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COMPARATIVE STUDY BETWEEN SOME ADDITIVES ON IMMUNE RESPONSE OF INFECTIOUS BURSAL DISEASE VACCINE IN BROILER FED DIET WITH AFLATOXIN-CONTAMINATED POISONS

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ABSTRACT
This study was carried out to determine the influence of vit. E with selenium, poultry star, levamezol and acetic acid on immune response to infections bursal disease in broiler fed diet with aflatoxin-contaminated aflatoxin poisons. 200 one-day old chicks were divided randomly into six equal groups, All groups (except 6th group) were fed diet with aflatoxin-contaminated feed (from 1 to 40 days old) as following: 1st group was given poultry star, 2nd was given vit. E and selenium, 3rd group was given levamezol, 4th group was given acetic acid, 5th group only fed aflatoxin (controle positive) and 6th group (control negative). Vit. E, poultry star, levamezol and acetic acid were given by drinking water (from 1 to 25 days old). All groups were vaccinated with IBD vaccine at 12 days old. Blood samples were collected to estimate the immune response by ELISA test. Samples from bursa of fabricius and spleen were taken for histopathological test at 15, 25and 35days old. The results revealed that aflatoxin at level 8ppb was caused significant reduction in Ab titers levels against IBD vaccine in all ages and caused histopathological lesion in lymphoid organs (bursa of fabricious and spleen) which showed marked depletion and reduction of lymophid follicle, while in spleen The lesion was showed depletion, congestion and hemorrhage also the results were showed that adding vit. E and selenium and poultry star lead to significant increasing in Ab titers level against IBD vaccine also reduced the histopathological lesion in bursa of fabricious and spleen which showed slight depletion and hyperplasia of lymphocyte in bursa of fabricius while in spleen showed slight hmg, congestion and slight depletion. It is conclude that aflatoxin in 8ppb in chicken fed had negative effect on IBD vaccination also cause histopathological change in bursa and spleen. On the other hand, using vit. E with selenium and poultry star were reduced the effect of aflatoxin on immune response and protect the lymphoid organs. Whereas, levamesole and acetic acid were act in less degree as compared with vit. E and poultry star concerning the reduction of the effect of aflatoxin in immune response and protection of the lymphoid tissue.

KEYWORDS: vitamin E with selenium, probiotic, prebiotic, levamasole, acetic acid, Aflatoxin.

INTRODUCTION

Due to a lack of biosecurity and management practice, free range chicken are more or less constantly exposed to the risk of immunosuppressant, like aflatoxin and infections bursal disease (IBDV)which might lead to increased susceptibility to disease and vaccinated failure[1]. Infection of bursal disease (IBD) is an acute, highly contagious viral infection of growing chickens. It is caused by double stranded, bisegmented RNA virus[2] IBD virus primarily attacks the lymphoid tissue with especial predilection for the bursa of fabricius resulting in depletion of B-cell by inducing apoptosis in severely immunodepressed chickens[3]. IBD is a serious menace in the development of poultry enterprise and has resulted in major worldwide economic losses[4] such outbreaks have caused colossal losses to poultry farmer in Iraq[5] and may be due to the occurrence of antigenic variant strain, interference by maternal antibodies or other immunosuprressive afflictions such as aflatoxicosis [6] Aflatoxins are a secondary fungal metabolites produce by some strain of *Aspergillus flavus and Aspergillus parasiticus*, these toxins are consider to be most important *Mycotoxins* because of wide range of host susceptibility, immunosuprrssion, hepatotoxicity and heat stable properties of aflatoxin and are potential hazard effecting

The poultry industry in heavy economic losses [7] Aflatoxins reduce complement activity which is the most sensitive aspect of cell-mediated immunity (CMI) and general immune suppression in chickens leading to poor vaccination response[8] measures used by livestock farmers to protect animals from the toxic effects of aflatoxins, includes grain testing, use of mold inhibitors, fermentation, microbial in activation, irradiation, ammoniation [9], ozone degradation [10] and adsorbents [11] In recent research which used additives added to feed and drinking water such as vitamins A, E, probiotic (biological material), levamezol (therapeutic drug) and acetic acid (organic acid) which are used to detoxified toxin contaminated feed[12-16] most of these substances act as immunomodnlators because it enhancement immune response in bird feed diet contain aflatoxin such as vit. E and levamesole [15, 17]. At present, the use of probiotics is the method of choice. Probiotic is a source of live micro-organisms that includes bacteria fungi and yeast [18] lactic acid bacteria such as lactobacilli, streptococci and Bifido. These microorganism detoxification by bind *Mycotoxin* to their cell wall component and inhibit mod to growth [19]

also act as immunomodulatory agent by activation specific and non specific host immune response in chick which help in turn in prevention and control. of various infection disease[20] preoiotic includes fructooligo-saccharide and mannan oligosaccharide (Mos. The Mos is extracted from The yeast cell wall of *Saccharomyces cerevisae* was found to have beneficial effects in poultry during mycotoxicosis which have consider able binding ability over several commonly occurring *Mycotoxins* [21] also has immunomodulatory properties [22] The presence of high levels of aflatoxin in diet was caused immune depressed, failure of vaccination and histopathological lesion in lymphoid tissue [23], so this study was conducted to compare among the effects of poultry star (probiotic plus prepiotic), Vit. E with selenium, levamezol and acetic acid on enhancement of immune response of broiler against infections bursal disease vaccine with presence of low level of aflatoxin in ratio.

MATERIALS & METHODS

The experiment was carried out in the animal house /department of pathology/ College of Veterinary Medicine / Baghdad University/Iraq.

200 chicks one day old broilers Ross unsexed, were weighted individually, wing banded and housed in floor pens with clean wood shaving under continuous fluorescent lighting. Feed and water were ad-libitim. Experimental period extended to 40 days. Feed was contaminated by aflatoxin at level 8ppb, which was measured by HPLC method [24]. The additives used in this experiment are as following:

1-Poultry star®: Is the product of Biomin® multispecies symbiotic through the combined action of selected probiotic microorganism and prebiotic fructooligo-saccrides with concentration 5×10^{12} cfu/kg.

2-Vit. E with selenium type KEPRO concentration of Vit.E 200 mg/Selenium 2.5 mg with doses 1g.

3-Acetic acid: commercial product with dose 1gm/L.

4-Levamezol: commercial product 10 mg/kg.

Chicks were divided randomly into six groups (35 bird/group) as follows:

1st: fed aflatoxin 8ppb and given poultry star® 0.2 g/L of drinking water.

2nd: fed aflatoxin 8ppb and given Vit. E with selenium 1 g/L of drinking water.

3rd: fed aflatoxin 8ppb and given levamezol/liters of drinking water

4th: fed aflatoxin 8ppb and given acetic acid 1 g/L of drinking water.

5th: fed aflatoxin 8ppb (control positive).

6th: control negative.

The birds of all groups except 6th were fed contaminated feed with aflatoxin from one day old to end the experiment, also poultry star®, Vit. E with selenium, levamesol and acetic acid were given to birds by drinking water from one day old to end the experiment, all groups were vaccinated with live attenuated infectious bursal disease vaccine (CEVA IBDL, winter field 2512 G-61, France) by drinking water method at 12 day of age against infectious bursal disease. On one day of age blood samples were taken from chicks to measured maternal immunity, at 15, 25 and35 days old, also blood sample were taken to measure the antibody titers against IBD by ELISA test (symbiotic USA). Specimens were taken from lymphoid organs of bird includes: bursa of fabricius and spleen at 15, 25 and 35 days for histopathological test. These organs were fixed in 10% buffer formaldehyde solution immediately after removed. The processing was routinely done and then slides were stained with H and E stain [25] to study the histopathological changes.

Data were subjected to analysis of variance (ANOVA) and means compared for significance using least significant difference (L.S.D) for comparative of means on a computer program [26].

RESULTS & DISCUSSION

Table (1) shows the results of ELISA test for chicks against infectious bursal disease vaccine: - the effect of the poultry star®, Vit. E with selenium, levamezol and acetic acid and aflatoxin on the ELISA antibody titer against IBDV. The results revealed that the differences were significant ($P< 0.05$) among all groups in Ab titer at 15, 25 and 35 days old chicks. 5th (control positive) fed aflatoxin alone showed significant decrease ($P< 0.05$) in Ab titer as compared with 6th (control negative) and with other groups in all ages.

This reduction in titer values is cleared induction of immunedepression effects of aflatoxin on humoral antibody response. These finding agree with previous reports [25, 27, 23] the reduction of antibody titer could be due to inhibition of DNA and protein synthesis by aflatoxin through impairment of amino acid transport and mRNA transcription resulting in low level of antibody[27]. Also aflatoxin causes regression of bursa fabricius therefore the low of antibody titer against Newcastle disease and infectious bursal disease may be attributed to the regression of this organ [29].

At 15 days old chicks the highest level of antibody titer was appeared in 2nd fed aflatoxin plus Vit. E with selenium, which increased significantly ($P< 0.05$) compared with 5th (control positive) and 6th (control negative) and with other groups followed by: The significant increase in Ab titer in 2nd could be attributed to the role of Vit. E and selenium in reducing aflatoxin by changing the toxic metabolic: increasing or activation the glutathione peroxidase (GSH-PH)[30]. Aflatoxin is inactivated by conjugation with glutathione-s-transferase (GST) to the aflatoxin glutathione and excreted through urine and bile[31]. They also reported that the Vit. E reduced the formation of aflatoxin adducts in the liver [32]. Various finding strongly suggested the enhancement of immune response due to Vit. E supplementation. Vit. E converted arachiodonic acid into prostaglandin, which plays an important role in enhancement of immune response [33]. This result is in agreement with [34, 35], who found that combination of Vit. E and selenium played a significant role in neutralizing aflatoxin effects and act as immunostimulatiors.

At age 25 days the results showed a significant difference ($p< 0.05$) among all groups in Ab titer. Highest mean value observed in the 1st (fed afltoxin and poultry star®) which increased significantly ($P< 0.05$) compared with 5th followed with 3rd and 4th but no significant with 6th .

The effect of aflatoxin in Ab titer in 1st group that given poultry star® by drinking water was reduced. This reduction could be attribute to probiotic (lactobacillus and

another beneficial bacteria) and prebiotic (Fructooligo-saccrides) as they represent a part of poultry star component. On the other hand, synergism could have promoted the growth of the beneficial microflora and consequently enhanced the immunity[36]. Both lactobacillus as a probiotic and fructooligosaccride as prebiotic have been shown to bind aflatoxin and prevented absorption of aflatoxin from intestine[37]. Also the improvement in immune response result from oral administration of probiotic lead to coloinization of lactobacillus in the gastrointestinal tract, activating immunocytes promoting the endogenous host defense mechanism and modulating the systemic and mucosal immune systems probiotic stimulate different subsets of immune system cells to produce cytokines which in turn play role in the induction of immune response [38, 37]. this results is agreement with [39] reported that probiotic treated birds had significantly more serum antibody than the birds that were not treated, this report is consider as the first on treatment aflatoxin detoxification and reduce the negative effect of aflatoxin on IBDV antibody virus by using poultry star® so there is no compare the current results with other work that has investigated the effect of poultry star® on enhanced the immune response of bird fed aflatoxin.

At 35 days old chicks the highest level of Ab was observed in 2nd and 6th groups with significant different (P< 0.05) as compared with other groups. 1st was significantly increased (P< 0.05) compared with 5th (control positive) but was not differ significantly as compared with 3rd and 4th .

This result reflected the role of Vit. E in reducing the accumulative effect of aflatoxin due to antioxidant properties of it protect cells involved in immune response such as lymphocyte, macrophages and plasma cells against oxidative damage and enhance the function and proliferation of these cells [40].

As the Vit. E has a role in detoxification aflatoxin and enhance immune response, the 2nd group reached to the normal condition same as 6th that fed basal diet without aflatoxin, followed by 1st group, which was not differ significantly with 6th, 3rd and 4th due to the growth of normal flora (such as lactobacillus) reached to maximum level and could caused a detoxification in aflatoxin and enhance immunity [41].

In 3rd group, that given levamezol by drinking water there was a significant (P< 0.05) reduction in negative effect of aflatoxin as a result of an improvement in Ab titer compared with 5th (control positive) and 4th (aflatoxin plus acetic acid) this results attributed to that levamezol is synthetic and helminthes agent with immune modulatory properties [42] levamezol have been use in an attempt to enhance protective immune response of chickens for prevention or control of infectious disease including Newcastle disease [43] and infectious bursal disease [44]. It's able to enhance both normal and cellular immune response by activation of the T-cell help interferon production, activation of macrophage and act as antistress[45]. Although, levamezol could reduce the accumulative effect of aflatoxin on immune response, our results revealed that Ab titer level in the 3rd group did not reach to same levels in other groups such as 6th , 2nd and 1st because is not act direct on aflatoxin. This result agree with [15] who found that levamezol given to birds fed aflatoxin lead to reduce immunosupression of aflatoxin on immune response of broiler against Newcastle disease vaccine.

The less effect of acetic acid in reducing effect of aflatoxin attributed to the toxicity of aflatoxin was minimized by acidic treatment [46] this result agree with [47] that found treated aflatoxicated chicks by yeast extract 3% and acetic acid 3% showed a significant improvement in the serum protein.

TABLE 1: Antibody titers against IBD vaccine (Mean±SE)

Age Group	15 days	25 days	35 days
1st	3357.7±209.86	1617.1±65.64	7136±336.49
	B	A	B
2nd	5967.1±576.98	1226.3±71.74	7632.4±363.70
	A	B	A
3rd	2131.1±131.55	1322.7±62.91	6292.2±207.56
	CD	B	B
4th	2293.2±88.58	940.2±32.58	5772.9±264.04
	C	C	B
5th	1533.7±39.43	772.8±34.28	3165.3±99.75
	D	D	C
6th	2651.5±112.82	1491.7±62.02	7936.3±344.31
	BC	A	A

Large litters mean significant differences (p≤0.o5) between groups.

Histopathological finding

Section from 6th group did not revealed any lesion at all ages and as consider normal (fig. 1), on 15 days of age section from bursa of 5th group (control positive) revealed slight to moderate depletion of medulary lymphocyte from bursal follicles (fig. 2) on 25 days of age revealed lesion similar to age 15 days with increase depletion of lymphocyte from bursal follicles (fig. 3)and on 35 days of age revealed market depletion (fig. 4) and present of vaculation with fibroblastic tissue (fig. 5). Same lesion were reported by [48, 49] also these results agreement with [50] who found that low level of aflatoxin in feed which causes depletion and reduction of bursal follicles, these results confirm the immunsupressive effect of aflatoxin in this group fed aflatoxin alone by recording lowest Ab level.

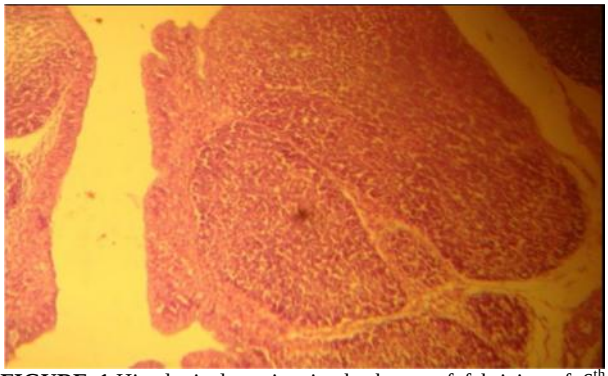

FIGURE 1:Histological section in the bursa of fabricius of 6th group (control negative) showed no clear lesion(H&E stain 10x).

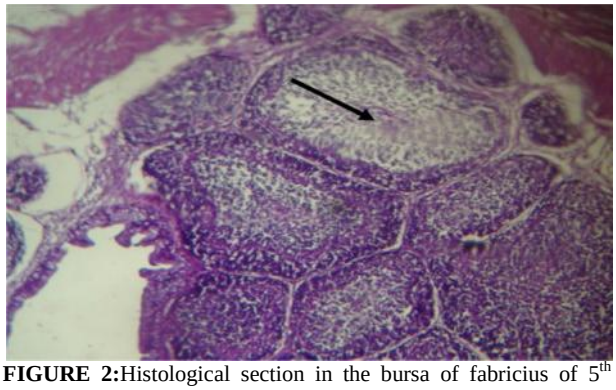

FIGURE 2: Histological section in the bursa of fabricius of 5th (control positive) group at age 15 day old showed slightly to moderate depletion (arrow). (H&E stain 40X).

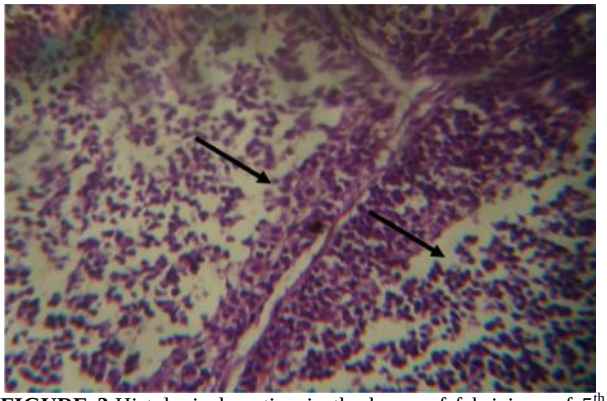

FIGURE 3:Histological section in the bursa of fabricious of 5th group at 25 day old showed marked depletion of lymphocyte of bursal follicles arrow (H&E stain 40X).

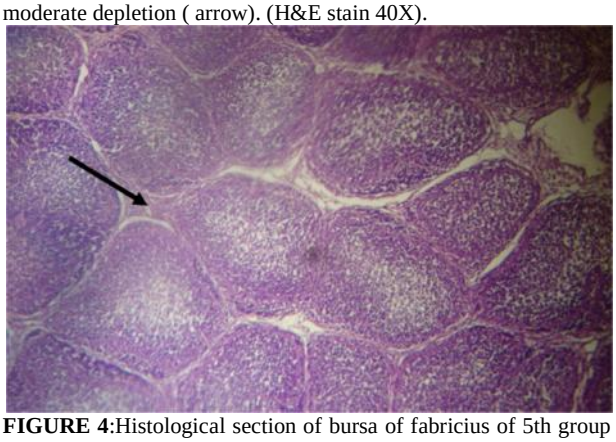

FIGURE 4:Histological section of bursa of fabricius of 5th group at 35 day old showed mild fibrosis with reduction of follicles size arrow (H&E stain 10X).

FIGURE 5: Histological section in the bursa of fabricius of 1st, 2nd , 3rd groups at 15 days showed normal to slight depletion of lymphocyte of bursal follicles arrow. (H&E stain 10X).

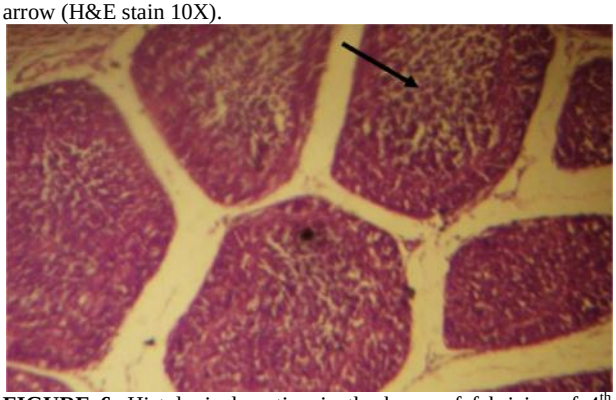

FIGURE 6: Histological section in the bursa of fabricius of 4th group at 15 days showed slight depletion of lymphocyte (H&E stain 10X).

While in 1st, 2nd and 3rd groups on 15 days of age revealed normal to slight depletion (fig. 6) in 5th group revealed depletion more than other groups (fig. 7), on 25 and 35 days of age 1st and 2nd groups revealed hyperplasia of lymphocyte (fig. 8). This lesion showed in 3rd and 4th group but in less degree. These results revealed high reduction effect of aflatoxin on lymphoid organs by using symbiotic (poultry star®), Vit. E and levamezol and in less degree acetic acid and these results agreement with [17] who found that chicks given Vit. E with selenium in combination or alone showed less severe lesion as compared to aflatoxin fed.. Our results also consistent with [51] who found that using symbiotic (protein and inulin) was reduced the histotoxic effect of Mycotoxins on immune organs, and with[47] who reported that pathological effect and toxicity of aflatoxin on immune system were minimized by using acetic acid. The role of levamezol in enhancement of immune response did not come from detoxifying of the aflatoxin, but from immune stimulation and activation of lymphocyte cell. These result is consistent with[15].The highest level of Ab titer in 1st and 2nd group confirmed the protection of immune system from histotoxic effect of aflatoxin. This occurs by decreased depletion of lymphocyte and increased proliferation of lymphocyte. The role of Vit. E and poultry star® represented in detoxification of aflatoin by binding with it and act as immunmodulator or immunstimulation of immune organs [31, 35, 37, 38]. Section from 6th group did not revealed any lesion at all age (fig. 9), while the lesion was showed in 5th group at 15 days of

age illustrated a depletion of lymphocyte, dilted of blood vessels, congestion with hyperplasia of endothial cell [10] same lesion but in less degree was showed in 1st , 2nd , 3rd and 4th groups (fig. 11). On 25 and 35 days of age the lesion shows marked depletion and infiltration of mononeuclear cell in 5th group (fig. 12 and fig. 13).

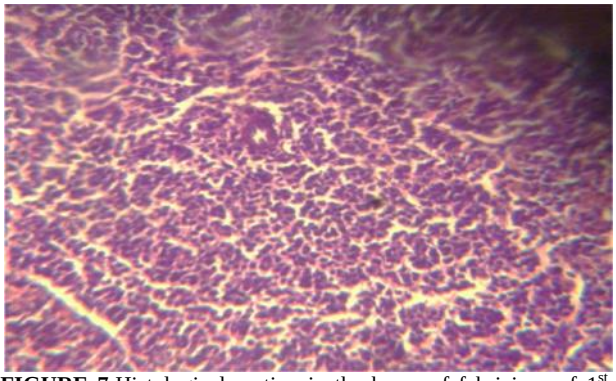

FIGURE 7:Histological section in the bursa of fabricious of 1st and 2nd groups at 25 and 35 day old showed hyperplasia of lymphocyte with formation of germ center (H&Estain 40X).

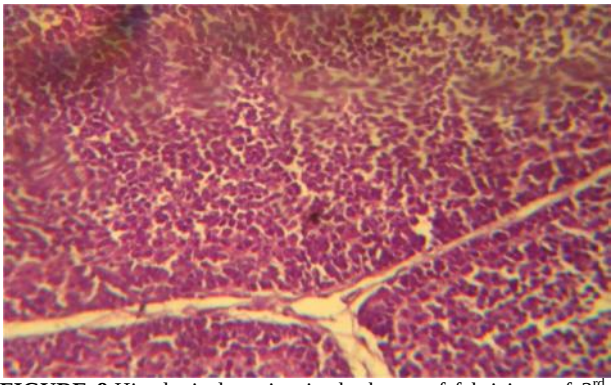

FIGURE 8:Histological section in the bursa of fabricious of 3rd and 4th groups at 25and 35 day old showed hyperplasia of lymphocyte. (H&E stain 40X).

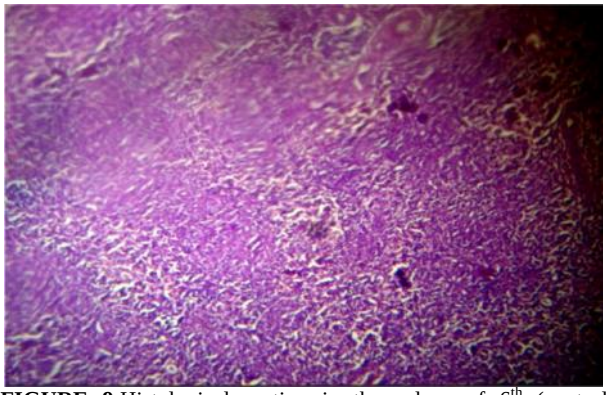

FIGURE 9:Histological section in the spleen of 6th (control negative) showed no clear lesion. (H&E stain 40X).

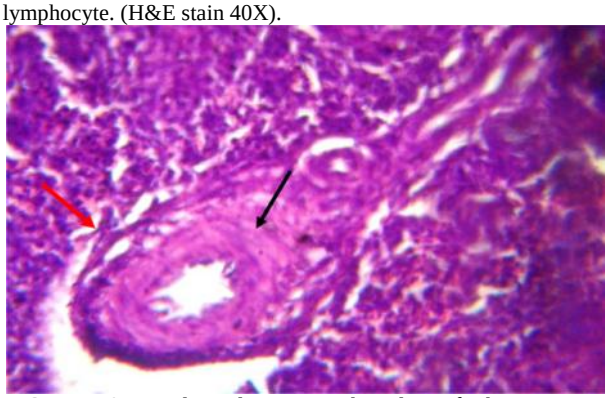

FIGURE 10: Histological section in the spleen of 5th group at 15 day old showed depletion (red arrow), congestion with hyperplasia of endothelial cell (black arrow).(H&E stain 40X).

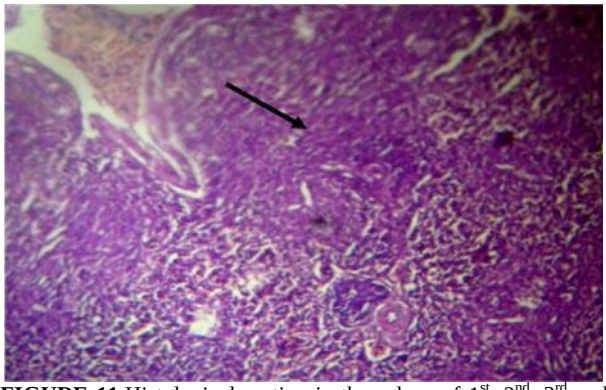

FIGURE 11:Histological section in the spleen of 1st, 2nd, 3rd and 4th groups at 15 day old showed slight depletion arrow.(H&E stain 40X).

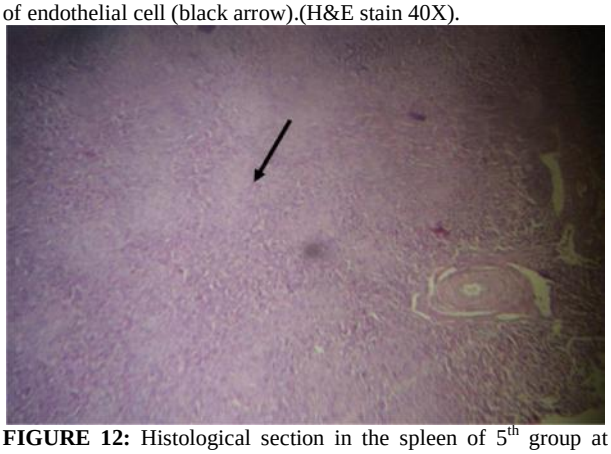

FIGURE 12: Histological section in the spleen of 5th group at 25&35 day old showed marked depletion arrow. (H&E stain 10X).

While in first, second, third and fifth groups the lesion shows slight depletion with infiltration of lymphocyte cell (fig. 14). The present results are in agreement with [52, 53] who showed that aflatoxin affected the organs belonged to the immune and reticuloendithelial system. Also the result was agreed with (54) who confirmed that low level of aflatoxin caused degeneration and necrosis of lymphocyte and this lead to decreased level of Ab titer against IBDV vaccine.

In conclusion: our results confirmed that present of low level of aflatoxin 8ppb caused immuneosupressive by lowering the Ab titer against infectious bursal disease vaccine and caused moderate to marked depletion on lymphoid tissue. Also, the using of Vit. E with selenium and poultry star® could reduce immunsupressive and histotoxic effect of aflatoxin more than levamezol and acetic acid.

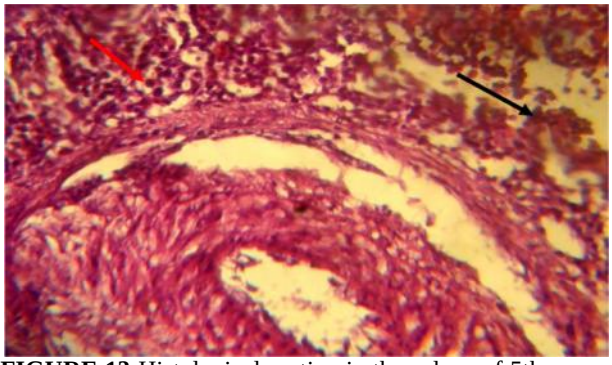

FIGURE 13: Histological section in the spleen of 5th group at 25 &35 day old showed marked depletion (black arrow) and infiltration of mononuclear cell(red arrow).(H&E stain 40X).

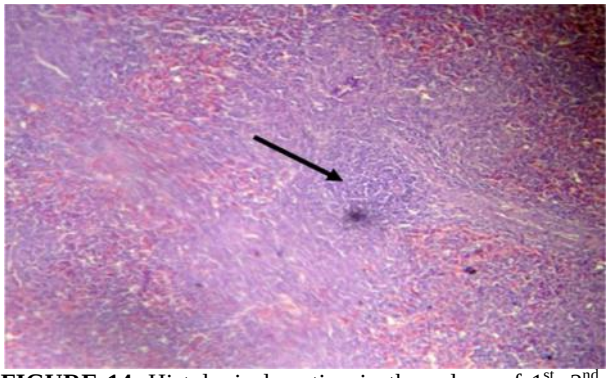

FIGURE 14: Histological section in the spleen of 1st, 2nd, 3rd and 4th groups at 25&35 daye old showed lymphocyte hyperplasia arrow. (H&E stain 10X).

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