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Dr. T Prasada Rao
Assist. Professor's, Depart. of Veterinary Biochemistry, College of Veterinary Science, Proddatur, Andhra Pradesh, India

Dr. V Manasa
Assist. Professor's, Depart. of Veterinary Biochemistry, College of Veterinary Science, Proddatur, Andhra Pradesh, India

Dr. TV Sai Kumar
Assist. Professor's, Depart. of Veterinary Biochemistry, College of Veterinary Science, Proddatur, Andhra Pradesh, India

Dr. Kumar Kutty
Librarian, College of Veterinary Scienece, Proddatur, Andhra Pradesh, India

Correspondence
Dr. T Prasada Rao
Assist. Professor's, Depart. of Veterinary Biochemistry, College of Veterinary Science, Proddatur, Andhra Pradesh, India

Efficacy of *Saccharomyces cerevisiae* in reducing the effects of ochratoxicosis in broiler chicks

Dr. T Prasada Rao, Dr. V Manasa, Dr. TV Sai Kumar and Dr. Kumar Kutty

Abstract
Ochratoxin A (OTA) is one of the most common mycotoxin causes ochratoxicosis in poultry. It poses a great threat to the lives of poultry, animals and humans. It causes nephrotoxicity, immune suppression and teratogenicity. Dried Yeast culture (*Saccharomyces cerevisiae*) has the ability to suppress the effect of OTA and enhances the performance of broiler chicks. The present study was designed to evaluate the ameliorative effects of dried yeast culture against induced ochratoxicosis in broilers. For this day old broilers were divided into three equal groups and were given OTA 2 ppm and dried yeast culture 0.1% for six weeks. Results indicated that feeding OTA alone caused reduction in body weight gain, poor FCR, increased gross lesions, altered relative organ weights and serum biochemical values. Feeding dried yeast culture along with OTA did not ameliorate the OTA induced alterations in body weight, FCR, gross lesions and serum biochemical parameters.

Keywords: Broiler chicks, OchratoxinA, Dried Yeast Culture (*Saccharomyces cerevisiae*), Serum biochemical parameters, body weight gains and Feed conversion ratio, Histopathological studies

Introduction
Ochratoxins are the most common and dangerous mycotoxins in the poultry feed which causes the ochratoxicosis leads to the huge economic losses to the poultry industry due to increased mortality and reduced body weight gain. Several toxigenic fungal strains of *Aspergillus* (Ghosh *et al*. 2015) [16] and *Penicillium*, the most important being *Aspergillus ochraceus*, are being involved in the production of ochratoxin. Ochratoxin A displays a multiple toxicity, including immunotoxicity. Ochratoxins are considered to be the main reason for causing a serious anomaly namely Balkan Endemic Nephropathy (BEN) in humans exposed to its dietary exposure (Zahoor-ul-Hassan *et al*. 2012; Solcan *et al*. 2015; Iftikar *et al*. 2015; Ben Salah-Abbes *et al*. 2015) [46, 39, 22, 4].
Ochratoxin A (OTA) is produced mainly by *Aspergillus ochraceus* in tropical and warmer region and *Penicillium verrucosum* in temperate and cold areas. The family of ochratoxins consists of three members, viz. Ochratoxin A, B and C but OTA is the most toxic one. OTA is an isocumarin derivative linked through the carboxyl group to a L-β-Phenylalanine. Ochratoxin is absorbed into the body and is distributed at a high concentration in the kidney. It shows renal toxicity by inhibiting various enzyme activities in the kidney (Stoev *et al*. 2000; Stoev *et al*. 2002; Elaroussi *et al*. 2006) [41, 42, 11].
Live yeast addition to animal feed has been known to improve the nutrient quality of feed and performance of animals (Santin *et al*. 2002; Brake, 1991; Moore *et al*. 1994; Pagan, 1990; Day, 1997; Onifade and Babatunde, 1996) [37, 6, 29, 32, 7, 31]. Whole yeast products or yeast cell wall components have been used to improve growth and affect the physiology, morphology and microbiology of the intestinal tract of turkey (Badley *et al*, 1994; Hooge, 2004b; Huff *et al*, 2007; Rosen, 2007b; Soils De Los Santos *et al*. 2007; Huff *et al*. 2010) [5, 19, 20, 36, 20]. Therefore, yeast culture in dried form is used in the present work to evaluate its effects on the performance, biochemical parameters and the internal organ weights of broiler chicks when fed on OTA contaminated diets.

2. Materials and Methods
2.1 Production, extraction and quantification of OTA: The culture used for production of OTA *A.ochraceus* was obtained from MMTC (Institute of Microbial Technology), Chandigarh. The OTA was grown on oat meal agar slants at 28˚C for 2 weeks on large scale as per Trenk *et al*. (1971) [43].

OTA was extracted and quantified using column chromatography and TLC, respectively as per AOAC (1995).

2.2 Experimental design

A total of 112 male day old commercial broiler chicks, were divided at random into 4 groups. In each group 4 replications each of 7 birds was maintained. All the chicks were fed with basal diet from 0-3 weeks and finisher diet from 4-6 weeks. The three groups of chicks were fed the following 3 diets at random.

1. Control
2. Ochratoxin A 2ppm
3. Ochratoxin A 2ppm + Yeast culture 0.1%

2.3 Serum Biochemical Profile

Blood was collected in non-heparinized tubes from the birds in each treatment during 0, 21 and 42 days of age. Serum was collected after 8-10 hours and was stored at -20°C for further analysis. The serum was used to estimate the following parameters.

Total proteins and Albumin (Biuret and BCG dye binding method), Cholesterol (Wybenga and Pileggi method), Creatinine (Alkaline Picrate method), Uric acid (Phospho Tungestic Acid Method), Glucose (O-Toluidine method), ALKP (King and Kings method), AST (Reitmann and Frankle method), ALT (Reitmann and Frankle method),GGT (Kinetic colorimetric method), Triglycerides (GPO method),Calcium (O-Cresolthalein comlexone method) and Phosphorus(Kinetic colorimetric method)

Histopathological studies

At the end of 6 weeks of age, two birds from each treatment were taken.

3. Results and Discussion

3.1 Production of Ochratoxin A (OTA)

The colony characteristics of the *A.ochraceus* on oat meal agar slants confirmed to those described for a pure culture and estimated by HPTLC (AOAC, 1995). The OTA content in the culture material was found to be 1200ppm comparable to the results of Raju (1998) [34].

3.2 Performance of Broiler Chicks

The effective concentration was incorporated into the poultry diet and its effect on serum biochemical profile and vital organs was studied. The mitogen, ochratoxin A (OTA) was used at the rates of 2 ppm 2.0mg/kg in this study for a period of 42 days and the selection of this level was made on the basis of different available studies (Santin *et al*, 2002; Elaroussi *et al*, 2006; Khatoon *et al*, 2013; Abidin *et al*, 2013; Marin and Taranu, 2015) [37, 11, 23, 28, 1].

3.2.1 Body weight gains

The data on the Feed consumption and body weight of broilers in different treatments from 1-6 weeks of age is presented in Table 1.The weight gains of broiler chicks from 1-6 weeks were gradually increased on control diet than diets on Yeast culture alone and OTA + Yeast culture. The weight gains were significantly ($P \leq 0.01$) affected by different diets as well as different periods. Feeding OTA to birds caused an adverse effect on the growth of the birds which could be well attributed by a decreased body weight and poor FCR as observed in this study in OTA treated birds. Decreased body weights and poor FCR in OTA intoxicated birds have been reported by many scientists (Kubena *et al.* 1988; Ramadevi 1993; Verma *et al*. 1995; Raju 1998; Stoev *et al*, 2000; Rajeev *et al*, 2003; Verma *et al*, 2004; Koynarski *et al*, 2007) [25, 35, 44, 34, 41, 33, 45, 24]. The adverse effects of OTA on growth performance have been related with a decrease in protein and energy utilisation, probably as a consequence of a deterioration of the digestive and metabolic efficiency of the birds (Daofeng *et al*. 2017) [8].

3.3 Serum biochemical profile

Serum biochemical parameters of all the groups fed on OTA, OTA+Yeast culture have been presented in Table **2**.Regarding serum biochemical alterations, significant increase in creatinine, ALT AST, ALP, GGT, GST, glucose and uric acid levels in OTA intoxicated birds has also been reported by many workers (Kubena *et al.*, 1988; Bailey *et al.*, 1989; Gentles *et al*., 1999; Stoev *et al*., 2002) [25, 2. 15, 42]. Similarly, reduction in serum total proteins, albumin and globulin, cholesterol, triglycerides, calcium and phosphorus concentrations in OTA treated birds has also been well reported previously (Bailey *et al*, 1989; Gentles *et al*, 1999; Stoev *et al*, 2002; Garcia *et al*, 2003; Koynarski *et al*, 2007) [2, 15 15, 42, 13, 24]. The mechanism by which OTA produced hypoproteinaemia and hypo albuminaemia is due to inhibition of phenylalanyl transfer-RNA-synthetase with phenylalanine and renal leakage of albumin resulting from kidney lesions induced by OTA. Feeding dried yeast culture did not result in the significant reduction of OTA induced alterations in serum biochemical parameters. One possible reason in this regard might be the non-polar nature of ochratoxin and secondly, up till now no single binder has been proved efficient against all the mycotoxins present in field (Denli and Perez, 2010; Sirhan *et al*, 2012) [9, 38].

3.4 Histopathological studies

A significant increase in relative kidney and liver weights in OTA treated birds as observed in this study has also been reported by different scientists (Huff *et al*,1974; Manning and Wyatt, 1984; Rama devi, 1993; Santin *et al*, 2002; Elaroussi *et al*., 2006) [17, 27, 35, 37, 11]. As liver and kidney are the primary organs being involved in the elimination of toxic materials from the body so the increased sizes of these organs might occur due to the damages caused during the elimination of OTA from the body through kidney and liver (Fuchs *et al*, 1988) [12]. Feeding of yeast culture has no significant effect on OTA induced alterations in relative liver and kidney weights. Reduced size of spleen and bursa in OTA treated birds has also been related by many authors (Dwivedi and Burns, 1984; Rama devi, 1993; Stoev *et al*, 2000; Rajeev *et al*, 2003; Kumar *et al*, 2004; Elaroussi *et al*, 2006) [10, 35, 41, 33, 11]. Immuno suppression, a major alteration associated with ochratoxicosis, might occur due to reduced sizes of immunological organs as observed in this study after feeding OTA to birds. OTA disturbs the functions of proximal tubules resulting in the reduction of a primary renal organic anion, the para-aminohippuric acid (PAH), transport leading to glucosuria and enzymuria (Gekle and Silbernagl, 1994). Gross lesion on different visceral organs as observed in this study has also been observed by many scientists (Elaroussi *et al*., 2006; Hanif *et al*, 2008) [11, 18, 21]. Upon feeding of Yeast culture along with OTA did not ameliorate the OTA associated gross lesions on different organs. Santin *et al*., (2002) [37] reported similar observations when birds were given 2 mg/kg OTA along with 0.25% HSCAS in feed. Similarly, Nedeljkovic Trailovic *et al*., (2015) [30] presented similar results in birds given OTA along with 2 g/kg modified zeolite.

Table 1

Period (Weeks)	Parameter	Control	Ochratoxin A	OTA+Yeast Culture
1 to 6 Mean±SE	Feed Consumption(g)	436.75±54.34	230±31.15	263.83±53.12
1 to 6 Mean±SE	Weight gain (g)	268.42±12.56	114.46±5.34	148.13±7.33

Table 2

Biochemical parameter	Mean ±SE		
	C	OA	OA +YC
Total Protein (g %)	3.83±0.87	2.39±0.1	2.76±0.18
Albumin (g %)	1.67±0.12	0.99±0.1	1.14±0.01
Globulins (g %)	2.63±0.81	1.51±0.2	1.65±0.16
A: G ratio (g %)	0.72±0.14	0.66±0.14	0.71±0.1
Cholesterol (mg %)	138.08±13.67	85.00±18.7	83.75±3.13
Triglycerides (mg %)	128.58±18.1	84.15±13.21	98.75±7.22
AST(IU/L)	119.91±3.07	128.75±3.83	119.17±5.21
ALT(IU/L)	26.69±0.82	30.16±2.66	29.01±2.49
GGT(IU/L)	9.55±2.57	11.87±3.29	11.48±3.15
GST?(U/g)	2.04±0.02	4.09±0.01	3.89±0.1
ALP(KA units)	51.08±3.79	57.45±5.43	58.67±6.01
Creatinine (mg %)	0.17±0.1	0.25±0.04	0.23±0.03
Uric acid (mg %)	12.68±1.2	15.32±1.65	14.06±1.42
Glucose (mg %)	98.17±2.7	115.54±8.5	112.03±9.3
Calcium (mg %)	12.59±1.05	10.42±0.41	11.26±0.7
Phosphorus (mg %)	8.36±0.77	6.99±0.42	7.02±0.28

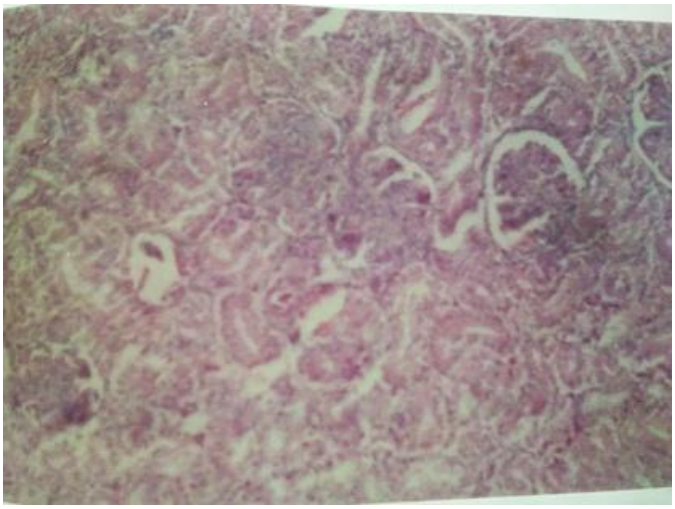

Fig 1: Kidney showing mild degenerative changes H&E X80

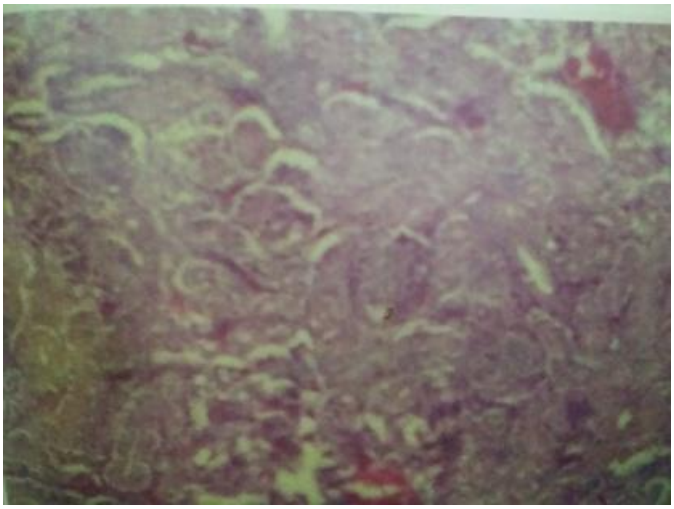

Fig 2: Kidney showing marked congestion, interstitial haemorrhages

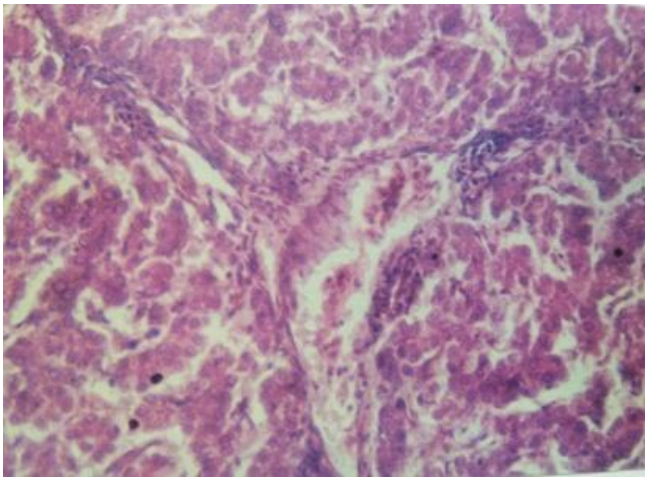

Fig 3: Liver showing mild congestion and bile duct hyperplasia H&E X80

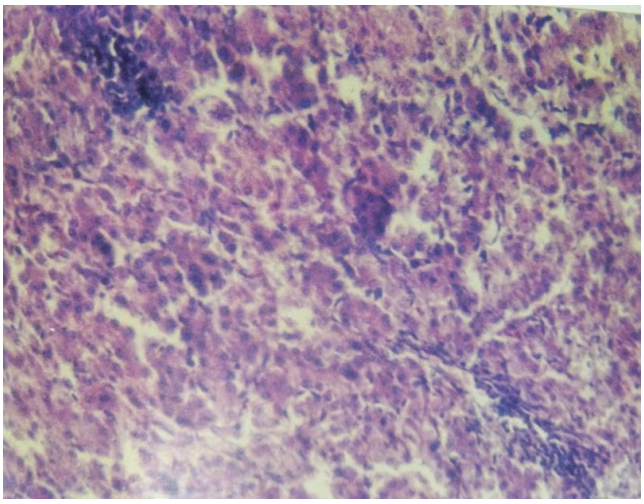

Fig 4: Liver showing focal areas of lymphoid aggregates and dilated sinusoidal spaces

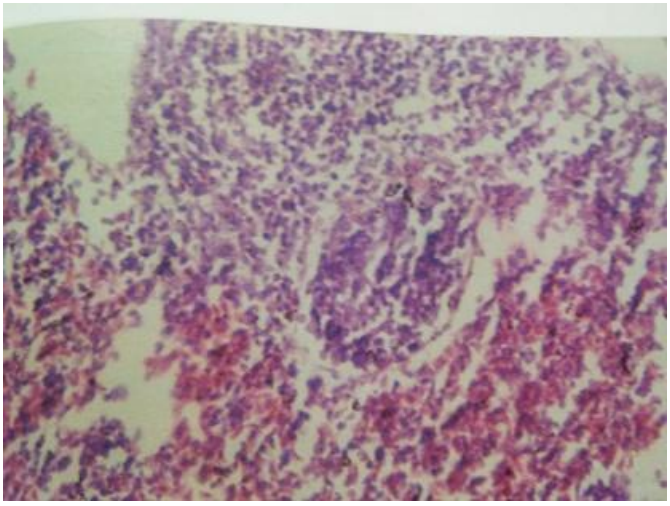

Fig 5: Spleen showing mild depletion of germinal center, marked congestion of trabecular arteries H & E X 100

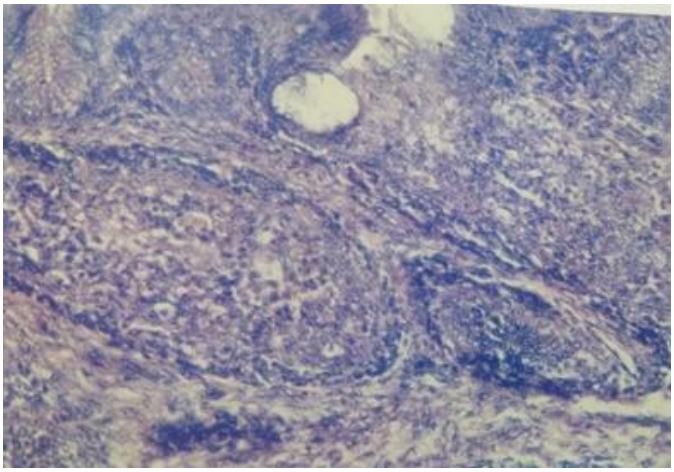

Fig 6: Bursa of Fabricius showing cystic spaces in the epithelium of follicles H&E X 100

4. Conclusions

From this study it can be well concluded that feeding dried yeast culture (0.5, 1 and 2 percent of feed) did not ameliorate ochratoxin A (2.0 mg/kg feed) induced toxic pathological and serum biochemical alterations in broilers.

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