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Evaluation of the Efficacy of *Saccharomyces cerevisiae* Cell Wall to Ameliorate the Toxic Effects of Aflatoxin in Broilers

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Abstract: A study was carried out to evaluate the efficacy of *Saccharomyces cerevisiae* (*SC*) as an inhibitor of aflatoxicosis in broilers. Clinic, pathologic, immune and productive aspects of broilers fed with aflatoxin in the diets were studied. The results showed that the feed intake and weight gain were negatively affected ($P < 0.01$) for aflatoxin in diet (1000 ppb). The *SC* (0, 20%) added to diet did not ameliorated the negatively aflatoxin effect at broiler weight gain, however the *SC* improve the feed conversion in this birds. The humoral immune response of broilers vaccinated against Newcastle virus disease (NDV) was reduced for aflatoxin in diet, as also the liver, kidneys and bursa of Fabricius were negatively affected for this toxin at diet. The *SC* did not improve the hemagglutination inhibiting antibody titers in birds fed with aflatoxin in diet, however improve the immune response of broilers at challenge with strain velogenic of NDV.

Key words: Broilers, aflatoxicosis, cellular wall *S. cerevisiae*, immune response

Introduction

Aflatoxin are toxic metabolites produced for fungi of *Aspergillus* species. Several studies have been related to the negative effects of aflatoxins in birds including reduction in performance, pathologic alterations in important organs as liver and kidneys and also the interference in immune system of birds (Kubena *et al*., 1990; Santin, 2000).

Recently, many research have been look forward ways that minimize the losses associated with aflatoxin in diet and the utilization of adsorbents has been suggested for several research (Boulton *et al*., 1981; Kubena *et al*., 1990; Miazzo *et al*., 2000; Rosa *et al*., 2001). The adsorbents most utilized in commercial poultry are clays and actually the yeast has also been suggested. Stanley *et al*. (1993) utilized different levels of a yeast, *Saccharomyces cerevisiae* (*SC*), in broilers diet with 5ppm of aflatoxin and observed improve in performance of intoxicated birds. This research suggested that the SC might be ameliorated the aflatoxins effects for adsorption of aflatoxin for the cell wall of *SC*, with elimination this toxic agent for the feces in intestinal tract.

Recent study of Santin *et al*. (2001) showed the cell wall of *SC* improve the intestinal mucosa aspects and suggested that it might be the explanation for the improve in performance of broilers supplemented with cell wall of *SC* observed in the same study.

In this context, the present study was carried out to evaluated the utilization of cell wall of SC in prevention of aflatoxicosis in broilers and ameliorate of immune response against Newcastle disease virus.

Materials and Methods

Were utilized 320 males Cobb™ one-day-old broiler housed in 16 boxes in a experimental housed and received feed and water *ad libitum*. Birds were distributed into four treatments with four repetitions of 18 birds per floor pen, as fellow: T_1-Control (0,0% of cell wall *S. cerevisiae* - CWSC e 0 ppm of aflatoxin); T_2-0,2% of CWSC; T_3-1 ppm of aflatoxin; T_4-0,2% of CWSC + 1 ppm of aflatoxin. The diet was based on corn and soybean meal, according to the recommendations of the NRC (1994). These diets were free from zearalenone and ochratoxin, according to thin-layer chromatography method (Soares and Rodriguez-Amaya, 1989).

The aflatoxin was produced according to Shotwel *et al*. (1966) modified for West *et al*. (1973). The level of aflatoxin was analyzed by thin-layer chromatography method, according to the technique described by Soares and Rodriguez-Amaya (1989) and was incorporated to broilers diet at level of 1 ppm, according with each experimental group.

The cell wall of *SC* utilized was var. *Calsberg*. All experimental groups received a standardized, recently manufactured Newcastle disease virus (NDV) vaccine by intra-conjunctival instillation at seven and 21 days of age. This lyophilized vaccine was prepared using the lentogenic strain LaSota of the NDV and the vaccine titer obtained by determining the 50% infecting dose (DIE_{50}) in embryonated eggs of Specific-Pathogen-Free (SPF) breeders at day eight to ten of incubation. The titer of vaccine virus was $10^{7,20}$/0, 1ml. Blood samples from eight birds per experimental group were taken at seven, 18, 25 and 35 days of age via wing vein puncture. Serum was separated by centrifugation at 1000g for 15

Table 1: Feed intake, weight gain and feed conversion of broilers chickens reared under experimental conditions (1-42 days of age)

Group / Treatment	Aflatoxin(ppm)	SC*(%)	Feed intake (g)	Weight gain(g)	Feed conversion (g/g)
T_1	0	0	4,281	2,326	1.841
T_2	0	0.20	4,272	2,371	1.801
T_3	1	0	3,766	1,998	1.886
T_4	1	0.20	3,773	2,071	1.823
C.V.[1] (%)			7.15	8.41	2.62
Principal Effects					
S. cerevisiae (+)			4,023	2,221	1.812
S. cerevisiae (-)			4,023	2,162	1.863
Aflatoxin (+)			3,770	2,035	2.035
Aflatoxin (-)			4,276	2,349	2.349
			---------- Probabilities ----------		
S. cerevisiae (S)			0.991	0.218	0.024
Aflatoxin (A)			0.001	0.001	0.119
S*A			0.906	0.746	0.582

[1]Coefficient of variation. **Saccharomyces cerevisiae*.

Table 2: Number of mitotic cells in the bursa of Fabricius of broilers necropsied at 21 and 42 days of age in different treatment/group. Cell count obtained using image analysis system (Video Plan, Carl Zeiss, Germany)

Group / Treatment	Aflatoxin (ppm)	SC* (%)	21 days	42 days
T_1	0	0	89	32
T_2	0	0.20	86	35
T_3	1	0	58	18
T_4	1	0.20	61	22
Principal effects				
S. cerevisiae (+)			73	28
S. cerevisiae (-)			73	25
Aflatoxin (+)			59	20
Aflatoxin (-)			87	33
Probabilities				
S. cerevisiae (S)			0.938	0.696
Aflatoxin (A)			0.001	0.005
S*A			0.700	0.516

**Saccharomyces cerevisiae*

minutes, inactivated at 56 °C for 30 minutes and stored at -20 °C until tested.

A Hemagglutinating inhibition antibody to NDV, using the method ß, standardized by Cunningham (1971). Antibody titers were obtained by transforming the values of the last dilutions which produced total inhibition of hemagglutination and were expressed as the logarithm to the base 2.

On 21 and 42 days of life, eight birds from each group, were necropsied and bursa of Fabricius and liver were dissected and individually weighed. Weights were expressed as percentage of body weight, thus obtaining the relative weight of organs. Samples of these organs were taken into 10% phosphate-buffered formalin, pH 7.0, for histological examinations. The samples were processed under conventional histological techniques and stained by hematoxylin-eosin method (HE). For analysis of histopathological change in the liver, the score of intensity of hepatocyte vacuolation was adopted (score 1-mild vacuolation; score 2-intermediate vacuolation; score 3-severe vacuolation). Mitotic cells were counted in bursal follicles by image analysis system (Video Plan, Carl Zeiss, Germany). In addition, the occurrence of picnotic nuclei and the reduction of follicle cell number (follicle depletion) were noted.

On 43 days of the life, eight birds of each group were challenged with velogenic strain of NDV by intra-conjunctival instillation, according with Code of Federal Regulations (1993). The resistance to challenge was expressed in percentage of total protection and was implicated the absence of clinic signs and mortality observed during 10 days post challenge. For control of NDV pathogenicity was used a group of SPF birds from whom was made the isolation and identification of NDV.

A random design with factorial arrangement (2 x 2) was used. Data were submitted to analysis of variance.

Results and Discussion

The results of performance observed in this study were in Table 1. The aflatoxin in diet reduced the weight gain and the feed intake of birds ($P < 0.01$) as had been related in other studies (Kubena *et al.*, 1990; Miazzo *et al.*, 2000). But these parameters were not affected for the cell wall of *S. cerevisiae* (CWSC). Did not have interaction between the aflatoxin and CWSC for these parameters in statistical analysis. The feed conversion was not modified ($P > 0.05$) for aflatoxin, however, it is improve significantly ($P < 0.05$) for the presence of CWSC in diet of animals. Santin *et al.* (2001) also showed significant improve in feed conversion for broilers submitted to diet with CWSC and suggested that the increased of villus height observed in intestinal mucosa, in that study, was a possible explanation for these results.

Table 3: Geometrical means of titers (GMT) of heamoagglutination inhibiting antibodies (HI) from serum at 7, 18, 25 and 35 days of age (expressed as logarithm on base 2) in the different treatments

Group / Treatment	Aflatoxin(ppm)	SC*(%)	Geometrical means of HI titers (Log_2)			
			Bird age (days)			
			7	18	25	35
T_1	0	0.00	4.50	7.50	7.75	7.50
T_2	0	0.20	3.37	7.50	7.60	7.00
T_3	1	0.00	3.00	5.75	6.25	5.87
T_4	1	0.20	3.50	6.37	7.17	5.50
Principal effects						
S. cerevisiae (+)			3.44	6.93	6.68	6.25
S. cerevisiae (-)			3.75	6.62	7.00	6.69
Aflatoxin (+)			3.25	6.06	6.12	5.69
Aflatoxin (-)			3.93	7.50	7.56	7.25
			Probabilities			
*S. cerevisiae (*S)			0.366	0.493	0.547	0.375
Aflatoxin (A)			0.053	0.003	0.009	0.003
S*A			0.224	0.493	0.904	0.898

**Saccharomyces cerevisiae*.

The birds submitted to diet with aflatoxin with or without CWSC showed, at necropsy, liver yellowish color with higher relative weight than birds did not exposed to toxin (P = 0, 05). The bursa of Fabricius of birds exposed to aflatoxin showed lower relative weight than birds did not exposed to aflatoxin in diet. In histological analysis the liver from birds intoxicated showed severe vacuolation (score 3), megalocytosis of hepatocytes and hyperplasia of biliary ephitelio. Birds exposed to aflatoxin in diet showed bursa of Fabricius with lower number of mitotic cells as compared to birds not fed the aflatoxin (Table 2). In addition, the bursae of birds fed diets containing aflatoxin frequently exhibited lymphofollicular depletion, with some picnotic nuclei and degenerate cells, also described for Santin (2000). The presence of CWSC did not modify the tissue changes in birds exposed or not to aflatoxin.

In the serology analysis (Table 3), although all groups had hemagglutination inhibiting antibodies against NDV, the highest hemagglutination inhibiting antibodies titers were obtained from birds not exposed to aflatoxin ($P < 0.01$) at 18, 25 and 35 days of age, indicating that the aflatoxin impact negatively on the humoral immune response of broiler vaccinated against NDV, as were observed for Gabal and Azzam (1998).

The CWSC did not influence significant the humoral immune response of broilers against NDV. At challenge with the velogenic strain of the VDN, at 43° days of age, all birds exposed to diet with aflatoxin (100%) showed NDV clinic signs, as green diarrhea, apathy and death like was observed in SPF birds. In the other way, any of the birds did not exposed to aflatoxin showed NDV clinic signs, establishing that the aflatoxin interfere negatively the immune response of broiler vaccinated. This immune suppression in broilers exposed to aflatoxin was described for Qureshi *et al.* (1998), that suggesting the aflatoxin interference with protein metabolism as the cause of this damage.

The presence of CWSC in diet of birds fed with 1ppm of aflatoxin ameliorated in 50% the protection against VDN challenge, however the CWSC did not show significant improvement in hemagglutination inhibiting antibodies titers. According with Paulillo *et al.* (1982), the challenge with NDV is most important to characterize the total immune response of bird to this virus than humoral immune response only. In this aspect, the 50% of protection observed in birds exposed to aflatoxin and CWSC could suggest that although the CWSC did not ameliorated all the aspect of birds exposed to aflatoxin, they have some results in immune response and feed conversion of birds. Theses results suggest that probably, this substance acts improving the quality of intestinal tract (TGI) of animals as was observed for Bradley *et al.* (1993). This Researchers found lower goblet cells number and crypt depth in ileal mucosa of broiler supplemented with *Saccharomyces cerevisiae*, demonstrating reduced of mucosa turnover, suggesting that this could be a result of lower number of bacteria ant toxins in the mucosa. Eshdat *et al.* (1978) reported that bacterial attachment in gut is often mediated by biding of bacterial lectins to receptors containing D-manose, thus, the use of products containing manose-based carbohydrate could be useful to reduce colonization by enteropathogenic bacteria. In the other hand, the immune system have a innate immunity response to sugars that are common to almost all cell wall of bacteria and yeast (Abbas *et al.*, 2000) and for this way, the cell wall of *S. cerevisiae* could attracted the immune

cells and other immune factors to gastrointestinal tract resulting in a barrier to antigens on intestinal tract. It is important to observe that all this possible mechanism could improve the performance of the animals, as was observed by Bradley *et al.* (1993) Santin *et al.* (2001) and this way is possible that the bird would be in the best organic conditions for respond to NDV challenge even if that was exposed to aflatoxin.

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