# EVALUATION OF THE EFFICACY OF A COMMERCIAL HYDRATED SODIUM CALCIUM ALUMINOSILICATE TO REDUCE THE TOXICITY OF T-2 TOXIN IN BROILER CHICKS.

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#### INTRODUCTION

Fungal contamination of agricultural products is often unavoidable and of worldwide concern, because the products frequently contain toxic metabolites that produce significant economic losses to the poultry industry. Mycotoxins cause a wide variety of adverse clinical signs, depending on the nature and concentration of toxins in the diets, animal species, age; and nutritional and health status at the time of exposure to contaminated feed. (6) The presence of mycotoxins in poultry feeds is of concern because it has resulted in economic losses due to the reduced performance and health.

T-2 toxin (T-2) is a naturally occurring mycotoxin from the group of the trichothecenes, which are produced by *Fusarium* spp. mainly before harvesting. Trichothecene toxins have been most often characterized by oral lesions and reduced growth in chickens, as well as the inhibition of protein synthesis, responsible for the negative effects on rapidly dividing cells such as those of the oral cavity, gastrointestinal tract, and lymphoid tissues (10, 15). In addition, Burditt et al. (3) found that T-2 toxin produced a dose related feed refusal, suggesting that it may have been due to the irritative properties of trichothecenes.

Practical methods to detoxify mycotoxins contaminated grain or feed on a large scale and in a cost-effective manner are not currently available. At present, one of the most practical approaches consist on using adsorbent materials in the diet to reduce the absorption of mycotoxins from the gastrointestinal tract. Hydrated sodium calcium aluminum silicate (HSCAS) represents an important group of products that have been used with success worldwide with this purpose. In the scientific literature, HSCAS has been reported to be effective against aflatoxin and ochratoxin, but not against T-2. (2, 4, 8, 9, 10)

The objectives of this research were to determine the efficacy of Myco-Ad<sup>®</sup> to ameliorate the toxic effects of T-2 present in broiler diets and to demonstrate that the addition of Myco-Ad<sup>®</sup> would not negatively affect broiler performance.

# MATERIALS AND METHODS

Two experiments were conducted using chicks obtained from a commercial hatchery. The chicks were individually caged and reared under uniform management conditions in each experiment, with feed and water provided *ad libitum*. Feed in both experiments was experimentally contaminated with synthetic T-2 toxin from Sigma Chemical Company, St. Louis, MO. A commercial HSCAS, Myco-Ad<sup>®</sup>, produced in Texas (Special Nutrients, Miami, FL, USA) was used in these experiments.

# **Experiment 1**

This experiment was conducted at the Institute of Veterinary Medicine in Debrecen, Hungary. A total of 75 day-old straight-run Arbor Acres broiler chicks were used in this experiment for 40 days. Birds were fed a basal starter (1-21 days), grower (22-35 days) and finisher (36-40 days) corn-wheat-soybean meal mash diets that contained or exceeded the levels of nutrients recommended by the NRC (11) and were found to be below detection limits for T-2, diacetoxyscirpenol, HT-2 toxin, deoxynivalenol, aflatoxin B1, ochratoxin A, and zearalenone. The chicks were randomly distributed into three treatments with 25 replications each. Dietary treatments were as follows: 1) control diets; 2) control diets with the addition of 1 mg of T-2 / kg of feed (1 ppm); and 3) control diets with 1 mg T-2 / kg of feed plus 0.25% Myco-Ad. Chickens were weighed individually and scored for oral lesions at 21, 28, 35 and 40 days of age. At the end of the experiment total feed consumption was recorded and all birds were sacrificed to record liver, spleen and heart weight and to carry out histopathological analysis in those organs. On days 21 and 28 four birds per treatment were sacrificed to record liver, spleen, and bursa weight and to perform histopathological analysis.

Oral lesion score consisted of a four point scoring system ranging from 0 to 3. A lesion score 0 indicate no visible lesions; a lesion score of 1 (mild) was seen as one or two mouth lesions clearly visible on either the lower or upper mandible; a lesion score of 3 (severe) was seen as large lesions occurring at several sites within the mouth, mainly on the upper and lower mandibles, the corners of the mouth, and on the tongue; lesions scored as 2 (moderate) were intermediate in appearance to lesion scored 1 or 3.

# Experiment 2

This experiment was conducted at the Instituto Internacional Investigacion Animal, Queretaro, Mexico. A total of 32 five-day-old Ross males broiler chicks were used in this experiment for 33 days. Birds were fed a basal sorghum-soybean meal mash diets that contained or exceeded the levels of nutrients recommended by the NRC (11). The chicks were randomly distributed into four treatments with 8 replications each. Dietary treatments were as follows: 1) control diets; 2) control diet plus 0.25% Myco-Ad; 3) control diets with the addition of 1.25 mg of T-2 / kg of feed; and 4) control diets with 1.25 mg T-2 / kg of feed plus 0.25% Myco-Ad. Chickens were weighed individually, total feed consumption recorded, and scored for oral lesions at 38 days of age. At the end of the experiment five birds from treatments 1, 3 and 4 were sacrificed to carry out

histopathological analysis of bursa, thymus, spleen, liver, kidney, tongue and gizzard. Bone ash was measured in all broilers from treatment 1 and 2, following the A.O.A.C. method of analysis (1)

Oral lesion score consisted of a four point scoring system ranging from 0 to 3, including lesions detected at several sites within the mouth, mainly on the upper and lower mandibles, the corners of the mouth, and on the tongue. A lesion score 0 indicates no visible lesions; score 1 was seen as one mouth lesion; score 2 was seen as up to two lesions; and a lesion scored as 3 indicated more than two lesions.

Data were evaluated with ANOVA for a complete randomized design, using the general linear models procedure of SAS software; SAS Institute (14). When the ANOVA showed significance, Duncan's significant-difference test was applied. Statistical significance was accepted at  $P \le 0.05$ .

#### **RESULTS**

# **Experiment 1.**

Results presented in Table 1 demonstrate the significant negative effect of feeding 1 ppm T-2 on body weight and oral lesions of broilers at 21, 28 and 35 days of age; and on the relative bursa weight at 21 and 28 days of age. The relative weights of liver and spleen were not altered by the dietary inclusion of T-2 at 21 and 28 days of age (data not shown). The most severe oral lesions and the largest reduction of the bursa occurred at 21 and 28 days of age, respectively. Addition of 0.25% Myco-Ad to the T-2 contaminated diet significantly improved body weight, oral lesions and relative bursa weight that were not statistically different from the chickens fed the control diets.

The incidence and severity of all oral lesions per treatment are shown in Figure 1. A high percentage of birds were affected with mild (27%), moderate (16%) and severe (20%) lesions when receiving diets containing T-2, whereas a small percentage of chickens presented mild (9.5%), moderate (1%) and no severe lesions when the adsorbent was added to the diets.

At the end of the experiment, Myco-Ad significantly prevented the impaired performance and the reduction in the relative spleen weight observed in broilers fed 1 ppm of T-2. The relative weights of liver and heart were not affected by the addition of the toxin. (Table 2)

The histopathological analyses reported necrosis of the skin tissue at the corner of the mouth and in the mucosa of the oral cavity with infiltration of inflammatory cells in the border between necrotic and healthy tissue in chicks that consumed T-2. These birds also presented reduced thymus lobes and reduced bursal follicles with a decreased amount of lymphocytes. No microscopic changes were reported in birds from the control and Myco-Ad added treatments.

### **Experiment 2.**

The effects of dietary treatments on chick performance from day 5 to 38 as well as oral lesions and bone ash at 38 days of age are presented in Table 3. Consumption of T-2 contaminated feed resulted in significant reduction in body weight gain (18%), poorer feed efficiency (14%), and increased amount of oral lesions. Supplementation of 0.25% Myco-Ad to the diet contaminated with 1.25 ppm T-2 significantly improved gain, feed efficiency and reduced the incidence of oral lesions. Body weight gain, feed consumption, efficiency of feed utilization, oral lesion scores, and bone ash were not significantly influenced by the adsorbent in the absence of added T-2.

The report from the histopathological analyses indicated that at 38 days of age 80% of the birds showed moderate microscopic damage of the bursa, gizzard, spleen, liver and kidney; and all of them presented severe lesions in thymus and tongue when fed a diet with 1.25 ppm T-2. Addition of 0.25% Myco-Ad to the T-2 contaminated diet markedly reduced the degree and number of lesions in those organs, to a level comparable to the one reported for the control diet.

#### DISCUSSION

The addition of 2.5 kg of Myco-Ad per ton of feed significantly diminished the adverse effects of T-2 in broiler chicks. This is the first report showing the *in vivo* effectiveness of an adsorbent against T-2. All previous attempts to prevent the toxicity of T-2 in broilers using inorganic or organic adsorbents have failed. (2, 8, 9, 13). The protective action of this HSCAS appears to involve sequestration of T-2 so that it is not available for gastrointestinal tract absorption by the chicks, as suggested by Phillips et al. (12) for aflatoxin.

In spite of the effectiveness of Myco-Ad in preventing the decreased broiler performance and organs damage produced by the addition of T-2, mild oral lesions were observed in some chickens fed the adsorbent. Probably, they are the results of the direct caustic effect of T-2 in the mouth (7), where the lack of appropriate conditions, especially high humidity and low pH, prevent the action of the adsorbent.

The results of Experiment 2 are in agreement with those previously obtained by Casarin et al. (4), indicating that Myco-Ad does not interfere with the absorption of nutrients.

Results obtained in these experiments demonstrate that 1.0 or 1.25 mg of T-2 per kg of feed can produce, in general, the typical signs of an acute T-2 toxicosis in broilers, similar to those reported in the literature (5, 7, 16) but using levels of T-2 greater than 2 mg/kg. According to Hoerr (7), the severe ulcerative stomatitis produced by T-2 leads to decreased feed intake, reduced gain and decreased feed efficiency.

#### CONCLUSIONS

- 1. The addition of 2.5 kg of Myco-Ad per metric ton of feed was effective in preventing the deleterious effects of toxin T-2 in broiler chickens.
- 2. The addition of 2.5 kg of Myco-Ad per metric ton of feed did not show any statistical difference in performance and bone ash of broilers compared to those from the control diet, demonstrating its lack of nutrients absorption.

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#### **ABSTRACT**

Two experiments were conducted to study the efficacy of a low inclusion commercial HSCAS (Myco-Ad<sup>®</sup>) in preventing the deleterious effects of T-2 toxin (T-2) in broiler chicks. Feed in both experiments was experimentally contaminated with synthetic T-2 from Sigma Labs, USA. In Experiment 1, 75 1-d-old Arbor Acres straight-run broilers individually caged were randomly distributed into three dietary treatments with 25 replications each. T I was a corn-wheat-soybean meal control diet, T II control + 1 ppm T-2, and T III control + 1 ppm T-2 + 2.5 kg/mt Myco-Ad<sup>®</sup>. At 40 d of age, birds fed 1 ppm T-2 contaminated diet showed significant lower body weight, poorer feed conversion, smaller bursa and severe macroscopic oral lesions than chicks fed the control diet. The addition of Myco-Ad<sup>®</sup> significantly prevented the impaired performance, bursa damage and the severe oral lesions observed in chicks fed T-2. In Experiment 2, 32 5-dold Ross male chicks were randomly divided into four dietary treatments with 8 replications each. T I was a sorghum-soybean meal control diet, T II control + 2.5 kg/mt Myco-Ad<sup>®</sup>, T III control + 1.25 ppm T-2, and T IV control + 1.25 ppm T-2 + 2.5 kg/mt Myco-Ad<sup>®</sup>. Feeding T-2 contaminated diet plus Myco-Ad<sup>®</sup> resulted in statistically significant heavier and more efficient broilers, with substantially reduced gross oral lesions and microscopic organs lesions (tongue, gizzard, thymus, bursa, spleen, liver, and kidney) than those fed 1.25 ppm T-2 at 38 d of age. The addition of 2.5 kg/mt of Myco-Ad<sup>®</sup> to chick diets did not show any statistical difference in performance and bone ash compared to the control diet, demonstrating its lack of nutrients absorption. These results indicated that Myco-Ad® at 2.5 kg/mt was effective in preventing the toxic effects of T-2 in broilers chicks.

Key Words: Myco-Ad, Toxin T-2

Table 1. Effects of 0.25% Myco-Ad on body weight (BW), oral lesion score, and bursa development of broiler chicks at different ages in Experiment 1.

Treatment	21 days			28 days			35 days	
	BW (g)	Oral lesion	Bursa % BW	BW (g)	Oral lesion	Bursa % BW	BW (g)	Oral lesion
Control	538 <sup>a</sup>	$0^{a}$	$0.30^{a}$	932 <sup>a</sup>	$0^{a}$	$0.45^{a}$	1446 <sup>a</sup>	$0^{a}$
Control + 1 ppm T-2	463 <sup>b</sup>	1.84 <sup>b</sup>	$0.20^{b}$	788 <sup>b</sup>	1.63 <sup>b</sup>	$0.20^{b}$	1148 <sup>b</sup>	$0.96^{b}$
Control + T-2 + Myco-Ad	543 <sup>a</sup>	$0.36^{a}$	$0.28^{a}$	938 <sup>a</sup>	$0.21^{a}$	$0.40^{a}$	1451 <sup>a</sup>	$0.04^{a}$

 $<sup>^{</sup>a,\,b}$  Means within columns with no common superscripts differ significantly (P $\leq$  0.05)

Table 2. Effects of 0.25% Myco-Ad on body weight, feed intake, feed conversion ratio (FCR), and organs development of broilers at 40 days of age in Experiment 1.

Treatment	Body Weight (g)	Feed Intake (g)	FCR	Spleen % BW	Liver % BW	Heart % BW
Control	1791 <sup>a</sup>	3690 <sup>a</sup>	2.06 <sup>a</sup>	$0.12^{a}$	2.5 <sup>a</sup>	$0.6^{a}$
Control + 1 ppm T-2	1381 <sup>b</sup>	2928 <sup>b</sup>	$2.12^{b}$	$0.09^{b}$	2.5 <sup>a</sup>	$0.6^{a}$
Control + T-2 + Myco-Ad	1840 <sup>a</sup>	3717 <sup>a</sup>	2.02 <sup>a</sup>	$0.12^{a}$	$2.4^{a}$	$0.6^{a}$

<sup>&</sup>lt;sup>a, b</sup> Means within columns with no common superscripts differ significantly ( $P \le 0.05$ )

Table 3. Effects of Myco-Ad on average daily gain (ADG), average daily intake (ADI), feed conversion ratio (FCR) and oral lesion score of 38 day-old broilers exposed to test diets for 33 days in Experiment 2.

Treatment	ADG (g)	ADI (g)	FCR	Oral lesion	Bone ash %
Control	54.8 <sup>a</sup>	105.3 <sup>a</sup>	1.92 <sup>a</sup>	0.25 <sup>a</sup>	45.95 <sup>a</sup>
Control + 0.25% Myco-Ad	51.3 <sup>a</sup>	103.0 <sup>a</sup>	2.01 <sup>a</sup>	$0.25^{a}$	45.65 <sup>a</sup>
Control + 1.25 ppm T-2	44.9 <sup>b</sup>	98.4 <sup>a</sup>	$2.19^{b}$	2.75 <sup>c</sup>	-
Control + 1.25 ppm T-2 + 0.25% Myco-Ad	53.5 <sup>a</sup>	101.4 <sup>a</sup>	1.90 <sup>a</sup>	1.75 <sup>b</sup>	-

 $<sup>^{</sup>a,\,b,\,c}$  Means within columns with no common superscripts differ significantly (P $\leq$  0.05)

Figure 1. Incidence and severity of oral lesions in Experiment 1

