Effect of *Saccharomyces boulardi* on Afflation and Ochratoxin Toxicosis in Broilers: Haemato-Biochemical Observations

P. V. Meshram[1], R.B. Ambade[2], S.Y. Shirale[3] and S.S.Chavan[4]

1,2,3&4. Bombay Veterinary College, Parel, Mumbai MAFSU Nagpur

Corresponding author e-mail:

Corresponding author e-mail: pramodvetpath@gmail.com

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ABSTRACT

Our hundred and twenty, day old boiler chicks were randomly divided in to four groups viz. Group A (control), B (received aflatoxin @ 0.5 ppm and ochratoxin 0.5 ppm in diet), C (normal diet with probiotic, Sacchromyces boulardi @ 10 mg/ kg feed) and D (toxins as in group B with probiotic as in group C), containing 30 birds each, for a period of 6 weeks. Significant decrease in hemoglobin and PCV was observed in toxin fed birds along with serum proteins, albumin, globulin and A: G ratio when compared to the controls. Serum creatinine and SGPT levels were high in toxin fed birds. The genomic mean of antibody titers organist New Castle Disease were significantly low in toxin fed birds indicating immunosuppression. Sacchromycesboulardi appeared to act as probiotic and helped in combating the adverse effects of toxins, when compared with the haematobiochemical parameters.

Key words: *Aflatoxicocis; ochratoxicosis; broilers; probiotic (Sacchromyces_Boulardi);*

Broiler industry in India have poised a major breakthrough and occupied a respectable position with a phenomenal expansion. The primary challenge before the industry is the control of diseases. In recent years, health hazards due to mycotoxicoses are posing major threat to the poultry industry. These are large and diverse group of toxic metabolic byproducts, formed by moulds and fungi, growing on grains and feed causing diseases when ingested. There are about 100 species of fungi known to produce mycotoxins (*Das, 1992*). So far, more than 350 different mycotoxins have been identified as being present in the nature, though all are not harmful to poultry *(Harris, 1998).*

Food grains getting damaged are common in India due to poor handling and storage facilities, high temperature and humidity. Aflatoxins and ochratoxins are the toxic compounds produced by various species of ubiquitous genera, Aspergillus and Penicillium. The toxicopathological spectrum of AFB1 and OA is very wide, encompassing different kinds of toxicities as acute, chronic, carcinogenicity, teratogenicity and immunotoxicity. AFB1 is primarily hepatotoxic, whereas OA is primarily nephrotoxic. They have significant impact on economic returns due to decreased productivity, immunosuppression and increased mortality due to secondary infections.

Fungal contamination of agricultural products and also the poultry feed is often unavoidable and is of worldwide concern. The quality of feed plays an important role in the performance of broilers. Dual occurrence of aflatoxin and ochratoxin in poultry diets is a severe threat as they interact synergistically to become more toxic when they occur as simultaneous contaminants of poultry diet (*Huff and Doerr, 1982*)

To control the toxic effects of these mycotoxins, several physical, chemical and biological methods are used viz., storing cereals or feed at moisture content less than 15%, gamma irradiation, use of various toxin binders as Hydrated Sodium Calcium Alluminosilicate (HSCAS), zeolite, bentonite, Mannon Oligosaccharide (MOS), activated charcoal etc. Use of probiotics in disease control and prevention, is gaining importance. They exert a beneficial effect via wide variety of actions. *Sacchromyces_boulardii* is a type of yeast species blessed with multifaceted probiotic activity via their antagonistic action against intestinal pathogens.

Studies indicated that *Sacchromyces_boulardii* secrets "Protease" a toxin binding enzyme, which bind the toxin receptors on the epithelial cells of gastrointestinal tract and enzymatically modifies them. Literature on the efficacy of *Sacchromyces_boulardii* in combating mycotoxicoses in poultry is meager. Hence the present studies were undertaken to evaluate the efficacy of *Sacchromyces_boulardii* in broilers induced with combined aflatoxin and ochratoxin toxicities in combination, through haemato-biochemical parameter.

METHODOLOGY

One day old, one hundred and twenty broiler chicks were randomly divided in four groups as A, B, C and D, with 30 chicks each. Group A served as control and was given normal broiler diet. Group B received the diet containing aflatoxin @0.5 ppm and ochratoxin @ 0.5 ppm in combination. Group C was given normal diet supplemented with *Sacchromyces_boulardii* premix @10.0 mg/kg feed, while group D received toxins as in group B in a supplemented diet as in group C. All the birds were maintained as per the standard procedure adopted at the farm, uniformly with routine health and management practices. Six birds from each group were sacrificed on day 4th, 28th and 42nd of the experiment. Blood was collected via cardiac puncture in 1% EDTA for haematological studies and in sterile test tubes for serum, making its slant and separated in sterile vials for biochemical and immunological testes and stored at -20°c for further use. Blood was analyzed for hemoglobin (Sahli's method) and PCV (microhaematocrit tube method). Biochemical estimations, viz., serum proteins and albumin (BCG and dye binding method), SGPT (Reitmen and Frankels method) were conducted using commercial reagent kits (Qualigen fine chemicals). Humoral immune response was evaluated by using Beta procedure of haemaglutination inhibition (*Allen and Gough, 1974*) using Luxbro 'U' micro titration plates and 25 microlitre dropping pipettes against Ranikhet (New Castle) Disease.

The data generated from various parameters was subjected to statistical analysis using completely randomized design (*Snedecor and Cochran, 1980*) (www.icargoa.res.in) and using CD values compared the treatment means.

RESULTS AND DISCUSSION

Mean ± SE values of the Hb, PCV, serum proteins, albumin, globulin, A:G Ratio, serum creatinine, SGPT are depicted in Table 1 and HI antibody titers in Table 2.

Mean ± SE values of hemoglobin and PCV were significantly low in toxin fed birds of group B and D as

Table: 1.Values of mean ± SE Haemato-biochemical parameters at different interval of study.

Parameters of the study	Days and Groups of experiment											
	Day 14				Day 28				Day 42			
	A	B	C	D	A	B	C	D	A	B	C	D
Hemoglobin	8.8^{b}	7.2^{a}	8.9^{b}	7.3^{a}	8.8	8.0	9.9	8.1	9.9^{b}	7.1^{a}	10.1^{b}	7.1^{a}
(g%)	± 0.33	± 0.30	± 0.41	± 0.25	± 0.73	± 0.41	± 0.62	± 0.34	± 0.35	± 0.29	± 0.18	± 0.30
PCV	27.76^{c}	21.53^{a}	27.98^{c}	23.08^{b}	29.08^{b}	24.16^{a}	29.63^{b}	25.13^{a}	30.21^{c}	24.25^{a}	30.70^{c}	26.83^{b}
(%)	± 0.42	± 0.63	± 0.21	± 0.36	± 0.38	± 0.62	± 0.59	± 0.38	± 0.56	± 1.12	± 0.65	± 0.73
Serum Proteins	1.48^{a}	1.10^{b}	1.55^{a}	1.17^{b}	2.07^{c}	1.62^{a}	2.12^{c}	1.81^{b}	2.02^{b}	1.73^{a}	2.09^{b}	1.95^{b}
(g% dl)	± 0.04	± 0.05	± 0.04	± 0.08	± 0.03	± 0.04	± 0.03	± 0.06	± 0.06	± 0.07	± 0.05	± 0.02
Serum Albumin	0.87^{bc}	0.62^{a}	0.92^{c}	0.67^{ab}	0.96^{b}	0.68^{a}	1.00^{b}	0.78^{a}	0.97	0.79	1.05	0.92
(g% dl)	± 0.051	± 0.066	± 0.062	± 0.058	± 0.044	± 0.045	± 0.037	± 0.043	± 0.080	± 0.069	± 0.053	± 0.056
Serum Globulin	0.61^{ab}	0.48^{a}	0.63^{b}	0.50^{a}	1.10	0.94	1.12	1.03	1.04	0.94	1.04	1.03
(g% dl)	± 0.009	± 0.028	± 0.064	± 0.044	± 0.055	± 0.070	± 0.043	± 0.046	± 0.052	± 0.065	± 0.084	1 0.046
A : G	1.42	1.30	1.47	1.33	0.87	0.72	0.90	0.76	0.93	0.84	1.01	0.89
Ratio	± 0.097	± 0.203	± 0.308	± 0.159	± 0.090	± 0.104	± 0.063	± 0.048	± 0.119	± 0.120	± 0.145	± 0.083
Serum Creatinine	0.34^{ab}	0.46^{c}	0.30^{a}	0.38c	0.51^{a}	0.69^{b}	0.46^{a}	0.54^{a}	0.52^{a}	0.72^{b}	0.54^{a}	0.68^{b}
(mg/dl)	± 0.019	± 0.016	± 0.014	± 0.010	± 0.053	0.067	± 0.027	± 0.044	0.030	± 0.046	± 0.030	0.030
SGPT	18.00	21.50	18.50	19.83	23.16^{a}	33.50^{b}	25.5^{a}	34.16^{b}	29.83^{a}	40.16^{c}	27.83^{a}	36.66^{b}
(kg/ml)	1.54	1.28	1.80	1.74	1.35	1.38	1.43	1.01	0.90	0.65	0.87	0.66

Values (n=30; Mean ± SE) in the same row bearing at least one common superscript do not vary significantly (P d≤0.05).

Table: 2. Average geometric means of antibody titers.

Days of Experiment	Groups of experiment A	B	C	D
Day 14	20	14	18	16
Day 28	23	12	24	15
Day 42	17	10	17	12
Average	20 [b]	12 [a]	19.66 [b]	14.33 [a]

Values in the same row bearing at least one common superscript do not vary significantly (P d≤0.05).

compared to those in groups A and C at 14th 28th and 42nd day of experiment. On 28th day those of Hb were non significantly low. Slight improvement in values due to probiotics in groups C and D was observed as an effect of probiotic over the period. Reduced values of Hb due to mycotoxicoses were reported by *Huff et al,(1979,1988), Mishra (1999), Prakash (2001)* and *Sawale (2007)*. Decrease in hemoglobin might be due to anaemia developed as a result of depressed erythropoiesis by combined effect of aflatoxin and ochratoxin. Adverse effect of toxin, especially ochratoxin, might had resulted in to renal damage and hampered the formation of erythropoietin and protein synthesis due to hepatopathy.

Higher Hb concentration in birds of group D indicated the beneficial effect of Sacchromycesboulardi, due to less absorption of toxin as described earlier and effect as probiotic. Significant decrease in packed cell volume due to mycotoxicoses were earlier reported by *Reddy et al,(1984), Mishra (1999)* and *Kurkure (2002)* which supported the observations during the present studies.

Lowered PCV might be due to anaemia as a result of defective protein metabolism due to hepatopathy and low erythropoietin due to nephropathy.

Hb, PCV, total serum proteins, albumin, globulin and A: G ratio in groups B and D were low as compared to those in groups A and C. The levels were slightly high in group D than in group B indicating the beneficial effect of the probiotic. Reduction in serum proteins could be due to inhibition of protein synthesis, following competing by mycotoxins with phenylalanine for binding site on phenylalanine transfer RNA synthetase enzyme (*Creepy et al,1979*), leakage of albumin due to renal lesions, induced by the toxins (*Huff et al,1988*) and hepatopathy.

Improvement in all there parameters in group D, as compared to Group B, was probably due to less absorption of toxin. Increased values in group C than in Group A indicated increased protein synthesis as an effect of probiotic *Sacchromyces boulardii.*

Sebum creatinine levels were siguificantly high in toxin fed birds of group B and D as compared to corresponding groups A and C over the period of experiment. The levels in birds of group D were low than in group B. Group C supplemented with medicine resulted in low serum creatinine levels as compared to control birds of group A.

Increased levels of creatinine, due to renal involvement, were reported earlier by *Huff et al (1979), Prakash (2000), Sakhare (2001)* and *Bhanuprakash (2002)* due to simultaneous and combined effect of aflatoxin and ochratoxin as observed in the present study.

Increased in sebum creatinine concentration in toxin fed birds, might be due to nephrotoxic action of mycotoxins, particularly OA, which causes renal impairment by destruction of epithelial cells of proximal and distal convoluted tubules and tubular damage causing excretion of toxin via kidney. The histological lesions in kidneys also supported the findings.

Decreased levels of serum creatinine, in birds of group D, as compared to toxin fed group B, indicated moderate improvement due to low adsorption of toxin.

The serum glutamate pyruvate transaminase (SGPT) levels were significantly high in toxin fed birds of group B and D on 28th and 42nd day of experiment and non significantly high on 14th day , than in the corresponding control groups. Probiotic supplementation revealed an improvement with low levels in group D then in B.

Increased SGPT levels in mycotoxicosis were reported earlier by *Rainaet al (1991), Singh et al, (1994)* and *Prakash (2001)*. The high SGPT levels were attributed to the damage of hepatocytes and subsequent leakage of enzymes (*Dafalla et al, 1987*). The damage to hepatocytes is due to adversary effect of toxic metabolites of mycotoxins, which acts over its nucleus and subsequently distracts the DNA. The histological legions in liver supported these findings.

Low SGPT levels of toxin administered and probiotic, *Sacchromyces boulardii,* treated group D, indicated the reduction of adverse effect due to low absorption of toxin. The SGPT level in control with medicine remained high, though not significantly, which could not be explained and needs further elucidation.

The average geometric means of antibody titers as depicted in Table no 2 of mycotoxicosis induced birds of groups B and D were significantly low than the corresponding groups A and C. Though the probiotic supplementation improved the titers in infected group (D over B) the effect was not seen in controls (C over A).

Suppression of humoral immune response to New castle disease virus in mycotoxicosis had been reported by Sakhare*et al* (2002) with aflatoxin @ 0.2 PPM and ochratoxin @ 0.2 PPM in combination. *Ramadevi et al, (1993)* and *Pathak (2002)* also reported the reduced antibody titers.

Immunosupressi on observed in the present study could be due to inflammation and depletion of lymphoid organs as the mycotoxins enter the nucleus of the lymphocytes, causes damage to DNA and depletion of lymphocytes as described earlier.

CONCLUSION

Thus, it could be concluded from the present study that the combined dose of toxins (AF and OA) caused significant reduction in hemoglobin, packed cell volume, decrease in serum proteins, albumin, globulin and A:G ration and increase in concentration of serum glutamate pyruvate transaminase and creatinine. It caused remarkable immunosuppression, resulting in inadequate response to vaccination as indicated by reduced values of HI titers against NDV in infected groups. Serum biochemical profiles are good indicators of heath status of liver and kidneys in birds.

REFERENCES:

Allen, W. H. and Gough, R. E. (1974).A standard of haemaglutination inhibition test for New Castle Disease, a comparison of macro and micro methods. *Vet. Rec.*, **95**:120-123.

Bhanuprakash, C. V. S. (2002). Pathology of experimental Aflatoxicosis, ochratoxicosis and assessment of the efficacy of a toxin binder in broiler chickens. M.V.Sc. Thesis, U.A.S., Bangalore.

Creepy, E. E., Lungnier, A.A.J., Fasiolo, F., Heller, K.,Roschenthaler, R. and Dirheimer, G. (1979).In vitro inhibition of yeast phenyl alanylt RNAsynthetase by ochratoxin A Chem. *Biol. Interact.* **24**: 257-64.

Dafalla, R., Yogi, A. I. and Adam, S.E.I. (1987).Experimental Aflatoxicosis in hybridized chicks. Sequential changes in growth and serum constituents and histo-pathological changes. *Vet. Hum. Toxicol.* **29**:222-226.

Huff, W.E., Doer, J.A. and Hamilton, P.B. (1979). Decreased glycogen mobilization during ochratoxicosis in broiler chickens.*Appl. and Environ. Micro.* **37***:122-126*

Huff, W.E., Kubena, L.F. and Harvey, R.B. (1988). Progression of ochratoxicosis in broiler chickens. *Poultry Sci.* **67**:1139-1136.

Kurkure, N.V. (2002). Study on pathology of Aflatoxicosis in broilers."Ph.D. Thesis, Maharashtra Animal and Fishery Sciences University, Nagpur.

Mishra, C.S. (1999). Pathology of fungal and bacterial infested feed in broilers: Effect of mycotoxin binder and hepatoprotectant. M.V.Sc. Thesis, KonkanKrishiVidyapeeth, Dapoli.

Pathak, M.A (2002).Evaluation of neutralizing activity of herbal extracts during simultaneous mycotoxicosis in broilers. M.V.Sc. Thesis, M.A.F.S.U., Nagpur.

Prakash, G.C. (2001). Pathology of combined effects of aflatoxin. M.V.Sc. Thesis. U.A.S., Bangalore.

Raina, J.J. and Singh, B. (1991).Prevalence and pathology of mycotoxicosis in poultry in Punjab. *Indian J. anim. Sci.,* **61**:671- 676.

Ramadevi, V., Naidu, N.R.G. and Ravikumar, P. (1993). An assessment of protective effect of Bentonite on ochratoxicosis in broilers with reference to certain haematochemical profiles. *Indian Vet. J.,* **77**:303-306

Reddy, N.D., Rao, P.V., Reddy, V.R.andYadgalli, B.(1984). Effect of selected levels of dietary aflatoxin on the performance of broile chickens. *Indian J. Anim. Sci.,* **54**:68-73.Sakhare, P.S (2001).Evaluation of herbal product during Aflatoxicosis and ochratoxicosis in broilers. M.V.Sc. Thesis. P.D.K.V., Akola.

Sakhare, P.S., Harne, S, D., Kalorey, D.R., Warke, S. Bhandarkar, A.G. and Kurkure, N.V. (2002).Immunopathology of individual and simultaneous mycotoxicosis in broilers. Compendium of XIX Annual Conference of I.A.V.P., Palampur, 6.15

Sawale, G.K (2002).Studies on ameliorating affect of arbomineral toxin binders I aflatoxin and ochratoxin in layers. M.V.sc. Thesis, I.G.K.V.V., Raipur.

Singh, H., Sandhu, B.S. and Singh B. (1994).Prevalence and pathology of mycotoxicosis in Poultry".*Indian J. Poult. Sci.* **29:**101-103.

Values (n=30; Mean ± SE) in the same **row** bearing at least one common superscript do not vary significantly (P d" 0.05).

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