

Effect of probiotic containing *Saccharomyces boulardii* on experimental ochratoxicosis in broilers: hematobiochemical studies

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In the present investigation, the toxicopathological effects of ochratoxin A of 0.5 ppm on hematobiochemical parameters of broilers were studied with efficacy of dietary concentration of probiotic containing yeast culture *Saccharomyces boulardii* of 10 mg/kg of feed. One hundred twenty day old chicks were randomly divided into four groups, thirty chicks each. Groups A and C chicks were offered normal feed and that added with probiotic *Saccharomyces boulardii* respectively. The birds in group B were fed ochratoxin A of 0.5 ppm of feed. Where as, the birds of group D, were fed with ochratoxin A of 0.5 ppm along with probiotic *Saccharomyces boulardii* of 10 mg/kg of diet. Hematological studies carried revealed significant decrease in the haemoglobin and packed cell volume in birds of group B and reduced effect in birds of group D due to probiotic. Biochemical profiles revealed significant improvement in probiotic treated group D when compared with decreased values of Total protein, albumin, globulin and increased levels of serum creatinine and SGPT in birds of groups B.

Key words: *Saccharomyces boulardii*, ochratoxin A, hematological, biochemical

Introduction

In India, poultry industry has developed leaps and bound from a small-scale backyard venture to the status of full-fledged, modernized, agro-based industry. India ranks 4th in egg production and 19th in broiler production with annual turnover of Rs. 65 billion [5]. One of the most effective ways for a profitable poultry industry is to reduce the input cost. Feed is the major input in poultry production constituting 70-75% of total cost of broiler production. Poor quality or damaged feed may results in poor production and discarding of such feed will be additional monetary loss.

The mycotoxins are considered as serious obstacle in

realizing the full genetic potential of the poultry. Several species of fungi infect grain and forage crops growing in the field, during harvest, transportation and while in the storage and produces mycotoxins. More than 300 different types of mycotoxins have been identified and many more are undiscovered. One species of mould can produce different mycotoxins. Conversely, different moulds can produce the same mycotoxin [11]. Among the mycotoxins, ochratoxin and aflatoxin occupy important position in causing mycotoxicosis in poultry.

Reports on ochratoxicosis are frequent in India and it is understood as an emerging problem for human, livestock and poultry, requiring proper attention [7,9]. Ochratoxicosis decrease the profitability in poultry industry by decreasing growth rate, egg production and increasing susceptibility to diseases. Several methods have been tried in past to detoxify the feed ingredients from toxic fungal metabolites, [16]. This includes physical, chemical, nutritional and biological methods. Advances made in the field of biotechnology, in last decades, have resulted in development of newer strategies for tackling the problem of mycotoxins [1,12,19]. Practical and cost effective methods to prevent ochratoxicosis in poultry field are in great demand.

Studies indicate that *Saccharomyces boulardii* is effective against ochratoxicosis in poultry [3,4]. The same was tried against ochratoxin A to ascertain its efficiency in reducing its adverse effect in broilers.

Materials and Methods

The present research work was conducted at Department of Pathology, Bombay Veterinary College, Parel, Mumbai, India.

Production of Ochratoxin

Source of organism: *Aspergillus ochraceus* NRRL 3147 culture maintained at the Department of Pathology, Nagpur Veterinary College, Nagpur, India was used as source.

Procedure of ochratoxin production: Ochratoxin was produced on broken wheat by using *Aspergillus ochraceus*

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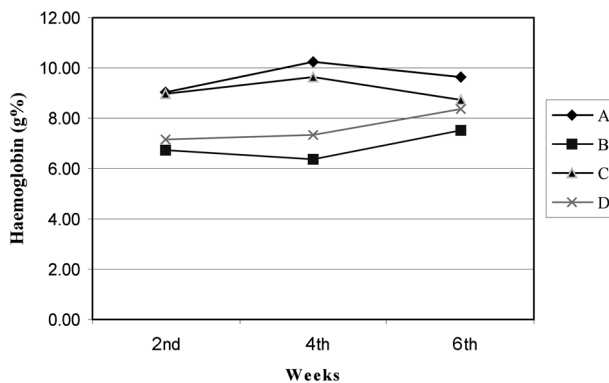
Table 1. Haemoglobin concentration (%) of different groups at different periods

Sr. No.	Week of experiment											
	2nd week				4th week				6th week			
	A	B	C	D	A	B	C	D	A	B	C	D
1	10.4	6.5	8.6	7.2	10.2	6.4	8.6	8.3	9.8	6.0	10.5	6.0
2	7.2	7.0	8.4	6.4	9.7	7.5	8.7	8.7	10.5	8.0	9.2	11.0
3	8.6	6.2	9.4	7.8	9.2	9.6	8.4	8.3	10.8	6.5	9.0	6.0
4	8.7	7.0	10.0	7.4	8.5	7.6	8.7	7.5	9.6	5.0	8.5	6.5
5	8.7	7.4	9.0	7.0	10.5	6.6	9.2	8.3	10.0	6.3	10.0	7.3
6	10.5	6.4	8.4	7.0	9.6	7.5	8.7	9.0	10.6	6.3	10.6	7.3
Mean	9.02 ^a	6.75 ^a	8.97 ^b	7.13 ^a	9.62 ^c	7.53 ^a	8.72 ^b	8.35 ^{ab}	10.22 ^b	6.35 ^a	9.63 ^b	7.35 ^a
S.E. ±	0.50	0.18	0.26	0.19	0.29	0.46	0.10	0.20	0.19	0.39	0.35	0.76

Means with at least one common superscript do not differ significantly, *- Significant at 5 %, N.S.- Non Significant.

Analysis of variance:

Week of experiment	Source	Sum of square	Degree of freedom	Mean sum of square	F-calculated	C.D. value at 5% level
2nd	Treatment	25.66333	3	8.554444	14.31706*	1.4
	Error	11.95	20	0.5975		
	Total	37.61333	23			
4th	Treatment	13.43458	3	4.478194	8.44544*	0.87
	Error	10.605	20	0.53025		
	Total	24.03958	23			
6th	Treatment	60.75458	3	20.25153	14.8626*	1.4
	Error	27.25167	20	1.362583		
	Total	88.00625	23			

**Fig. 1.** Average haemoglobin concentration (%).

NRRL 3147 culture as suggested by Trenk *et al.* [22].

Overnight soaked broken wheat (50 g + 25 ml tap water) was autoclaved at 121°C for 20 minutes and inoculated with fungal spore suspension. The inoculum was incubated for 12 days at room temperature in dark place with vigorous shaking once a day to break the brown mycelial mass. By using sterile wireloop, the mycelial growth from flask was collected and inoculated on SDA (Sabraoud Agar) plate for isolation and identification of *Aspergillus ochraceus*.

Colonies of *Aspergillus ochraceus* were observed on SDA plate. Staining with lactophenol cotton blue stain did microscopic examination. The fermented wheat was autoclaved to kill the spores and dried at 80 °C in hot air oven, overnight. The dried material was powdered and stored in the dark place for further use.

Quantification of Ochratoxin: The representative samples of feed were analyzed for the quantification of ochratoxin A, by thin layer chromatography (TLC) [2].

Procedure

Steps of quantification of ochratoxin A are as follows

1. Collect 40 - 50 gram broken wheat (sample) in beaker.
2. Add 10 gram cellite, 2 gram NaCl, 110 ml methanol and 90 ml distil water in it.
3. Shake it for half an hour.
4. Filtrate it through Whatman filter paper No.1.
5. Collect 50 ml filtrate.
6. Put it in separating funnel.
7. Add 50 ml hexane in it.
8. Shake it for five minutes in separating funnel.
9. After shaking collect the lower feed sample layer in beaker.

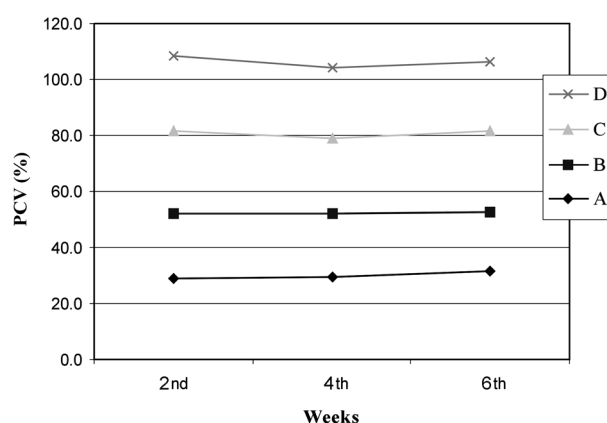
Table 2. Packed cell volume percentage of different groups at different periods

Sr. No.	Week of experiment											
	2nd week				4th week				6th week			
	A	B	C	D	A	B	C	D	A	B	C	D
1	27.1	21.4	28.5	27.1	32.0	24.8	30.2	24.5	31.0	16.0	31.5	22.5
2	27.1	24.2	30.0	28.0	28.5	20.6	26.5	26.0	30.4	22.5	24.3	25.0
3	28.5	25.7	27.1	25.7	31.5	26.4	24.2	26.5	32.5	24.0	30.5	20.0
4	28.5	22.5	31.4	25.8	27.5	18.2	28.0	23.5	30.0	22.4	25.0	25.5
5	34.2	21.4	27.1	24.5	28.0	21.6	29.4	27.0	33.5	20.2	32.4	26.0
6	28.5	25.30	31.4	28.5	29.0	23.6	25.0	24.0	31.2	21.0	30.5	28.6
Mean	29.0 ^{bc}	23.4 ^a	29.3 ^c	26.6 ^b	29.4 ^c	22.5 ^a	27.2 ^{bc}	25.3 ^b	31.4 ^c	21.0 ^a	29.0 ^c	24.6 ^b
S.E. ±	1.08	0.75	0.80	0.62	0.76	1.21	0.97	0.58	0.54	1.13	1.41	1.21

Means with at least one common superscript do not differ significantly, Significant at 5 %, N.S.- Non Significant.

Analysis of variance:

Week of experiment	Source	Sum of square	Degree of freedom	Mean sum of square	F-calculated	C.D. value at 5% level
2nd	Treatment	134.0833	3	44.69444	10.69288*	2.46
	Error	83.59667	20	4.179833		
	Total	217.68	23			
4th	Treatment	154.1446	3	51.38153	10.12394*	2.71
	Error	101.505	20	5.07525		
	Total	255.6496	23			
6th	Treatment	386.58	3	128.86	16.89821*	3.32
	Error	152.52	20	7.6258		
	Total	539.1	23			

**Fig. 2.** Average packed cell volume (%).

10. Add 30 ml chloroform in it.
11. Put it in separating funnel.
12. Shake it for five minutes in separating funnel.
13. After shaking collect lower chloroform layer.
14. Keep it in beaker.
15. Evaporate it on moist heat up to dryness.
16. Cool it at room temperature.
17. After cooling, add 1ml chloroform in it.

18. Transfer it into test tube.
19. Again evaporate it.
20. Add 1ml benzoacetonitrile in it.
21. Spot 20 µl of it, on TLC plate
22. Spot the Standard of ochratoxin A on TLC plate with dilutions, 100 µl, 20 µl, and 10 µl
21. Run the TLC plate in solution containing 95 ml chloroform and 5 ml acetone for half an hour.
23. Dry it at room temperature.
24. Observe under UV light.

Measurement

1 µl of standard ochratoxin contains 10 ppb ochratoxin A. When spot of test feed sample match with standards spot containing 5 µl quantity of ochratoxin A, then it means test sample contains 50 ppb ochratoxin A. When it matches with standards spot containing 10 µl, 20 µl and 100 µl, it means test sample contains 100 ppb, 200 ppb and 1000 ppb ochratoxin A respectively (1 ppm = 1000 ppb).

Experimental chicks: Day old broiler chicks of breed White Leghorn were procured from a reputed hatchery. These chicks were weighed individually and reared with deep litter system under optimum condition of brooding and

Table 3. Total serum protein (%) of different groups at different periods

Sr. No.	Weeks of experiment											
	2nd week				4th week				6th week			
	A	B	C	D	A	B	C	D	A	B	C	D
1	2.50	1.83	2.64	1.80	2.67	1.87	2.81	1.86	2.96	1.93	2.98	1.96
2	2.45	1.87	2.44	1.88	2.77	1.91	2.85	1.91	2.91	1.95	3.10	1.95
3	2.60	1.65	2.74	1.83	2.70	1.85	2.76	1.96	3.00	1.95	3.12	2.05
4	2.65	1.95	2.60	1.85	2.74	1.88	2.78	1.87	2.97	1.89	2.95	2.00
5	2.54	1.81	2.68	1.82	2.65	1.90	2.82	1.90	3.10	2.00	2.99	1.99
6	2.56	1.89	2.64	1.86	2.79	1.87	2.74	1.89	2.90	1.95	2.98	2.10
Mean	2.55 ^b	1.83 ^a	2.62 ^d	1.84 ^a	2.72 ^b	1.88 ^a	2.79 ^c	1.89 ^a	2.97 ^c	1.94 ^a	3.02 ^d	2.00 ^b
S.E. ±	0.02	0.04	0.04	0.01	0.02	0.00	0.01	0.01	0.02	0.01	0.02	0.02

Means with at least one common superscript do not differ significantly, *- Significant at 5 %, N.S.- Non Significant.

Analysis of variance:

Weeks of experiment	Source	Sum of square	Degree of freedom	Mean sum of square	F-calculated	C.D. value at 5% level
2nd	Treatment	3.391267	3	1.130422		
	Error	0.133267	20	0.006663	169.6482*	0.098
	Total	3.524533	23			
4th	Treatment	4.532479	3	1.510826		
	Error	0.032617	20	0.001631	926.4137*	0.048
	Total	4.565096	23			
6th	Treatment	4.532479	3	1.510826		
	Error	0.032617	20	0.001631	926.4137*	0.048
	Total	4.565096	23			

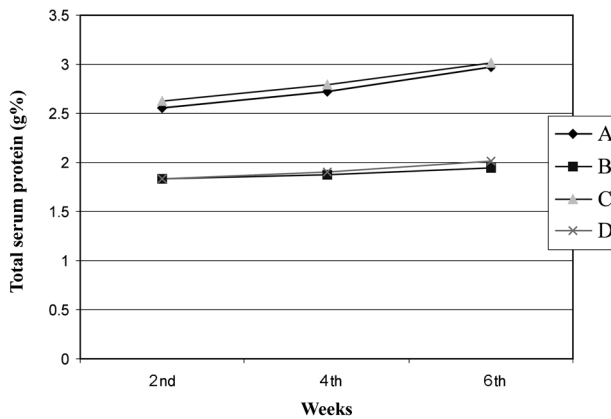


Fig. 3. Average total serum protein (%).

management.

Experimental feed: Broiler starter and finisher feed were procured from Central Poultry Breeding Farm (CPBF), Aarey colony, Mumbai.

Toxin feed: Powdered ochratoxin A was incorporated in feed to maintain the level of 0.5 ppm in the feed.

Medicine: Drug (Probiotic) containing yeast *Saccharomyces boulardii* was obtained from UNI-Sankyo limited, Gaganpahad, Hyderabad-501323. It was in powdered form containing 20 billion CFU/GM.

Experimental procedure: A total of 120 chicks day old chicks were randomly divided into different dietary treatment groups as detailed below.

Group	No. of Birds	Treatment
A	30	Normal feed
B	30	Ochratoxin A of 0.5 ppm of the feed
C	30	Normal feed + Probiotics containing <i>Saccharomyces boulardii</i> of 10 mg/kg of feed.
D	30	Ochratoxin of 0.5 ppm + Probiotics containing <i>Saccharomyces boulardii</i> of 10 mg/kg.

Hematology: Six birds from each group were sacrificed on 14th, 28th, and 42th day of experiment. Prior to sacrifice, blood was collected by cardiac puncture for hematobiochemical studies.

1. Haemoglobin concentration was determined by using Sahli's method (Acid haematin).

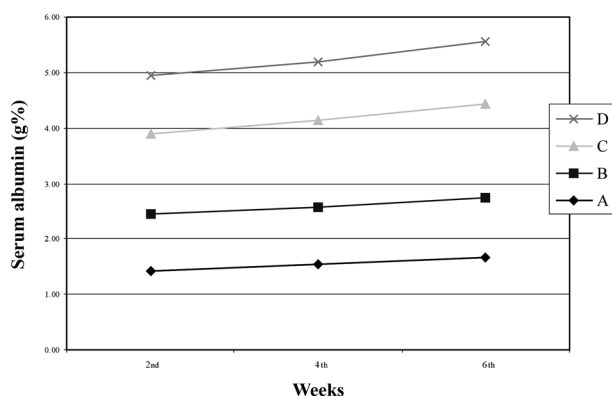
Table 4. Serum albumin concentration (%) of different groups at different periods

Sr. No.	Weeks of experiment											
	2nd week				4th week				6th week			
	A	B	C	D	A	B	C	D	A	B	C	D
1	1.41	1.02	1.45	1.02	1.49	1.06	1.55	1.02	1.76	1.06	1.68	1.11
2	1.44	1.01	1.46	1.02	1.56	1.02	1.56	1.12	1.71	1.11	1.72	1.12
3	1.41	1.00	1.45	1.08	1.59	1.05	1.59	1.09	1.69	1.09	1.71	1.12
4	1.42	1.01	1.48	1.02	1.49	1.02	1.59	1.09	1.62	1.09	1.69	1.13
5	1.42	1.01	1.49	1.05	1.54	1.03	1.54	1.03	1.54	1.06	1.67	1.09
6	1.47	1.02	1.46	1.02	1.53	1.03	1.53	1.02	1.69	1.04	1.73	1.12
Mean	1.43 ^c	1.01 ^a	1.47 ^d	1.04 ^b	1.53 ^b	1.04 ^a	1.56 ^b	1.06 ^a	1.67 ^b	1.08 ^a	1.70 ^b	1.12 ^a
S.E. ±	0.009	0.003	0.007	0.010	0.016	0.007	0.010	0.02	0.032	0.011	0.01	0.006

Means with atleast one common superscript do not differ significantly, *- Significant at 5 %, N.S.- Non Significant.

Analysis of variance:

Weeks of experiment	Source	Sum of square	Degree of freedom	Mean sum of square	F-calculated	C.D. value at 5% level
2nd	Treatment	1.080933	3	0.360311	965.119*	0.023
	Error	0.007467	20	0.000373		
	Total	1.0884	23			
4th	Treatment	1.494283	3	0.498094	457.6672*	0.039
	Error	0.021767	20	0.001088		
	Total	1.51605	23			
6th	Treatment	2.090513	3	0.696837	376.8387*	0.051
	Error	0.036983	20	0.001849		
	Total	2.127496	23			

**Fig. 4.** Average serum albumin (%).

2. Packed Cell Volume was determined by using Wintrobe method.

Serum biochemistry: During each screening blood samples were collected in non-heparinized tubes. The serum was separated after eight hours and stored at -20°C for subsequent analysis.

1. Serum proteins, albumin and globulin were estimated

by Biuret and BCG dye binding method, respectively using commercial reagent kits (Qualigens Fine Chemicals, India).

2. Serum Glutamate Pyruvate Transaminase (SGPT) was determined by Reitman and Frankels method [17], using commercial reagent kits (Qualigens Fine Chemicals, India).

3. Serum Creatinine was determined by alkaline picrate method, using commercial reagent kits (Qualigens Fine Chemicals, India).

Statistical analysis: Statistical analysis was done by using two way ANOVA method as suggested by Snedecor and Cochran [20].

Results

Production of Ochratoxin (OA)

Mycelial growth started to appear on broken wheat by third day with condensation of moisture inside the flask, after inoculation of spore suspension in it. The wheat media gradually turned to brown in colour, which became dark brown after 12 days of inoculation. SDA plate revealed colonies fungus *Aspergillus ochraceus*. Microscopic examination was shown punctuate hypae along with spores of *Aspergillus ochraceus*.

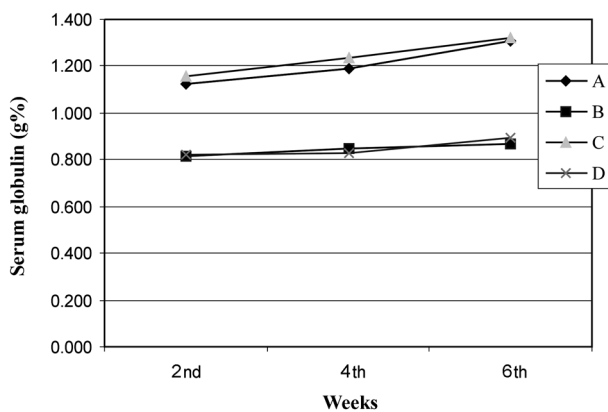
Table 5. Serum globulin concentration (%) of different groups at different periods

Sr. No.	Weeks of experiment											
	2nd week				4th week				6th week			
	A	B	C	D	A	B	C	D	A	B	C	D
1	1.09	0.82	1.19	0.78	1.18	0.81	1.26	0.84	1.20	0.87	1.30	0.85
2	1.01	0.85	0.98	0.86	1.21	0.89	1.29	0.79	1.20	0.84	1.38	0.83
3	1.19	0.64	1.29	0.82	1.11	0.80	1.17	0.87	1.31	0.86	1.41	0.93
4	1.23	0.93	1.12	0.83	1.25	0.86	1.19	0.78	1.35	0.80	1.26	0.87
5	1.12	0.78	1.19	0.79	1.11	0.87	1.28	0.87	1.56	0.94	1.32	0.90
6	1.09	0.87	1.18	0.84	1.26	0.84	1.21	0.87	1.21	0.91	1.25	0.98
Mean	1.12 ^b	0.81 ^a	1.15 ^c	0.82 ^a	1.18 ^b	0.84 ^a	1.23 ^b	0.83 ^a	1.30 ^b	0.870 ^a	1.32 ^b	0.89 ^a
S.E. ±	0.032	0.041	0.042	0.012	0.027	0.014	0.02	0.017	0.057	0.02	0.026	0.023

Means with at least one common superscript do not differ significantly, *- Significant at 5 %, N.S.- Non Significant.

Analysis of variance:

Weeks of experiment	Source	Sum of square	Degree of freedom	Mean sum of square	F-calculated	C.D. value at 5% level
2nd	Treatment	0.628146	3	0.209382	30.36354*	0.100
	Error	0.137917	20	0.006896		
	Total	0.766062	23			
4th	Treatment	0.824446	3	0.274815	111.8272*	0.059
	Error	0.04915	20	0.002457		
	Total	0.873596	23			
6th	Treatment	1.116013	3	0.372004	50.79133*	0.103

**Fig. 5.** Average serum globulin (%).

Ochratoxin Quantification

The broken wheat inoculated with *Aspergillus ochraceus* was quantified by thin layer chromatography (TLC) method. Spot of test feed sample 20 µl matched with standard spot of ochratoxin A containing 100 µl ochratoxin A. So, it yielded ochratoxin A of 5 ppm of broken wheat.

Discussion

Hemoglobin percentage was significantly ($p < 0.05$) low in toxin fed birds of groups B and D as compared to A and C

for second and sixth week of experiment. At the end of fourth week, they were significantly ($p < 0.05$) low in birds of group B than control groups A and C and nonsignificantly low than group D.

Reduction in hemoglobin percentage in ochratoxicosis in birds was observed by Ramadevi *et al.* [16].

Decrease in hemoglobin percentage in ochratoxin fed birds of groups B and D might be due to anemia as a result of depressed erythropoiesis. Nephrotoxicity by the ochratoxin [8] probably resulted in hampered formation of erythropoietin and ultimately erythropoiesis. Higher hemoglobin concentration of group D than B indicated beneficial effect of *Saccharomyces boulardii*. It secretes "Protease", a toxin binding enzyme, that binds to toxin receptor on the epithelial cells of the gastro intestinal tract and enzymatically modifies them. Thus, toxin of pathogen cannot damage the epithelium and adsorbed on epithelium of gastrointestinal tract [4]. Hence due to less absorption, there was less effect of OA.

Packed cell volume was low in OA administered birds of groups B and D over the period. It was significantly ($p < 0.05$) low in birds of groups B as compared to other groups. Addition of probiotic resulted in significant ($p < 0.05$) high PCV in the birds of group D as compared to group B, though it was lower than the groups A and C.

Reduction in PCV due to ochratoxicosis, observed in the present study, is in agreement with the earlier findings by

Table 6. Serum glutamate pyruvate transaminase (U/ml) of different groups at different periods

Sr. No.	Weeks of experiment											
	2nd week				4th week				6th week			
	A	B	C	D	A	B	C	D	A	B	C	D
1	28	41	29	40	31	40	27	36	31	42	26	34
2	31	39	31	42	29	38	26	39	29	38	26	37
3	32	38	31	36	31	39	27	39	32	38	31	37
4	26	38	30	35	33	42	30	38	26	41	29	39
5	33	42	28	37	27	42	31	35	27	42	26	41
6	27	42	27	37	28	40	26	35	28	42	27	35
Mean	29.5 ^a	40.00 ^b	29.33 ^a	37.83 ^b	29.83 ^a	40.16 ^c	27.83 ^a	37 ^b	28.83 ^a	40.5 ^c	27.5 ^a	37.1 ^b
±S.E.	1.17	0.77	0.66	1.07	0.90	0.65	0.87	0.77	0.94	0.80	0.84	1.04

Means with at least one common superscript do not differ significantly, *- Significant at 5 %, N.S.- Non Significant.

Analysis of variance:

Weeks of experiment	Source	Sum of square	Degree of freedom	Mean sum of square	F-calculated	C.D. value at 5% level
2nd	Treatment	555.6667	3	185.2222	34.4066*	2.79
	Error	107.6667	20	5.383333		
	Total	663.3333	23			
4th	Treatment	612.4583	3	204.1528	52.01345*	2.38
	Error	78.50	20	3.925		
	Total	690.9583	23			
6th	Treatment	721.3333	3	240.4444	47.77042*	2.70
	Error	100.6667	20	5.033333		
	Total	822	23			

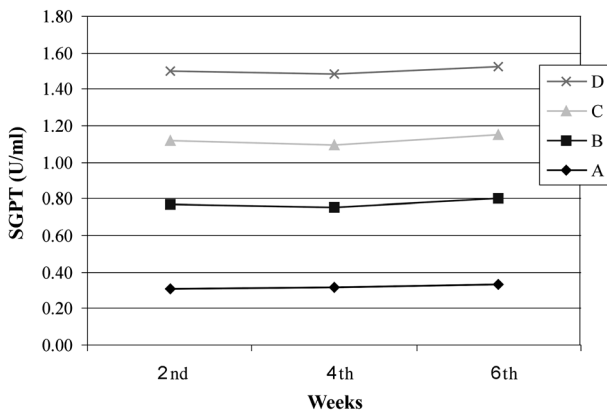


Fig. 6. Average serum glutamate pyruvate transaminase (U/ml).

Pawar [14]. Lowered PCV in OA fed birds might be due anemia developed in ochratoxicosis as described earlier and the values in group D indicated that probiotic, *Saccharomyces boulardii*, was effective in reducing of the adverse effect of OA on hemopoietic system as described earlier, probably by low absorption of toxin.

Total serum proteins were significantly ($p < 0.05$) low in OA administered groups B and D as compared to groups A and C over the period. It was significantly ($p < 0.05$) high in

birds of group D than B but considerably low than in groups A and C. Reduction in total serum proteins due to OA were reported by Prior *et al.* [15]. Low total serum proteins, might be due to inhibition of protein synthesis. OA inhibits the protein synthesis, through competitive inhibition of phenylalanine-t-RNA synthesis with phenylalanine as reported by Creepy *et al.* [6] and leakage of albumin due to nephrotoxicity induced by OA Huff *et al.* [10].

Improvement in protein values in group D as compared to B and in birds of group C as compared to group A, indicated increased protein synthesis as an effect of probiotic *Saccharomyces boulardii* along with reduced absorption of OA as discussed earlier.

Serum albumin concentrations were significantly ($p < 0.05$) low in birds of group B and D as compared to group A and C over the period of experiment. In birds of group D, it was high than group B. Lowered serum albumin levels, due to ochratoxicosis had been recorded by Manning and Wyatt [13]. Decrease in serum albumin concentrations in birds of group B and improvement in group D could be due to the factors related with protein synthesis, as described earlier.

Serum globulin concentrations were low in birds of group B and D than control groups A and C over the period of experiment. Lowered serum globulin concentrations, due to

Table 7. Serum creatinine (mg/dl) of different groups at different periods

Sr. No.	Weeks of experiment											
	2nd week				4th week				6th week			
	A	B	C	D	A	B	C	D	A	B	C	D
1	0.26	0.39	0.31	0.42	0.34	0.45	0.31	0.43	0.35	0.52	0.27	0.41
2	0.35	0.51	0.29	0.38	0.27	0.41	0.27	0.36	0.37	0.39	0.31	0.37
3	0.34	0.46	0.38	0.41	0.36	0.39	0.32	0.42	0.26	0.52	0.37	0.4
4	0.27	0.48	0.31	0.39	0.29	0.47	0.38	0.37	0.31	0.47	0.33	0.36
5	0.31	0.46	0.37	0.37	0.3	0.51	0.36	0.34	0.35	0.45	0.34	0.41
6	0.32	0.47	0.41	0.35	0.34	0.4	0.42	0.4	0.34	0.51	0.44	0.32
Mean	0.30 ^a	0.46 ^c	0.34 ^{ab}	0.38 ^b	0.31 ^a	0.43 ^{bc}	0.34 ^{ab}	0.38 ^{bc}	0.33 ^a	0.47 ^b	0.34 ^a	0.37 ^a
S.E. ±	0.014	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.01	0.02	0.02	0.01

Means with at least one common superscript do not differ significantly, *- Significant at 5 %, N.S.- Non Significant.

Analysis of variance:

Weeks of experiment	Source	Sum of square	Degree of freedom	Mean sum of square	F-calculated	C.D. value at 5% level
2nd	Treatment	0.077946	3	0.025982	17.64478*	0.046
	Error	0.02945	20	0.001472		
	Total	0.107396	23			
4th	Treatment	0.050979	3	0.016993	8.971257*	0.0524
	Error	0.037883	20	0.001894		
	Total	0.088862	23			
6th	Treatment	0.079046	3	0.026349	11.99027*	0.0564
	Error	0.043950	20	0.002198		
	Total	0.122996	23			

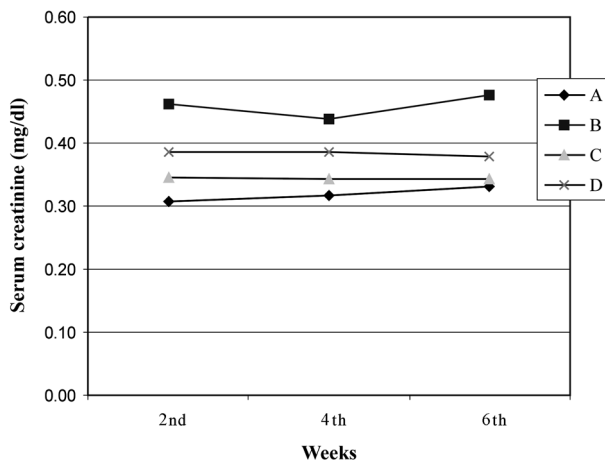


Fig. 7. Average serum creatin (mg/dl).

ochratoxicosis, were also observed by Huff *et al.* [10]. Decrease in serum globulin in toxin fed group B might be due to the adverse effect of ochratoxin A on synthesis of total proteins and globulin.

The results coincided with the tune of total proteins and albumin. Slight improvement in serum globulin concentrations in birds of group D, indicated effectiveness of probiotic

Saccharomyces boulardii in globulin synthesis by the birds with poor absorption of OA as described earlier.

The SGPT levels of groups B and D remained high as compared to groups A and C over the period. The SGPT levels of group D were significantly ($p < 0.05$) low than the group B. Increased SGPT levels in birds due to ochratoxicosis had been reported earlier by Sremannarayana *et al.* [21]. The SGPT levels high in toxin fed group B indicated damage to the hepatocytes and release of enzymes after the damage.

Low SGPT levels of toxin administered and probiotic treated group D birds indicated reduction of adverse effect of OA due to its less absorption in the body.

The levels of serum creatinine were high in toxin fed birds of groups B and D as compared to groups A and C over the period. The levels were significantly ($p < 0.05$) high in group B than D (except fourth week). Increase in serum creatinine concentrations, due to OA, had been reported earlier by Sakhare [18].

Increase in serum creatinine concentration in toxin fed birds might be due to nephrotoxic action of OA, which causes renal impairment by destruction of epithelial cells of proximal and distal convoluted tubules and tubular damage. Significant ($p < 0.05$) decrease in levels of serum creatinine

in group D as compared to toxin fed group B indicated, reduction in adverse effect of OA on kidneys, in presence of probiotic *Saccharomyces boulardii* which reduces absorption of OA in the body as described earlier.

On the basis of literature reviewed, results of present experiment and a foresaid discussion, it could be concluded that ochratoxin A 0.5 ppm of feed, adversely affected the broilers. It resulted in significant reduction in hemoglobin and packed cell volume percentage, decrease in total serum proteins, albumin and globulin but increase in concentration of creatinine and SGPT.

Probiotic *Saccharomyces boulardii* along with ochratoxin A at dose rate 10 mg/kg of feed, employed in present study, resulted in significant improvement related to hematobiochemical parameters in broilers.

In ochratoxin A contaminated feed, use of probiotic, containing yeast *Saccharomyces boulardii* is an alternative in field conditions to reduce the adverse effect and economic losses. Similar studies with other toxins may exhibit its spectrum of activities.

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