

Protecting Consumers from Mycotoxins & Parameters to Assess the Mycotoxin Binder Performance

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INTRODUCTION

The worldwide occurrence of mycotoxin contamination of feedstuffs and the severity of mycotoxicosis in farm animals shows a tendency to increase in recent years. Many factors contribute to this increase such as the global climate change and increased international trading of feedingstuffs from different geographical origins. In addition to reduced feed intake, lower weight gain and in some cases reduced feed efficiency, mycotoxins can also be deposited in meat, eggs and milk. This transfer of mycotoxins into food products poses a threat to human health. Increasingly food safety standards across the world are putting more emphasis on the control of mycotoxins in food and animal feed. Mycotoxin control requires proactive HACCP-based strategies to prevent fungal growth on crops on the fields, at harvest, during processing, transport and storage. In addition, the use of mycotoxin binders is also an established practice in many parts of the world for further reduction of mycotoxin-related risks in animal production.

The objective of the present study was to determine the efficacy of a mycotoxin binder on several animal performance and health parameters when broilers are fed a diet contaminated with a mixture of mycotoxins (Aflatoxins, Ochratoxin A, T-2 toxin and Citrinin). The current study also addresses all the primary mechanisms that are affected during mycotoxicosis by studying animal performance, immunology, organ morphology and mycotoxin excretion.

OBJECTIVE

The objective of the present study was to determine the efficacy of mycotoxin binder on several animal performance and health parameters when broilers are fed a diet contaminated with a mixture of mycotoxins (Aflatoxins, Ochratoxin A, T-2 toxin and Citrinin). The current study also addresses all the primary mechanisms that are affected during mycotoxicosis by studying animal performance, immunology, organ morphology and mycotoxin excretion.

MATERIAL AND METHODS

192 sexed Vencobb broilers (day-old) were weighed individually and randomly distributed among 4 groups with 4 replicates in each (2 males x 2 females). The birds were housed in cages for 35 days under similar experimental conditions and maintained with feed and water available *ad libitum*. The experimental design is given in Table 1.

Table 1. Experimental design

Treatment	Description
1	Control (basal diet)
2	Control + Toxin Binder, 0.3%
3	Control + Aflatoxins, 250 ppb + Ochratoxin A, 250 ppb + Citrinin, 250 ppb + T-2, 250 ppb
4	Control + Aflatoxins, 250 ppb + Ochratoxin A, 250 ppb + Citrinin, 250 ppb + T-2 250 ppb + Toxin Binder, 0.3%

The control group with the mycotoxin binder, but no mycotoxins (treatment 2) was included in the trial to check whether or not the toxin binder binds the essential nutrients, which could result in reduced growth. Furthermore this group can be used to identify an undesirable growth promoting effect, in case if better bird performance is observed in comparison to treatment 1. Growth promotion may hide mycotoxicosis and therefore can increase food safety risks because the animal may still absorb mycotoxins.

A corn-soya based mash diet was formulated according to specifications of NRC (1984). The basal diet was analyzed for mycotoxins and was found to contain levels that were below the detection limits (less than 10 µg/kg) for Aflatoxins, Ochratoxin A, Citrinin and T-2 toxin. The inclusion level of contaminants in the diet was based on total levels of Aflatoxin, Ochratoxin A, Citrinin and T-2 toxin in moulded rice and wheat powder, which was incorporated into the basal diet to provide the desired level of toxins. Most of the mycotoxins were added above levels known to be toxic for broilers.

The parameters listed below were measured to check the efficacy of the toxin binder in terms of performance, organ morphology and immunology.

- | | |
|------------------------------------|----------------------------------------------------------------------------------------------|
| 1. <i>Performance</i> | Body weight and weight gain
Feed consumption and feed conversion ratio (FCR)
Mortality |
| 2. <i>Organ morphology</i> | Organ weight – liver and kidney at age 35 days
Visual observation – liver photographs |
| 3. <i>Mycotoxin excretion</i> | Mycotoxin recovery in faeces |
| 4. <i>Immunological parameters</i> | Haemagglutination inhibition after New Castle Disease vaccination |

Chicks were weighed every week individually. Weight gain, weekly feed intake, FCR and mortality were recorded for each replication. At day 35 one bird from each replication was submitted to *post-mortem* evaluation of the organs by visual observation and by measurement of organ weight. Furthermore, any changes from the regular organ morphology in terms of size, weight and colour were recorded. The organ weights were converted to percentage of body weight for further comparison. The data were subjected to ANOVA and Duncan's multiple range tests, and all statements of significance were based on the 0.05 level of probability.

RESULTS AND DISCUSSION

Zootechnical performance, nutrient binding and growth promoting effects

The overall performance of broilers at day 35 is shown in the Table 2.

Table 2. Effect of combined mycotoxins on performance of commercial broilers at day 35 *

Treatments	Weight gain, g	Feed consumption, g	FCR	Mortality, %
1	1564 ^a	2554	1.63 ^a	2
2	1568 ^a	2514	1.60 ^a	4
3	1455 ^b	2557	1.76 ^b	8
4	1581 ^a	2486	1.57 ^a	4

* Rows with different superscripts indicate statistically significant differences (p<0.05)

The toxin binder in the diet free from mycotoxins (treatment 2) did not show growth promotion nor nutrient binding effects since the toxin binder is a selective binder of wide range of mycotoxins only. This group showed similar weight gain and FCR as that of positive control treatment 1 proving that the toxin binder did not bind essential nutrients such as vitamins, minerals and amino acids. Furthermore there were no growth promoting effects that are able to hide mycotoxin absorption by the animal and consequently might hide the food safety risk.

Bird performance in the negative control group (treatment 3) was significantly worse than the basal diet in terms of body weight gain and FCR. Considerable improvement in weight gain and feed conversion was observed for the group that received the mycotoxin cocktail in the diet with additional toxin binder supplementation (1455 g and 1.76 vs. 1581 g and 1.57 respectively). The observed numerical differences were also statistically significant (Figure 1).

Feed consumption was more or less at the same level in all treatments varying within ~3% between the highest level of 2557 g observed in the treatment 3 and the lowest – 2486 g in the treatment 4 with mycotoxins and the toxin binder.

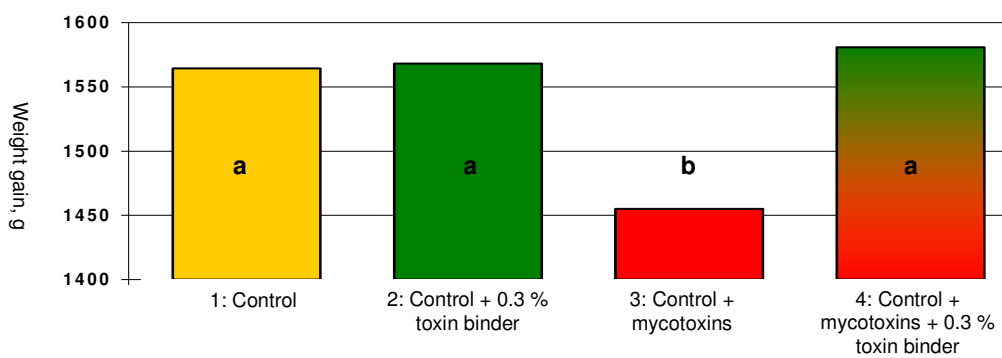


Figure 1. Effect of the toxin binder on weight gain of broilers at 35 days

Liveability of chicks in treatment 3 was reduced by mycotoxins causing 8% mortality. In contrast, the treatments 1, 2 and 4 showed only 2-4% mortality, which corresponds to the normal rate. Thus, the toxin binder in the treatment 4 reduced negative impact of mycotoxicosis on zootechnical performance and improved chicken liveability.

Organ morphology

The relative weight of the 32-day old chickens' liver and kidney as a percentage of live weight are shown in Table 3.

Table 3. Organ weight of broilers at day 32, (% of body weight) *

Treatment	Liver	Kidney	Spleen
1	1.96 ^a	0.62 ^a	0.12 ^a
3	2.74 ^b	0.85 ^b	0.13 ^a
4	2.02 ^a	0.63 ^a	0.12 ^a

* Rows with different superscripts indicate statistically significant differences (p<0.05)

Liver weight is the most sensitive indicator of mycotoxicosis. The liver weight of the mycotoxin treatment 3 was significantly increased by 0.78% as absolute or 40% as relative dimension comparing to control (2.74% vs. 1.96%). A significant reduction in liver relative weight was found in broilers that received the toxin binder supplementation. Statistically, the group treated with the toxin binder had the same organ weight as the control (p<0.05). Visual evaluation of the liver (Figure 2) confirmed that the toxin binder successfully eliminated mycotoxicosis due to the binding of the different mycotoxins, which makes them unavailable for absorption through the gut wall.

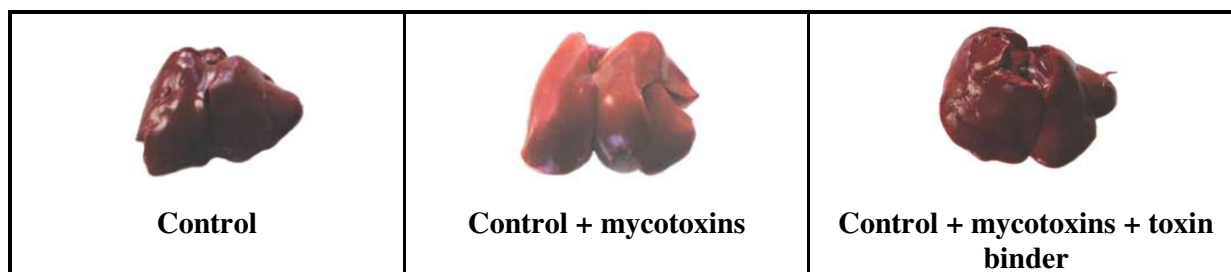


Figure 2. Effect of the toxin binder on liver health

There was also a clear negative influence of mycotoxins on kidneys. The group including only mycotoxins (treatment 3) showed 0.85% of kidney weight whilst in the control group and treatment with the toxin binder the kidney weight was 0.62% and 0.63% respectively. The differences were statistically significant. The negative impact of the mycotoxin cocktail on kidney weight within the present study can be attributed to the known nephropathological effects of Ochratoxin A and Citrinin. The relative weight of spleen in this experiment was not affected by mycotoxins.

Mycotoxin excretion

In order to evaluate the mycotoxin binding power of the toxin binder, a balance study was conducted. Mycotoxin levels in the excreta were analysed and compared with levels given to birds through the feed. Results are shown in the Table 4. Only about 30% of mycotoxins were excreted in the treatment 3. That means 70% of the mycotoxins remained in the chicken, apparently available for absorption and retention in animal products. In the group fed with mycotoxins plus the toxin binder the majority of the toxins were excreted. Overall binding performance of the toxin binder was found in this study to be 76%. Consequently 24% of the mycotoxins could not be recovered, probably due to metabolism by microorganisms, rather than absorption. This conclusion is based on the fact that it is known that several microorganisms are able to metabolise mycotoxins and on the observation that the visual evaluation of organs confirmed the healthy state of the organs indicating only minimal absorption.

Table 4. Excretion of mycotoxins

Treatment	Aflatoxins		Ochratoxin A		Citrinin		T-2	
	ppm	%	ppm	%	ppm	%	ppm	%
1	0	-	0	-	0	-	0	-
3	0.09	36	0.06	24	0.07	28	0.08	32
4	0.20	80	0.18	72	0.20	80	0.19	76

Immunological parameters

The possible negative impact of mycotoxins on the efficacy of Newcastle Disease vaccination was investigated. Antibodies against the viral protein responsible for haemagglutination can prevent haemagglutination. This principle is used as the basis for the haemagglutination-inhibition test (HI). The HI titre value was found to be the lowest in the mycotoxin fed group (Table 5), which indicates that vaccination was not successful. Addition of the toxin binder to the diet levelled the HI titre value in the treatment 4 to the control mean.

Table 5. Haemagglutination inhibition (HI) titre values against Newcastle Disease (ND) antigen

	Treatment			
	1	2	3	4
HI titre value	5.75	5.67	2.00	6.00

CONCLUSION

Inclusion of the toxin binder in a broiler diet contaminated with a mixture of four harmful mycotoxins resulted in a complete recovery of zootechnical performance. The addition of a treatment without mycotoxins, but with the toxin binder shows that the binders do not bind essential nutrients. Furthermore this treatment also proves that the toxin binder does not show growth promoting effect to compensate mycotoxicosis, hence, it cannot be used to hide mycotoxin problems. Consequently all beneficial effects are attributed to mycotoxin binding, which prevents mycotoxin absorption through the gut wall. The recovery of the organ health status and restored immunity show that the toxin binder prevents mycotoxin absorption. However, the only direct proof for the mycotoxin binding properties of the toxin binder remains the excretion study, which shows that on average over 76% of the mycotoxins are excreted when the toxin binder is added to the diet compared to only 30% for the negative control group.

A few criteria which have been developed from a food safety perspective will strongly assist in making animal products safer for human consumption. However, compliance to these criteria will also assure that profitability of animal production is optimized to the maximum as all negative effects of a wide range of mycotoxins will be eliminated. These criteria can be proposed to assess the performance of mycotoxin binders from a food safety perspective. They are: Proof of broad-spectrum mycotoxin binding performance; Maintain availability of essential nutrients to the animal; Evaluate growth promoting effects as growth promotion potentially masks mycotoxicosis; Improvement of zootechnical performance; Recovery of organ status - Protection of organ health from mycotoxin; Data on mycotoxin excretion via faeces and Recovery of the immune status.

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