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Immunological Parameters from Broiler Chickens Supplemented with Adsorbents and Challenged with Mycotoxins

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Abstract: Mycotoxins are one of the most immunosupressive factors in animal diets. It is important to consider that the consumption of certain mycotoxins, at levels that are not cause overt clinical mycotoxicosis, suppresses immune function and decrease resistance infectious disease. In this sense, mycotoxin adsorbents that are effective both in combating the damage caused by these metabolites and in protecting and supporting animal health are increasingly essential in the animal nutrition industry. It has been evaluated the effect of mycotoxin adsorbents, with different compositions, on the immune parameters in broiler chickens challenged with mycotoxins. We have utilized 300 broilers, distributed in a completely randomized design with four treatments and six replications. Three mycotoxin adsorbents with different compositions were tested, which were added 2.5kg/kg, in diets that were contaminated with 1.0 pp aflatoxin + 50.0 ppm fumonisin + 25.0 ppm DON. Evaluating a control diet without contamination (T1), contaminated diet + YES - FIX HP (T2), contaminated diet + adsorbent A (T3), contaminated diet + adsorbent B (T4). On the last day of the experimental period, blood samples were collected from eight birds per treatment for the evaluation of phagocytosis by flow cytometry and the cecal tonsils were collected and determined the expression of interleukin 6 pro-inflammatory (IL 6) and interleukin 10 anti-inflammatory (IL 10) by quantifying the gene expression by RT-qPCR. In the phagocytosis assay, a significant difference was observed between the control and T4 treatments, with a lower percentage of phagocytic monocytes for treatment 4, which also presented the lowest value of IL-6 (pro-inflammatory). In the phagocytosis assay, a significant difference was observed between the control and T4 treatments, with a lower percentage of phagocytic monocytes for treatment 4, which also presented the lowest value of IL-6. Among the adsorbents evaluated, YES FIX HP provided the highest average production of IL-10, which suggests a greater balance in the immune response of animals to the challenge with mycotoxins, possibly contributing to the greater resistance of this group of animals to the main challenges faced in the field and that require a response from the defense system.

Keywords: Beta-Glucans, Immunity, Interleukin, Silymarin, Organic Selenium

1. Introduction

Globally, food and feeds have been seriously contaminated with mycotoxins which aflatoxin, zearalenone, fumonisin, deoxynivalenol, and ochratoxin are the most found [23].

In farm animals the consumption of mycotoxins, at levels that do not cause clinical mycotoxicosis, suppress immune functions and may decrease resistance to infectious disease [1]. There are several relevant parameters for assessing the immune status of animals, including phagocytosis assays and

quantification of interleukins.

According to Sattler et al. [24], phagocytosis has become a central process of the immune system's first line of defense against pathogens. Professional phagocytes take up microbes, kill and digest them, and activate the downstream immune response.

Essentially, cytokines represent an integrated network of cellular mediators capable of eliciting diverse biological effects influenced by the prevailing state of the organism [2]. Avian cytokines, similar to their mammalian counterparts, are influential in host immune response to pathogenic infection.

Usually categorized as being either pro-inflammatory or anti-inflammatory, cytokines are secreted by diverse cell populations upon stimulation [21]. Notably, both pro-inflammatory and anti-inflammatory types elicit distinct responses to immunogens at different stages of an infection [3].

IL-6 is considered an early and sensitive indicator of inflammatory reactions. It is the basic stimulator of acute phase protein synthesis in the liver. In inflammatory conditions, the concentration of IL-6 in the serum of patients increases many times [5].

Interleukin IL10 is one of the main anti-inflammatory cytokines. Suppressing distinct functions of Natural Killers cells and T lymphocytes primarily, it prevents the production of IL-12 and other pro-inflammatory cytokines (such as TNFα, IL-6 and IL-1β) by APCs [20, 27]. IL-10 prevents the increased expression of several genes in phagocytic and dendritic cells that are normally induced by stimulation of TLRs (toll like receptors) [27].

The use of mycotoxin detoxifiers as feed additives aims to reduce mycotoxin toxicity in contaminated feed ingredients, enabling their use for animal feed formulation [16]. There is a wide array of mycotoxin detoxifiers, with an equivalent myriad of claims [12].

The aim of this study was to evaluate the effect of different mycotoxin adsorbents, with different compositions, on the immune parameters of broiler chickens challenged with the mycotoxins aflatoxin, fumonisin and DON.

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.288.274.

It was used 300 male broilers of Cobb's 500-line, one day old and average weight of 41.88 grams. The experimental test was conducted in an experimental room, 22 m^2, with negative pressure, acclimatized. The animals were housed in experimental cages, each with a width of 0.5m, a length of 0.5m and a height of 0.33m, arranged in four overlapping levels, each level with two cages. Each cage had a trough type feeder, nipple type drinker with height adjustment.

2.2. Experimental Design and Diets

The birds were distributed in a completely randomized design with five treatments and six replications, totaling 30 experimental units, each cage being an experimental unit composed of ten birds.

The experimental diets were formulated to meet the nutritional requirements, according to the recommendations of NRC [22]. The diets were iso caloric, 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.

Table 1. *Composition of diets.*

Ingredients	**%**
Corn	63.00
Soybean bran	29.80
Soybean oil	3.00
Lysine	0.10
Methionine	0.04
Dicalcium phosphate	2.00
Calcitic Limestone	1.00
Salt	0.46
Premix[1]	0.60
Calculated composition	
Crude protein	20%
Metabolizable Energy	3050 Kcal/Kg
Met+ Cist	0.95%
Lysine	1.19 %
Calcium	0.95 %
Phosphorus available	0.48 %
Sodium	0.22%

1 guarantee levels per kg of product: Acid Folic: 140 mg/kg; Acid Pantot 1700 mg/kg; biotin: 15 mg/kg; Calcium: 30/130 g/kg; copper: 1410 mg/kg; choline: 40 g/kg DL-methionine: 260 g/kg; Enramycin: 1333 mg/kg; Iron: 8500 mg/kg; Iodine: 150 mg/kg; Lysine: 50 g/kg; Manganese: 12 g/kg; Niacin: 5930 mg/kg; Selenium: 45 mg/kg; vit. A: 1800000 UI/kg, Vit. B1: 580 mg/kg; vit. B12: 3000 mcg/kg; vit. B2: 960 mg/kg; vit. B6: 730 mg/kg; vit. D3: 300000 UI/kg; vit. E: 3750 UI/kg; vit. K3: 300 mg/kg, Zinc: 9170 mg/kg.

Birds received feed and water ad libitum during the experimental period (1 – 21 days). Three mycotoxin adsorbents with different compositions were tested, which were added 2.5kg/kg, in diets that were contaminated with mycotoxins, using 1.0 ppm aflatoxin + 50.0 ppm fumonisin + 25.0 ppm DON. 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% e G2: 0,7%. Fumonisin (B1 and B2) were obtained from the cultivation of a toxin strain of *Fusarium moniliforme,* and the concentrations used were 95.8% of B1 and 4.2% of B2. And the mycotoxin deoxynivalenol (DON) was obtained from the cultivation of a toxin strain of *Fusarium graminearum*.

We evaluated a control diet without contamination, contaminated diet with mycotoxins without absorbent, contaminated diet + YES - FIX HP®, contaminated diet + adsorbent A, contaminated diet + adsorbent B. The YES - FIX HP® adsorbent presents in its composition: 1.3 and 1.6 beta-glucans, polycationic bentonite, activated charcoal, organic molecule, silymarin and organic selenium. And, as informed by

the manufacturers, in the respective packaging, adsorbent A contains the following composition: dry brewery yeast (glucomannans), Na aluminosilicate and Ca, and oyster flour. While adsorbent B is composed of: bentonite, diatomite, Eubacterium sp, seaweed flour, inactivated yeast, dried chicory pulp.

2.3. Gene Expression of Interleukins IL 6 and IL 10

At the end of the experimental period, the cecal tonsils were collected and determined the expression of pro-inflammatory (IL 6) and anti-inflammatory (IL 10) interleukins by quantifying the gene expression by RT-qPCR.

The quantification of gene expression is performed by RT-qPCR, using specific initiators (primers) for each target. In this type of analysis, each combination target and sample generates a threshold value, Ct (threshold cycle), a measure of the target-specific messenger RNA (mRNA) concentration in the sample. The Ct value needs to be normalized as a function of previously chosen reference genes generating a deltaCt value (dCt) (Ct target-Ct reference gene).

For RNA extraction, approximately 100mg of tissue was homogenized in TissueLyser (Qiagen), and the total RNA was purified by extraction with TRI® Reagent (Sigma) – chloroform. The extracts were treated with turboDNaseI (Ambion) and the RNA was quantified with NanoDrop (Thermo Scientific). cDNAs were synthesized using the High-capacity cDNA Reverse Transcription kit (Applied Biosystems), using 1 ug of RNA per reaction. cDNAs were diluted 5x in sterile MilliQ water, and targets quantified using Bright-Green PCR Master Mix (Biotium) in a QuantStudio 3 thermocycler (Thermo). The cycling used was 95°C 10min, followed by 40 cycles of 95°C 15s and 60°C 1min. The Primer Express 3.0 program was used to design the oligonucleotide primers. The GAPDH and ACTB genes were used as internal controls, and the relative gene expression was determined using the 2-ΔΔCt method [17].

2.4. Statistical Analysis

Data were subjected to analysis of variance and comparison of means by the Tukey test at 5%.

3. Results and Discussion

The results of gene expression of interleukins IL-6 and IL-10 are shown in Figure 1. It was observed lower expression of IL-6 in birds that received the diet contaminated with mycotoxin + adsorbent B, differing from the other treatments. Which, when unbalanced, it could indicate immunosuppression of the birds' defense system. The other treatments did not differ statistically from each other.

Immune response to pathogens involves the rapid activation of pro-inflammatory cytokines that serve to initiate host defense against microbial invasion. However, excess inflammation can give rise to systemic metabolic and hemodynamic disturbances harmful to the host. As a result, the immune system has evolved parallel anti-inflammatory mechanisms that serve to curb the production of pro-inflammatory molecules to limit tissue damage and to maintain or restore tissue homeostasis.

The effect of mycotoxins on the bird immune system can be summarized in the following: depressed T- or B- lymphocyte activity (regressed bursa and thymus), suppressed immunoglobulin and antibody production, reduced complement or interferon activity, impaired macrophage-effector cell function, and reduced antibody titers and serum concentration of antibiotics [11].

Basically, cytokines mediate the turnout of an effective immune response and serve as an interface between the two arms (i.e., innate and adaptive elements) of an otherwise complex immune system [9].

IL-6 also has a pyrogenic effect. Together with IL-1, TNF and INF, this cytokine can significantly increase body temperature by stimulating prostaglandin production. Increased IL-6 production and sustained high serum concentration of this cytokine promote the passage of an acute inflammatory reaction into the chronic phase [5].

The largest expression of the IL-10, considering the scenario of contamination and adsorbent addition, was observed in the challenged group and supplemented with the adsorbent YES - FIX HP. This fact is probably related to its composition and resulted from the synergy between its active ingredients. In addition to the raw materials responsible for the adsorption of the main mycotoxins found in the field, this adsorbent contains principles known to have immunomodulating, antioxidant and anti-inflammatory action: 1.3 and 1.6 beta-glucans from the yeast Sacharomyces cerevisiae, organic selenium and milk thistle extract (silymarin) differing from the other analyzed adsorbents.

IL-10 is an immunoregulatory cytokine whose primary function is to limit inflammatory responses [6], with potent anti-inflammatory properties that plays a central role in limiting host immune response to pathogens, thereby preventing damage to the host and maintaining normal tissue homeostasis [13].

According to De Smedt et al. [7], IL-10 plays an essential role in controlling the immune response, balancing the response between Th1 and Th2 through the regulation of cytokine synthesis by antigen-presenting cells and reducing tissue damage.

The function of beta-glucan present in Yes-Fix HP is primarily the adsorption of mycotoxins, especially zearalenone [30]. Additionally, β-glucans are known as immune system modulators, acting mainly on macrophages, exerting a beneficial effect against a variety of bacteria, viruses, fungi and parasites [19], which can reduce the release of pro-inflammatory cytokines [28].

Because b-glucans are not present within animal cells, they are seen as ‘‘foreign’’ by the immune system and act as a major microbe-associated molecular pattern (MAMP), that primarily activates members of innate immunity [26].

However, it is noteworthy that the immunomodulatory attributes of these molecules, which induce regulatory responses of greater or lesser intensity, are probably related to

their degree of purification and biotechnologies involved in their production process. According to Zabriskie et al. [31], the beta-glucan-mediated immunoregulation mechanism depends on their interaction with immune cells located in the intestine, which recognize these oligosaccharides.

An excessive inflammatory response is associated with oxidative stress [15]; Selenium regulates the activation of NF-κB, a transcription factor, which plays a pivotal role in the regulation of inflammatory pathways [14]. Selenium can inhibit NF-κB from binding the inflammation-related genes which eventually reduce the expression of pro-inflammatory cytokines [8]. The anti-inflammatory function of Se might be due to the presence of specific selenoproteins, such as glutathione peroxidase (GPx) which reduces the oxidation induced inflammatory changes in the liver [18].

In addition to β-glucan and organic selenium, another particularity of the composition of Yes – Fix HP is the presence of silymarin. Silymarin is a natural product, extracted from the seeds and fruits of the plant Silybum marianum (milk thistle), and its effectiveness has been attributed to the antioxidant, anti-inflammatory and immunomodulatory mechanisms acting on several cells signaling pathways [25, 4]. Wang et al. [29], observed a reduction in the expression of pro-inflammatory interleukins with the administration of silymarin prior to the challenge with triploid-induced acute hepatotoxicity. According to Esmaeila et al. [10], silymarin inhibits factor-kappaB activation through suppression of inhibitory kappa B (IκB) degradation and suppresses inflammatory response, oxidative stress. Also, silymarin by suppression of STAT3 and ERK1/2 signaling pathways, inhibits oncogenesis, cell proliferation, cell migration and iNOS gene expression.

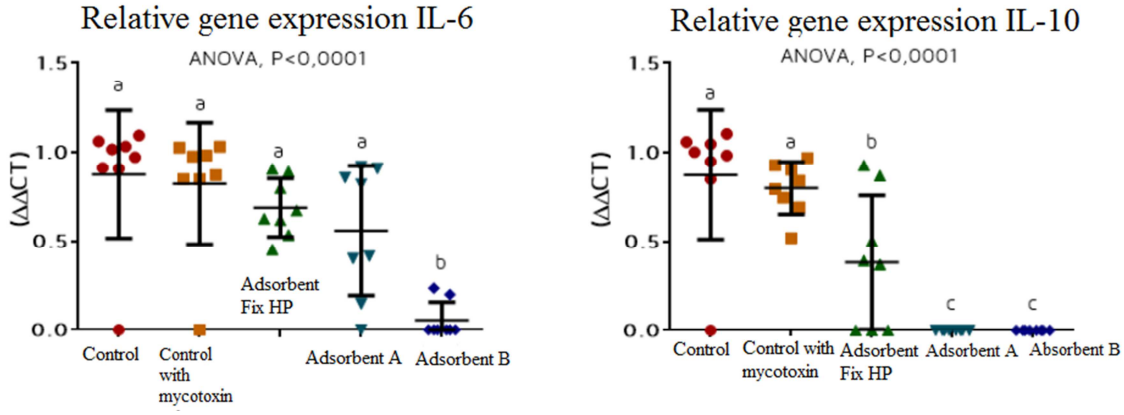

Figure 1. *Gene expression of interleukins IL-6 and IL-10 in cecal tonsil of broiler chickens challenged with mycotoxins and different adsorbents.*

4. Conclusion

Supplementation with the adsorbent YES - FIX HP® allowed greater expression of IL-10, which is possibly related to its composition and resulted from the synergy between its active principles. In addition to the raw materials responsible for the adsorption of the main mycotoxins found in the field, this adsorbent contains principles known to have immunomodulating, antioxidant and anti-inflammatory action, which can contribute to strengthening the health of animals.

References

[1] Aly, E., Madbouly, Y. (2016). The Effect of Mycotoxins on Immune Response of Broilers to the Live ND Vaccines Applied by Different Routes. *Middle East Journal of Applied Sciences,* 06 (01), 51-58.

[2] Arseniy E. Y., Anton G. K. (2014). *Interleukins in Cancer Biology: Their Heterogeneous Role*. Elsevier; London, UK: Academic Press; Cambridge, MA, USA.

[3] Bello R. O., Chin V. K., Abd Rachman Isnadi MF et al. (2018). The Role, Involvement and Function(s) of Interleukin-35 and Interleukin-37 in Disease Pathogenesis. *International Journal of Molecular Sciences*. 19 (4).

[4] Bijak, M. (2017). Silybin, a Major Bioactive Component of Milk Thistle (Silybum marianum L. Gaernt.)—Chemistry, Bioavailability, and Metabolism. *Molecules,* 22, 1-11.

[5] Borzecki, P., Borzecka, A., Chylinska-Wrzos, P., Lis-Sochocka, M., Wawryk-Gawda, E., Jodlowska-Jedrych. (2019). Evaluation of interleukin-6 concentration in the liver of Albino Swiss mice after intoxication with various doses of patulin. Curr. *Issues Pharm. Med. Sci*., 32 (1), 34-39.

[6] Cho, M. J., Ellebrecht, C. T., Payne, A. S. (2015). The dual nature of interleukin-10 in pemphigus vulgaris. *Cytokine*, 73 (2), 335-341.

[7] De Smedt, T., van Mechelen, M., De Becker, G., Urbain, J., Leo, O. and Moser, M. (1997). Effect of interleukin-10 on dendritic cell maturation and function. *European Journal of Immunology*. 27, 1229-1235.

[8] Duntas, L. H. (2012). The evolving role of selenium in the treatment of Graves'disease and ophthalmopathy. *J Thyroid Res*, 736161.

[9] Duque A. G., & Descoteaux A. (2014). Macrophage Cytokines: Involvement in Immunity and Infectious Diseases. *Front. Immunol*, 5, 491.

[10] Esmaeila, N., Anarakib, B. S., Gharagozlooc, M., Moayedia, B. (2017). Silymarin impacts on immune system as an immunomodulator: One key for many locks. *International Immunopharmacology,* 50, 194-201.

[11] Hofstetter, U. (2007). The negative effects of various mycotoxins on the immune system of poultry. *BIOMIN Newsletter*, 5-46.

[12] Holanda, D. M., Kim, S. W. (2020). Efficacy of Mycotoxin Detoxifiers on Health and Growth of Newly-Weaned Pigs under Chronic Dietary Challenge of Deoxynivalenol. *Toxins*, 12, 311.

[13] Iyer, S. S., Cheng, G. (2012). Role of Interleukin 10 Transcriptional Regulation in Inflammation and Autoimmune Disease. *Crit Rev Immunol*. 32 (1), 23-63.

[14] Khan, A. Z., Khan, I. U., Khan, S. …Ran L. (2019). Selenium-enriched probiotics improve hepatic protection by regulating pro-inflammatory cytokines and antioxidant capacity in broilers under heat stress conditions *Journal of Advanced Veterinary and Animal Research.* 6 (3), 355–361.

[15] Kim, S. H., Oh, D. S., Oh, J. Y., Son, T. G., Yuk, D. Y., Jung, S. (2016). Silymarin prevents restraint stress-induced acute liver injury by ameliorating oxidative stress and reducing inflammatory response. *Molecules* 21, 443.

[16] Lauwers, M., Croubels, S., Letor, B., Gougoulias, C., Devreese, M. (2019). Biomarkers for exposure as a tool for efficacy testing of a mycotoxin detoxifier in broiler chickens and pigs. *Toxins*, 11, 187.

[17] Livak, K. J., Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2 (-Delta Delta C(T)) *Method. Methods*, 25 (4), 402-8.

[18] Liu Y, Liu Q, Ye G, Khan A, Liu J, Gan F, et al. (2015). Protective effects of Selenium-enriched probiotics on carbon tetrachloride-induced liver fibrosis in rats. *J Agric Food Chem*; 63, 242.

[19] Mantovani, M. S., Bellini, M. F., Angeli, J. P., Oliveira, R. J., Silva, A. F., Ribeiro, L. R. (2008). Beta-Glucans in promoting health: prevention against mutation and cancer. *Mutat Res*., 658 (3), 154-61.

[20] Moore, K. W., Waal Malefyt, R. de, Coffman, R. L., & O'Garra, A. (2001). Interleukin-10 and the interleukin-10 receptor. *Annual review of immunology*, 19, 683-765.

[21] Ning X., Jian Z., Wang W. (2015). Low Serum Levels of Interleukin 35 in Patients with Rheumatoid Arthritis. *Tohoku J. Exp. Med*., 237, 77-82.

[22] National Research CouncilL. Nutrient requirements of poultry. 9 th ed. Washington, D.C.: National Academy of Science, 1994. 154 p.

[23] Qu D., Huang X., Han J., Man N. (2017). Efficacy of mixed adsorbent in ameliorating ochratoxicosis in broilers fed ochratoxin A contaminated diets. Ital *J Anim Sci.* 16, 573–579.

[24] Sattler N., Monroy R., Soldati T. (2013) Quantitative Analysis of Phagocytosis and Phagosome Maturation. In: Eichinger L., Rivero F. (eds) Dictyostelium discoideum Protocols. Methods in Molecular Biology (Methods and Protocols), vol 983. Humana Press, Totowa, NJ. https://doi.org/10.1007/978-1-62703-302-2_21

[25] Shahbazi, F., Dashti-Khavidaki, S., Khalili, H., Lessan-Pezeshki, M., (2012). Potential renoprotective effects of silymarin against nephrotoxic drugs: A review of literature. *Journal of Pharmacy and Pharmaceutical Sciences*, 15, 112-123.

[26] Soltanian, S., Stuyven, E., Cox, E., Sorgeloos, P. and Bossier, P. (2009) Beta-glucans as immunostimulant in vertebrates and invertebrates. *Crit. Rev. Microbiol*., 35, 109-138.

[27] Trinchieri, G. (2007). Interleukin-10 production by effector T cells: Th1 cells show self-control. *The Journal of experimental medicine*, 204 (2), 239-43.

[28] Tzianabos, A. O. (2000). Polysaccharide immunomodulators as therapeutic agents: structural aspects and biologic function. *Clinic. Microbiol*., 13, 523-533.

[29] Wang L, Huang QH, Li YX, et al. Protective effects of silymarin on triptolide-induced acute hepatotoxicity in rats. Mol Med Rep. 2018; 17 (1): 789-800. doi: 10.3892/mmr.2017.7958.

[30] Yiannikouris A., François J., Poughon L., Dussap C. G., Bertin G., Jeminet G., Jouany J. P. (2004). Adsorption of zearalenone by beta-D-glucans in the Saccharomyces cerevisiae cell wall. *J. Food Prot*. 67, 1195-1200.

[31] Zabriskie, Hannah A., Julia C. Blumkaitis, Jessica M. Moon, Brad S. Currier, Riley Stefan, Kayla Ratliff, and Chad M. Kerksick (2020). Yeast Beta-Glucan Supplementation Downregulates Markers of Systemic Inflammation after Heated Treadmill Exercise. *Nutrients*, 12 (4), 1144.