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Mycotoxin Adsorbent Improves the Performance and Health of Broilers Challenged Simultaneously with Aflatoxins and Fumonisins

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Abstract: In poultry production, aflatoxins and fumonisins stand out as being two of the main mycotoxins that compromise the performance and health of animals, with serious damage to the producer. It should be noted that, in most cases, the losses are potentiated because the rations are contaminated, simultaneously, by two or more mycotoxins, giving rise to additive or synergistic effects, which means that the general toxicity is not just the sum, but the multiple the individual toxicities of mycotoxins, increasing the damage. We have utilized 540 broilers, distributed in a completely randomized design with three treatments and six replications. A broad-spectrum mycotoxin adsorbent (YES – FIX HP®) was tested, added at 2.5kg/ton in diets contaminated with 1.0ppm aflatoxin + 50.0ppm fumonisin. Evaluating a control diet without contamination (T1), a contaminated diet without adsorbent (T2), and a contaminated diet + adsorbent (2.5kg/ton). The following parameters were evaluated: feed intake, body weight, daily weight gain, feed conversion, and productive efficiency index. On the last day of the experimental period, the birds were slaughtered for the evaluation of the following parameters: relative liver weight, jejunal histomorphometry, intestinal absorption area, and histopathology of the Fabricius bursa and liver. The birds in the treatment challenged with the inclusion of the adsorbent had greater live weight and daily weight gain, a greater relationship between villus height and crypt depth, greater intestinal absorption area, and less frequent and more frequent histopathological lesions in the liver and bursa of Fabricius, light compared to the contaminated treatment without the adsorbent. Given the observed data, it is concluded that the tested adsorbent was able to mitigate the deleterious effects caused by mycotoxins in relation to the productive performance, in addition to exerting a positive action on the health status of the animals.

Keywords: Bursa of Fabricius, Feed Conversion, Health, Histopathology, Immunology, Liver, Organic Selenium, Silymarin, Weight Gain

1. Introduction

Occurrence of mycotoxins is widespread throughout the world. Their acute and chronic dietary exposures can induce a variety of adverse health effects in humans and animals, making these chemically diverse substances highly relevant agricultural contaminants [1].

These metabolites have varying bio-availabilities, while some are more rapidly absorbed, others get further along the gastrointestinal tract before their absorption can take place [2]. In poultry production systems, the aflatoxins negatively affect body weight gain and feed conversion and causes histological changes in the liver as well as immunosuppression and increased mortality rates [3], and fumonisins cause decreased feed consumption and body weight gain, increased relative weights of liver and kidney, and liver necrosis [4, 5].

However, this scenario is made more concerning by the fact that food and feed are often co-contaminated with two or more mycotoxins and their interactions may exert additive or synergistic effects [6]. Therefore, the toxicity studies of

mycotoxins in animals require an understanding of the effects of multiple mycotoxins. Different combinations of mycotoxins have a synergistic effect on environmental toxicity, while some combinations cause antagonistic effects [7].

In this scene, the combination of two or more adsorbents to detoxify mycotoxin-contaminated poultry feeds could be more effective in reducing the effect of the toxins on nutrient digestibility because, most of the adsorbing agents appear to bind to only a limited group of mycotoxins while showing very little or no affinity to others [8, 9].

The objective of this study was to evaluate the effect of a broad-spectrum adsorbent on the productive performance and health parameters of broilers challenged, simultaneously, with aflatoxin and fumonisin in the diets.

2. Material and Methods

2.1. Animals

This project was approved by the Commission on ethics in the use of animals (CEUA) of the company SAMITEC – CEUA/SAMITEC, under number 019.01.22 AFDF01 – 22.

It was used 540 male broilers of Cobb's 500-line, one day old, with an average weight of 45,08 grams. The experimental test was conducted in an experimental room, 22m^2, with negative pressure, acclimatized. The animals were housed in 18 boxes, with an area of 3m^2 each, on a bed of rice husk. Each cage had a trough-type feeder and nipple-type drinker with height adjustment.

2.2. Experimental Design and Diets

The birds were distributed in a completely randomized design with three treatments and six replications, totaling 30 experimental units.

The experimental diets were formulated to meet the nutritional requirements, according to the recommendations of [10], as shown in Table 1. The diets were isocaloric, iso-protein, and iso-vitamin, according to the composition shown in Table 1. The raw materials and experimental diets were analyzed for the presence of mycotoxins (aflatoxins, deoxynivalenol, diacetoxyscirpenol, fumonisin, ochratoxin A, T-2 toxin, and zearalenone), and no mycotoxin was detected in the raw materials used.

Birds received feed and water ad libitum during the experimental period (1 – 42 days). The mycotoxin adsorbent was added 2.5kg/ton, in diets that were contaminated with mycotoxins, using 1.0 ppm aflatoxin + 50.0 ppm fumonisin. Aflatoxins (B1, B2, G1, and G2) were obtained from the cultivation of a toxin strain of Aspergillus parasiticus, and the concentrations used were B1: 93.8%, B2: 2.1%, G1: 3.4% and G2: 0.7%. Fumonisin (B1 and B2) was obtained from the cultivation of a toxin strain of Fusarium moniliforme, and the concentrations used were 93.8% of B1 and 2.1% of B2.

We evaluated a control diet without contamination, a contaminated diet with mycotoxins without adsorbent, and a contaminated diet + YES - FIX HP®[1]

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Table 1. *Nutritional levels of diets provided to broilers during the experimental period.*

Ingredients	Initial (1 - 21 days)	Growth (22 - 35 days)	Termination (36 - 42 days)
	Kg	Kg	Kg
Corn	58.500	60.700	65.000
Soybean meal	35.350	32.100	28.000
Soybean oil	1.600	3.200	3.470
Dicalcium phosphate	2.000	2.000	1.570
Limestone	1.000	1.066	0.991
Salt	0.460	0.375	0.350
Methionine	0.240	0.010	0.150
Lysine	0.200	0.040	0.160
Mineral and vitamin premix	0.600	0.500	0.300
Total	100.0	100.0	100.0

Chemical composition			
Crude protein, %	**22**	**20**	**19**
Metabolizable energy, Kcal/kg	2990	3030	3.100
Methionine + Cystine, %	0.91	0.74	0.78
Lysine, %	1.35	1.20	1.17
Calcium, %	0.99	0.98	0.84
Useful phosphorus, %	0.50	0.48	0.39
Sodium %	0.24	0.18	0.17

2.3. Productive Performance Parameters

Weekly, all birds, and leftover feed were weighed to evaluate the average weight, weight gain, feed intake, and feed conversion. The productive efficiency index was obtained between daily weight gain, viability, and feed conversion. Temperature and humidity were recorded daily and kept within the comfort range.

2.3.1. Liver Weight, Intestinal Integrity and Histopathology of the Liver and Bursa of Fabricius

At the end of the last experimental day (42nd day), the birds were slaughtered for the following evaluations:

2.3.2. Relative Weight of the Liver

The relative weight of the liver was obtained by calculating the weight of the carcass (without blood and feathers) and its respective liver.

2.3.3. Jejunal Histomorphometry

For histomorphometric analysis, five villus heights were measured per jejunum/animal segment, from the basal region coinciding with the upper limit of intestinal crypts to its apex, in addition to measuring the width of their respective villi. The depth of the crypts was also measured at five points per segment/animal evaluated. The analyzes were performed using an Opticam 0600R trinocular microscope, associated with the OPTHD 3.7 Microscope Imaging Software program, in the 10x objective, with capture by the Nikon Eclipse E200 and TCCapture Image Software, in the 4x objective. The data were obtained from the sampling of 12 birds/treatment.

2.3.4. Intestinal Absorption Surface Area

From the measurement of three histological parameters (villus height and width and crypt depth), the intestinal absorption area was calculated, following the formula

described by Kisielinsk K. et al in their study [11].

2.3.5. Histopathological Evaluation of the Liver

For histopathological evaluation of the liver, the integrity of hepatocytes and possible inflammatory and degenerative changes were evaluated. The alterations of microvacuolar hepatocellular degeneration and random necrosis of hepatocytes were graded in intensities into discrete, moderate, and severe.

2.3.6. Histopathological Evaluation of the Bursa of Fabricius

To evaluate the Bursa of Fabricius, lymphoid depletion levels were analyzed, such as the reduction in the height of the folds, the morphology of lymphoid follicles, decrease in lymphoid cells, and follicular necrosis, characterized by pyknotic, karyorrhexis lymphocytes and cellular debris. The classification of lymphoid depletion followed the parameters: absent; mild when less than 25% of follicles were affected; moderate when 25 to 75% of follicles were affected and severe when more than 75% of lymphoid follicles were affected.

3. Statistical Analysis

For the analysis of data with normal or approximate distribution and homogeneity of variances, descriptive statistics were used: mean, standard deviation, and confidence interval (95%). In this case, the means were compared by analysis of variance (ANOVA) using Duncan's test ($P \leq 0.05$). Non-parametric data were described by their median and their quartiles (25% and 75%). Medians were compared using the Kruskal-Wallis test. Ordinal data, such as those from histopathology analyses were compared using the Chi-Square test. The presence of outliers in the parametric statistics was verified through the studentized residuals. The Stratigraphic Centurion XV® software, version 15.1, was used.

Table 2. *Average of the productive performance results of broilers challenged with aflatoxins and fumonisins, with or without the inclusion of the mycotoxin adsorbent in the diets, at 42 days of age.*

Treatments	CR[4], g	PV[5], g	GPD[6], g	CA[7], kg/kg	IEP[8]
T1[1]	5092.700[a] ± 104.914	3197.84[a] ± 106.26	75.065[a] ± 2.532	1.625[b] ± 0.054.	429.923 ± 49.008
T2[2]	4892.090[b] ± 116.292.	2910.95[b] ± 122.36.	68.235[b] ± 2.916.	1.709[a] ± 0.046	374.902 ± 26.601
T3[3]	4988.290[ab] ± 121.499	3109.91[a] ± 110.37	72.972[a] ± 2.630	1.648[ab] ± 0.579	389.667 ± 36.616
P-Value	0.0275	0.0017	0.0017	0.0391	0.0658

[a,b,c] different letters in the same column differ by Duncan's test at 5% significance [1] Control treatment, no challenge, and no adsorbent; [2]Treatment with challenge and without adsorbent, [3] Treatment with challenge and with the inclusion of YES – FIX HP. 4= feed intake, 5 = live weight, 6 = daily weight gain, 7 = feed conversion, 8 = productive efficiency index.

4. Results and Discussion

Contamination with mycotoxins reduced feed consumption (-3.93%) and the inclusion of the adsorbent attenuated this effect (-2.05%) in relation to the control treatment. High concentration of aflatoxins in broiler feeds has been reported to repress feed intake [12].

The challenged group without the presence of the adsorbent in the diet had the worst live weight and daily weight gain compared to the other groups, in addition to the worst feed conversion.

The negative interference of mycotoxins on nutrient absorption and protein synthesis can influence weight gain in challenged animals [13]. Direct disturbance in the functions of some organs, especially the liver, and the partition of nutrients for activities other than body growth also increase a challenged animal's demands for protein and energy [14]. Thus, according to a study carried out by Andretta, I. et al, 2011 [15], the explanation for disturbance in challenged broiler growth is the association of reduced feed intake with alterations in protein deposition efficiency.

As a result of the worst zootechnical performance, proven through the parameters discussed above, numerically, the worst index of productive efficiency was also presented by the challenged group without adsorbent. Most toxicity mechanisms are specific for each type of mycotoxin. The metabolites derived from aflatoxins react with cellular macromolecules such as DNA and RNA, interfering with the functional properties of the liver and protein synthesis [16, 17]. On the other hand, the proposed action mode of fumonisins relates its toxicity to interferences on sphingolipid biosynthesis [18].

It is noteworthy that an important factor interfering in the effect of mycotoxin concentrations is the incidence of combined mycotoxins in the same diet, and this is the scenario most commonly found in animal production, because several mycotoxins may be produced concomitantly in a given substrate and ingredients may be cross-contaminated in feed mills [19, 20]. Interactions between mycotoxins are complex and may result from the association of individual toxic properties or additive effects [20]. The same concentration of mycotoxin may cause worse performance responses when animals are challenged by combined mycotoxins.

In this sense, the use of broad-spectrum adsorbents becomes an essential tool in mitigating the negative impacts of mycotoxicosis in animal production.

The adsorbent evaluated in this study is composed of 1.3 and 1.6 beta-glucans, polycationic bentonite, activated charcoal, and organic molecule. These are recognized efficient active principles in the adsorption of the main mycotoxins found in the field. In addition, it has silymarin and organic selenium in its composition, as informed by the

manufacturer, which has proven hepatoprotective and antioxidant functions, respectively.

It is postulated that the excellent results found in favor of the group challenged with the inclusion of the adsorbent compared to the group that did not receive the additive, can be attributed to the synergistic effect between the adsorbent components.

This occurs due to the ability of the adsorbents to adhere to the surface of mycotoxins, forming a mycotoxin and adsorbent complex and thus it is possible to eliminate them together with the feces, not being available for absorption by the animal organism during the passage through the gastrointestinal tract [21].

Table 3. *Mean of the Relative Liver Weight Results of Broilers Challenged with Aflatoxins and Fumonisins, with or Without the Inclusion of the Mycotoxin Adsorbent in the Diets, at 42 Days of Age.*

Treatments	Relative weight of the liver, %
T1[1]	$1.848^{b} \pm 0.308$.
T2[2]	$1.941^{a} \pm 0.296$
T3[3]	$1.878^{ab} \pm 0.252$
P-Value	0.0189

[a,b,c] distinct letters in the same column differ by Duncan's test at 5% significance. [1] Control treatment, without challenge and without adsorbent; [2]Treatment with challenge and without adsorbent, [3] Treatment with challenge and with the inclusion of YES – FIX HP®

The inclusion of YES – FIX HP® attenuated the deleterious effects of mycotoxins on the liver of birds, as can be seen by the decrease in the weight of this organ in the contaminated group that received the adsorbent, compared to the challenged contaminated group without the adsorbent. In a study carried out by Abreu, A. P (2008) [22], was reported that when they have a diet with mycotoxins, animals start developing mycotoxicosis, which is characterized by a diffuse syndrome, which can cause damage to several organs, such as the liver, kidneys, or even the central nervous system. The liver is affected by most mycotoxins. Alterations in liver functions are observed in broilers challenged by aflatoxins [23], and the increase in this organ weight can be attributed to fat degeneration [24]. Furthermore, some alterations in blood circulation in the liver can be related to cardiac hypertrophy [25].

The recent focus on herbal alternatives has created new hope for safe and effective solutions such as substances that demonstrate hepatoprotective effects against the harmful impact of mycotoxins on metabolism in particular on liver function and health [26].

One of the great differentials of the evaluated adsorbent is the presence of silymarin in its composition. It has been demonstrated that silymarin protects the structure and function of hepatocytes through scavenging free radicals, activating related antioxidant genes, restoring damaged tissues, and producing new hepatocytes [27] leading to this suggestion that silymarin could be used as an ideal agent for the comparison of hepatoprotective bioactive components [28].

Metabolically, Silymarin stimulates the hepatic cells and induces the synthesis of ribosomal RNA to promote protein production [29].

In addition to silymarin, the presence of organic selenium in the adsorbent composition probably contributed to the decrease in the severity of histopathological lesions in the liver and bursa of the animals, as shown in Tables 4 and 5, respectively.

Selenium regulates the activation of NF-κB, a transcription factor, which plays a pivotal role in the regulation of inflammatory pathways. Selenium can inhibit NF-κB from binding to the inflammation-related genes which eventually reduces the expression of pro-inflammatory cytokines [30]. The anti-inflammatory function of Se might be due to the presence of specific selenoproteins, which reduces the oxidation-induced inflammatory changes in the liver [31].

The bursa of Fabricius is intimately connected to the cloaca and the intestinal system. It is well-known as a primary lymphoid organ in the chicken and a major channel through which environmental antigens stimulate the immune system and is the site for B-cell lymphopoiesis, lymphocyte maturation, and differentiation and development of the antibody repertoire [32].

According to a study carried out by Fernandes, J. I. M. et al (2011) [33], microbial challenges or immunosuppressive agents such as mycotoxins can induce lymphoid tissue hyperplasia to increase the production of antibodies and immune defense signals. The consumption of mycotoxins, at levels that do not cause clinical mycotoxicosis, suppresses immunity functions and can decrease resistance to infectious diseases.

The mechanism of protection of bursal cells by selenium is related to the preservation against oxidative damage induced by mycotoxins, especially AFB1, through increased activity of the antioxidant enzymes catalase, superoxide dismutase, and glutathione peroxidase, in addition to the ability to scavenge radicals and a decrease in the level of malondialdehyde (a marker of oxidative stress) [34].

Table 4. *Mean of the Results of the Severity of Histopathological Lesions in the Liver of Broilers Challenged with Aflatoxins and Fumonisins, with or Without the Inclusion of the Mycotoxin Adsorbent in the Diets, at 42 Days of Age.*

Treatments	Score	Lymphoid depletion	Necrosis	No injury	With injury
T1[1]	0	5	12	17	7
	1	6	0		
	2	1	0		
	3	0	0		
T2[2]	0	2	4	6	18
	1	7	5		
	2	2	3		
	3	1	0		

Treatments	Score	Lymphoid depletion	Necrosis	No injury	With injury
T3[3]	0	5	10	15	7
	1	5	1		
	2	1	0		
	3	0	0		

Contrasts	P-Value
T1 x T2	0.0015
T1 x T3	0.8452
T2 x T3	0.0033

[1] Control treatment, without challenge and without adsorbent; [2]Treatment with challenge and without adsorbent, [3] Treatment with challenge and with the inclusion of YES – FIX HP®.

Table 5. *Mean of the severity results of the histopathological lesions of the bursa of Fabricius of broilers challenged with aflatoxins and fumonisins, with or without the inclusion of the mycotoxin adsorbent in the diets, at 42 days of age.*

Treatments	Score	Fat degeneration	Periportal heterophilic infiltrate	Multifocal mononuclear inflammatory infiltrate	No injury	With injury
T1[1]	0	5	12	9	38	10
	1	7	0	3		
	2	0	0	0		
	3	0	0	0		
T2[2]	0	0	0	0	7	41
	1	5	12	10		
	2	7	0	2		
	3	0	0	0		
T3[3]	0	3	7	0	21	23
	1	7	4	10		
	2	1	0	1		
	3	0	0	0		

Contrasts	P-Value
T1 x T2	<0.0001
T1 x T3	0.0017
T2 x T3	0.0006

[1] Control treatment, without challenge and without adsorbent; [2]Treatment with challenge and without adsorbent, [3] Treatment with challenge and with the inclusion of YES – FIX HP®.

Table 6. *Mean histomorphometric results and surface area of intestinal absorption of broilers challenged with aflatoxins and fumonisins, with or without the inclusion of mycotoxin adsorbent in the diets, at 42 days of age.*

Treatments	Villus height, µm	Crypt depth, µm	Villus height/crypt depth ratio	Intestinal absorption area, (μm^2)[3]
T1[1]	$1148.85^{ab} \pm 109.45$	$78.67^{b} \pm 20.13$	$14.92^{a} \pm 14.07$	$25.74^{a} \pm 3.45$
T2[2]	$1097.26^{b} \pm 164.96$	$94.84^{a} \pm 17.99$	$11.92^{c} \pm 2.46$	$23.85^{b} \pm 4.49$
T3[3]	$1170.43^{a} \pm 160.82$	$89.53^{a} \pm 85.68$	$13.32^{b} \pm 2.78$	$26.36^{a} \pm 4.69$
P-Value	0.0255	<0.0001	<0.0001	0.0049

[a,b,c] distinct letters in the same column differ by Duncan's test at 5% significance. [1] Control treatment, without challenge and without adsorbent; [2]Treatment with challenge and without adsorbent, [3] Treatment with challenge and with the inclusion of YES – FIX HP.

Challenged animals that received the adsorbent had higher villus height ($p \leq 0.0255$) and intestinal absorption area ($P \leq 0.0049$) when compared to the challenged group without the adsorbent inclusion. Upon ingestion of contaminated food or feed, the gastrointestinal tract is particularly affected by mycotoxin. Generally, the intestinal barrier in the GI tract functions as a filter against harmful mycotoxin [35].

Studies have shown that mycotoxins increase the permeability of the intestinal epithelial layer in numerous species, which can result in excessive/uncontrolled leakage of substances into the animal, as well as affecting intestinal cell viability. Mycotoxins can also reduce cell proliferation, thus reducing the ability of the intestine to repair and replenish itself [36], directly impacting the decrease in nutrient absorption and, consequently, the performance of the animals.

5. Conclusions

Our findings suggest that YES - FIX HP® mitigated the deleterious effects resulting from the contamination of broiler diets by aflatoxin and fumonisin, proving its broad spectrum of action. In addition, the adsorbent contributed to improving the health of the animals, by reducing the lesions in the liver and Bursa of Fabricius and improving the parameters of intestinal integrity, even in birds under challenge.

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