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# Evaluation of the supplementation of a feed additive as a potential protector against the adverse effects of 2.5 ppm T-2 toxin on growing broiler chickens

[*Avaliação da suplementação de um aditivo alimentar como um protetor potencial contra os efeitos adversos de 2,5ppm de toxina T-2 em frangos em crescimento*]

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## ABSTRACT

A trial was conducted to evaluate a feed additive containing epoxidase activity from a bacterium (Mycofix-S) as a potential protection against the adverse effects of 2.5 ppm dietary T-2 toxin in male growing broiler chickens. A total of 144 one-day-old Ross 308 male chicks were individually wing-banded and allotted into each of the four experimental groups. Group 1: negative control, no T-2 toxin or additive; group 2: Mycofix-S, 2.5 g/kg; group 3: positive control, 2.5 ppm T-2 toxin; group 4: 2.5 ppm T-2 toxin + 2.5 g/kg Mycofix-S. Feed and water were provided *ad libitum* for 28 days (days 1 to 28 of age). Each experimental treatment was replicated 6 times, with 6 birds per replicate pen. Response variables included performance parameters, serum activity of alkaline phosphatase (ALP) and amylase, relative weight of selected organs and histology of the upper digestive system. T-2 toxin at 2.5 ppm significantly ($P = 0.016$) decreased the 28-day body weight gain and cumulative feed intake without affecting feed conversion. The feed additive counteracted these adverse effects. Serum enzyme activities were not significantly ($P>0.05$) affected for the four experimental groups but when data from the groups receiving T-2 toxin was pooled and compared against the pooled data from groups without the toxin a significant decrease in amylase activity was observed in chickens receiving T-2 toxin. The histological examination of the upper digestive system revealed lesions in mouth, esophagus, proventriculus, gizzard and duodenum in the chickens fed T-2 toxin without the additive. Chickens fed T-2 toxin plus the additive showed lesions in the same tissues except in the duodenum. The results of the present study show that the addition of 2.5 g/kg of the feed additive tested protects against adverse effects on performance and also the integrity of the duodenal mucosa.

Keywords: *ALP, amylase, epoxidase, mycotoxin decontamination, trichothecenes*

## RESUMO

*Foi realizado um experimento com o objetivo de avaliar um aditivo alimentar contendo atividade de epoxidase de uma bactéria (Mycofix-S) como proteção potencial contra os efeitos adversos de uma dieta com 2,5ppm de toxina T-2 em frangos de corte machos. Um total de 144 pintos machos Ross 308 de um dia de idade foram marcados na asa individualmente e alocados em um de quatro grupos experimentais: grupo 1: controle negativo, sem toxina T-2 ou aditivo; grupo 2: 2,5g/kg de Mycofix-S; grupo 3: controle positivo, 2,5ppm de toxina T-2; grupo 4: 2,5ppm de toxina T-2 + 2,5g/kg de Mycofix-S. Alimento e água foram fornecidos* ad libitum *por 28 dias (dias um a 28 de idade). Cada tratamento experimental foi replicado seis vezes, com seis pintos por gaiola de replicação. As variáveis de resposta incluíram parâmetros de desempenho, atividade sérica de fosfatase alcalina (ALP) e amilase, peso relativo de órgãos selecionados e histologia do sistema digestivo superior. A toxina T-2 a 2,5ppm diminuiu significativamente ($P = 0.016$) o ganho de peso corporal aos 28 dias e o consumo de alimento acumulado, sem afetar a conversão alimentar. O aditivo diminuiu os efeitos adversos. As atividades séricas das enzimas não foram afetadas significativamente ($P>0.05$) nos quatro grupos experimentais,*

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*porém, quando os dados dos grupos que receberam a toxina T-2 foram combinados e comparados com o* pool *de dados dos grupos sem toxina, foi observado um decréscimo significativo da atividade de amilase nos frangos que receberam a toxina T-2. O exame histológico do sistema digestivo superior revelou lesões em boca, esôfago, pró-ventrículo, moela e duodeno nos frangos alimentados com toxina T-2 sem aditivo. Frangos alimentados com toxina T-2 mais aditivo mostraram lesões nos mesmos tecidos, exceto no duodeno. Os resultados do presente estudo mostram que a adição de 2,5g/kg do aditivo alimentar testado protege contra os efeitos adversos sobre o desempenho e a integridade da mucosa duodenal.*

*Palavras-chave: ALP, amilase, epoxidase, descontaminação de micotoxinas, tricotecenos*

## INTRODUCTION

Mycotoxins are frequent contaminants of human foods and animal feeds, produced by specific fungal strains. Mycotoxins are capable of affecting the health and performance of domestic animals, decrease the immune response and even cause death when their levels are high enough (Murugesan *et al*., 2015). The trichothecenes are a large group of mycotoxins with the same basic chemical structure. The toxicology of trichothecenes for domestic species was reviewed by Eriksen and Pettersson (2004). Although the number of known trichothecenes is over 100, few of them occur naturally; the most important are deoxynivalenol (DON), nivalenol (NIV), T-2 toxin, HT-2 toxin and diacetoxyscirpenol (DAS), being DON the most prevalent found in grains (Marin *et al.*, 2013). The major effect of T-2 toxin in poultry is an inflammatory focal reaction in the oral cavity that progresses to necrosis and invasion of the normal microflora. Other effects of exposure to dietary T-2 toxin at levels between 1 and 4 ppm and different exposure times include decreased feed intake and decreased body weight gain (Diaz *et al.*, 1994; Diaz, 2002; Diaz *et al.*, 2005).

One way of ameliorating the adverse effects of the tricothecenes in poultry is by removing the contaminated feed and providing support therapy. Some aluminosilicates are capable of adsorbing aflatoxins but they have no activity against trichothecenes such as DAS or T-2 toxin (Diaz, 2002; Di Gregorio *et al*., 2014). Aravind *et al.* (2003) suggest that esterified glucomannans can counteract the adverse effect of some mycotoxins, including T-2 toxin. However, the trials conducted with this type of feed additive have shown no satisfactory results. It is clear, therefore, that new strategies to counteract the adverse effects of trichothecenes on commercial poultry species are needed. A novel strategy is based on the detoxification of the trichothecene through an epoxidase enzyme that removes the 12,13-epoxy ring found in all trichothecenes. A bacterium of the genus *Eubacterium* isolated from bovine ruminal fluid and named BBSH has demonstrated *in vitro* that it can detoxify DON and structurally related trichothecenes due to its epoxidase activity (Fuchs *et al.*, 2002). This bacterium has been used to prepare a commercial product (Mycofix-S, Biomin Gesunde GmbH, Austria). The aim of the present trial was to evaluate through an *in vivo* model the possible protective effect of a feed additive based on the BBSH bacterium (Mycofix-S) against the adverse effects of 2.5 ppm T-2 toxin in growing broiler chickens (days 1-28 of age).

## MATERIALS AND METHODS

The experiment was approved by the Ethical and Animal Welfare Committee of the College of Veterinary Medicine, National University of Colombia. A total of 144 Ross 308 one-day-old chickens were individually weighed and wing-banded and allotted at random within the different experimental groups. The experimental design consisted of a completely randomized 2 x 2 factorial arrangement of treatments with a negative control (no toxin or feed additive), a group with only the feed additive, a positive control (2.5 ppm T-2 toxin) and a group with the feed additive plus the T-2 toxin. The four experimental treatments consisted of the same mash broiler starter ration modified as described below. The experimental diet was analyzed for aflatoxins B1, B2, G1 and G2 (Diaz *et al.*, 2001), DON (Trucksess *et al.*, 1998), zearalenone (Diaz and Céspedes, 1997), T-2 toxin and HT-2 toxin (Pascale *et al.*, 2003) and no detectable levels of any of these mycotoxins were found. The experimental groups were; Group 1: negative control, no T-2 toxin or additive; group 2: Mycofix-S, 2.5g/kg; group 3: positive control, 2.5 ppm T-2 toxin; group 4: 2.5 ppm T-2 toxin +

2.5g/kg Mycofix-S. Feed and water were provided *ad libitum* for 28 days (days 1 to 28 of age). Each experimental treatment was replicated 6 times, with 6 birds per replicate pen for a total of 36 birds per treatment.

Semipurified T-2 toxin obtained from *Fusarium* culture material (Biopure Lot # Q621-Q629) was provided by Biomin Holding GmbH, Herzogenburg, Austria. This material, dried over silicagel, contained 20.9 mg of T-2 toxin per g and was added to an aliquot of each of the experimental diets 3 and 4 in the amount needed to obtain a final concentration of 2.5 ppm. The contaminated aliquot was blended with an adequate amount of diet in a stainless steel mixer, and samples of the blended diet were taken for T-2 toxin analysis by high-performance liquid chromatography with fluorescence detection (Pascale *et al.*, 2003). The analysis revealed the presence of 2630 and 2520 ppb T-2 toxin for diets 3 and 4, respectively. These same diets were also found to contain 192 and 202 ppb HT-2 toxin, respectively.

The feed additive (Mycofix-S, lot 29962) was provided by Biomin Holding GmbH, Herzogenburg, Austria, and was added to the experimental diets 2 and 4 at 2.5g/kg. The chickens were housed in battery cages in a temperature-controlled room with a photoperiod of 23 hours of light and 1 of darkness. The response variables measured during and at the end of the experiment included the following: body weight (days 1, 7, 14, 21 and 28), body weight gain (days 7, 14, 21 and 28), feed intake (days 7, 14, 21 and 28), feed conversion (days 7, 14, 21 and 28), serum activity of the enzymes alkaline phosphatase (ALP) and amylase (days 13 and 23, 12 observations per treatment) and relative weight of selected organs: liver, heart, proventriculus, gizzard, pancreas and bursa (day 28, 12 observations per treatment). Performance variables were analyzed using the replicate pen as experimental unit (n = 6). On day 28, samples were taken in buffered formalin from 3 birds taken at random from each experimental treatment for histological examination of tongue, esophagus, crop, proventriculus, gizzard and small intestine (duodenum).

Data from all response variables were analyzed by one-way analysis of variance (ANOVA) for a completely randomized experimental design using Statistix® for Windows version 9 (2008). The significance level used was $P \leq 0.05$. When the ANOVA analysis showed a significant P value, differences between means were analyzed using the Tukey test.

## RESULTS

Table 1 summarizes the weekly body weight of the 4 experimental treatments. No significant differences were observed among the experimental treatments in any of the days measured except on day 28 when the birds receiving 2.5 ppm T-2 toxin without feed additive (group 3) had a body weight significantly lower than that of the other 3 groups. Weekly feed intake and 28-day cumulative feed intake is shown in Table 2. Significant differences were observed for weeks 2, 3 and 4 and for the 28-day cumulative feed intake. Feed intake in the birds fed the diet containing only T-2 toxin (group 3) was significantly lower than that of the control from week 2 and for the cumulative 28-day feed intake.

Table 1. Individual and combined effect of the dietary supplementation of 2.5 ppm T-2 toxin and/or a feed additive on the weekly body weight of growing broiler chickens[1]

| Group | T-2 toxin (ppm) | Additive and inclusion level (kg/t) | Days of exposure | | | | |
|---|---|---|---|---|---|---|---|
| | | | 0 | 7 | 14 | 21 | 28 |
| 1 | 0 | 0 | 42.9±0.6a | 150.2±3.1a | 398.5±5.5a | 785.6±15.0a | 1365.3±22.4a |
| 2 | 0 | Mycofix-S, 2.5g/kg | 43.7±0.5a | 153.0±4.0a | 387.9±6.7a | 751.3±10.2a | 1327.2±12.8a |
| 3 | 2.5mg/kg | 0 | 43.2±0.5a | 155.5±4.0a | 381.8±7.3a | 748.3±17.5a | 1267.2± 18.4b |
| 4 | 2.5mg/kg | Mycofix-S, 2.5g/kg | 42.9±0.5a | 160.8±4.1a | 393.3±7.9a | 777.1±14.9a | 1331.9±23.5a |
| P | | | 0.722 | 0.272 | 0.382 | 0.219 | 0.017 |

[1]Values are means±S.E.M. of 6 replicate pens per treatment.
Within a column, means with different superscripts differ significantly (P<0.05).

Table 2. Individual and combined effect of the dietary supplementation of 2.5 ppm T-2 toxin and/or a feed additive on the weekly feed intake of growing broiler chickens[1]

| Group | T-2 toxin (ppm) | Additive and inclusion level (kg/t) | Days of exposure | | | | |
|---|---|---|---|---|---|---|---|
| | | | 1-7 | 8-14 | 15-21 | 22-28 | Total (1-28) |
| 1 | 0 | 0 | 119.2±1.4a | 333.2±4.0a | 599.9±11.5a | 944.3±12.7a | 1996.6±11.6a |
| 2 | 0 | Mycofix-S, 2.5g/kg | 119.6±2.6a | 313.4±9.3ab | 574.2±7.6ab | 923.5±23.0ab | 1930.7±23.6ab |
| 3 | 2.5 mg/kg | 0 | 115.0±3.0a | 293.6±6.7b | 535.7±13.6c | 843.0±14.7c | 1787.3±24.3c |
| 4 | 2.5 mg/kg | Mycofix-S, 2.5 g/kg | 119.9±2.8a | 315.4±4.0ab | 555.7±11.3bc | 879.1±10.9bc | 1870.1±12.9b |
| P | | | 0.488 | 0.003 | 0.005 | 0.001 | 0.000 |

[1]Values are means ± S.E.M. of 6 replicate pens per treatment.
Within a column, means with different superscripts differ significantly (P<0.05).

Feed intake in the birds fed the diet containing T-2 toxin plus the feed additive (group 4) was significantly lower than that of the control group during weeks 3 and 4 and for the cumulative 28-day feed intake. The cumulative 28-day feed intake was significantly higher in the birds from group 4 (T-2 toxin plus feed additive) compared with group 3 (T-2 toxin only). Birds in group 2 (feed additive only) did not differ from the control group at any of the sampling times evaluated. Feed conversion was significantly lower in both groups receiving T-2 toxin during weeks 1 and 3 only (data not shown). No significant differences for this variable were seen on weeks 2 or 4 or for the 28-day cumulative feed conversion.

Tab. 3 summarizes the performance variables for the 4-week experimental period, presented both as absolute values and as relative values compared to the control group. Body weight gain was not significantly different between the control group and the two groups receiving the feed additive, with or without T-2 toxin (groups 2 and 4); however, body weight was significantly lower in the group receiving 2.5ppm T-2 toxin without feed additive (group 3). Body weight gain in the birds fed T-2 toxin at 2.5ppm was 7.5% lower compared with the control group. Cumulative 28-day feed intake was significantly lower than the control in both groups receiving T-2 toxin (groups 3 and 4); however, in the group receiving only T-2 toxin (group 3) the cumulative feed intake was significantly lower than that in the group receiving T-2 toxin plus the feed additive (group 4). Compared with the control group, the lowest feed intake was that of the group fed T-2 toxin without feed additive (10.5% lower). Cumulative 28-day feed conversion did not differ significantly among the four experimental groups.

Table 3. Individual and combined effect of the dietary supplementation of 2.5 ppm T-2 toxin and/or a feed additive on 28-day performance variables in male broiler chickens[1]

| Group | T-2 toxin (ppm) | Additive and inclusion level (kg/t) | Weight gain(g) | | Feed intake (g) | | Feed conversion (g:g) | |
|---|---|---|---|---|---|---|---|---|
| | | | Mean ± S.E.M. | % of control | Mean ± S.E.M. | % of control | Mean ± S.E.M. | % of control |
| 1 | 0 | 0 | 1322.4±22.3a | 100.0 | 1996.6±11.6a | 100.0 | 1.397±0.020a | 100.0 |
| 2 | 0 | Mycofix-S, 2.5g/kg | 1283.6±13.0a | 97.1 | 1930.7±23.6ab | 96.7 | 1.395±0.013a | 99.9 |
| 3 | 2.5mg/kg | 0 | 1224.0±18.1b | 92.5 | 1787.3±24.3c | 89.5 | 1.358±0.015a | 97.2 |
| 4 | 2.5mg/kg | Mycofix-S, 2.5g/kg | 1289.0±23.5a | 97.5 | 1870.1±12.9b | 93.7 | 1.351±0.017a | 96.7 |
| P | | | 0.016 | | 0.000 | | 0.125 | |

[1]Values are means ± S.E.M. of 6 replicate pens per treatment.
Within a column, means with different superscripts differ significantly (P<0.05).

Serum ALP and amylase measurements on days 13 and 23 of age are shown in Tab. 4. Even though no significant differences were observed at any sampling time for any enzyme, there was a trend towards lower amylase values in the birds receiving dietary T-2 toxin. When data from groups without toxin (1 and 2) was combined and compared against the combined data for the groups with toxin (3 and 4), a significant difference in amylase activity (P<0.05) was observed.

Table 4. Individual and combined effect of the supplementation of 2.5 ppm T-2 toxin and/or a feed additive on the serum activity of alkaline phosphatase (ALP) and amylase in male broiler chickens measured on days 13 and 23 of age[1]

| Group | T-2 toxin (ppm) | Additive and inclusion level (kg/t) | Day 13 | | Day 23 | |
|---|---|---|---|---|---|---|
| | | | ALP (U/l) | Amylase (U/l) | ALP (U/l) | Amylase (U/l) |
| 1 | 0 | 0 | 1637±143a | 457±25a | 635±36a | 465±36a |
| 2 | 0 | Mycofix-S, 2.5 g/kg | 1175±98a | 547±61a | 622±30a | 484±58a |
| 3 | 2.5 mg/kg | 0 | 1303±59a | 449±53a | 644±41a | 398±30a |
| 4 | 2.5 mg/kg | Mycofix-S, 2.5 g/kg | 1390±152a | 386±26a | 671±46a | 385±24a |
| P | | | 0.059 | 0.105 | 0.833 | 0.213 |

[1]Values are means ± S.E.M. of 12 observations per treatment.
Within a column, means with different superscripts differ significantly (P<0.05).

Average serum amylase activity on day 13 for the birds without dietary T-2 toxin was 506 U/l whereas for the birds exposed to 2.5 ppm T-2 toxin it was 417 U/l; on day 23 the values for the same groups were 475 and 391 U/l, respectively. No significant differences in relative organ weights were observed (data not shown).

The histological examination of the tissues analyzed showed no pathological changes in the birds from groups 1 or 2; however, in the birds from group 3 (T-2 toxin) there was heterophile infiltration and severe ulceration in the tongue, esophagus and crop. There was also necrosis in the tongue, proventriculus and gizzard, and congestion, microhemorrhages, cell sloughing and enterocyte necrosis in the duodenum. The birds from group 4 (T-2 toxin plus feed additive) showed similar lesions but less severe and, in contrast with group 3, no lesions were seen in the duodenum. Birds from group 4 showed ulceration and heterophile infiltration of the mucosa and submucosa in the upper digestive system, cell sloughing and superficial lesions in crop, and small, well circumscribed foci of epithelial superficial necrosis in the gizzard mucosa.

## DISCUSSION

Mycotoxins in general, and trichothecenes in particular, are well known for their potential deleterious effects on commercial poultry performance (Eriksen and Pettersson, 2004; Murugesan *et al*., 2015). In broiler chickens, T-2 toxin causes a dose-response dependent reduction in feed intake and body weight gain, oral lesions and immunological dysfunction; it is also a potent inhibitor of the protein synthesis and tissues with high cell division rate such as the intestinal mucosa and the liver (Eriksen and Pettersson, 2004).

The results of the present study showed that growing broiler chickens can tolerate 2.5 ppm T-2 toxin in their diet for 21 days without showing significant adverse effects on their health or performance but after 28 days of exposure, adverse effects on performance parameters (body weight and feed intake) are observed. Supplementation of 2.5 g/kg of the feed additive based on the BBSH bacterium counteracted the adverse effects on performance caused by the T-2 toxin. This finding is in agreement with a previous study in which a feed additive containing epoxidase activity protected against the adverse effects of 2 ppm dietary T-2 toxin (Diaz *et al.*, 2005). Interestingly, both in the present trial as well as in the study reported by Diaz *et al.* (2005), the ratio of T-2 toxin to feed additive was 1:1000. This observation could be relevant given that under field conditions feed additives used for mycotoxin control are dosed empirically, without scientific support. In the particular case of a feed additive with epoxidase activity it could be recommended to add the additive into the contaminated feed at a ratio of 1 to 1000, once the level of T-2 toxin (or the total sum of type-A trichothecenes in the feed) has been determined.

Even though no significant differences were observed among the experimental groups for the serum activities of ALP and amylase, ALP activity was lower by about half on day 23 compared with day 13 in all four groups. This finding confirms the observations of Tanabe (1962) who found that ALP serum activity gradually decreases starting at 1.5 weeks of age in male chicks and keeps decreasing until week 95 of age. In regards to amylase activity, pancreatic enzyme secretion increases from the first day after hatching due to the higher feed intake and gut weight (Noy and Sklan, 1995);

even in turkey poults without access to feed, amylase, lipase and trypsin activities increase with age (Corless and Sell, 1999). In the present trial, an increase in amylase activity on day 23 versus day 13 was not observed, possibly because of the short interval between sampling. However, a clear trend towards a decreased amylase activity in birds receiving T-2 toxin (groups 3 and 4) compared with those that did not receive the toxin (groups 1 and 2) was observed; in fact, when data from groups 1 and 2 was pooled and compared against the pooled data from groups 3 and 4, a significantly lower amylase activity was found in the birds exposed to the toxin. These results suggest that measurement of serum amylase activity could be useful as a biomarker of exposure to T-2 toxin and possibly other trichothecenes. It would be interesting to evaluate the effects of T-2 toxin on amylase and other pancreatic enzymes in larger trials and to determine whether the supplementation of an epoxidase-based feed additive could eventually counteract potential adverse effects on enzyme activities.

The lack of significant differences in relative organ weights observed in the present study corroborates the findings of Diaz (2002) who did not find differences for this variable in growing broiler chickens exposed to 1 or 2 ppm dietary DAS for 28 days.

The histologic examination of selected organs and tissues showed similarities and differences only in the groups receiving T-2 toxin (3 and 4). While both groups showed lesions in the upper digestive tract, lesions and an inflammatory process with heterophile infiltration of the duodenum were observed only in group 3 (T-2 toxin without additive). This finding suggests that the feed additive was capable of inactivating the toxin before it was able to reach the small gut, therefore preventing its necrotic action and possibly its systemic absorption during digestion. This issue needs to be further investigated, preferably using radio-labeled mycotoxins.

## CONCLUSIONS

The results of the present study demonstrate that growing broiler chickens can tolerate dietary levels of T-2 toxin of 2.5 ppm during 21 days without showing adverse effects on performance parameters. A minimum of 28 days of exposure at 2.5 ppm T-2 toxin is required to observe a significant decrease in the most important performance parameter (body weight). The experimental model was successful in causing T-2 toxin-induced adverse effects on body weight, body weight gain and feed intake, and it was demonstrated that the feed additive evaluated is capable of counteracting these adverse effects. Further, the feed additive protected against the adverse effects of T-2 toxin on the duodenal mucosa and its supplementation did not cause any adverse effect on the response variables tested.

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