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LOW LEVEL OF AFLATOXIN IN BROILER AT EXPERIMENTAL CONDITIONS. USE OF CELL WALL YEAST AS ADSORBENT OF AFLATOXIN

(*Aflatoxina em baixos níveis para frangos em condições experimentais. Uso de parede celular de levedura como adsorvente de aflatoxina)*

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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 aflatoxin in broilers from 1 to 42 days of age. A total of 320 broilers were distributed in four treatments with four replicates of 20 birds each: T1- control; T2 – 0.1% CWSC; T3 – 500 ppb of aflatoxin; T4 – 0.1% CWSC + 500 ppb of aflatoxin. The parameters evaluated were feed intake; weight gain, feed conversion; relative weights of liver, kidneys and bursa of Fabricius. Although aflatoxin has decreased ($P<0.05$) the feed conversion of birds at 21 days of age, the levels provided did not affect ($P>0.05$) other performance parameters when the birds were at 21 and 42 days of age. The CWSC improved the feed conversion of birds exposed or not to aflatoxin at 42 days of age. The aflatoxin and / or CWSC in the diet did not affect the relative weight of evaluated organs. No interaction between CWSC and dietary aflatoxin was found. These results demonstrate that, at the levels given in this study, aflatoxin do not cause deleterious effects in the performance of broilers when in experimental conditions.

Key words: Aflatoxin, broilers, cell wall of *Saccharomyces cerevisiae*.

RESUMO – Este estudo foi realizado para avaliar a eficácia da parede celular de *Saccharomyces cerevisiae* (PCSC) como inibidor dos efeitos tóxicos da aflatoxina em frangos de corte de 1 a 42 dias de idade. Foram utilizados 320 frangos distribuídos em quatro tratamentos com quatro repetições de 20 aves cada: T1- controle; T2 – 0.1% PCSC; T3 – 500 ppb de aflatoxina; T4 – 0.1% PCSC + 500 ppb de aflatoxina. Os parâmetros avaliados foram consumo de ração; ganho de peso; conversão alimentar; peso relativo de fígado, rins e bursa de Fabricius. O nível de Aflatoxina da dieta afetou ($P<0,05$) a conversão alimentar das aves aos 21 dias de idade, porém, não afetou ($P>0,05$) outros parâmetros de desempenho aos 21 e 42 dias de idade. A PCSC melhorou a conversão alimentar das aves expostas ou não à aflatoxina aos 42 dias de idade. A aflatoxina e/ou PCSC da dieta não afetaram o peso relativo dos órgãos avaliados. Não foram observadas interações entre aflatoxina e PCSC. Os resultados demonstram que, nos níveis utilizados no presente estudo, a aflatoxina não causa efeitos deletérios às aves em condições experimentais.

Palavras chave: Aflatoxina, frangos de corte, parede celular de *Saccharomyces cerevisiae*.

Introduction

Aflatoxin are mycotoxins produced by a series of *Aspergillus* and *Penicillium* species (SANTIN, 2000). These mycotoxins occur in several parts of the world, contaminating cereals that are traditionally used in poultry feeds. Aflatoxin is classified as the first most important mycotoxin in terms of economic losses worldwide (HESSELTIDE, 1986). Intoxication of birds by aflatoxin in higher levels in experimental conditions results in reduced weight gains, and impaired feed efficiency (HUFF *et al.*, 1986), and also immunosuppression in broilers (SANTIN *et al.*, 2001).

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Lately, the more promising and practical approaches to counteract mycotoxins are the use of adsorbents. A live yeast, *Saccharomyces cerevisiae* was found to alleviate the adverse effects of aflatoxicosis in poultry (STANLEY *et al*., 1993), and these beneficial effects have been attributed to the cell wall of *Saccharomyces cerevisiae* (CWSC). In this aspect, the present study was aimed to evaluate the effect of the cell wall yeast in preventing performance losses in broilers exposed to aflatoxin.

Material 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 the following four treatments: T1 - control (0.0% CWSC, and 0 of ochratoxin), T2 - 0.1% of CWSC, T3 - 500 ppb of aflatoxin and T4 – 0.1% of CWSC and 500 ppb of aflatoxin. 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 practised 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: For the statistical analysis a completely random experimental design in a factorial (2 X 2) arrangement was used. Data were subjected to analysis of variance.

Aflatoxin production and cell wall of Saccharomyces cerevisiae: Aflatoxin was produced by the fermentation of rice with *Aspergillus parasiticus*, strain NRRL 2999, according to WEST *et al.* (1973). After fermentation, the rice was dried in an oven at 100º C for 12 hours and ground. The level of aflatoxin was analysed 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 37º C for 48 hours. Then the solid phase was separated by centrifugation at 3000 x for 30 min and lyophilised 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

Aflatoxin might cause significant losses to poultry industry due to reduced performance and health problems in the exposed birds. In the present study, although, the level of aflatoxin used affected the feed conversion of birds at 21 days of age no other effect of aflatoxin on performance of broilers was observed. The results in TABLE 1,2 and 3 showed that aflatoxin in diet did not cause a significant decrease in feed intake, weight gain and feed conversion as compared to the control group ($P>0.05$), except for feed conversion at 21 days of age. Actually, DOERR *et al.* (1983) reported that the level of aflatoxin that could affect the performance of birds is related with the comfort, what means that if the animal is exposed to less stress it is more resistant to aflatoxin. In this way, it could be suggested that the good conditions of nutrition and management which the birds were exposed to in the present study might be reduced the sensitivity of theses birds to low level of aflatoxin added to experimental diet.

TABLE 1 – FEED INTAKE, BODY WEIGHT GAIN AND FEED GAIN OF BROILERS AT INITIAL STAGE OF EXPERIMENT (1 TO 21 DAY).

Treatments	CWSC* (%)	Aflatoxin (ppb)	Feed intake (g)	Body weight gain (g)	Feed:gain (g/g)
T1	0.00	0	1,136	694	1.642
T2	0.10	0	1,136	723	1.575
T3	0.00	500	1,063	699	1.520
T4	0.10	500	1,078	726	1.487
C.V. (%)			2.45	2.33	1.42
			Main		
Aflatoxin (+)			1,070	712	1.503
Aflatoxin (-)			1,136	708	1.608
CWSC (+)			1,057	724	1.531
CWSC (-)			1,099	696	1.581
			Probabilities		
Aflatoxin (A)			0.148	0.476	0.050
CWSC (C)			0.916	0.051	0.068
A x C			0.820	0.364	0.214

*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* (%)	Aflatoxin (ppb)	Feed intake (g)	Body weight gain (g)	Feed:gain (g/g)
T1	0.00	0	3,555	1,782	1.998
T2	0.10	0	3,432	1,813	1.903
T3	0.00	500	3,543	1,717	2.063
T4	0.10	500	3,452	1,781	1.941
C.V. (%)			3.20	3.89	1.45
			Main		
Aflatoxin (+)			3,497	1,749	2.002
Aflatoxin (-)			3,493	1,797	1.950
CWSC (+)			3,442	1,797	1,922
CWSC (-)			3,549	1,749	2,030
			Probabilities		
Aflatoxin (A)			0.412	0.312	0.065
CWSC (C)			0.061	0.478	0.045
A x C			0.412	0.356	0.520

*CWSC – Cell wall of *Saccharomyces cerevisiae*

Addition of CWSC to diet showed improvement in feed conversion of birds fed with or without mycotoxin in the diet at 42 days of age (TABLE 2 and 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 improvement in performance of broilers supplemented with CWSC in the same study. The cell wall of yeast is normally constituted of mannan oligosaccharides, and the use of this substance has improved feed conversion of birds in some reports independent of mycotoxins in diet (SAVAGE and ZAKRZEWSKA, 1997, FRITTS and WALDROUP, 2003).

TABLE 3 – FEED INTAKE, BODY WEIGHT GAIN AND FEED GAIN OF BROILERS AT TOTAL PERIOD OF EXPERIMENT (1 TO 42 DAY).

Treatments	CWSC* (%)	Aflatoxin (ppb)	Feed intake (g)	Body weight gain (g)	Feed:gain (g/g)
T1	0.00	0	4,691	2,475	1.896
T2	0.10	0	4,573	2,536	1.807
T3	0.00	500	4,600	2,417	1.905
T4	0.10	500	4,530	2,506	1.808
C.V. (%)			4.41	5.47	2.45
			Main		
Aflatoxin (+)			4,565	2,461	1.856
Aflatoxin (-)			4,632	2,505	1.851
CWSC (+)			4,551	2,521	1.900
CWSC (-)			4,645	2,446	1.807
			Probabilities		
Aflatoxin (A)			0.082	0.105	0.245
CWSC (C)			0.075	0.112	0.048
A x C			0.415	0.352	0.825

*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* (%)	Aflatoxin (ppb)	Liver (g/100g BW)	Kidney (g/100g BW)	Bursa of Fabricius (g/100g BW)
T1	0.00	0	3.17	0.74	0.26
T2	0.10	0	3.21	0.86	0.30
T3	0.00	500	3.15	0.94	0.25
T4	0.10	500	3.31	1.03	0.21
C.V. (%)			14.00	26.00	42.00
			Main		
Aflatoxin (+)			3.23	0.98	0.23
Aflatoxin (-)			3.19	0.80	0.28
CWSC (+)			3.26	0.94	0.25
CWSC (-)			3.16	0.84	0.25
			Probabilities		
Aflatoxin (A)			0.354	0.128	0.145
CWSC (C)			0.254	0.212	0.972
A x C			0.412	0.315	0.314

*CWSC – Cell wall of *Saccharomyces cerevisiae*

Although aflatoxin has been reported to cause increase in relative weight gain of liver and kidneys of poultry (LEESON *et al*., 1995), the level of mycotoxin used was relatively higher than the one used in the present study and perhaps it could be an explanation for the results in TABLE 4 and 5, as no differences between the experimental groups in relative weight of organs are seen.

Conclusions

At the light of the results of the present study and other studies, by the same authors it might be suggested that aflatoxin, in the experimental condition and at levels used (500 ppb), did not cause losses in performance of broilers, but in field situation it could be different.

The use of CWSC improves the feed conversion of bird with or without aflatoxin in the diet.

TABLE 5 – RELATIVE WEIGHTS OF LIVER, KIDNEY AND BURSA OF FABRICIUS IN BROILERS AT 42 DAYS OF AGE.

Treatments	CWSC* (%)	Aflatoxin (ppb)	Liver (g/100g BW)	Kidney (g/100g BW)	Bursa of Fabricius (g/100g BW)
T1	0.00	0	2.05	0.69	0.17
T2	0.10	0	1.74	0.74	0.17
T3	0.00	500	2.31	0.63	0.12
T4	0.10	500	2.51	0.72	0.15
C.V. (%)			9.64	42.00	14.00
			Main		
Aflatoxin (+)			2.41	0.67	0.13
Aflatoxin (-)			1.89	0.71	0.17
CWSC (+)			2.12	0.73	0.16
CWSC (-)			2.18	0.66	0.14
			Probabilities		
Aflatoxin (A)			0.215	0.279	0.186
CWSC (C)			0.345	0.174	0.275
A x C			0.186	0.412	0.412

*CWSC – Cell wall of *Saccharomyces cerevisiae*

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