ISSN 1682-8356
ansinet.org/ijps

INTERNATIONAL JOURNAL OF

POULTRY SCIENCE

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International Journal of Poultry Science 2 (6): 465-468, 2003

Evaluation of Cell Wall Yeast as Adsorbent of Ochratoxin in Broilers Diets

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Abstract: This study aimed to evaluate the efficacy of cell wall of *Saccharomyces cerevisiae* (CWSC) as an inhibitor of the toxic effects of ochratoxin in broilers from 1 to 42 days of age. A total of 320 broilers was distributed into four treatments with four replicates of 20 birds each: T_1 - control; T_2 - 0.1% CWSC; T_3 - 500 ppb of ochratoxin; T_4 - 0.01% CWSC + 500 ppb of ochratoxin. The parameters evaluated were feed intake; weight gain, feed conversion; relative weights of liver, kidneys and bursa of Fabricius. Ochratoxin in diet negatively affected ($P > 0.05$) the feed intake and weight gain when the birds were 21 and 42 days of age, and affected the feed conversion at 42 days of age. Was no interaction between CWSC and dietary ochratoxin. The CWSC improve the feed conversion of birds exposed or not to ochratoxin at 42 days of age. The ochratoxin or CWSC in diet did not affect the relative weight of organs evaluated. These results reflect that ochratoxin in the level used in diet impaired the feed intake, weight gain and feed conversion of the birds and the CWSC did not ameliorate these parameters in the presence of the ochratoxin.

Key words: Broilers, performance, cell wall of *Saccharomyces cerevisiae*, ochratoxin

Introduction

Ochratoxin are mycotoxins isolated from *Aspergillus ochraceus* but can also be produced by a series of *Aspergillus* and *Penicillium* species. Of this group of isocumarins, only ochratoxin A has been naturally isolated from cereals, and it is most toxic mycotoxin for birds (Gibson *et al*., 1990). These mycotoxins occur in several parts of the world, contaminating cereals that are traditionally used in poultry feeds. Ochratoxin A is classified as the second most important mycotoxin in terms of economic losses worldwide, aflatoxin being the first (Hesseltide, 1986). Intoxication of birds by ochratoxin results in reduced weight gains, impaired feed efficiency, reduced egg production and quality (Huff *et al*., 1974; Prior *et al*., 1980; Burns and Dwiveldi, 1986), and also immunosuppression in broilers (Santin *et al*., 2002).

Lately, the more promising and practical approaches to counteract mycotoxins are the use of adsorbents. In this way, however studies showed that aluminosilicates can reduce the effects of aflatoxin due to capacity to bind aflatoxin (Phillips *et al*., 1988; Kubena *et al*., 1990), theses adsorbents did not ameliorate the deleterious effects of ochratoxin (Santin *et al*., 2002), probably due to variation in the structure of mycotoxin.

By the other hand a live yeast, *Saccharomyces cerevisiae* was found to alleviate the adverse effects of aflatoxicosis in poultry (Stanley *et al*., 1993), and theses beneficial effects have been attributed to cell wall of *Saccharomyces cerevisiae*. In this aspect, the present study was aimed to evaluate the effect of cell wall yeast in preventing performance losses in broilers exposed to ochratoxin.

Materials and Methods

Experimental birds and management: A total of 320 sexed (male) Cobb 1-day-old broilers were housed in 16 cages in an experimental house and received feed and water *ad libitum*. Birds were distributed into following four treatments: T_1 - control (0.0% cell wall *Saccharomyces cerevisiae* - CWSC and 0 of ochratoxin), T_2 - 0.1% of CWSC, T_3 - 500 ppb of ochratoxin and T_4 - 0.1% of CWSC and 500 ppb of ochratoxin. The diet was based on corn and soybean meal, containing or exceeding the National Research Council (1994). The corn used was free from aflatoxin, ochratoxin and zearalenone as determined by thin-layer chromatography (Soares and Rodriguez-Amaya, 1989). The husbandry was similar to that practiced in commercial flocks.

Birds and residual feed were weighted at the beginning and end of each experimental stage (initial stage: one to 21 day and final stage: 22 to 42 days). Feed conversion was obtained by dividing total feed intake by the weight gain of each pen added to the weights of dead birds.

Statistical Analysis: The statistical analysis used a completely random experimental design in a factorial (2 X 2) arrangement. Data were subjected to analysis of variance, and when significant differences were obtained, Tukey´s test was used.

Ochratoxin Production and Cell wall of *Saccharomyces cerevisiae*: Ochratoxin was produced by the fermentation of wheat with *Aspergillus ochraceus*, strain NRRL 3174, according to Manning and Wyatt (1984). After fermentation, the wheat was dried in an

Table 1: Feed intake, body weight gain and feed:gain of broilers at initial stage of experiment (1 to 21 day)

Treatments	CWSC*(%)	Ochratoxin(ppb)	Feed intake(g)	Body weight gain (g)	Feed:gain(g/g)
T_1	0.00	0	1,136	694	1.642
T_2	0.10	0	1,136	723	1.575
T_3	0.00	500	1,071	667	1.606
T_4	0.10	500	1,040	660	1.578
	C.V. (%)		7.22	7.85	3.12
			---------- Principal effects ----------		
	Ochratoxin (+)		1,055	663	1.592
	Ochratoxin (-)		1,136	708	1.608
	CWSC (+)		1,088	691	1.576
	CWSC (-)		1,103	680	1.624
			---------- Probabilities ----------		
	Ochratoxin (O)		0.015	0.032	0.101
	CWSC (A)		0.598	0.289	0.068
	A x O		0.854	0.952	0.923

* CWSC-Cell wall of *Saccharomyces cerevisiae*

Table 2: Feed intake, body weight gain and feed:gain of broilers at final stage of experiment (22 to 42 day)

Treatments	CWSC*(%)	Ochratoxin(ppb)	Feed intake(g)	Body weight gain (g)	Feed:gain(g/g)
T_1	0.00	0	3,555	1,782	1.998
T_2	0.10	0	3,432	1,813	1.903
T_3	0.00	500	3,325	1,653	2.005
T_4	0.10	500	3,459	1,701	2.034
	C.V. (%)		8.54	9.92	2.83
			---------- Principal effects ----------		
	Ochratoxin (+)		3,392	1,677	2.019
	Ochratoxin (-)		3,493	1,797	1.950
	CWSC (+)		3,445	1,757	1,968
	CWSC (-)		3,440	1,717	2.001
			---------- Probabilities ----------		
	Ochratoxin (O)		0.018	0.042	0.050
	CWSC (A)		0.279	0.415	0.151
	A x O		0.240	0.872	0.520

* CWSC – Cell wall of *Saccharomyces cerevisiae*

oven at 100 °C for 12 hours and ground. The level of ochratoxin A and was analyzed by the thin-layer chromatography method, according to the technique described by Soares and Rodriguez-Amaya (1989).

Saccharomyces cerevisiae strain *Calsberg* was cultivated in simple agar, after growth was replicated in liquid peptonate water with 2% of dextrose and incubated at 37 °C for 48 hours. Then the solid phase was separated by centrifugation at 3000 x for 30 min and lyophilized to be incorporated in feed.

The inocula (500 ppb of ochratoxin and 0.1% of cell wall yeast) were weekly mixed into the feed, as dictated by each treatment, from the first day until the end of the trial (1 to 42 days).

Necropsy and macroscopic and morphometric analysis of the organs: On days 21 and 42 of the experimental period, four birds from each group were slaughtered by cervical dislocation to perform macroscopic observations of the bursa of Fabricius, liver and kidneys. These organs were immediately dissected and individually weighed. Weights were expressed as a percentage of body weight, thus obtaining the relative weight of organs.

Results and Discussion

Ochratoxin might cause significant losses to poultry industry due to reduced performance and health problems in the exposed birds as was observed in the present study. The results in Table 1 showed that ochratoxin in diet had a significant decrease in feed intake and weight gain as compared to the control group. Birds exposed to ochratoxin, at initial stage (1 to 21 days), final stage (22 to 42 days) or in total period of experiment (1 to 42 days) had lower average feed intake and weight gain ($P < 0.05$). At final stage of experiment (22 to 42 days) and in total period of experiment (1 to 42 days of age) the ochratoxin reduce the feed conversion of birds. Prior *et al.* (1980) also showed reduction in performance of birds using the same levels of ochratoxin in broilers.

Table 3: Feed intake, body weight gain and feed:gain of broilers at total period of experiment (1 to 42 day)

Treatments	CWSC*(%)	Ochratoxin(ppb)	Feed intake(g)	Body weight gain (g)	Feed:gain(g/g)
T_1	0.00	0	4,691	2,475	1.896
T_2	0.10	0	4,573	2,536	1.807
T_3	0.00	500	4,400	2,325	1.906
T_4	0.10	500	4,500	2,360	1.906
	C.V. (%)		11.02	8.91	3.48
			----------Principal effects ----------		
	Ochratoxin (+)		4,450	2,342	1,906
	Ochratoxin (-)		4,632	2,505	1.851
	CWSC (+)		4,536	2,448	1,856
	CWSC (-)		4,545	2,400	1.901
			---------- Probabilities ----------		
	Ochratoxin (O)		0.032	0.012	0.049
	CWSC (A)		0.386	0.108	0.050
	A x O		0.320	0.812	0.922

* CWSC - Cell wall of *Saccharomyces cerevisiae*

Table 4: Relative weights of liver, kidney and bursa of Fabricius in broilers at 21 days of age

Treatments	CWSC* (%)	Ochratoxin (ppb)	Liver (g/100g BW)	Kidney (g/100g BW)	Bursa of Fabricius (g/100g BW)
T_1	0.00	0	3.17	0.74	0.26
T_2	0.10	0	3.21	0.86	0.30
T_3	0.00	500	3.35	1.00	0.21
T_4	0.10	500	3.23	1.01	0.27
	C.V. (%)		12.00	25.12	32.00
			---------- Principal effects ----------		
	Ochratoxin (+)		3.29	1.00	0.24
	Ochratoxin (-)		3.19	0.80	0.28
	CWSC (+)		3.22	0.93	0.28
	CWSC (-)		3.26	0.87	0.23
			---------- Probabilities ----------		
	Ochratoxin (O)		0.215	0.418	0.125
	CWSC (A)		0.287	0.452	0.218
	A x O		0.780	0.919	0.415

* CWSC - Cell wall of *Saccharomyces cerevisiae*

Table 5: Relative weights of liver, kidney and bursa of Fabricius in broilers at 42 days of age

Treatments	CWSC* (%)	Ochratoxin (ppb)	Liver (g/100g BW)	Kidney (g/100g BW)	Bursa of Fabricius (g/100g BW)
T_1	0.00	0	2.05	0.69	0.17
T_2	0.10	0	1.74	0.74	0.17
T_3	0.00	500	2.24	0.85	0.14
T_4	0.10	500	2.12	0.60	0.15
	C.V. (%)		25.00	33.19	9.16
			---------- Principal effects ----------		
	Ochratoxin (+)		2.18	0.72	0.14
	Ochratoxin (-)		1.89	0.71	0.17
	CWSC (+)		1.93	0.67	0.16
	CWSC (-)		2.14	0.77	0.15
			---------- Probabilities ----------		
	Ochratoxin (O)		0.092	0.920	0.112
	CWSC (A)		0.184	0.072	0.218
	A x O		0.820	0.312	0.801

* CWSC - Cell wall of *Saccharomyces cerevisiae*

Addition of CWSC to diet containing the mycotoxin did not minimize the effects of ochratoxin but showed improve in feed conversion of birds fed with or without mycotoxin in diet when the total period of experiment (1 to 42 days) was analyzed (Table 3). Recent study from Santin *et al*. (2001) showed the CWSC improve the intestinal mucosa aspects and suggested that it might be the explanation for the improve in performance of broilers supplemented with CWSC in the same study. Actually, the cell wall yeast used in the present study showed no efficacy to reduce the effects of ochratoxicosis however they improve the feed conversion of animal with or without mycotoxins which suggested that probably the effect of the CWSC used was not as adsorventes of mycotoxin but as a supplement that could improve the feed conversion of birds. The cell wall of yeast is normally constituted for mannan oligosaccharides and the use of theses compost have been showed improve in feed conversion of birds in some reports (Savage and Zakrzewska, 1997; Fritts and Waldroup, 2003).

Although ochratoxin had been reported to cause increase in relative weight gain of liver and kidneys of poultry (Santin *et al*., 2002), the level of mycotoxin used was relatively higher than used in present study and perhaps it could be an explanation for the results in Table 4 and 5 did not show differences between the experimental groups as relative weight of organs.

In the light of the results of the present study and other published studies, might be suggested that ochratoxin cause losses in performance of broilers and that CWSC used did not was efficient in reduce theses losses caused by this mycotoxin.

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