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Effect of Zinc-selenium Complex (Selcon®) Supplementation in Broiler in Prevention of Infectious Bursal Disease

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Abstract: A zinc-selenium complex (Selcon®) was evaluated for the prevention of IBDV infection in broilers. Chicks were reared in relative isolation with optimum temperature, water and feeds. They were divided into 4 different groups named A₁, B₁, A₂ and B₂; having 3 replications each. Among them Groups A₁ and B₁ were uninfected group of which Group B₁ was supplemented with Selcon®. The rest 2 groups (A₂ and B₂) were Infected (challenge) group of which Group B₂ were supplemented with Selcon® as a preventive measure. At 28 days of age all the birds of challenge Groups (A₂ and B₂) were inoculated with field homogenates of Infectious Bursal Disease (IBD) virus. The birds were observed for clinical signs, morbidity and mortality at every 6 h interval upto 10 days post infection. Three birds from each of the four groups of three replications were sacrificed at 0, 3 and 6 days post infection and bursa/body weight ratio was determined. Part of bursal tissues were fixed in formalin and processed for bursal lesion scoring along with spleen, thymus, liver and kidney tissues by histopathological study. Selcon® treated IBDV challenged birds of Group B₂ revealed 44.44% overall cumulative morbidity and 2.22% overall cumulative mortality, while Selcon® untreated IBDV challenged birds of Group A₂ revealed 60.00% overall cumulative morbidity and 11.11% overall cumulative mortality. Results also showed significant difference ($p>0.05$) in bursa/body weight ratio between Selcon® treated and untreated groups, that overall bursa/body weight ratio in birds of Group B₂ was highest (1.30 ± 0.44) and lowest (1.23 ± 0.42) in those of Group A₂ had at 6 days post infection. The bursal lesion scores of Group B₂ were lower than Group A₂. There was no significant variation among the histopathological lesions of spleen, thymus, liver and kidneys of different groups. It may be concluded that supplementation of Selcon® to broiler diets as a preventive measure provides a satisfactory level of protection against morbidity and mortality in IBD.

Key words: Zinc-selenium, broiler diets, morbidity, mortality

INTRODUCTION

Nutrition has long been known to affect the ability of the host responding to infectious diseases (Beck, 1999). Malnourishment results in impaired immune response and increased susceptibility to the infectious diseases. Several minerals act as the trace elements of nutrients among which Zinc is an essential one. It is found in almost every cells of body and participates in many physiological mechanisms including wound healing, sensory function, T cell division and differentiation and thus promoting healthy immune status (Prasad, 1995; Prasad *et al.*, 1997; Solomons, 1998). Zinc is the structural component of wide variety of proteins and dependent enzymes like superoxide dismutase and act as an essential component of antioxidant defense system (Baum *et al.*, 2000).

Selenium is another essential element which is a component of selenocysteine containing protein. It is involved in many aspects of cell biochemistry and cell function and influences the immune system. It is also an important part of antioxidant enzymes that protect cells against the effects of free radicals. As a constituent of selenoproteins, selenium is required for the functioning

of neutrophils, macrophages, NK cells and T lymphocytes (Ferencik and Ebringer, 2003). In addition, adequate selenium may enhance resistance to infections through modulation of interleukin production and subsequently the Th1/Th2 mediated immune response. Selenium supplementation regulates IL-2 production and increases activation, proliferation and differentiation of T helper cells (Arthur *et al.*, 2003). Thus zinc and selenium are two important micronutrients that supports a healthy immune system (Prasad, 1995; Prasad *et al.*, 1997; Ferencik and Ebringer, 2003).

Infectious Bursal Disease Virus (IBDV) is an RNA virus of Birna viridae. It is an acute and highly contagious viral infection of young chickens of 3-6 wks age characterized by severe damage in the bursa of Fabricius and associated immuno suppression (Cosgrove, 1962; Cheville, 1967; Allan *et al.*, 1972; Lukert and Saif, 1997). So the bursa/body weight ratio and the histopathological bursal lesion score are the indication of the severity of the disease (Chauhan and Roy, 2000) and considered as an accurate indication of the pathogenicity of infectious bursal disease virus (Rossels *et al.*, 1989; Tsukamoto *et al.*, 1995).

Selcon^(R) (Zeus Biotech Limited, Mysore-570016, India) is a zinc-selenium complex with yeast nucleotides, yeast mannoglucans and enzymes. It is claimed that the product prevents the devastating effects of the RNA viral infections by maintaining normal selenium reserves in the mucosal tissues which produces toxic effect to the viruses and protect bird from severity. Considering the above mentioned claim, the objective of present work was taken to observe the efficacy of zinc-selenium complex (Selcon^(R)) in the prevention of experimental IBDV infection.

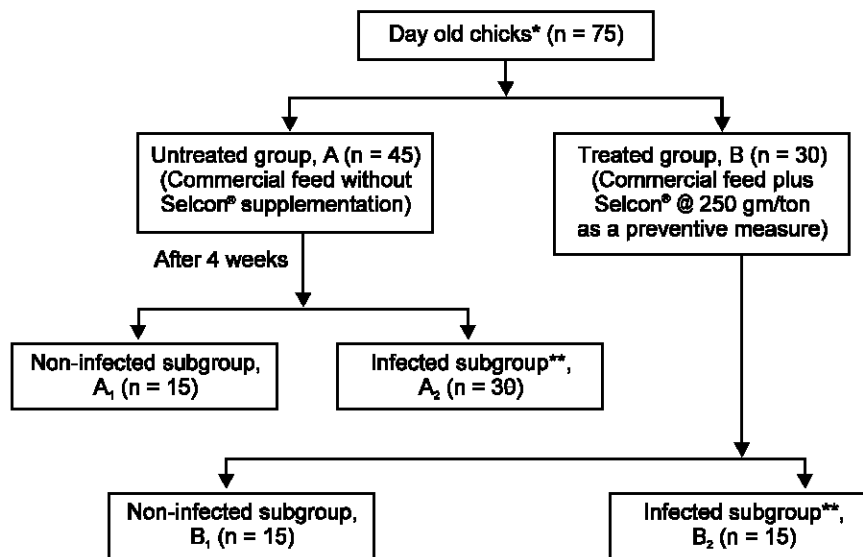
MATERIALS AND METHODS

The experiment was carried out in the Department of Pathology, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh. Two hundreds and twenty five apparently healthy day-old broiler chicks (Hubbard-classic) of Dhaka Hatchery were purchased from the local agent in Mymensingh and reared in two separate sheds, one for infected (challenge) birds and another for non-infected birds.

Shed preparation: The sheds were cleaned and disinfected properly before rearing the chicks. At first all the darts were removed from the sheds, then the floor and walls were rubbed with a brush and rinsed properly with tap water. The sheds were disinfected with bleaching powder and allowed to dry by leaving the room unused for two days. Then the rooms were sprayed with a disinfectant solution (virkon-s^(R) @ 5 gm/liter of water) properly. All the feeders, waterers and other necessary appliances were also properly cleaned and then disinfected by using KMnO₄ (Potassium permanganate) solution. The double strength of the fumigation was done due to history of previous outbreak of Ranikhet disease in the same sheds.

Management: The birds were reared in the above mentioned isolated poultry sheds. Strict bio-security was maintained. Entry of visitors to the poultry shed was extremely restricted. Only the attendant and research supervisor were allowed to enter into the shed by taking necessary precautionary measures. Rubber boots, foot dipping in disinfectant containing footbath and hand spray (with virkon-s^(R) on hands @5 gm/litter of water) were compulsory for the workers during entry and exit. All the chicks were reared in the same brooder house up to 3 weeks of age under proper chick management. For the first week brown paper was placed above the litter within the chick guard, which was changed regularly. Optimum temperature in the chick guard was maintained using electric bulbs in required number and at required distances. Optimum ventilation and lighting were also ensured in the brooder. For the first four days the birds were maintained on suji (a coarse flour of wheat) which was then replaced by commercial starter feed. Feed and water were supplied *ad libitum*. Vitamins, minerals and amino acids except vitamin E and selenium were given at a routine schedule in water. No vaccine was given to the birds during the course of the experiment. zinc-selenium complex (Selcon^(R)) was supplied to broiler diets @ 250 gm/ton of feed in group B1 and B2 where A1 and A2 was not supplemented with it.

Challenge inoculation: At 28 days of age the birds of Groups A₂ and B₂ were given IBDV infection using the homogenates of naturally infected bursa of Fabricius. The infected bursal homogenates (inocula) were prepared from bursa of naturally infected chicks. All the birds of infected groups were inoculated with 100 µl of bursal homogenate each, 50% by intranasal and 50% by intraocular route. The experiment was carried out under the following design:



* 3 replications of the experiment

** Inoculation by ocular and nasal route

Parameters studied following challenged with field bursal homogenates:

- Clinical signs, morbidity and mortality (Frequent observation)
- Collection of tissues (liver, kidney, bursa, spleen and thymus) for pathological study (both gross and microscopic lesions)
- Bursa/Body weight (B/BW) ratio (Debnath *et al.*, 2005)
- Bursal lesion scoring (Raue *et al.*, 2004)

Clinical signs, morbidity and mortality: After infection on 28th days of age, all the birds of different groups were observed at every six (6) hours interval for studying clinical signs, morbidity and mortality upto 10 days Post infection, till the end of the experiment (38 days of age). The morbidity and mortality at that time were recorded.

Gross pathology: Three birds from each replication of different groups were sacrificed at the day of infection (28 days of age), 3 days post infection (31 days of age) and 6 days post infection (34 days of age). All the dead and sacrificed birds were examined for gross lesions. During necropsy gross lesions in various organs were recorded.

Histopathology: During post-mortem examination the bursa, spleen, thymus, liver and kidney of each sacrificed or dead bird were collected and fixed in 10% neutral buffered formalin for histopathological study. After fixation, the tissues were processed, sectioned and stained as per standard procedure (Luna, 1968). All the samples from each group and each replication were used for histopathological studies.

Bursa/body weight ratio (B/BW ratio): Each bird was weighed before sacrifice and the bursa of Fabricius was collected and weighed after sacrifice. The average Bursa/body weight ratio (B/BW ratio) was determined by using following formula described by Debnath *et al.* (2005). The formula is given below.

$$\text{B/BW ratio} = \frac{\text{Bursal weight of individual bird in gm}}{\text{Body weight of individual bird in gm}} \times 1000$$

Bursal lesions scoring: The slides prepared from the tissues were studied under microscope using low and high power objectives. The scoring of the bursal lesions was done on the basis of the following criteria (Raue *et al.*, 2004).

- Score 0 = Apparently normal lymphoid follicles.
- Score 1 = Mild lymphoid depletion indicated by just thinning of lymphocyte population without any sign of focal necrosis or remarkable edema.
- Score 2 = Moderate lymphoid depletion along with focal necrosis and interfollicular edema.

Score 3 = Severe lymphoid depletion virtually leaving no lymphocyte but only reticular cells and proliferating fibrous tissue

Score 4 = Atrophy of follicles usually with cystic spaces, infolding of epithelium and marked fibroplasias.

Statistical analysis: Data were analyzed by Analysis of Variance (ANOVA) techniques by using Micro Statistics (MSTATC) package program. The significance of differences between means was also tested by DMRT (Duncan's Multiple Range Test) at the level of $p > 0.05$ (Gomez and Gomez, 1984).

RESULTS

Clinical manifestations, morbidity and mortality: After field viral challenge at 28 days of age, the first clinical signs were observed after 24 h of infection (1 day p.i.) in two birds of Group A₂ to which no Selcon[®] supplementation was given. Clinical signs (reduced feed and water intake, depression, ruffled feathers, drowsiness and severe prostrations) were also observed after 48 h of infection (2 days p.i.) among the birds of Group B₂ to which Selcon[®] supplementation was given as a preventive measure. The overall cumulative morbidity and mortality were 60.00% and 11.11% respectively in Group A₂ (without Selcon[®] supplementation) whereas those were 44.44% and 2.22% respectively in Group B₂ (Selcon[®] supplementation as preventive measure) (Table 1 and Fig. 1).

Bursa/body weight (B/BW) ratio: The bursa/body weight ratio (B/BW ratio) was determined on 0 (day of infection), 3 and 6 day post infections to compare infection condition in different groups. The reduction of bursa/body weight ratio was highly remarkable in all the birds of infected Groups A₂ (infected birds without Selcon[®] supplementation) and B₂ (infected birds with Selcon[®] supplementation as a preventive measure) than the non-infected Groups (A₁ and B₁) at 6 days post infection (Table 2). But the overall B/BW ratio of Group B₂ was higher (1.30±0.44) than Group A₂ (1.23±0.42). The variations of B/BW ratio among the infected (A₂ and B₂) and non-infected (A₁ and B₁) groups at 6 days post infection were significant.

Gross pathology: All the dead birds of Group A₂ (infected birds without Selcon[®] supplementation) and B₂ (infected birds with Selcon[®] supplementation as a preventive measure) showed typical lesions of IBD. The most usual lesions that recorded were severe nephritis, edematous and swollen bursa with hemorrhage and mucus in the duodenum. The bursae of all sacrificed birds were swollen and edematous after 3 days post infection but were atrophied at 6 days post infection.

Table 1: Overall cumulative morbidity and mortality pattern in birds of group A₂ (infected bird without Selcon® supplementation and B₂ (infected birds with Selcon® supplementation as a preventive measure)

Days of post infection	Infected birds without Selcon® supplementation (A ₂)					Infected birds with Selcon® supplementation as a preventive measure (B ₂)				
	Total birds (A ₂)	Affected	Dead	Cumulative morbidity (%)	Cumulative mortality (%)	Total birds (B ₂)	Affected	Dead	Cumulative morbidity (%)	Cumulative mortality (%)
0*	45	0	0	0	0	45	0	0	0	0
1	36	1	0	2.22	0	36	0	0	0	0
2	36	+9	2	22.22	4.44	36	4	0	8.89	0
3*	34	+4	+1	31.11	6.67	36	+6	1	22.22	2.22
4	24	+11	+2	55.56	11.11	26	+10	0	44.44	2.22
5	22	+1	0	57.78	11.11	26	0	0	44.44	2.22
6*	22	+1	0	60.00	11.11	26	0	0	44.44	2.22
7	13	0	0	60.00	11.11	17	0	0	44.44	2.22
8	13	0	0	60.00	11.11	17	0	0	44.44	2.22
9	13	0	0	60.00	11.11	17	0	0	44.44	2.22
10	13	0	0	60.00	11.11	17	0	0	44.44	2.22

3 birds (total 9) were sacrificed from each replication. **Compiling 3 replications

Table 2: Overall** bursa/body weight (B/BW) ratio (mean±SD) of birds of different groups at different days post infection

Days post infection	A ₁ (non-infected birds without Selcon®)	B ₁ (non-infected birds with Selcon® as a preventive measure)	A _{2a} (infected birds without Selcon®)	B ₂ (infected birds with Selcon® as a preventive measure)
0 day post infection	2.88±0.21	3.30±0.08	3.11±0.57	2.84±0.42
3 days post infection	3.19±0.36	2.81±0.19	3.35±0.32	3.23±0.56
6 days post infection	2.93±0.24 ^a	2.51±0.46 ^b	1.23±0.42 ^c	1.30±0.44 ^c

Different superscripts in the same row indicate significant difference (p>0.05). **Compiling 3 replications

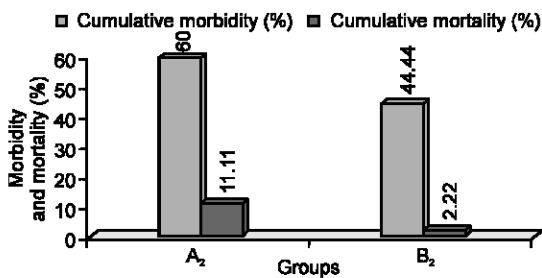


Fig. 1: A cumulative morbidity and mortality pattern of Selcon® non supplemented (A₂) and Supplemented (B₂) groups

Histopathology

Bursa of Fabricius and bursal lesion scores:

Histopathological lesions in the bursa of Fabricius of all the infected birds were variable in intensity among Groups A₂ (without Selcon® supplementation) and B₂ (with Selcon® supplementation as a preventive measure) at different days post infection. At 3 days post infection, the bursae of Group A₂ showed severe lymphoid depletion with marked haemorrhage within the follicles (Fig. 2) but those of Group B₂ showed mild or moderate to severe lymphoid depletion (Fig. 3). At 6 days post infection the birds of Group A₂ showed infolding of epithelia and follicular atrophy with cystic space within the follicles (Fig. 4). But those of Group B₂ showed only severe lymphoid depletion (Fig. 5). The bursae of the non-infected Groups (A₁ and B₁) showed no histopathological lesion.

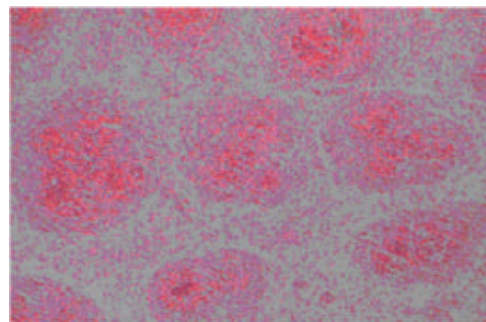


Fig. 2: Severe lymphoid depletion in bursa of Fabricius of bird of group A₂ at 3 days post infection (H and E, x84)

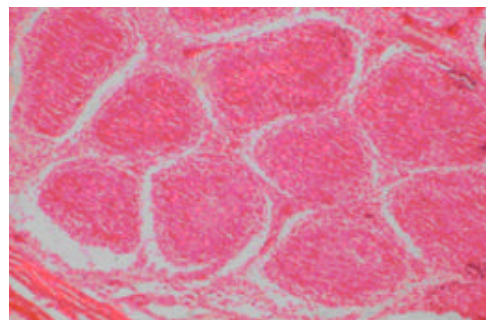


Fig. 3: Mild lymphoid depletion in bursa of Fabricius of bird of group B₂ at 3 days post infection (H and E, x84)

Table 3: Bursal lesion scores at different days post infection

Groups	Bursal lesion score		
	0 days post infection	3 days post infection	6 days post infection
A ₁ (non-infected birds without Selcon®)	0,0,0,0,0,0,0,0,0	0,0,0,0,0,0,0,0,0	0,0,0,0,0,0,0,0,0
A ₂ (infected birds without Selcon®)	0,0,0,0,0,0,0,0,0	3,3,3,3,3,3,3,3,3	4,4,4,4,4,4,4,4,4
B ₁ (non-infected birds with Selcon® as a preventive measure)	0,0,0,0,0,0,0,0,0	0,0,0,0,0,0,0,0,0	0,0,0,0,0,0,0,0,0
B ₂ (infected birds with Selcon® as a preventive measure)	0,0,0,0,0,0,0,0,0	3,3,1,1,3,3,2,1,2	3,3,3,3,4,3,3,3,3

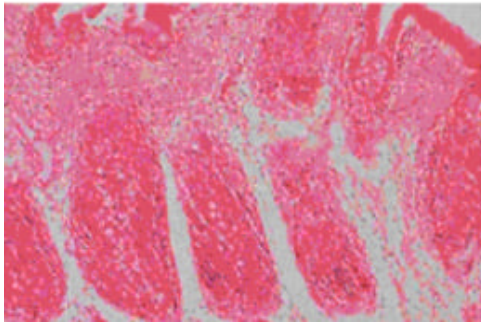


Fig. 4: Infolding of the epithelium with cystic space within the follicle in bursa of Fabricius of bird of group A_{2a} at 6 days post infection (H and E, x84)

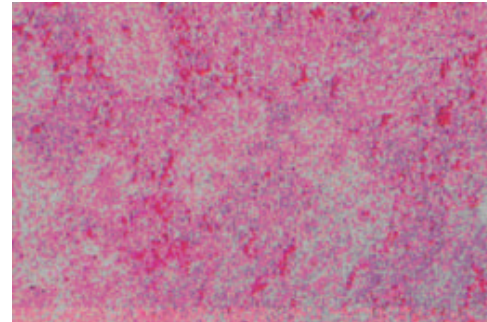


Fig. 6: Hemorrhage in spleen of bird of group B₂ at 6 days post infection (H and E, x84)

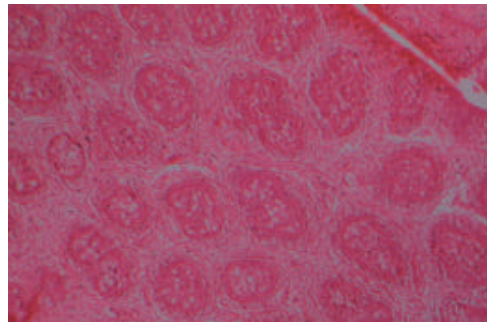


Fig. 5: Severe lymphoid depletion in bursa of Fabricius of bird of group B₂ at 6 days post infection (H and E, x84)

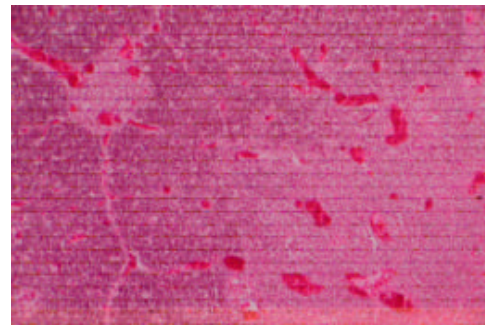


Fig. 7: Haemorrhage in thymus of bird of group B₂ at 6 days post infection (H and E, x84)

Bursal lesion score was calculated on the basis of criteria described by Raue *et al.* (2004). Although there was no remarkable variation in lesion scoring among the birds of same sacrificed day within a group but variations were remarkable between Groups A₁ (non-infected birds without Selcon®), A₂ (infected birds without Selcon®), B₁ (non-infected birds with Selcon® as a preventive measure) and B₂ (infected birds with Selcon® as a preventive measure) (Table 3).

Spleen and thymus: In spleen the histopathological lesions in birds of Group A₂ (infected birds without Selcon® supplementation) and B₂ (infected birds with Selcon® supplementation as a preventive measure) were almost similar and were characterized mainly by haemorrhage (Fig. 6). Like spleen the histopathological

lesions in birds of Group A₂ (infected birds without Selcon® supplementation) and B₂ (infected birds with Selcon® supplementation as a preventive measure) were also characterized by haemorrhage in the thymus (Fig. 7).

Kidneys and liver: The histopathological lesions of kidneys were mainly characterized by haemorrhage and congestion and infiltration of reactive cells (Fig. 8). The lesions were almost similar in birds of Group A₂ (infected birds without Selcon®) and B₂ (infected birds with Selcon® as a preventive measure). Like kidneys, the livers of infected birds were also characterized mainly by haemorrhage and congestion and infiltration of reactive cells (Fig. 9). The histopathological lesions were almost similar in birds of Group A₂ (infected birds without Selcon®) and B₂ (infected birds with Selcon® as a preventive measure).

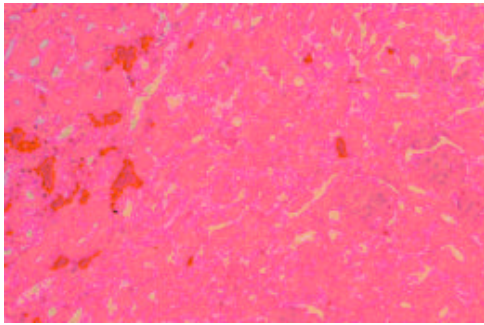


Fig. 8: Congestion and reactive cell proliferation in kidney of bird of Group B₂ at 6 days post infection (H and E, x84)

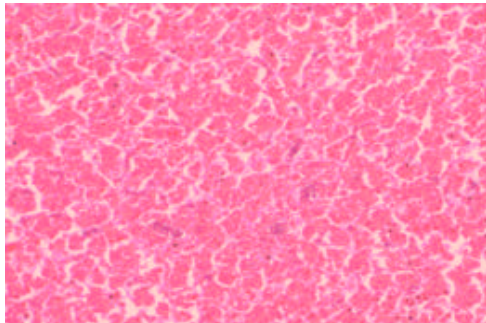


Fig. 9: Reactive cell proliferation in liver of bird of group B₂ at 6 days post infection (H and E, x84)

DISCUSSION

The zinc-selenium complex Selcon[®] was observed for its effects on clinical features, bursa body weight ratio, histopathology study and bursal lesion scoring on experimentally IBDV infected birds to justify the claim that it decreases the severity of RNA viral infection.

Clinical observation upto 10 days following infection revealed the highest overall cumulative morbidity (60.00%) among the birds of Group A₂ to which no Selcon[®] supplementation was given and the lowest (44.44%) in Selcon[®] supplemented Group B₂. The overall cumulative mortality was also highest (11.11%) in Group A₂ and the lowest in Group B₂ (2.22%).

The lowest overall cumulative morbidity (44.44%) and mortality (2.22) in the Selcon[®] supplemented Group B₂ may be due to less immunosuppressive effect of IBDV as improved selenium status stimulates the cell-mediated immunity and cause higher CD4+ cell counts in the bursa of Fabricius (Leng *et al.*, 2003). This result supports the fact that the host nutriture can influence the genetic makeup of the pathogen (Specially for an RNA virus) and alter its virulence (Levander, 1997; Beck, 1999).

The overall bursa/body weight ratio was almost similar in all bird groups at 0 day post infection (at the day of

infection). But it was drastically reduced among the birds of infected (Challenged) Groups (A₂ and B₂) at 6 days post infection which is similar to the result of Debnath *et al.* (2005). At 6 days post infection the bursa/body weight ratio of Group A₂ to which no Selcon[®] supplementation was given was significantly ($p>0.01$) reduced than that of Groups A₁ (non-infected birds without Selcon[®]), B₁ (non-infected birds with Selcon[®]) and B₂ (infected birds with Selcon[®] as a preventive measure). At 6 days post infection the overall bursa/body weight ratio was lowest (1.23 ± 0.42) in Group A₂ to which no Selcon[®] supplementation was given (Group A₂) and the highest (1.30 ± 0.44) in Group B₂ to which Selcon[®] supplementation was given as a preventive measure.

The lowest overall bursa/body ratio among the birds without Selcon[®] supplementation (group A₂) indicates that they suffered most severely from Gumboro disease whereas Group B₂ (Selcon[®] supplemented) suffered least as they possess the highest overall bursa/body weight ratio. This result also supports the findings of Debnath *et al.* (2005). It may be due to the fact that adequate selenium enhances resistance to infections through modulation of interleukin production and subsequently the Th1/Th2 response (Arthur *et al.*, 2003). The protective effect of Selcon[®] in IBDV infection is also supported by the Bursal lesion score which is an accurate indication of the pathogenicity of infectious bursal disease virus (Rossels *et al.*, 1989; Tsukamoto *et al.*, 1995).

The results of Bursal lesion scores in the experimental groups indicate that Selcon[®] supplementation as preventive measure reduces the severity of bursal lesions. At 6 days post infection, the bursal lesion score of infected birds of Group A₂ (without Selcon[®] supplementation) was 4 but the score was almost 3 in Group B₂ (Selcon[®] supplemented as a preventive measure). The high bursal lesion score of Group A₂ (without Selcon[®] supplementation) indicates that the bursa affected most severely from Gumboro disease (Debnath *et al.*, 2005) whereas the lower bursal lesion score of Group B₂ (with Selcon[®] supplementation as a preventive measure) indicates Selcon[®] supplemented birds suffered least severely from Gumboro disease.

There was no significant variation among the histopathological lesions of spleen, thymus, liver and kidneys of different groups.

From the above mentioned discussion it may be concluded that supplementation of zinc-selenium complex (Selcon[®]) to broiler diets as a preventive measure provides a satisfactory level of protection against morbidity and mortality in IBD. Based on this experiment it may be recommended that supplementation of Selcon[®] to broiler diets @ 250 gm/ton of feed can be used as a preventive measure to prevent IBD in broilers. However, the protocol needs to be tested in the field condition.

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