EVALUATION OF THE EFFICACY OF A COMMERCIAL HYDRATED SODIUM CALCIUM ALUMINOSILICATE TO REDUCE THE TOXICITY OF AFLATOXIN AND OCHRATOXIN IN BROILER CHICKS.

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INTRODUCTION

Aflatoxins, the first type of mycotoxins identified, are highly toxic and carcinogenic substances produced by the genera *Aspergillus* and *Penicillium.* Aflatoxin B1 (AFB) is the most toxic and carcinogenic of all Aflatoxin metabolites, and the presence of contaminated feedstuffs as well as the problems associated with it, has been recognized for many years worldwide. On the other hand, Ochratoxin A (OCA), identified later in 1965, is considered the most toxic mycotoxin for domestic fowl, as demonstrated by lethality studies (5). The addition of Hydrated Sodium Calcium Aluminum Silicate (HSCAS) to the diet is one of the methods used to control mycotoxins' deleterious effects, as was initially demonstrated by Taylor and Phillips (10). Several studies (8, 6, 7, 12) have shown the efficacy of different types of HSCAS to prevent reduced performance, change in organ weights, serum chemistry, and macroscopic/microscopic lesions caused by AFB. Traditionally, nutritionists have been reluctant to use high inclusion HSCAS in the diet (0.5-1.0%) since these products do not have a nutritional value and take a valuable space in feed formulation. This situation is critical in broiler diets due to the importance of keeping high energy and amino acid levels to meet the nutritional requirements of fast-growing modern genetic lines. Therefore, the advantages of using a low inclusion HSCAS are twofold: inclusion of a smaller volume which will reduce the possibility of adsorbing critical nutrients like vitamins and minerals; and a lower inclusion cost when products with a similar price are compared.

Before considering a HSCAS as a mycotoxins binder candidate, *in vitro* evaluations have been emphasized (3). It is commonly accepted that products showing above 70% net adsorption are good candidates for *in vivo* trials, although exceptionally few products showing a low *in vitro* absorption can be very effective when used *in vivo*. That is, there is not always a good correlation between *in vivo* and *in vitro* results because lab conditions are very different from the ones present in the animal. On the other hand, the effect of an adsorbent is difficult to evaluate, since it is problematic to calculate how much mycotoxin has been bound. Therefore, the animal performance and measurement of mycotoxins concentration and/or degree of damage in tissues and organs is necessary to determine how effective the product is.

In vitro studies performed in several laboratories with Myco-Ad at 0.25% inclusion level have demonstrated an average net adsorption capacity of 98 % (11) and 96% (1) when tested against 5 ppm of AFB. When the same inclusion level was tested against 5ppm of OCA, the net adsorption reported was 64% (1).

One of the purposes of this study was to demonstrate the efficacy of Myco-Ad, a commercial low inclusion HSCAS, when used in rations experimentally contaminated with AFB and OCA. A second objective was to determine if the addition of Myco-Ad to the diet caused any effect on broiler performance.

MATERIALS AND METHODS

Birds and facilities. Male broilers (Arbor Acres, Glastonbury, Connecticut, US) were transported from a commercial hatchery to a experimental farm and brooded in the floor for 4 days (experiment 1) and 7 days (experiment 2), then selected with an average body weight (BW) of 78 g and 84 g respectively. The birds were housed in battery cages (one bird per cage) at the Instituto Internacional de Investigación Animal (IIIA), in Querétaro, México and kept in a building under positive pressure, with a constant humidity (55%) and a controlled temperature (26C) during the whole experiment. All birds received artificial light for 16 hours daily.

Feed. A basal sorghum-soybean meal mash diet was provided *ad libitum* in both studies.

Mycotoxins. Artificial AFB (lot # 123H4038) produced by *Aspergillus flavus*, and artificial OCA were bought from Sigma Labs, St. Louis, Missouri, US.

Mycotoxin binder. A HSCAS sold as Myco-Ad , produced in Texas (Special Nutrients, Miami, Florida, USA) was imported from the U.S. and included in the diet at 0.25% (2.5 kg/mt), the standard commercial dose recommended in field conditions.

Lesions detection in internal organs. Daily mortality was recorded and carcasses were examined in both experiments. Macroscopic lesions were classified as mild (small congested areas and small areas showing jaundice appearance), moderate (congested livers and large jaundice areas), and severe (large areas of paleness, jaundice appearance and hepatitis). In the kidneys, the presence of urates was reported as mild. Light congestion and hemorrhage were considered moderate, and the presence of congestion combined with widespread hemorrhage with nephritis was reported as severe. At the end of the trial, all birds were euthanized humanely, posted and their internal organs evaluated. In experiment 1, the liver-BW ratio was calculated at 24 day. In experiment 2, the liver and kidney- BW ratios were calculated at 28 days.

Experimental Design. Trial 1. 96 four-day-old broilers were divided in four dietary treatments with 24 replications each. Treatment 1 was a control diet, T II control plus Myco-Ad , T III control plus 7.5 ppm AFB, and T IV control plus Myco-Ad plus 7.5 ppm AFB.

Experimental design. Trial 2. 64 seven-day-old male broilers were divided in four dietary treatments with 16 replications each. Treatment 1 was a control diet, T II control plus Myco-Ad, T III control plus 2.0 ppm OCA, and T IV control plus Myco-Ad plus 2.0 ppm OCA.

Statistical analysis. Data were evaluated with ANOVA for a complete randomized design, using the general linear models procedure of SAS software (13). When the ANOVA showed significance, Duncan's significant-difference test was applied. Statistical significance was accepted at $P \leq 0.05$.

RESULTS

Experiment 1. The addition of Myco-Ad to the diet contaminated with AFB significantly prevented the impaired performance (BW 609 g versus 447 g; FC 1.62 versus 1.92) and the gross liver lesions observed in chicks fed AFB, as shown in table 1. The addition of the adsorbent in the absence of added AFB in the diet did not show any significant difference in BW, FC and daily weight gain. The control treatment plus AFB was the only one showing mortality in this experiment (8.3%) and a significantly higher liver weight than all the other treatments. Macroscopically, the treatment with AFB plus Myco-Ad showed mild to moderate liver lesions, while the group treated with AFB showed severe lesions. The control diet and the control diet plus Myco-Ad did not show any liver lesions.

Experiment 2. Feeding OCA contaminated diet plus Myco-Ad resulted in significantly heavier (770 g versus 706 g) and more efficient (FC 1.65 versus 1.78) broilers with markedly reduced macroscopic kidney lesions than those fed 2 ppm OCA, as shown in table 2. The diet free of OCA and the one treated with Myco-Ad showed similar (no statistical difference) kidney-BW ratio. When evaluating the liver-body weight ratio, no significant difference was observed in any of the treatments tested.

DISCUSSION

Experiment 1. The results obtained in this study are in agreement with previous reports (2, 6,7,8, 10) indicating the efficacy of several HSCAS in reducing the toxicity of AFB. We must emphasize that most HSCAS, in spite of their mineralogical differences, will be able to adsorb AFB, considering that this is a positively charged mycotoxin. It has been previously shown (10) that AFB binds to HSCAS containing a large number of negative charges. However, not all HSCAS will bind AFB because it may take multiple electrical sites to hold the AFB molecule or because the dosage necessary for some of these products to work is very high.

Experiment 2. The results reported in this trial indicate that Myco-Ad was effective in preventing the toxic effects of OCA in broiler chicks consuming a feed experimentally contaminated, as previously reported in the literature when using a similar product (2). Even though the lack of efficacy of a HSCAS to control OCA in broilers has been described (4,12); under the conditions of this trial, we obtained effective results as demonstrated by significantly better BW and FC; and reduced macroscopic kidney lesions in the broilers fed the diet treated with Myco-Ad and OCA. To explain this discrepancy, it is important to take into consideration the large number of different products that are included into the HSCAS classification and the fact that OCA, contrary to AFA is a dipolar molecule, which will be more difficult to bind. Possibly, as described in the literature (9, 14) when classifying the HSCAS properties, the fact that the product tested in this experiment has distinct characteristics, is the cause of his effectiveness in binding the OCA molecule present in the feed. Among these characteristics, cationic exchange, chemical composition, lack of expandability, slightly alkaline pH, and particle size, play an important role (14).

Regarding the interference with the adsorption of critical nutrients, the use of high inclusion expandable HSCAS with a high cationic exchange capacity, might have a detrimental effect in the animal diet by affecting the adsorption of important nutrients. In the two experiments reported here, using 2.5 kg of Myco-Ad/mt of feed, we did not observe any significant difference between the group treated with Myco-Ad and the diet free of mycotoxin and adsorbent, demonstrating its lack of negative effects on broiler performance. This finding has been already reported in the literature, using a HSCAS adsorbent (6).

CONCLUSIONS

1. The addition of 2.5 kg/mt of Myco-Ad to the chick diet was effective in preventing the toxic effects of Aflatoxin B1 and Ochratoxin A.

2. The addition of 2.5 kg/mt of Myco-Ad to the diets did not cause any statistical difference in broiler performance when compared to the control diet.

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ABSTRACT

Two experiments were conducted to study the efficacy of a low inclusion commercial HSCAS (Myco-Ad®) in preventing the deleterious effects of Aflatoxin B1 (AFB) and Ochratoxin A (OCA) in broiler chicks. Arbor Acres broiler males individually caged were used in both experiments. The feed was experimentally contaminated with synthetic AFB or OCA from Sigma Labs, USA. In Experiment 1, 96 4-day-old chicks were randomly assigned four dietary treatments with 24 replications each. T I was a sorghum-soybean meal control diet, T II control + 2.5 kg/mt Myco-Ad®, T III control + 7.5 ppm AFB, and T IV control + 7.5 ppm AFB + 2.5 kg/mt Myco-Ad®. At 24 days of age, birds fed 7.5 ppm AFB contaminated diet showed severe macroscopic liver lesions, higher mortality, lower body weight, poorer feed conversion, and higher liver weight than chicks fed the control diet. The addition of Myco-Ad® significantly prevented the impaired performance and the gross liver lesions observed in chicks fed AFB. In Experiment 2, 64 7-day-old chicks were randomly divided into four dietary treatments with 16 replications each. T I was a sorghum-soybean meal control diet, T II control + 2.5 kg/mt Myco-Ad®, T III control + 2 ppm OCA, and T IV control + 2 ppm OCA + 2.5 kg/mt Myco-Ad®. Feeding OCA contaminated diet plus Myco-Ad® resulted in statistically significant heavier and more efficient broilers, with markedly reduced macroscopic kidney lesions than those fed 2 ppm OCA at 28 days of age. In both experiments, the addition of 2.5 kg/mt of Myco-Ad® to chick diets did not show any statistical difference in performance compared to the control diet, demonstrating its lack of interference with the absorption of nutrients. These results indicated that Myco-Ad® at 2.5 kg/mt was effective in preventing the toxic effects of AFB and OCA in broilers chicks.

Key Words: Myco-Ad, Aflatoxin, Ochratoxin

Table 1 (Experiment 1). Effects of 0.25% Myco-Ad on average daily intake (ADI), average daily gain (ADG), feed conversion ratio (FCR), initial and final body weight (BW) in 24 day-old broiler chicks.

GROUP	ADI (g)	ADG (g)	FCR	Initial BW 4 d (g)	Final BW 24 d (g)	Liver % BW	Macroscopic lesions in the liver	Mortality (%)
Control	45.90 [a]	28.03 [a]	1.637 [a]	78.87 [a]	639.47 [a]	3.54 [a]	Negative	0
Control + MYCO-AD	47.82 [a]	27.83 [a]	1.717 [a]	78.35 [a]	634.95 [a]	3.18 [a]	Negative	0
Control + 7.5 ppm AFB	35.42 [b]	18.49 [b]	1.915 [b]	76.71 [a]	446.51 [b]	6.14 [b]	100% severe	8.3
Control + MYCO AD + 7.5 ppm AFB	43.01 [c]	26.48 [a]	1.623 [a]	78.90 [a]	608.50 [a]	3.83 [a]	25% negative 40% mild 25% moderate 10% severe	0

[a, b, c] Values within columns with different superscripts are significantly different ($P < 0.05$).

Table 2 (Experiment 2). Effects of 0.25% Myco-Ad on average daily intake (ADI), average daily gain (ADG), feed conversion ratio (FCR), initial and final body weight (BW) in 28 day-old broiler chicks.

GROUP	ADI (g)	ADG (g)	FCR	Initial BW 4d (g)	Final BW28d (g)	Liver % BW	Macroscopic lesions in liver	Kidney % BW	Macroscopic lesions in kidneys
Control	53.87 [a]	31.05 [a]	1.734 [a]	82.45 [a]	734.50 [a]	4.90	Negative	1.09 [a]	Negative
Control + MYCO-AD	54.85 [a]	31.12 [a]	1.762 [a]	85.36 [a]	738.80 [a]	4.96	Negative	1.19 [a]	Negative
Control + 2 ppm OC	52.91 [a]	29.67[b]	1.783 [a]	82.63 [a]	705.70 [a]	4.89	19% mild 63% moderate 18% severe	1.37 [b]	88% severe 6% mild 6% moderate
Control + MYCO AD + 2 ppm OC	53.89 [a]	32.63 [a]	1.651[b]	85.19 [a]	770.40 [a]	4.81	44% negative 19% mild 31% moderate 6% severe	1.33 [b]	62% negative 19% mild 6% moderate 13% severe

[a, b, c] Values within columns with different superscripts are significantly different ($P < 0.05$).
No mortality was reported in any of the groups tested.