www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2019; 8(2): 547-552

www.thepharmajournal.com
Received: 26-12-2018
Accepted: 30-01-2019

Pravesh Kumari
Department of Veterinary Pathology, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana, India

Pooja Kundu
Department of Veterinary Public Health and Epidemiology, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana, India

Sushma Kajal
Department of Veterinary Pathology, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana, India

G Narang
Department of Veterinary Pathology, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana, India

Vikas Nehra
Department of Veterinary Pathology, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana, India

Deepika Lather
Department of Veterinary Pathology, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana, India

Correspondence
Pooja Kundu
Department of Veterinary Public Health and Epidemiology, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana, India

Effect of *Spirulina* feeding on biochemical parameters in infectious bursal disease vaccinated chickens

Pravesh Kumari, Pooja Kundu, Sushma Kajal, G Narang, Vikas Nehra and Deepika Lather

Abstract
Infectious bursal disease (IBD) commonly known as Gumboro disease is a disease of global economic importance; manifested by inflammation and subsequent atrophy of the bursa of Fabricius and various degrees of immunosuppression. Various vaccination strategies have been applied in the field to control IBD. Live commercially available hot strains of vaccines for IBD lead to varying levels of immunosuppression which increases the bird's vulnerability to various infections. The present study was conducted to observe the effect of *Spirulina* feeding on biochemical parameters of Infectious bursal disease vaccinated broiler chickens. One hundred and two day old chickens were reared up to 38 day of age. At the age of 10 days, chickens were divided randomly into four groups (groups A (33), B (27), C (21) and D (21) having different number of chickens. From 10 to 20 days, feed of all the chickens of group B and D was supplemented with probiotic *Spirulina* at the dose rate of 1.0% of feed (i.e. 1.0 g/ 100 gm of feed) whereas all chickens of group A and C were given feed without *Spirulina* supplementation. All the chickens of group C and D were vaccinated with IBDV intermediate plus strain vaccine at the age of 17 days whereas no vaccine was given to the chickens of groups A, and B. There was significantly lower activity of serum aspartate transaminase (AST) in *Spirulina* fed group however significantly higher activity was observed in IBD vaccinated alone group indicating that *Spirulina* acted as hepatoprotective agent. Serum alanine transaminase (ALT) activity was also lower in *Spirulina* supplemented groups. ALT activity was significantly higher in IBDV vaccinated group. Serum creatinine concentration was significantly higher in the vaccinated groups but it was more pronounced in IBD vaccinated group chickens without *Spirulina*. Similarly blood urea nitrogen (BUN) concentration in both the vaccinated groups chickens was higher, but more pronounced in IBD vaccinated alone group. *Spirulina* feeding reduced the activity of Serum alkaline phosphatase (ALP) in IBD vaccinated chickens. On the basis of finding of present study it may be concluded that IBDV vaccination effect can be reduced by supplementation of *Spirulina* at 1.0 percent level in feed from 10 to 20 days of age along with IBD vaccine.

Keywords: infectious bursal disease vaccine, broiler chickens, alanine transaminase, creatinine

1. Introduction
Infectious bursal disease (IBD) commonly known as Gumboro disease is a disease of global economic importance [14]. IBD was first recognized as a distinct disease in 1957. Cosgrove described the disease as avian nephrosis on the basis of tubular degenerative lesions found in the kidneys of infected broiler chickens. The syndrome adopted the name Gumboro disease since the first outbreak occurred in and around the area of Gumboro, Delaware, USA [3]. The disease is characterized by the destruction of the lymphoid cells in the bursa of Fabricius as the virus replicates in differentiating B- lymphocytes [2]. Due to specific pathognomonic lesions produced in bursa of Fabricius, it was later termed as infectious bursal disease (IBD) [6]. The immunosuppressive effects of infectious bursal disease virus (IBDV) infections were first described by Allen *et al.* [1]. The infectious bursal disease virus (IBDV) is classified in the Avibirnavirus genus of the family Birnaviridae [4, 5]. The disease is produced in broiler chicks between 3-6 weeks of age and immuno-suppression is the main consequence in infected chicks [9].

Vaccination plays an important role in the successful control of the disease but vaccination failure due to appearance of variant or newer strains of the virus in the recent times has also been reported [13]. Moreover, various attenuation levels of commercially available live vaccines for IBD lead to varying levels of immunosuppression increasing the bird's vulnerability to various infections. *Spirulina*; a blue-green algae, is rich in essential amino acids, minerals,

Essential fatty acids and antioxidant [17]. *Spirulina* has been reported to have numerous health benefits, including antioxidant, immunomodulatory, anti-inflammatory, anticancer, anti-viral and antibacterial activity due to presence of phycocyanin and beta-carotene [7]. In addition to these *Spirulina platensis* also stimulate immunity and synthesis of blood cells [10, 15]. However, no such report regarding the interaction of *Spirulina* with IBD or its vaccine could be traced in the literature. Keeping in view the above facts, the present study has been planned to study the effect of *Spirulina* feeding on Infectious Bursal Disease Vaccine.

2. Materials and Methods

2.1 Experimental design

One hundred and two chicks were purchased. At the age of 10 days, chicks were divided randomly into four groups (groups A, B, C and D) having different number of chicks as detailed in the table 1. From 10 to 20 days, feed of all the chicks of group B and D was supplemented with probiotic *Spirulina* at the dose rate of 1% of feed (i.e. 1 g/ 100 gm of feed) whereas all chicks of group A and C were given feed without any *Spirulina* supplementation. All the chicks of group C and D were vaccinated with IBDV intermediate plus strain vaccine (M.B. strain) at the age of 17 days whereas no vaccine was given to the chicks of groups A and B. Serum was collected for biochemical parameters estimation from six chicks of different groups at different days of age (DOA) as mentioned in table 1.

Table 1: Design of experiment

Groups	Treatment	Day of age (doa) for sampling
A (33)	Control	10, 17, 24, 31, 38 doa
B (27)	*Spirulina* (10-20 doa)	17, 24, 31, 38 doa
C (21)	Vaccination alone	24, 31, 38 doa
D (21)	*Spirulina* (10-20 doa) + Vaccination (at 17 doa)	24, 31, 38 doa

2.2 Biochemical studies

Serum samples were analyzed for different biochemical parameters using semi-automatic biochemistry analyzer (Erba Mannheim Chem-5 Plus, Transasia) and different standard kits procured from ERBA diagnostics Mannheim GmbH (Transasia Bio-Medicals Ltd.) for different biochemical parameters as mentioned below:

2.2.1 Serum aspartate transaminase (AST) activity

Serum aspartate transaminase activity was estimated as per the method of Tietz [19] by the standard methods of International Federation of Clinical Chemistry using single step reagent by semi-automatic biochemistry analyzer.

2.2.2 Serum alanine transaminase (ALT) activity

Serum alanine transaminase activity was estimated as per the method of Wroblewski and La Due [20] by the standard methods of International Federation of Clinical Chemistry using single step reagent by semi-automatic biochemistry analyzer.

2.2.3 Serum creatinine concentration

Total serum protein concentration was analyzed as per the method of Tietz [18] using single step reagent by semi-automatic biochemistry analyzer.

2.2.4 Blood urea nitrogen (BUN)

Blood urea nitrogen was estimated by the standard method of International Federation of Clinical Chemistry using single step reagent employing Chemistry Analyzer.

2.2.5 Serum alkaline phosphatase (ALP) activity

Alkaline phosphatase activity was estimated by using single step reagent employing semiautomatic biochemistry analyzer [19].

2.3 Statistical Analysis

The data for various parameters were subjected to statistical analysis using analysis of variance technique through Post hoc-Duncan LSD Alpha (0.05).

3. Results

3.1 Serum aspartate transaminase (AST) activity

Mean serum aspartate transaminase (AST) activities of different groups are given in table 2 and illustrated in figure 1. Mean serum aspartate transaminase (AST) activity was found to be significantly ($P \leq 0.05$) lower in *Spirulina* fed chicks (group B) as compared to control group(group A) throughout the experiment. A significant ($P \leq 0.05$) increase in the serum AST activity was observed in the IBDV vaccinated group C as compared to control group A throughout the experiment. Amongst vaccinated groups (group C and D) the serum AST activity was found to be significantly ($P \leq 0.05$) lower in group D (IBDV vaccinated with *Spirulina* fed) as compared to group C throughout the experiment. The AST activity in group D (IBDV vaccinated with *Spirulina* fed) was almost equal to the control group A at 14 and 21 DPV, however at 7 DPV it was significantly ($P \leq 0.05$) lower as compared to control group (group A).

Table 2: Mean serum aspartate transaminase activities (IU/L) in different experimental groups at different intervals (Mean ± S.E.)

Mean value of serum aspartate transaminase activity of broiler chicks(IU/L)				
Groups	Days of age(doa) / Days Post vaccination (DPV)			
	17doa/0DPV	24doa/7DPV	31 doa/14DPV	38doa/21DPV
A	248.77^{b}±15.63	248.97^{b}±19.87	246.08^{b} ±3.69	244.73^{b}±5.69
B	235.00^{a}±18.88	234.77^{a} ±13.97	213.13^{a} ±19.23	233.93^{a}±7.72
C	-	279.08^{c} ±20.62	299.12^{c}±27.32	252.95^{c}±12.25
D	-	234.77^{a} ±6.90	236.80^{b}±7.95	240.15^{b}±5.04

a, b, c : Means with unlike superscript in the column differ significantly ($P \leq 0.05$)

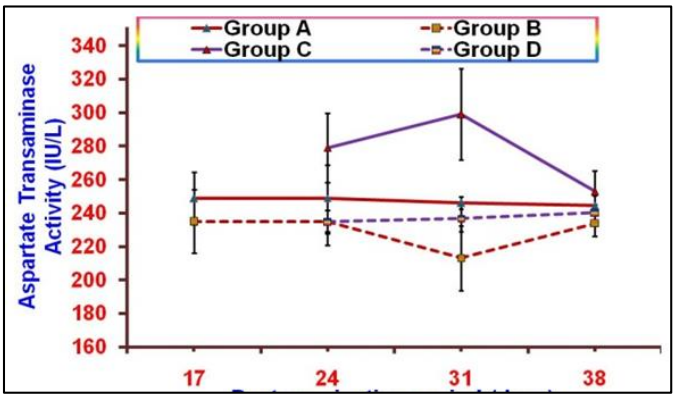

Fig 1: Post vaccination period (days)

3.2 Serum alanine transaminase (ALT) Activity

Mean serum alanine transaminase (ALT) activities of different groups are shown in table 3 and illustrated in figure 2. Non-significant lower level of ALT activity was observed in *Spirulina* supplemented group B as compared to control group A throughout the experiment.Serum ALT activity was found to be significantly($P \leq 0.05$) higherin group C (IBDV vaccinated) as compared to control group chicks (group A) till 14 DPV which become comparable at 21 DPV. Amongst vaccinated groups (group C and D) serum ALT activity was found to be significantly ($P \leq 0.05$) lower in group D (IBDV vaccinated with *Spirulina* fed) as compared to group C till 14 DPV. The ALT activity in group D was almost equal to the control group throughout the experiment.

Table 3: Mean serum alanine transaminase activities (IU/L) in different experimental groups at different intervals (Mean ± S.E.)

Mean value of serum alanine transaminase activity of broiler chicks(IU/L)				
Groups	Days of age(doa) / Days Post Vaccination (DPV)			
	17doa/0DPV	24doa/7DPV	31doa/14DPV	38doa/21DPV
A	12.37^{a}±1.44	12..63^{ab}±0.50	12.13^{ab}±0.98	11.35^{a}±0.58
B	11.38^{a}±0.94	11.38^{a} ±0.49	10.48^{a}±0.63	11.37^{a} ±0.50
C	-	16.39^{c}±0.19	14.39^{c}±0.33	11.72^{a} ±0.21
D	-	13.45 ^{b} ±0.26	13.23^{b}±1.01	11.70^{a} ±0.29

a, b, c : Means with unlike superscript in the column differ significantly ($P \leq 0.05$)

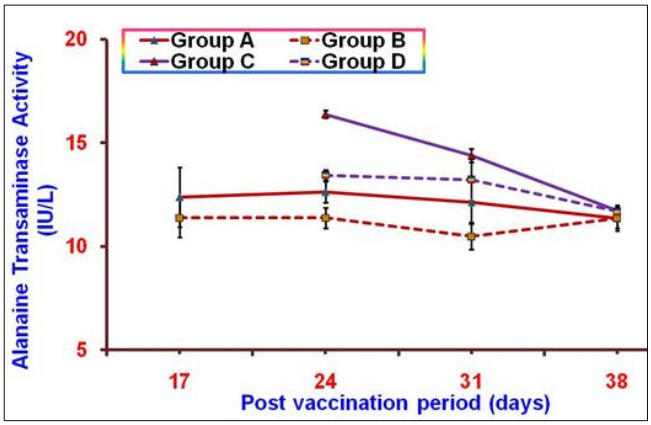

Fig 2: post vaccination period (days)

3.3 Serum creatinine concentration

Mean serum creatinine concentration of different groups at different intervals are shown in table 4 and illustrated in figure 3. *Spirulina* supplementation (group B) did not reveal any significant difference in serum creatinine concentration throughout the experiment as compared to control group (group A). A significant ($P \leq 0.05$) increase in creatinine concentration was observed in IBDV vaccinated group (group C and group D) as compared to control group A throughout the experiment.Amongst vaccinated groups (group C and group D) serum creatinine concentration was found to be significantly ($P \leq 0.05$) higher in group C (IBDV vaccinated) as compared to group D (IBDV vaccinated with *Spirulina* fed) throughout the experiment.

Table 4: Mean serum creatinine (mg/dl) concentration in different experimental groups at different intervals (Mean ± S.E.)

Mean value of serum creatinine concentration of broiler chicks (mg/dl)				
Groups	Days of age(doa) / Days Post Vaccination (DPV)			
	17doa/0DPV	24doa/7DPV	31 doa/14 DPV	38 doa/21DPV
A	0.70^{a}±0.01	0.73^{a}±.02	0.75^{a} ±.02	0.72 ^{a}±.02
B	0.72^{a}±0.02	0.74^{a} ±.01	0.74^{a} ±.04	0.70 ^{a} ±.01
C	-	0.96^{c} ±.05	0.94^{c} ±.02	0.85^{c}±.05
D	-	0.89^{b}±.02	0.82^{b} ±.02	0.76^{b}±.02

a, b, c : Means with unlike superscript in the column differ significantly ($P \leq 0.05$)

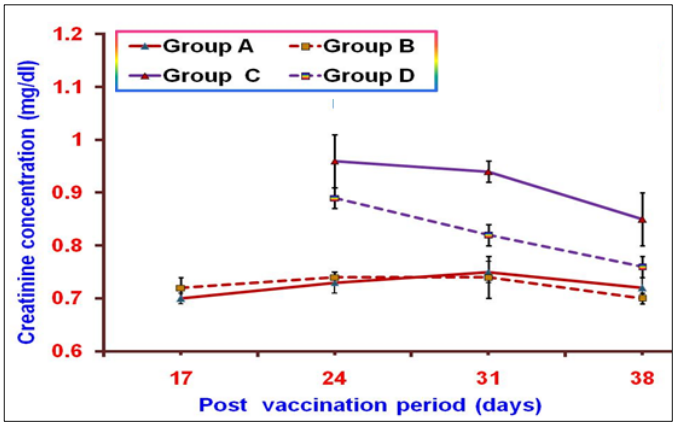

Fig 3: Post vaccination period (days)

3.4 Blood urea nitrogen (BUN)

Mean blood urea nitrogen concentration (BUN) in different experimental groups are given in table 5 and presented in figure 4. A decrease in blood urea nitrogen concentration was observed in *Spirulina* fed chicks (group B) as compared to control group (group A) throughout the experiment; although the difference was significant ($P \leq 0.05$) only at 31 doa. BUN concentrations were slightly higher in the vaccinated groups (group C and D) as compared to the control group A throughout the experiment but without statistical difference. Amongst vaccinated groups (group C and D) little higher values of serum BUN level was found in group C as compared to group D throughout the experiment but without statistical difference.

Table 5: Mean of blood urea nitrogen (mg/dl) concentration in different experimental groups at different intervals (Mean ± S.E.)

Mean value of blood urea nitrogen concentration of broiler chicks (mg/dl)				
Groups	**Days of age(doa) / Days Post Vaccination (DPV)**			
	17doa/0DPV)	**24doa/7DPV**	**31doa/14DPV**	**38doa/21DPV**
A	5.26^{b}±0.11	5.80^{a}±0.48	6.10^{b}±0.52	6.10^{a}±0.65
B	5.20^{b} ±0.52	5.75^{a} ±0.46	5.85^{a} ±0.25	6.05^{a} ±0.28
C	-	6.29^{a} ±0.25	6.66^{b} ±0.36	6.43^{a} ±0.48
D	-	6.00^{a} ±0.19	6.40^{b}±0.22	6.19^{a} ±0.58

a, b : Means with unlike superscript in the column differ significantly ($P \leq 0.05$)

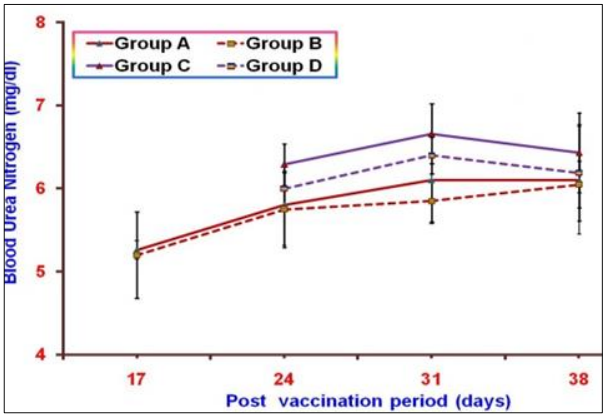

Fig 4: Post vaccination period (days)

3.5 Serum alkaline phosphatase (ALP)

Mean serum ALP activity in different experimental groups are given in table 6 and presented in figure 5. Statistically non-significant decrease in serum alkaline phosphatase (ALP) activity was observed in *Spirulina* supplemented group B as compared to control group A throughout the experiment. Serum ALP activity was found to be significantly ($P \leq 0.05$) higher in group C (IBDV vaccinated) as compared to control group (group A) till 14 DPV and it was comparable at 21 DPV. Amongst both the vaccinated groups (group C and D) serum ALP activity was found to be significantly ($P \leq 0.05$) lower in group D (IBDV vaccinated with *Spirulina* fed) as compared to group C only at 7 DPV and later at 14 and 21 DPV serum ALP activity in group D was comparable to the control group A.

Table 6: Mean serum alkaline phosphatase activity (IU/L) in different experimental groups at different intervals (Mean ± S.E.)

Mean value of serum alkaline phosphatase activity of broiler chicks (IU/L)				
Groups	**Days of age (doa) / Days Post Vaccination (DPV)**			
	17doa/0DPV	**24 doa/7DPV**	**31doa/14DPV**	**38doa/21DPV**
A	390.1^{a}±15.94	400.0^{ab}±14.01	412.0^{ab}±17.00	415.0^{ab}±12.13
B	380.9^{a}±16.01	387.0^{a}±13.21	407.6^{a}±14.00	413.0^{a}±13.99
C	-	425.0^{c}±11.12	423.0^{c}±17.00	421.5^{b}±14.34
D	-	418.0^{b}±14.34	416.0^{bc}±13.00	415.4^{ab}±14.67

a, b, c : Means with unlike superscript in the column differ significantly ($P \leq 0.05$)

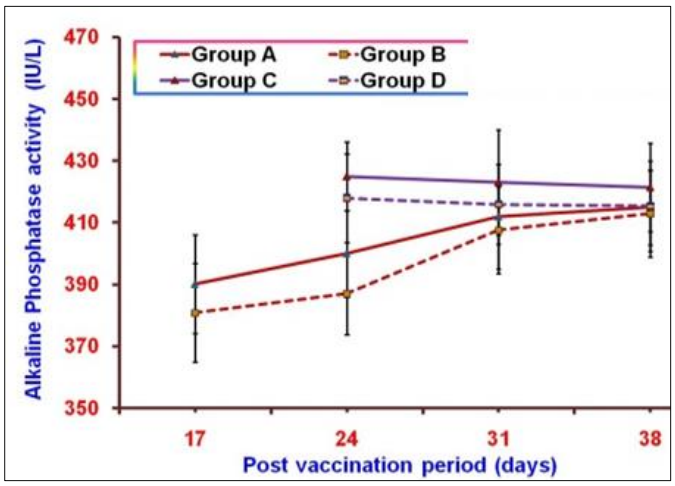

Fig 5: Post vaccination period (days)

4. Discussion

Serum aspartate transaminase (AST) concentration was significantly higher in IBDV vaccinated group C as compared to control group A throughout the experiment. There was significant decrease in activity of serum AST in group D as compared to group C throughout the experiment and the concentration was almost equal to the control group A chickens. Since this enzyme is present in large quantity in hepatic cells, the increased activity of this enzyme in serum might be due to damage of hepatocytes induced by IBDV vaccine as evident from the histopathological results. Zeweil H. *et al* [21]. also reported that *Spirulina* and vitamin E feeding significantly reduces the AST concentration in heat stressed broiler chickens indicating its hepato-protective effect.

Mean serum alanine transaminase (ALT) activity was lower in *Spirulina* supplemented group B as compared to control group A throughout the experiment. Serum ALT activity was found to be significantly higher in group C (IBDV vaccinated) as compared to control group chickens (group A) till 14 DPV which become comparable at 21 DPV. Amongst vaccinated groups (group C and D) serum ALT activity was found to be significantly lower in group D (IBDV vaccinated with *Spirulina* fed) as compared to group C till 14 DPV. The ALT activity in group D was almost equal to the control group throughout the experiment. *Spirulina* supplementation in IBDV vaccinated group (group D) was able to reduce the ALT activity as compare to IBDV vaccinated birds without *Spirulina* (group C). *Spirulina* seems to reduce the hepatic damage induced by IBDV vaccine to some extent. In a study by Shanmugapriya *et al* [16]. 1% of *Spirulina* platensis supplementation significantly increased body weight gain, decreased feed conversion ratios and increases the villi height. The *Spirulina* platensis offers a good natural alternative to improve poultry production. 1.5% *Spirulina* feeding resulted in lower final bodyweight when compared with feeding 0.5 or 1% of *S. platensis*. Excessive intake of *S. platensis* resulted in metabolic disturbances and affected the liver function leading to retarded growth rate in broilers.

Spirulina supplementation (group B) did not show any

significant difference in mean serum creatinine concentration throughout the experiment as compared to control group (A). A significant higher creatinine concentration was observed in IBDV vaccinated groups (C and D) as compared to control group A throughout the experiment. Amongst vaccinated groups (group C and group D) serum creatinine concentration was found to be significantly higher in group C (IBDV vaccinated) as compared to group D (IBDV vaccinated with *Spirulina* fed) throughout the experiment. Creatinine is a chemical waste product in the blood that passes through the kidneys to be filtered and eliminated in urine. The chemical waste is a by-product of normal muscle function. The more muscle an organism has, the more creatinine they produce. Levels of creatinine in the blood reflect both the amount of muscle an organism has and their kidney function. In case of *Spirulina* feeding along with IBDV vaccine it reduced the renal damage to some extent. Malau-Aduli and Holman [12] studied the effect of *Spirulina* supplementation on plasma metabolites in crossbred and purebred Australian Merino lambs. Creatinine levels were indicative of muscularity and lambs supplemented at low *Spirulina* levels had the highest muscularity. High *Spirulina* supplementation levels resulted in the highest glucose concentrations indicative of available energy for driving protein metabolism and other metabolic pathways including gluconeogenesis.

Mean blood urea nitrogen concentration (BUN) was lower in *Spirulina* fed chicks (group B) as compared to control group A throughout the experiment; although the difference was significant only at 31 doa. BUN concentrations were slightly higher in the vaccinated groups (C and D) as compared to the control group A throughout the experiment but without statistical difference. Amongst vaccinated groups (C and D) little higher values of serum BUN level was found in group C as compared to group D throughout the experiment but without statistical difference. BUN is also a waste product that passes through kidney. Its level increases when there is an effect on kidney. *Spirulina* feeding in IBDV vaccinated chickens seems to reduce the renal damage to a certain level. Kuhad *et al* [11]. studied the effect of *Spirulina* on gentamicin induced oxidative stress and renal dysfunction in rats. Renal injury was assessed by measuring serum creatinine, blood urea nitrogen and creatinine clearance and serum nitrite levels.

Serum alkaline phosphatase (ALP) activity was lower in *Spirulina* supplemented group B as compared to control group A throughout the experiment. Serum ALP activity was found to be significantly higher in group C (IBDV vaccinated) as compared to control group A till 14 DPV and it was comparable at 21 DPV. Amongst both the vaccinated groups (group C and D) serum ALP activity was found to be lower in group D as compared to group C at all intervals but significantly lower only at 7 DPV and later at 14 and 21 DPV serum ALP activity in group D was comparable to the control group A. James *et al* [8]. studied the effect of dietary *Spirulina* on reduction of copper toxicity and improvement of growth, blood parameters and phosphatase activity in fresh water fish carp.

5. Conclusion

Spirulina supplementation in IBDV vaccinated chickens was able to reduce the AST activity, indicating that *Spirulina* acted as hepatoprotective agent. Serum ALT activity was also lower in *Spirulina* supplemented groups. ALT activity was significantly higher in IBDV vaccinated group; however *Spirulina* feeding was able to decrease the activity of ALT in vaccinated chickens. Serum creatinine concentration was significantly higher in the vaccinated groups but it was more pronounced in IBD vaccinated group chickens without *Spirulina*. Similarly BUN concentration in both the vaccinated groups chickens was higher, but more pronounced in IBD vaccinated alone group. Serum ALP activity was significantly higher in IBD vaccinated chickens. *Spirulina* feeding reduced the activity of ALP in IBD vaccinated chickens and activity was almost equal to the control group chickens. On the basis of finding of present study it may be concluded that IBDV vaccination lead to immunosuppression as evident by biochemical parameters changes in broiler chickens. Supplementation of Spirulina at 1.0 percent level in feed from 10 to 20 days of age along with IBD vaccine helped in reducing the immunosuppressive effect of hot strain of vaccines.

6. References

1. Allan WH, Faragher JT, Cullen GA. Immunosuppression by the infectious bursal agent in chickens immunised against Newcastle disease. Veterinary Record. 1972; 90(18):511-512.
2. Cheville NF. Studies on the pathogenesis of Gumboro disease in the bursa of Fabricius, spleen, and thymus of the chicken. The American journal of pathology. 1967; 51(4):527-551.
3. Cosgrove AS. An apparently new disease of chickens: avian nephrosis. Avian diseases. 1962; 6(3):385-389.
4. Dobos P, Hill BJ, Hallett R, Kells DT, Becht H, Teninges D. Biophysical and biochemical characterization of five animal viruses with bisegmented double-stranded RNA genomes. Journal of virology. 1979; 32(2):593-605.
5. He X, Wei P, Yang X, Guan D, Wang G, Qin A. Molecular epidemiology of infectious bursal disease viruses isolated from Southern China during the years 2000–2010. Virus genes. 2012; 45(2):246-255.
6. Hitchner SB. Infectivity of infectious bursal disease virus for embryonating eggs. Poultry science. 1970; 49(2):511-516.
7. Hoseini SM, Khosravi-Darani K, Mozafari MR. Nutritional and medical applications of *Spirulina* microalgae. Mini reviews in medicinal chemistry. 2013; 13(8):1231-1237.
8. James R, Sampath K, Nagarajan R, Vellaisamy P, Manikandan MM. Effect of dietary *Spirulina* on reduction of copper toxicity and improvement of growth, blood parameters and phosphatases activities in carp, Cirrhinus mrigala IJEB. 2009; 47(09).
9. Kibenge FS, Dhillon AS, Russell RG. Biochemistry and immunology of infectious bursal disease virus. Journal of General Virology. 1988; 69(8):1757-1775.
10. Kozlenko R, Henson R. *Spirulina*: Nutrition and Health Studies, Healthy & Nat. J, 1997. (http://www.*Spirulina*. com/ SPLNews96.html).
11. Kuhad A, Tirkey N, Pilkhwal S, Chopra K. Effect of *Spirulina*, a blue green algae, on gentamicin-induced oxidative stress and renal dysfunction in rats. Fundamental & clinical pharmacology. 2006; 20(2):121-128.
12. Malau Aduli AE, Holman BW. Effect of Spirulina supplementation on plasma metabolites in crossbred and purebred Australian Merino lambs. International Journal of Veterinary Science and Medicine. 2015; 3(1-2):13-20.

13. Mohamed MA, Elzanaty KE, Bakhit BM, Safwat MM. Genetic Characterization of Infectious Bursal Disease Viruses Associated with Gumboro Outbreaks in Commercial Broilers from Asyut Province, Egypt. ISRN veterinary science, 2014, Article ID 916412.
14. Pitcovski J, Gutter B, Gallili G, Goldway M, Perelman B, Gross G, *et al.* Development and large-scale use of recombinant VP2 vaccine for the prevention of infectious bursal disease of chickens. Vaccine. 2003; 21(32):4736-4743.
15. Rasool M, Sabina EP. Appraisal of immunomodulatory potential of *Spirulina* fusiformis: an in vivo and in vitro study. Journal of natural medicines. 2009; 63(2):169.
16. Shanmugapriya B, Babu SS, Hariharan T, Sivaneswaran S, Anusha MB. Dietary administration 0f *Spirulina platensis* as probiotics on growth performance and histopathology in broiler. Int J Recent Sci Res. 2015; (2): 2650-265.
17. Somchit MN, Mohamed NA, Ahmad Z, Zakaria ZA, Shamsuddin L, Omar Fauzee MS *et al*. Anti-inflammatory and anti-pyretic properties of *Spirulina platensis* and *Spirulina lonar*: a comparative study. Pakistan journal of pharmaceutical sciences. 2014; 27(5):1277-1280.
18. Tietz NW. Fundamentals of Clinical Chemistry. W.B. Saunders Co. London, Philadelphia, 1986a.
19. Tietz NW. Fundamentals of Clinical Chemistry. W.B. Saunders Co. London, Philadelphia, 1986b.
20. Wroblewski F, Ladue JS. Serum glutamic pyruvic transaminase in cardiac and hepatic disease. Proceedings of the Society for Experimental Biology and Medicine. 1956; 91(4):569-571.
21. Zeweil H, Abaza IM, Zahran SM, Ahmed MH, Haiam M, Asmaa AS. Effect of *Spirulina platensis* as dietary supplement on some biological traits for chickens under heat stress condition. Asian Journal of Biomedical and Pharmaceutical Sciences. 2016; 6(56): 8-12.