlier reports (Dhama, 2002; Schat, 2003; Bhatt et al., 2013). It is hypothesized that this decrease in the Hb, PCV, TEC and TLC levels in CIAV infected chicks might be due to destructive effect of CIAV on erythroid and myeloid tissues of bone marrow where it causes suppression of differentiation and proliferation of haemopoietic precursor cells, affecting erythropoiesis and myelopoiesis leading to anaemia and panleukopenia (Pope, 1991; Schat, 2003; Dhama et al., 2008; Bhatt et al., 2013).

The levels of haematological parameters in comparison to group II (virus positive control) were significantly higher (P<0.05) in chicks of group III which were supplemented with Multimune for 21 days in drinking water. The reason for this could be that the protein and other ingredients of 'Multimmune' which possess immunostimulant properties and thus limited the progression of panleukopenia and immunosuppression during CIAV infection.

Biochemical Parameters

The biochemical analysis results of the chicks of all the three groups are summarized in Table 1. Assessment of activities of ALT, AST and ALP enzymes, and uric acid was

Table 1: Haematological and biochemical parameters of healthy, CIAV challenged and 'Multimune' treated groups of chicks (Mean±SE)

Parameters	Groups	Days of observation				
		7th day	14th day	21st day	28th day	35th day
Hb (g/L)	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	103.67±0.24^{Ba}	94.67±0.47^{Bb}	70.00±0.20^{Bc}	103.33±0.48^{Ba}	104.67±0.30^{Ba}
PCV (L/L)	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}	0.29±0.01^{Ba}	0.26±0.00^{Bb}	0.29±0.00^{Ba}	0.31±0.01^{Ba}
TEC (x10^{12}/L)	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	3.00±.012^{Ba}	2.49±0.13^{Bb}	2.09±0.09^{Bc}	3.08±0.12^{Ba}	3.18±0.12^{Ba}
TLC (x10^{9}/L)	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	19.23±0.14^{Ba}	19.03±0.35^{Ba}	17.60±0.35^{Bb}	19.00±0.29^{Ba}	19.43±0.27^{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	53.00±0.58^{Ba}	53.67±1.20^{Ba}	53.67±1.20^{Ba}	54.67±0.88^{Ba}	55.67±2.18^{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	39.00±0.58^{Aa}	39.00±0.58^{Ba}	39.67±1.20^{Ba}	38.67±0.67^{Ba}	38.00±1.00^{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	4.67±0.34	4.67±1.20	4.00±1.20^{AB}	5.00±0.00^{A}	4.67±0.58^{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	3.33±0.33^{A}	3.33±0.33^{AB}	3.33±0.33
AST (IU/L)	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	186.77±2.13^{Bc}	204.77±3.25^{Bb}	221.43±2.19^{Ba}	206.17±2.05^{Bb}	172.83±1.92^{Bd}
ALT (IU/L)	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	38.07±0.42^{Bc}	42.89±0.31^{Bb}	46.97±0.88^{Ba}	43.56±0.53^{Bb}	33.63±0.27^{Bd}
ALP (IU/L)	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	503.73±2.15^{Bc}	515.00±1.32^{Bb}	534.67±1.32^{Ba}	523.67±3.17^{Bb}	490.17±3.21^{Bd}
UA (mg/dl)	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	6.23±0.04^{Bd}	6.74±0.06^{Bb}	7.18±0.03^{Ba}	6.52±0.03^{Bc}	6.09±0.03^{Be}

The 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.

significantly higher (P<0.05) in the CIAV inoculated birds (group II), which could be associated with the damage to the organs like liver, kidneys and muscles due to CIAV infection (McNulty, 1991; Dhama et al., 2008). AST, ALT and ALP are found in the liver and additionally ALP is also found in the kidney (Kaneko et al., 1997). CIAV infections are known to result in damage and focal necrosis of liver, kidney and spleen (McNulty, 1991; Dhama et al., 2008). The AST value of healthy group was found to be 172-175 IU/L, and CIAV infection was found to elevate it on 7th (228.10±5.13), 14th (291.83±3.77), 21st (322.00±4.44), 28th (296.50±2.84) and 35th (190.87±4.12) days post infection

Table 2: ND HI titre (log_2) in healthy, CIAV challenged and 'Multimune' treated groups of chicks (Mean±SE)

Groups	Days of observation				
	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}	8.00±0.58^{Ba}	7.67±0.33^{Aa}	7.00±0.00^{Aab}	6.00±0.58^{Ab}

The values (Mean±SE) having atleast one common superscript (Capital letters in columns and small letters in rows) does not differ significantly (P<0.05)

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

Groups	Days of observation					
	0 day	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	39.50±0.75^{Ae}	72.90±3.68^{Be}	133.20±4.68^{Bd}	223.65±8.86^{Bc}	324.50±7.32^{Bb}	578.00±17.56^{Ba}

The values (Mean±SE) having atleast one common superscript (Capital letters in columns and small letters in rows) does not differ significantly (P<0.05)

Table 4: Organ: body weight ratios of all lymphoid organs in healthy, CIAV challenged and 'Multimune' treated groups of chicks (Mean±SE)

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	0.14±0.01^{Bab}	0.12±0.01^{Bc}	0.13±0.00^{Bbc}	0.13±0.01^{Bbc}	0.15±0.02^{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±0.01^{Bb}	0.14±0.01^{Ab}	0.16±0.00^{Aa}	0.17±0.00^{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	0.13±0.00^{Bb}	0.13±0.00^{Bb}	0.13±0.01^{Bb}	0.13±0.00^{BCb}	0.14±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	3.68±0.02^{Bc}	3.88±0.03^{Bb}	4.04±0.03^{Ba}	3.99±0.01^{Ba}	3.60±0.03^{Bc}

The values (Mean±SE) having at least one common superscript (Capital letters in columns and small letters in rows) does not differ significantly (P<0.05) for a parameter

(DPI), respectively. But immunomodulator (Multimune) group showed significantly (P<0.05) improved values of AST on 7th (186.77±2.13), 14th (204.77±3.25), 21st (221.43±2.19), 28th (206.17±2.05) and 35th (172.83±1.92) DPI, respectively. The normal value of ALT (33-34 IU/L) in healthy chicks was increased to 71.06 IU/L in CIAV infected chicks, and the improvement was found in Multimune treated group (46.97 IU/L) on 21st DPI. Similar to above mentioned enzymes, ALP value was also found to be significantly (P<0.05) higher in group II (606.83 IU/L) as compared to the normal healthy group (489.40 IU/L) and immunomodulator supplemented group (534.67 IU/L) on the day of peak infection. The normal value of uric acid (UA) was found to be 5-6 mg/dl in healthy chicks. CIAV inoculation was found to increase the uric acid values on 7th (7.08 mg/dl), 14th (7.91 mg/dl), 21st (8.98 mg/dl) and 28th (8.37 mg/dl) DPI. Immunomodulator treatment was found to significantly reduce the effect of CIAV induced increase in the value of uric acid.

ND Antibody Titre (HI Test)

The humoral immune response (HIR) to ND vaccination in chicks of the three groups of birds was measured by Haemagglutination inhibition (HI) test at weekly intervals, i.e. day 7, 14, 21, 28 and 35. The mean HI antibody titers in chicks of all three groups obtained at different days post vaccination, expressed as HI titre (log_2) are presented in Table 2. A significant decrease (P<0.05) in the mean HI ND antibody titre was seen on 14th, 21st, 28th and 35th days post vaccination in chicks of group II challenged with

CIAV, while statistically no significant difference was observed in the mean HI antibody titres in all chicks on 7th day of the experiment. The group III chicks supplemented with 'Multimune' showed significantly higher values of HI titres as compared to group II CIAV challenged birds only which might be due to immunomodulatory potential of protein and immunoglobulins present in the immunomodulator used. This effect could be due to suppression of the population of both helper ($CD4^+$) and cytotoxic ($CD8^+$) T-Lymphocytes in the thymus due to CIAV infection, as suggested by Hu et al. (1993) and Adair (2000). Herbal and protein 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 observations of mean body weights recorded in all three groups of chicks are presented in Table 3. The healthy group I chicks showed highest weight gain in comparison to CIAV infected groups II and III. The findings show that chicks of CIAV affected group II revealed minimum weight gain, while the immunomodulator supplemented group III showed significantly ($P<0.05$) higher weight gains from group II chicks on all the experimental days, which may be due to growth stimulation with the immunomodulator used. The mean live body weights of all the groups on 21st day were in the range of 194.38±8.75 and 256.32±9.77 g and on 35th day were in the range of 443.00±18.83 and 730.00±19.64 g. The healthy group showed maximum weight gain as compared to CIAV infected group, whereas group III showed significantly ($P<0.05$) higher weight gain than group II. 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 Dhama (2002).

Organ: Body (O: B) Weight Ratios

The mean percent ratios of the lymphoid organs (thymus, bursa, spleen and liver) determined in chicks of all 3 groups are summarized in Table 4. The group II chicks revealed 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 PI, with the maximum decline on day 21. The CIAV challenged group 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 intervals studied. These observations are in accordance with the experimental CIAV infection studies as documented by Dhama (2002) in day old chicks. The 'Multimune' supplemented group III of chicks showed minimal alterations in mean percent ratios of lymphoid organs as compared to group II chicks, and had significantly ($P<0.05$) improved ratios for lymphoid organs. These findings of the organ: body weight ratios in the present investigation are in accordance that CIAV causes atrophy of the thymus, bursa and spleen, and increases the size of liver (Goryo et al., 1987; Dhama, 2002; Sommer and Cardona, 2003). Dhama (2002) and Vachhani (2005) also reported similar findings in accordance with the present observations. The 'Multimune' supplemented group III of chicks exhibited minimal alterations in mean percent ratios of lymphoid organs as compared to CIAV inoculated group II chicks which can be attributed to the presence of proteins in the immunomodulatory agent used.

Immunomodulatory, growth performance enhancing and protective effects of the components of 'Multimune' *viz.* proteins like lactalbumins, betaglobulins, serum albumin, immunoglobulins and lactoferrins, vitamins (A, D_3, E, C, B) and selenium are well known ingredients which enhance the immunity and act as immunomodulator in livestock and poultry (Sahin et al., 2001; Konjufca et al., 2004; Wintergerst et al., 2007; Baker, 2009; Peric et al., 2009; Saad et al., 2009; Dhama et al., 2014a, 2014b). Therefore, 'Multimune' was selected to explore its immunomodulatory and ameliorative potential in CIA affected birds. CIAV infection suppresses the immunity and reduces the size of lymphoid organs in young birds with stunted growth performance, and in this situation the results of the present trial indicate that 'Multimune' could serve as a useful immunomodulatory and treatment formulation to ameliorate CIAV infection in broiler chicks.

CONCLUSION

Overall, our results revealed the immunomodulatory effects of "Multimune" during immunosuppressive condition (CIA) reducing the pathogenicity of CIAV and up surging the depressed immune response. Furthermore, there is need to assess the impact of "Multimune" supplementation on growth performance of the virus affected birds. Adoption of such alternative approach along with use of available effective vaccines is the most attractive way of minimizing economic losses being suffered by poultry producers due to CIAV infection. In view of the preliminary findings, we propose that supplementation of immunomodulators could be adopted to minimize the pathological effects and immunosuppressive potential of CIAV and other similar diseases in young chicks.

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

REFERENCES

- Adair BM (2000). Immunopathogenesis of chicken anaemia virus infection. Dev. Comp. Immunol. 24: 247-255.
- Allan WH, Gough RE (1974). A standard haemagglutination inhibition test for Newcastle disease: Vaccination and challenge. Vet. Rec. 95: 147-149.
- Baker DH (2009). Advances in protein-amino acid nutrition of poultry. Amino Acids. 37(1): 29-41. http://dx.doi.org/10.1007/s00726-008-0198-3
- Basaraddi MS, Dhama K, Wani MY, Sawant PM, Tiwari R, Kumar D, Singh SD, Singh R (2013). Downregulation in cytokines profiles and immunopathological changes in chicks infected with chicken infectious anaemia virus. Afr. J. Microbiol. Res. 7(21): 2464-2474.
- Bhatt P, Shukla SK, Mahendran M, Dhama K, Chawak MM, Kataria JM (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, Shukla SK, Wani MY, Tiwari R, Dhama K (2013). Amelioration of chicken infectious anaemia virus induced immunosuppression by immunomodulator and haematinic supplementation in chicks. Veterinarski Arhiv. 83(6): 639-652.
- Box PG, Holmes HC, Bushell AC, Finney PM (1988). Impaired response to killed Newcastle disease vaccine in chicken possessing circulating antibody to chicken anaemia agent. Avian Pathol. 17: 713-723.
- Bulow V Von, Schat KA (1997). Chicken infectious anemia. In: Diseases of Poultry, 10thedn. B.W. Calnek et al. (Editors), Iowa State University Press, Ames, IA, Ch. 30, pp. 739-756.
- De Boer GF, Van Roozelaar DJ, Moormann RJ, Jeurissen SHM, Van Den Wijngaard JC, Hilbink F, Koch G (1994). Interaction between chicken anaemia virus and live Newcastle disease vaccine. Avian Pathol. 23: 263-275.
- De Herdt P, Van Den Bosch G, Ducatelle R, Uyttebroek E, Schrier C (2001). Epidemiology and significance of chicken infectious anemia virus infections in broilers and broiler parents under nonvaccinated European circumstances. Avian Dis. 45: 706-708.
- Dhama K, Mahendran M, Somvanshi R, Chawak MM (2008). Chicken infectious anaemia virus: an immunosuppressive pathogen of poultry - A review. Indian J. Vet. Pathol. 32(2): 158-167.
- 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, Tiwari R, Khan RU, Chakraborty S, Gopi M, Karthik, K, Saminathan M, Desingu PA, Sunkara LT (2014a). 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(3): 129-159.
- Dhama K, Chakraborty S, Tiwari R, Verma AK, Saminathan M, Amarpal, Malik YS, Nikousefat Z, Javdani M, Khan RU (2014b). A concept paper on novel technologies boosting production and safeguarding health of humans and animals. Res. Opin. Anim. Vet. Sci. 4(7): 353-370.
- Farkas T, Maeda K, Sugiura H, Kai K, Hirai K, Otsuki K, Hayashi T (1998). A serological survey of chickens, Japanese quail, pigeons, ducks and crows for antibodies to chicken anaemia virus (CIAV) in Japan. Avian Pathol. 27: 316-320.
- Goryo M, Shibata Y, Suwa T, Umemura T, Itakura C (1987). Outbreak of anaemia associated with chicken anemia agent in young chicks. Jap. J. Vet. Sci. 49: 867-873.
- Goryo M, Suwa T, Umeura T, Itakura C, Yamashiro S (1989). Histopathology of chicks inoculated with chicken anaemia agent (MSB1-TK 5803 strain). Avian Pathol. 18: 73-89.
- Hagood LT, Kelly TF, Wright JC, Hoerr FJ (2000). Evaluation of chicken infectious anaemia virus and associated risk factors with disease and production losses in broilers. Avian Dis. 44: 803-808.
- Hu LB, Lucio B, Schat KA (1993). Depletion of CD4+ and CD8+ T lymphocyte subpopulations by CIA-1, a chicken infectious anemia virus. Avian Dis. 37: 492-500.
- Jain NC (1986). Schalm's Veterinary Haematology. 5thedn. Lea and Febiger, Philadelphia. Pp. 1221.
- Kaneko JJ, Harvey JW, Bruss ML (1997). Clinical Biochemistry of Domestic Animals. Fifth edn. Academic Press.
- Konjufca VK, Bottje WG, Bersi TK and, Erf GF (2004). Influence of dietary vitamin E on phagocytic functions of macrophages in broilers. Poultry Sci. 83: 1530-1534.
- Latheef SK, Dhama K, Wani MY, Samad HA, Tiwari R, Singh SD (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(4): 172-182.
- Liu ZG, Zhou ZY, Xu YB, Wang XR, Zheng SM, Yang LP (1997). Haematopathological changes in chicks with chicken infectious anaemia. Acta Veterinaria et Zootechnica Sinica. 28: 434-443.
- Liu ZG, Zheng SM, Yang LP, Xu YB, Zhou ZY (2001). Study on immunoprotective efficacy and mechanism post Newcastle disease vaccination of chicken infected with chicken anaemia virus. Sci. Agri. Sinica. 34: 81-83.
- Lucas AM, Jamroz C (1961). Atlas of Avian Haematology. Govt. Priner, Washington, D.C.
- McIlroy SG, McNulty MS, Bruce DW, Smyth JA, Goodall EA, Alcorn MJ (1992). Economic effects of clinical chicken anaemia agent infection on profitable broiler production. Avian Dis. 36: 566-574.
- McNulty MS (1991). Chicken anaemia agent: a review. Avian Pathol. 20: 187-203.
- Natt MP, Herrick CA (1952). A new blood diluent for counting the erythrocytes and leucocytes of the chicken. Poultry Sci. 31: 735-778.
- Otaki Y, Nunoya T, Tajima M, Kato A, Nomura Y (1988). Depression of vaccinal immunity to Marek's disease by infection with chicken anaemia agent. Avian Pathol. 17: 333-347.
- Peric L, Zikic D, Lukic M (2009). Application of alternative growth promoters in broiler production. Biotech. Anim. Husb. 25: 387-397.
- Pope CR (1991). Chicken anaemia agent. Vet. Immunol.

Immunopathol. 30: 51-65.
•Rosenberger JK, Cloud SS (1989). Immunosuppressive aspects of chicken anemia agent (CAA). In: Proceedings, 24th National Meeting on Poult. Hlth. and Condemnations, 19-20 Oct., 1989, Ocean City, M.D., pp. 154.
•Rosenberger JK, Cloud SS (1998). Chicken anaemia virus. Poult. Sci. 77: 1190-1192.
•Saad MB, Gertner LR, Bona TD, Santin E (2009). Selenium influence in the poultry immune response-review. Recent Pat. Food Nutr. Agric. 1(3): 243-247.
•Sahin N, Sahin K, Kucuk O (2001). Effects of Vitamin E and vitamin A supplementation on performance, thyroid status and serum concentrations of some metabolites and minerals in broilers reared under heat stress (32°C). Vet. Med. Czech. 46(11-12): 286-292.
•Sawant PM, Dhama K, Rawool DB, Wani MY, Tiwari R, Singh SD, Singh RK (2015). Development of a DNA vaccine for chicken infectious anaemia and its immunogenicity studies using high mobility group box 1 protein as a novel immunoadjuvant indicated induction of promising protective immune responses. Vaccine. 33(2): 333-340.
•Schat KA (2003). Chicken infectious anaemia. In: Diseases of Poultry, 11th ed. (Editors) Saif YM, Barnes HJ, Glisson JR, Fadly AM, Mcdougald LR and Swayne DE. Iowa State University Press. Ames, USA. Pp. 182-202.
•Schat KA (2009). Chicken infectious anaemia. Curr. Top. Microbiol. Immunol. 331: 151-183.
•Snedecor GW, Cochran WG (2004). Statistical Methods. Oxford and IBH Publications, New Delhi.
•Sommer F, Cardona C (2003). Chicken anaemia virus in broilers: dynamics of the infection in two commercial broiler flocks. Avian Dis. 47: 1466-1473.
•SPSS (2007). Statistical packages for Social Sciences. Version 16, SPSS Inc., Ilinois, USA
•Taniguchi T, Yuasa N, Maeda M, Horiuchi T (1982). Haematopathological changes in dead and moribund chicks induced by chicken anaemia agent. Natl. Instt. Anim. Hlth. Q. (Japan). 22: 61-69.
•Todd D, McNulty MS, Mankertz A, Lukert PD, Dale JL, Randles JW (2000). Family Circoviridae. In: Virus Taxonomy - Classification and Nomenclature of Viruses. Seventh Report of the ICTV. F.A. Murphy et al., (Editors), Academic Press, New York.
•Todd D (2000). Circoviruses: immunosuppressive threats to avian species: a review. Avian Pathol. 29: 373-394.
•Toro H, Gonzalez C, Creda L, Hess M, Reyes E, Geisse C (2000). Chicken anaemia virus and fowl adenoviruses: association to induce the inclusion body hepatitis/hydropericardium syndrome. Avian Dis. 44: 51-58.
•Vachhani KV (2005). Etio-immuno-pathological studies on chicken infectious anemia-gangrenous dermatitis syndrome in pullets. M.V.Sc. Thesis, Anand Agricultural University, Anand, Gujarat, India.
•Van Den Berg TP (1996). Immunodepressive viral diseases in poultry: current situation and perspectives. Poult. Advisor, Nov. issue. Pp. 17-25.
•Wani MY, Dhama K, Barathidasan R, Gowthaman V, Tiwari R, Bhatt P, Mahajan NK, Chawak MM, Singh SD, Kataria, JM (2013). Molecular detection and epidemiology of chicken infectious anaemia virus in India. South Asian J. Exp. Biology. 3(4): 145-151.
•Wintergerst ES, Maggini S, Hornig DH (2007). Contribution of selected vitamins and trace elements to immune function. Ann. Nutr. Metab. 51(4): 301-23.
•Zhang X, Wu B, Liu Y, Chen W, Dai Z, Bi Y, Xie Q (2015). Assessing the efficacy of an inactivated chicken anemia virus vaccine. Vaccine. 2015 Mar 8. pii: S0264-410X(15)00266-2. [Epub ahead of print] http://dx.doi.org/10.1016/j.vaccine.2015.02.066
•Zheng SM, Liu ZG (1996). Changes in the immunoglobulin content and the HI titre after Newcastle disease vaccination of chicks infected with chicken anaemia virus. ActaVeterinaria et Zootechnica Sinica. 27: 242-246.