

TOXIC AND IMMUNOLOGIC EFFECTS OF FUMONISIN B₁ ON QUAIL AND THE PROTECTIVE ROLE OF LACTOBACILLUS ACIDOPHILUS

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SUMMARY

To investigate the toxic and immunologic effects of fumonisin B₁ (FB₁) on quail and to test whether *Lactobacillus acidophilus* (LBA) could reduce its effects, this study was performed. Quail were divided into four groups. The first, second, and third groups were fed on diets contained 25 ppm FB₁ or 25 ppm FB₁ + 50 ppm LBA or 50 ppm LBA respectively, the fourth group served as a control. This treatment was started from the 2nd to the 6th week of age. Toxic effects of FB₁ were evident by the decrease of body weight gain, food consumption and food conversion and by the histopathological lesions of the intestine, liver, and kidney. Humoral and cell mediated immune responses were also affected. There were significant changes in the haemagglutination inhibition titer, electrophoretic pattern of serum protein, total and

differential leukocytic counts and the relative weights of spleen and bursa. Microscopic examination of both spleen and bursa showed pathological alterations. In the meanwhile LBA not only significantly reduced the toxic and immunosuppressive effects of FB₁ but also improved the nutritional and immune status of the normal birds.

INTRODUCTION

Food and feed contamination by mycotoxins is virtually inevitable particularly in tropic areas. Mycotoxins are unavoidable because they are naturally occurring compounds. They contaminate crops before harvest or invade foodstuffs during processing, transport or storage.

Fusarium species are common soil fungi, now they are recognized as major contaminant of many grains used for poultry diets. They produce a variety of mycotoxins having a biological and toxicological effects on humans and animals. Fumonisin are a class of mycotoxins produced by *Fusarium moniliforme*. Six types of fumonisins were identified, designated as fumonisin B₁, B₂, B₃, B₄, A₁ and A₂. Among them fumonisin B₁ (FB₁) is the most abundant metabolite and of toxicological significance (Norred, 1993) High levels of fumonisin B₁ were detected in commercial corn products obtained from Egypt, India and USA (Theil et al., 1992; Murphy et al., 1993 and Chatterjee and Mukherjee, 1994).

Toxic effects of FB₁ seem to vary with the species and doses. It is implicated as a causative agent in a variety of human and animal diseases including equine leukoencephalomalacia in horses (Wilson et al., 1990 and Sydenham et al., 1992) porcine pulmonary edema in swine (Harrison et al., 1990) hepato and renal toxicities in rats (Gelderblom et al., 1991) Epidemiological studies suggested that fumonisins may be partially responsible for the high incidence of human esophageal cancer (Rheeder et al., 1992). Fumonisin are linked with poor performance in poultry and increase the relative organ weights and mortality. Other toxic effects as myocardial alterations, leg shape deformities and immunosuppression were also documented (Engelhardt et al., 1989; Wu et al., 1991; Ledoux et al., 1992; Brown et al., 1992

and Weibking et al., 1993). The mechanism of action of fumonisins is due to the close molecular structural similarities between these mycotoxins and the natural substrates for the ceramide synthase enzyme (sphinganine and sphingosine). These substrates are highly bioactive compounds, its accumulation inhibit protein kinase C, phosphatidic acid phosphatase and large number of other cell regulatory systems. Fumonisin not only cause accumulation of sphinganine and sphingosine but also block *de novo* synthesis of complex sphingolipids. Sphingolipids have a regulatory pathway in the cells of immune system (Hannun et al., 1986; Wang et al., 1991; 1992 and Merrill et al., 1993).

Probiotic compounds regulate the microbial environment of the intestines. They influence gut pH thus reduce the growth and colonization of pathogenic microorganisms in chicken as *Salmonella* and *E. coli* (Panda et al., 2000 and Tellez et al., 2001). The traditional probiotics are lactic acid bacteria such as *Lactobacillus acidophilus*, *L. casei* and *L. streptococci*. *Lactobacilli* are known potent immunostimulant, directly activate macrophage functions and certain immuno potentiators (Sato, 1984). They inhibited the growth of some molds as *Fusarium*, *Penicillium*, *Aspergillus* and *Monilia* (Corsetti et al., 1998; Patel and Patel, 1998 and Styriak et al., 1998).

Great attention was paid lately towards quails farming as a trial to fulfill excessive demands of

increased population from animal protein. Fumonisin may cause heavy economic losses in quail industry due to its immunosuppressive effect. Immunosuppression subsequently increases the susceptibility to a wide variety of infectious diseases accompanied with failure in the therapeutic drugs and vaccinations.

Therefore the purpose of this research was to investigate and describe the major toxic and immunologic effects of FB₁ on quails fed on diet contained 25ppm from 2-6 weeks of age. In the meantime the prophylactic effect of *Lactobacillus acidophilus* against FB₁ toxicosis was evaluated.

MATERIALS AND METHODS

Materials:

- **Ground *Fusarium moniliforme*:** It was used as a culture material containing 4300, 1380 and 355 mg FB₁, FB₂ and FB₃/ kg was produced by the method described by Weibking et al., (1993) and was provided by National Research Center, Dokki, Cairo, Egypt. This cultural material was added to the commercial diet to obtain the desired level of FB₁ (25ppm). The diet was also contained trace amounts of the less toxic metabolites FB₂ and FB₃ (approximately 7.9 and 1.9 mg/kg respectively).

***Lactobacillus acidophilus*:** It was used as a commercial product under the name Lact-A-Bac Premix. It is tended for water and feed applications. It

contains a live (viable) naturally occurring microorganisms (0.8 billion CFU/g). Application of this product followed the manufacture's instructions (0.5g/kg ration).

- **New Castle disease virus (NDV) vaccine, La Sota Strain:** It was introduced by Intervet International, Holland, as a lentogenic strain has titer of 10⁹ EID₅₀.

- **Experimental birds:** Two weeks old quail chicks of both sexes weighing 20-27 g were obtained from Faculty of Agriculture, Cairo University. They were reared in cages under continuous lighting provided with water and balanced ration *ad libitum*. Ration was composed of 24% protein, 2.7% crude fiber and 3% fat with 3000kcal/kg). To avoid the synergistic effect between fumonisins and other mycotoxins, the diet was analyzed for the presence of some mycotoxins as aflatoxins and ochratoxins to be ensured that they were under the detection limits.

Experiment:

Birds were randomly assigned into 4 groups 60 of each. The first group fed on diet contained 25ppm FB₁. The second group fed on diet contained the same concentration of FB₁ and 0.5g/ kg LBA (50 ppm). The third group fed on diet contained only 50 ppm LBA, while the fourth group served as a control. For each group there were five replicates of twelve chicks.

Symptoms and mortality percent were recorded daily. Birds were weighed on an individual basis and feed consumption for each replicate was recorded. At the 3rd week of age ocular vaccination against ND was carried out on all birds. Every two weeks 10 quails (5 replicates of 2 quails each) from each group were collected and killed by cervical dislocation. Two blood samples were collected. The first sample was taken with addition of anticoagulant for determination of total and differential leukocytic count (Nemi, 1986). The second sample was taken for serum separation to estimate electrophoretic pattern of protein fractionation (Kaplan and Savory, 1965) and to assess the antibody titer against NDV by haemagglutination inhibition (HI) test (Majiyabe and Hitchner, 1977). Sacrificed birds were subjected to postmortem inspection. The relative weights of spleen and bursa of Fabricius were calculated. Tissue specimens of the intestines, liver, kidney, spleen and bursa were taken for histopathological examination (Drury and Wallington, 1980).

Statistical analysis was applied on the obtained data using one-way ANOVA test (Gad and Weil, 1982).

RESULTS AND DISCUSSION

Toxic effects:

Quail fed on diet contaminated with 25ppm FB_1 (gp1) exhibited depression and diarrhea all over the experimental period. Significant reduction in

body weight gains accompanied with significant reduction in feed consumption and conversion rate was recorded. High cumulative mortality percent (30%) was showed (table 1). Similar findings were reported due to FB_1 toxicosis in turkey and broiler chicks (Ledoux et al., 1992 and Kubena et al., 1995 and 1997). Reduction of body weight gains and poor feed conversion may be attributed to diarrhea and inflammation of intestinal mucosa recorded during postmortem inspection and confirmed by histopathological examination of intestines (fig.1), liver and kidney (figs 2 and 3). These lesions were most likely due to the direct irritative properties of FB_1 (Gelderblom et al., 1991). In this respect Wang et al., (1991) and Riely et al., (1993) found that decreased body weight and decreased efficiency of feed utilization due to fumonisin toxicosis were associated with inhibition of protein synthesis and disruption of sphingolipid biosynthesis.

Application of LBA as a probiotic to the contaminated diet (gp2) significantly alleviated the signs of fumonisin toxicosis in growing quail and reduced the mortality percent (16%). Signs of diarrhea and depression were also reduced. *Lactobacillus acidophilus* significantly improved the quail performance as manifested by the increased body weight gains and the rate of feed conversion. Moreover LBA not only increased the rate of absorbability, bioavailability and utilization of nutrients of the stressed birds, but also those of the healthy quail. The statistical analysis of the ob-

ained data showed that the performance of quail given only LBA (gp3) was much better than that of the untreated control. This finding was agreed with the results of Cho et al., (1992); Dhingra (1993) and Manickam et al., (1994). *Lactobacillus acidophilus* regulates the microbial environment of the intestines. Thus it decreases the digestive disturbance and improves the efficiency of feed conversion and increases the growth rate. It increases feed utilization and retards the excretion of endogenous nitrogen, so increases the absorptive capacity of the intestine (Windschitl, 1992 and Bohm and Srour, 1995). Although the actual mechanism of LBA against fumonisins is not known, Wu (1997) speculated that, as LBA im-

proved the colonization of the intestines, the bacterial wall material could bind the toxins and be flushed out as feces thus reducing the amount of toxins absorbed by the bird. Or that live bacteria introduced by LBA in gastrointestinal tract selectively destroy the mycotoxins. He also postulated a possible metabolic regulatory effect of LBA that potentiates the processes of detoxification and excretion of the mycotoxins. This postulation was confirmed in the present study by the mild histopathological lesions of the intestines, liver and kidney recorded in the quails fed on diet contained FB₁ and LBA.

Table (1): Mean values \pm S.E of the quail performance fed on contaminated ration with 25ppm fumonisin B₁ (FB₁) and /or supplemented with 50 ppm *Lactobacillus acidophilus* (LBA) from the 2nd-6th week of age¹.

Groups Paramters	Control	FB ₁	FB ₁ +LBA	LBA
Initial weight (g)	16.3 \pm 4.6 ^a	18.9 \pm 3.2 ^a	17.3 \pm 3.4 ^a	19.5 \pm 2.5 ^a
Final weight (g)	214.2 \pm 20.5 ^a	150 \pm 15.6 ^b	195.6 \pm 20.3 ^c	240.7 \pm 22.8 ^d
Total gain (g)	197.3 \pm 18.4 ^a	131.3 \pm 15.2 ^b	175.9 \pm 17.7 ^{a,b}	222.2 \pm 20.1 ^d
Total feed consumption (g)	490.9 \pm 35.8 ^a	389.4 \pm 29.2 ^b	449.3 \pm 32.3 ^{a,b}	530.9 \pm 39.2 ^{a,c}
Feed conversion ² (g:g)	2.49 \pm 0.04 ^a	2.97 \pm 0.03 ^b	2.57 \pm 0.04 ^c	2.38 \pm 0.03 ^d
Total mortality %	2	30	16	0

¹ Values represent the mean of 5 replicates per each dietary treatment

² Feed conversion = feed: gain ratio

Mean values in each row have different scripts are significantly different at $p < 0.05$.

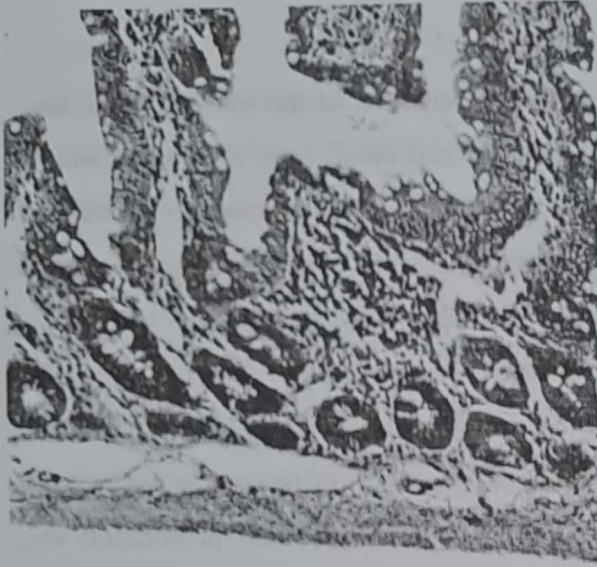


Fig (1): Intestine of quail fed on fumonisin B₁ contaminated ration showing depletion of lymphoid follicles in the lamina propria of mucosal layer (H&E X 40).

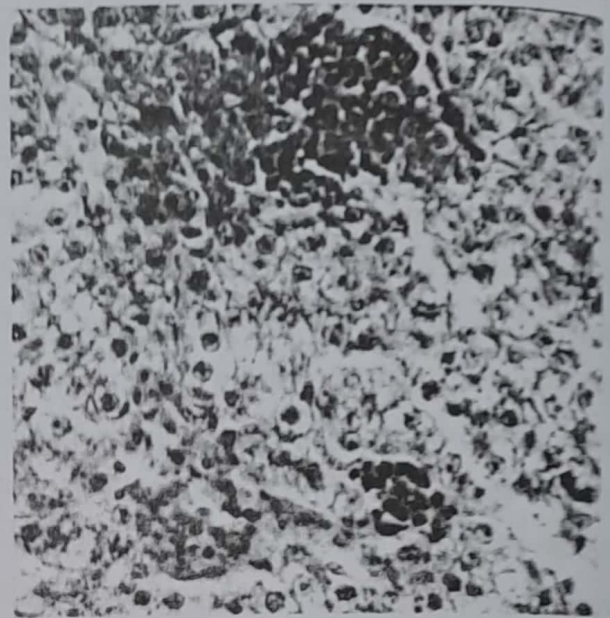


Fig (2): Liver of quail fed on fumonisin B₁ contaminated ration showing focal aggregation of lymphocytes, dilated central vein and intracytoplasmic vacuolar degenerated lymphocytes (H&E X 160).

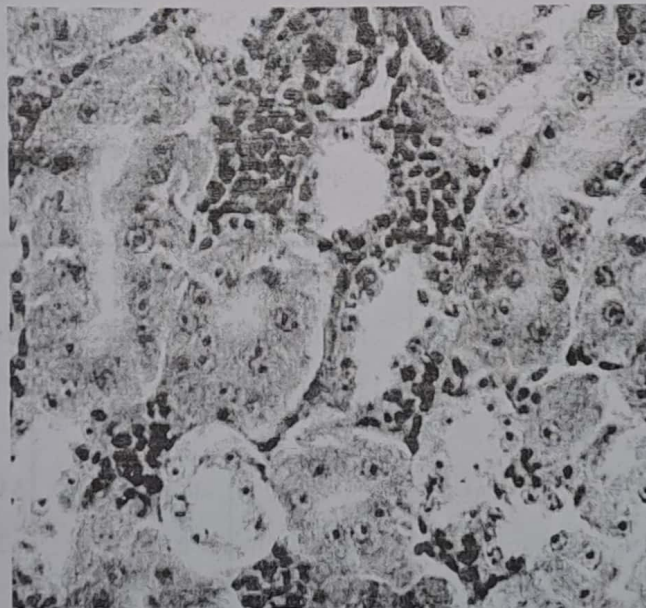


Fig (3): Kidney of quail fed on fumonisin B₁ contaminated ration showing hyperemia and focal extravasated red blood cells in between the degenerated renal tubules (H&E X 160).

Immunologic effect:

The present study showed that FB₁ induced immunosuppressive effect on the treated birds. It decreased the antibody titer against NDV, as measured by HI and increased the relative weights of spleen and bursa (table 2). Fumonisin B₁ is known to irritate the organs thus causing an increase in their relative weights (Gelderblom, et.al., 1991). This effect was established by the histopathological changes recorded in these organs which revealed edema and inflammatory infiltration (figs 4 and 5). Similar increase in the relative organ weights was recorded in chicken and in turkey poults. (Ledoux et al., 1992; Weibking et al., 1993; 1995 and Kubena et al., 1995). Outbreaks of ND were appeared on some quails treated with FB₁ although vaccination was carried

out on the usual scale. This could due to low titer of antibody against NDV. Fumonisin B₁ was the primary cause of the impaired humoral immunity and reduced resistance to ND infection. Birds of this group were in poor physical and nutritional conditions, thus assumed to be more susceptible for many infectious diseases. Decrease of antibody titer could be resulted from inhibition of its synthesis by β -lymphocytes. Concomitant leucocytopenia associated with lymphocytopenia, heterophilia, monocytosis, esinophilia and basophilia were recorded due to FB₁ (table 3). Lymphocytopenia was due to the histopathological effect of FB₁ on bursa and spleen, which are responsible for the humoral and cellular immunity. Lymphocytopenia could explain the diminished antibody response against NDV. Heterophilia and monocytosis were due to bacterial infection, as a

Table (2): Mean values \pm S.E of the relative organ weights and HI titer of quail fed on contaminated ration with 25 ppm fumonisin B₁ (FB₁) and/or supplement with 50ppm *Lactobacillus acidophilus* (LBA) from the 2nd -6th week of age.

Paramters	Weeks	Control	FB ₁	FB ₁ +LBA	LBA
Relative spleen weight (g/100g. B.wt)	2	0.112 \pm 0.03 ^a	0.113 \pm 0.04 ^a	0.112 \pm 0.03 ^a	0.115 \pm 0.03 ^a
	4*	0.111 \pm 0.01 ^a	0.165 \pm 0.02 ^b	0.132 \pm 0.01 ^b	0.113 \pm 0.02 ^a
	6	0.109 \pm 0.01 ^a	0.168 \pm 0.01 ^b	0.131 \pm 0.02 ^c	0.111 \pm 0.01 ^a
Relative bursal weight (g/100g. B.wt)	2	0.27 \pm 0.027 ^a	0.28 \pm 0.025 ^a	0.26 \pm 0.014 ^a	0.25 \pm 0.017 ^a
	4*	0.23 \pm 0.021 ^a	0.35 \pm 0.032 ^b	0.30 \pm 0.013 ^c	0.24 \pm 0.014 ^a
	6	0.23 \pm 0.032 ^a	0.38 \pm 0.031 ^b	0.34 \pm 0.021 ^c	0.26 \pm 0.019 ^a
HI (geometric mean)	2	0.67 \pm 0.058 ^a	0.58 \pm 0.089 ^a	0.63 \pm 0.079 ^a	0.68 \pm 0.073 ^a
	4*	4.5 \pm 0.39 ^a	3.3 \pm 0.28 ^b	4.5 \pm 0.32 ^c	6.5 \pm 0.48 ^d
	6	3 \pm 0.38 ^a	1.5 \pm 0.22 ^b	3 \pm 0.29 ^c	5.5 \pm 0.39 ^d

Mean values in each row have different scripts are significantly different at $p < 0.05$

*Vaccination by NDV vaccine at the 3rd week



Fig (4): Spleen of quail fed on fumonisin B₁ contaminated ration showing focal circumscribed round aggregation of mononuclear leukocytes in diffuse manner all over the splenic tissue with thickening in splenic capsule (H&E X 160).

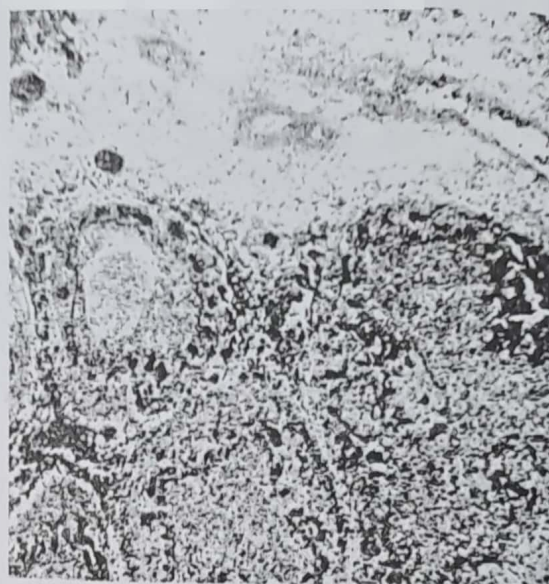


Fig (5): Bursa of quail fed on fumonisin B₁ contaminated ration showing hemorrhage of corticomedullary capillaries, edema and inflammation of interfollicular tissue (H&E X 160).

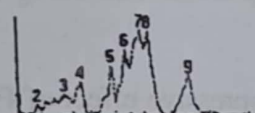
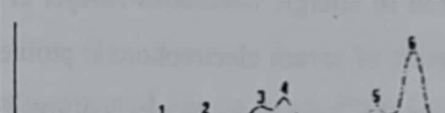
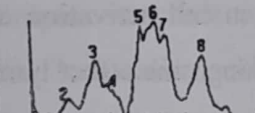
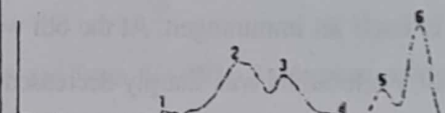
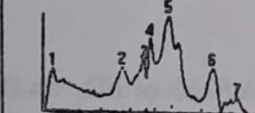

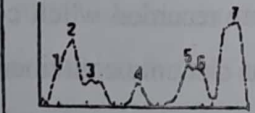

Table (3): Mean values \pm S.E of total and differential leukocytic counts of quail fed on contaminated ration with 25 ppm fumonisin B₁ (FB₁) and / or supplemented with 50 ppm *Lactobacillus acidophilus* (LBA) from the 2nd –6th week of age.

Paramters	Weeks	Control	FB ₁	FB ₁ +LBA	LBA
WBC 10 ³ /mm ³	2	16.5 \pm 0.92 ^a	18.2 \pm 0.73 ^a	17.4 \pm 0.82 ^a	18.8 \pm 1.2 ^a
	4*	18.9 \pm 0.88 ^a	10.4 \pm 1.4 ^b	15.3 \pm 1.2 ^c	22.5 \pm 1.9 ^d
	6	17.3 \pm 1.2 ^a	8.3 \pm 1.3 ^b	14.4 \pm 1.4 ^c	23.4 \pm 1.2 ^d
Heterophils %	2	28.8 \pm 1.9 ^a	31.3 \pm 2.1 ^a	30.4 \pm 2.4 ^a	29.3 \pm 2.4 ^a
	4*	26.6 \pm 1.7 ^a	37.4 \pm 1.3 ^b	31.3 \pm 1.9 ^c	25.6 \pm 1.3 ^a
	6	28.2 \pm 1.1 ^a	38.5 \pm 2.5 ^b	32.2 \pm 2.2 ^c	28.9 \pm 2.1 ^a
Lymphocytes %	2	65.4 \pm 4.2 ^a	63.4 \pm 5.1 ^a	64.3 \pm 4.6 ^a	65.5 \pm 7.3 ^a
	4*	65.3 \pm 3.9 ^a	53.2 \pm 4.4 ^b	59.4 \pm 4.3 ^c	69.4 \pm 3.4 ^a
	6	65.2 \pm 4.3 ^a	53.5 \pm 5.9 ^b	62.6 \pm 3.5 ^a	66.3 \pm 5.2 ^a
Monocytes %	2	2.0 \pm 0.21 ^a	2.1 \pm 0.19 ^a	2.5 \pm 0.23 ^a	2.4 \pm 0.25 ^a
	4*	2.1 \pm 0.19 ^a	3.2 \pm 0.21 ^b	3.1 \pm 0.18 ^b	1.9 \pm 0.21 ^a
	6	2.2 \pm 0.17 ^a	3.5 \pm 0.18 ^b	2.2 \pm 0.22 ^a	2.3 \pm 0.24 ^a
Eosinophils%	2	2.2 \pm 0.31 ^a	2.3 \pm 0.21 ^a	2.3 \pm 0.22 ^a	2.1 \pm 0.33 ^a
	4*	2.5 \pm 0.28 ^a	3.4 \pm 0.18 ^b	2.8 \pm 0.23 ^{a,b}	2.1 \pm 0.29 ^{a,c}
	6	1.8 \pm 0.21 ^a	3.1 \pm 0.19 ^b	2.4 \pm 0.24 ^a	1.6 \pm 0.28 ^{a,c}
Basophils%	2	0.48 \pm 0.051 ^a	0.53 \pm 0.052 ^a	0.54 \pm 0.059 ^a	0.53 \pm 0.041 ^a
	4*	0.52 \pm 0.062 ^a	1.14 \pm 0.049 ^b	0.84 \pm 0.063 ^c	0.48 \pm 0.032 ^a
	6	0.51 \pm 0.073 ^a	1.25 \pm 0.47 ^b	0.63 \pm 0.058 ^a	0.55 \pm 0.051 ^a

Mean values in each row have different scripts are significantly different at $p < 0.05$.

* Vaccination by NDV vaccine at 3rd week.

Table (4): Serum electrophoretic values of quail fed on contaminated ration with 25 ppm fumonisin B₁ (FB₁) and/or supplemented with 50 ppm *Lactobacillus acidophilus* (LBA) at the 4th and 6th week of age.

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Pk: peak
α, β, γ: globulins
Pos: position
T.alb: Total albumin (Postalbumin+albumin)
Ht: height
Amt: amount

sequence of reduced immunity. While eosinophilia and basophilia may be due to the allergic reaction induced by FB₁ as an immunogen. Eosinophils phagocytes antigen-antibody complex, while basophils secrete histamine and heparin, so they are elevated in allergic conditions (Meyer et al., 1992). Result of serum electrophoretic profile of this group at the 2nd and 4th weeks confirmed the antigenic response to FB₁. High level of γ -globulins of IgY and IgM was recorded (table 4) which was not accompanied with high antibody titer against NDV, as measured by HI. This indicates that FB₁ is itself an immunogen. At the 6th week the level of γ -globulins was sharply decreased indicating immunosuppressive effect of FB₁. This result was agreed with the result of Martinova and Merrill (1995) who recorded that FB₁ has diverse effects on the immune system causing stimulation and suppression of the response to foreign antigens and inducing an antigenic response in mice. They detected FB₁-binding immunoglobulins in the animal sera. The electrophoretic pattern of quail fed on diet contained FB₁ also revealed an increase in the amount of β -globulin (transferrin). Serum transferrin transport iron, its level is increased in case of iron deficiency. Iron deficiency may result from its malabsorption due to intestinal lesions recorded in this group. The amount of total albumin was drastically decreased when compared with that of the control and the other experimental groups. This could be resulted from depression of its synthesis by the liver or from its leakage through the glomerular filter or due to

malnutrition (Meyer et al., 1992). *In vitro* and *in vivo* studies, FB₁ showed an immunosuppressive effect and low antibody titers in chicken (Marijanovic et al., 1991; Wu et al., 1991; Qureshi and Hagler, 1992 and Chatterjee and Mukherjee, 1994) and in calves (Osweiler et al., 1993).

Immunosuppressive effect of FB₁ could be attributed to its effect on sphingolipid. Fumonisin cause disruption of sphingolipid metabolism leading to accumulation of sphinganine and sphingosine. Sphinganine inhibits protein kinase C which is involved in cell activation and for B-cells induction. Sphingosine affect lymphocyte migration and proliferation. Fumonisin inhibit the biosynthesis of sphingolipids which are involved in regulation of cell surface receptors, ion pumps and other systems vital for cell functions (Hannun et al., 1986; Wang et al., 1991 and 1992).

The diverse effects of FB₁ on the immune system of the growing quail were minimized by addition of LBA to the contaminated diet (gp 2). Significant difference in the relative weights of spleen and bursa with moderate to mild histopathological changes were recorded when compared with that group fed on diet only contained FB₁. Significantly high antibody titer against NDV was also recorded in this group. An increase in the total number of leucocytic cell count with marked increase in the percentage of lymphocytes was recorded. Eosinophils, basophils and monocytes percent were decreased to the normal values, beside that,

serum electrophoretic profile indicated an improve in the immune status. It was inferred from this result that LBA enhanced both cell mediated and humoral immunity in immunosuppressed birds resulted from fumonisin toxicosis. It has the advantage of regenerating the damaged lymphoid organs.

Lactobacilli directly activate macrophage functions, increase phagocytic activity and secretion of lysosomal enzymes. Moreover, it produces some compounds as organic acids (lactic and acetic acids) and other complex materials as lactolin, bulgarican, acidophilin, lactocidin, acidolin and nisin. All these substances are powerful antibacterial agents and play a role in rising the immune status and protect the host against many infections (Miake et al., 1985; Sato 1984 and Manickam et al., 1994).

Quail fed on diet contained only LBA (gp 3) recorded normal relative weights of spleen and bursa. Histological examination showed increases in the number of follicles with the presence of numerous plasma cell reactions. High and persistent antibody titer against NDV was detected 3 weeks postvaccination. This indicated that LBA improves immune response to vaccination in normal birds. High level of γ - globulins and total albumin were detected by serum electrophoretic analysis.

In conclusion, fumonisin B₁ caused heavy economic losses in quail due to high mortality and

consequences of immunosuppression. Its level in the diet should be paramount concerned. Lactic acid bacteria succeed to overcome the toxic and immunosuppressive state resulted from FB₁.

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REFERENCES

- Bohm, J. and Srour, A. (1995): An Austrian probiotic feed additive for Egyptian Buffalo and cattle production, 3rd Sci. Cong. Egypt Society for cattle diseases, 3-5 Dec.
- Brown, T.P.; Rottinghaus, G.E. and Williams, M.B (1992): Fumonisin mycotoxicosis in broilers: performance and pathology. *Avian Dis.* 36: 450-454.
- Chatterjee, D. and Mukherjee, S.K. (1994): Contamination of Indian maize with fumonisin B₁ and its effects on chicken macrophage. *Lett. Appl. Microbio.* 18: 251-253.
- Cho, K.H.; Lee, U.T.; Yang, C.K.; Ryu, D.Y.; Kim, Y.S. and Yoon, Y.D. (1992): The effect of *L. casei* for growth promotion in broiler chicken. *Korean J. Vet. Public Health.* 16: (1) 55-59.
- Corsetti, A.; Gobbetti, M.; Rossi, J.; Damiani, D. (1998): Antimould activity of sourdough lactic acid bacteria. *Appl. Microbiol. Biotechnol.* 50: (2) 253-256.
- Dhingra, M.M (1993): Probiotics in poultry diet. *Poultry Adviser.* 26 (8): 43-45.
- Drury, R. and Wallington, E. (1980): *Carlton Histological technique.* 4th Ed., Oxford Univ. Press, N.Y. pp. 25-32.

- Engelhardt, J.A.; Carlton, W.W. and Tuite, J.F. (1989): Toxicity of *Fusarium moniliforme* var. *subglutians* for chicks, duckling and turkey poults. *Avian Dis.* 33: 357-360.
- Gad, S. and Weil, C. (1986): Statistics for toxicologist. In Hayes, A.W. (ed.) *Principles and Methods and Toxicology*. 2nd ed. Raven Press, N.Y, pp. 273-360.
- Gelderblom, W.C. ; Krick, N.P.; Marasas, W.F. and Theil, P.G. (1991): Toxicity and carcinogenicity of *Fusarium moniliforme* metabolite, fumonisin B₁, in rats. *Carcinogenesis*, 12: 1247-1251.
- Hannun, Y.; Loomis, C.R.; Merrill, A.H. and Bell, R.M. (1986): Sphingosine inhibition of protein kinase C activity and of phorbol dibutyrate binding in vitro and in human platelets. *J.Biol.Chem.* 261: 12604-12609.
- Harrison, L.R.; Colvin, B.; Greene, J.T.; Newmon, I.E.; and Cole, J.R. (1990): Pulmonary edema and hydrothorax in swine produced by fumonisin B₁, a toxic metabolite of *Fusarium moniliforme*. *J.Vet. Diagn. Invest.* 2: 217-221.
- Kaplan, A. and Savory, J. (1965): Evaluation of a cellulose acetate electrophoresis. *Clin.Chem.* 11: 937-941.
- Kubena, L.F; Edrington, T.; Harvey, R. and Buckley, S. (1997): Individual and combined effects of fumonisin B₁ present in *Fusarium moniliforme* culture material and T-2 toxins or deoxynivalenol in broiler chicks. *Poultry Sci.* 67: 1239-1247.
- Kubena, L.F; Edrington, T.; Kamps, C. and Harvey, R. (1995): Effect of feeding fumonisin B₁ present in *Fusarium moniliforme* culture material and aflatoxin singly and in combination to turkey poults. *Poultry Sci.* 74: 1295-1303.
- Ledoux, D.R.; Brown, T.P.; Weibking, T.S. and Rottin-ghaus, G.E. (1992): Fumonisin toxicity in broiler chicken. *J.Vet. Diagn. Invest.* 4: 330-333.
- Majiyabe, K and Hitchner, S.B. (1977): Antibody response to strain combination of New Castle disease virus as measured by haemagglutination inhibition. *Avian Dis.* 21: 576-584.
- Manickam, R.; Viswanathan, K. and Mohan, M. (1994): Effect of probiotics in broiler performance. *Indian Vet. J.* 737-739.
- Marijanovic, D.R.; Holt, P.; Norred, W.P.; Bacon, C.W.; Vosse, K.A. and Stancel, P.C. (1991): Immunosuppressive effects of *Fusarium moniliforme* corn cultures in chickens. *Poultry, Sci.* 70: 1895-1901.
- Martinova, E.A. and Merrill, A.H (1995): Fumonisin B₁ alters sphingolipid metabolism and immune function in BALB/c mice: Immunological responses of fumonisin B₁. *Mycopathologia*, 130: 163-170.
- Merrill, A.H. ; Van Echten, G.; Wang, E and Sandhoff, K. (1993): Fumonisin B₁ inhibits sphingosine (sphinganine) N-acetyltransferase and *de novo* sphingolipid biosynthesis in cultured neurons *in situ*. *J. Bio.Chem.* 268: 27299-27306.
- Meyer, D.; Coles, E. and Rich, L. (1992): *Veterinary laboratory Medicine. Interpretation and diagnosis.* W.B. Saunders CO., Philadelphia, P.122.
- Miake, S.; Nomoto, K.; Yokokura, T.; Yoshikai, Y.; Mutai, M. and Nomoto, K (1985): Protective effect of *L. casei* on *Pseudomonas aeruginosa* infection in mice. *Infection and Immunity* 48: (2) 480-485.
- Murphy, P.A.; Rice, L.G. and Ross, P.E. (1993): Fumonisin B₁, B₂ and B₃ content in Iowa, Wisconsin and Illinois

- corn and corn screenings. *J. Agric. Food Chem.* 41: 263-266.
- Comi, C.J. (1986): Schalm's Veterinary Hematology. 4th ed., Lea and Febiger, Philadelphia, pp 14-19.
- Norred, W. P. (1993): Fumonisin: Mycotoxins produced by *Fusarium moniliforme*. *Fundam. Appl. Toxicol.* 10: 2-19.
- Dewiler, G.D.; Kehrt, M.E.; Stabel, J.R. and Wilson, T. M. (1993): Effect of fumonisin contaminated corn screenings on growth and health of feeder calves. *J. Anim. Sci.* 71: 459-466.
- Panda, A.K.; Reddy, M.R.; Rao, S.V.R.; Raju, M.V.L.N. and Praharaj, N. K. (2000): Growth, carcass characteristics, immunopetence and response to *Escherichia coli* of broilers fed diet with various levels of probiotic. *Archiv. Fur. Geflugelkunde.* 64: (4) 152-156.
- Patel, S.M and Patel, B.R. (1998): Growth inhibition of *Fusarium oxysporum* by milk bacteria. *Ann. Plant Protec.Sci* 6: (1) 97-99.
- Qureshi, M.A. and Hagler, W. M. (1992): Effect of fumonisin B1 on chicken macrophage functions *in vitro*. *Poultry Sci.* 71: 104-112.
- Rheeder, J.P; Marasas, W.F.; Theil, P.G.; Sydenham, E.W. Shephard, G.S and Van Schalkwyk, D.J. (1992) : *Fusarium moniliforme* and fumonisins in corn in relation to human esophageal cancer in Transkei, *Phytopathology*, 82: 353-357.
- Riely, R.T.; Norred, W.P. and Bacon, C.W. (1993): Fungal toxicity in foods: Recent concerns. *Ann. Rev. Nutr.* 13: 167-189.
- Sato, K. (1984): Enhancement of host resistance against *Listeria* infection by *Lactobacillus casei* : Role of macrophages. *Infection and Immunity* 44: (2) 445-451.
- Styriak, I.; Conkova, E.; Laciakova, A.; Bohm, J. (1998): Prevention of fumonisin production by microorganisms Czech. *J. Animal Sci.* 43(10) 449-452.
- Sydenham, E.W; Marasas, W.F.; Shephard, G.S.; Thiel, P.G and Hirooka, E. Y. (1992): Fumonisin concentration in Brazilian feeds associated with field outbreaks of confirmed and suspected animal mycotoxicosis. *J Agric. Food Chem.* 40: 994-997.
- Tellez, G.; Petrone, V.M.; Escorcia, M.; Morishita, T. Y.; Cobb, C.W. and Villasenor, L. (2001): Evaluation of avian - specific probiotic on cecal colonization and organ invasion of *Salmonella enteritidis* in broilers. *J. Food Protection*, 64: (3) 287-291.
- Theil, P.G.; Marasas, W.F.; Sydenham, E.W.; Shephard, G.S. and Gelderblom, W.C. (1992): The implication of naturally occurring levels of fumonisins in corn for human and animal health. *Mycopathologia* 117: 3-9.
- Wang, E.; Norred, W.P.; Bacon, C.W.; Riely, R.T. and Merrill, A.H. (1991): Inhibition of sphingolipid biosynthesis by fumonisins: Implications for diseases associated with *Fusarium moniliforme*. *J. Biol. Chem.* 266: 14486-14490.
- Wang, E.P.; Ross, P.F.; Wilson, T.M.; Riely, R.T. and Merrill, A. H. (1992): Increases in serum sphingosine and sphinganine and decreases in complex sphingolipids in ponies given feed containing fumonisins, mycotoxins, produced by *Fusarium moniliforme*. *J.Nut.* 122: 1706-1716.
- Weibking, T.S.; Ledoux, D .R.; Bermudez, A.J; Turk, J.R and Rottinghaus, G.E. (1995): Effects on turkey poult of feeding *Fusarium moniliforme* M-1325 culture

- material grown under different environmental conditions. *Avian Dis.* 39: 32-38.
- Weibking, T.S.; Ledoux, D.R.; Brown, T.P. and Rottenghaus, G.E. (1993): Fumonisin toxicity in turkey poult. *J. Vet. Diagn. Invest.* 57: 75-83.
- Wilson, T.M.; Ross, P.E.; Rice, L.G.; Osweiler, G.D.; Nelson, H.A.; Owens, D.L.; Plattner, R.D.; Reggiardo, C.; Noon, T.H. and Pickrell, J.W. (1990): Fumonisin B₁ levels associated with an epizootic of equine leukoencephalomalacia. *J. Vet. Diagn. Invest.* 2: 213-216.
- Windschitl, P.M (1992): Effect of probiotic supplementation of hullless barely and corn based diets on bacterial fermentation in continuous culture of ruminal contents. *Can J. Animal sci.* 72: 265-269.
- Wu, W. (1997): Counteracting *Fusarium proliferatum* toxicity in broiler chicks by supplementing drinking water with Poultry Aid Plus®. *Poultry Sci.* 76: 463-468.
- Wu, W.; Cook, M.E. and Sanalley, E.B. (1991): Decreased immune response and increased incidence of tibial dyschondroplasia caused by *Fusaria* grown on sterile corn. *Poultry Sci.* 70: 293-301.