

# Newsletter

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## > EDITORIAL

*Mycotoxins cause a wide range of harmful effects in poultry. The economic impact of reduced productivity, increased incidence of diseases due to immunosuppression, damage of vital organs and interferences with laying capacity is often greater than the impact caused by clinical acute symptoms or mortality due to mycotoxin poisoning. Immunosuppression in chickens can be caused by several different factors. Consumption of mycotoxins, at rather low levels that do not cause clinical mycotoxicosis, suppress immune functions and may decrease resistance to infectious disease. The sensitivity of the immune system to mycotoxin-induced immunosuppression arises from the vulnerability of the continually proliferating and differentiating cells that participate in immune mediated activities and regulate the complex communication network between cellular and humoral components. As nutrition forms the hub of efficient poultry farming we need - apart from optimal productivity - to feed the bird for 'immunity'. Probably no biological effects of the mycotoxins are more medically and economically important than their suppression of the immune responses and native resistance mechanisms of affected animals. To grasp this complicated subject, it is essential that we first understand how actually immune mechanisms operate in poultry. Therefore we decided to write a newsletter on the negative effects mycotoxins are causing on immunity in poultry to understand the relevance and complexity of this very important topic and to demonstrate the various detrimental effects on the immune system caused by different mycotoxins.*

Enjoy reading!

Ursula Hofstetter



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**The immune system is the defense mechanism of the body of which key organs are thymus, bursa, liver and spleen. In poultry bursa of Fabricius (located in the lower back of the chicken) and thymus (in the neck) are key sites for immune responses and hence termed as chief immune organs.**

## The negative effects of various mycotoxins on the immune system of poultry

It is common belief that the birds' immunity is solely due to the development of antibodies. However, it is only the half truth. It is often forgotten that protection of birds against infectious diseases is also imparted through special cells, the lymphocytes. The protection given through cells is known as cell-mediated immunity, which is equally important to that of antibody mediated (humoral) immunity. In the chicken, lymphocytes are normally about 60 per cent of the total number of white blood cells. In the incubating egg, lymphocytes are derived from the lymphoid stem cells (parent cells), which originate in the yolk sac membrane. These stem cells travel into two different directions between 5 to 7 days of incubation. One set of stem cells goes to bursa of Fabricius - a round, sac like structure just above the cloaca - while the other set of stem cells enters the thymus. Bursa and thymus reach their greatest size in the chick about 1-2 weeks after hatching and then gradually disappear. The stem cells which differentiate into lymphocytes in bursa are called B-lymphocytes ('B' for bursa) and those which differentiate into lymphocytes in thymus are called T-lymphocyte ('T' for thymus).

The B-lymphocytes on contact with pathogens are transformed into another type of cells known as 'plasma cells', instead of producing antibodies by themselves. The plasma cells are especially equipped to produce the antibodies (immunoglobulins, Ig). These antibodies then inactivate the pathogens and thus protect the body. Each plasma cell can produce up to 300 molecules of antibody per second. Antibodies are continuously replaced. One type of plasma cells produces only antibodies against one infectious agent. This means that different types of B-lymphocytes must be activated to produce different sets of plasma cells, specific for each disease. It is now believed that B-lymphocytes carry tiny ready-made antibodies (immunoglobulin M; IgM) on their surface against each infectious agent present in nature. When any organism enters into the body, it identifies the particular antibody on the surface of a specific B-lymphocyte acting as a receptor for it. The organism then attaches to the specific antibody present on its surface, like a lock and key arrangement. In other words, antibodies against one disease are derived from only one type of B-lymphocyte, which then multiplies and gives rise to a specific set of plasma cells. This is immunologically expressed as "antibodies are monoclonal in origin", thus they are derived from a single cell (B-lymphocyte), which then proliferates and forms a colony of plasma cells.

The T-cells are involved in cell-mediated immune responses, such as delayed hypersensitivity reaction and immune surveillance against foreign or altered cells. Several subpopulations of T-cells exist: cytotoxic T-cells, helper T-cells, and suppressor T-cells. It is now known that neither these cytotoxic T-cells nor the B-cells can function without the stimulation received through certain short acting soluble mediators (messenger molecules) known as "lymphokines". These lymphokines are produced by another class of specific lymphocytes known as "helper T-Lymphocytes". They are called helper cells because the messenger molecules secreted by them help both B- and T-lymphocytes in producing antibody and cell-mediated immune responses. The two lymphokines which influence the cytotoxic T-lymphocytes are interleukin-2 and gamma-interferon. If these lymphokines are not available from helper T-lymphocytes, both antibody and cellular immune responses will grind to a halt and no immune protection will be imparted to the bird.

Both B-lymphocytes and T-lymphocytes don't pick up the infectious organisms themselves. Pathogens are first taken up by a cell called "macrophage", which is derived from blood monocyte, a type of white blood cell like lymphocyte. Macrophages process and present the pathogen to B- and T-lymphocytes. Even then the immune response does not begin, unless these two cells are stimulated by the messenger molecules released by the macrophages. These are interleukin-1 and interleukin-6. They are called monokines because they are derived from monocytes.

#### Manifestations of Mycotoxin-induced immunosuppression

- 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.
- Reduced antibody titers and serum concentration of antibiotics

**Immunosuppression** in chickens can be caused by several factors such as nutritional, managerial, diseases, stress etc. Among nutritional causes consumption of mycotoxins, at levels that do not cause overt clinical mycotoxicosis, suppress immune functions and may decrease resistance to infectious disease. The sensitivity of the immune system to mycotoxin-induced immunosuppression arises from the vulnerability of the continually proliferating and differentiating cells that participate in immune mediated activities and regulate the complex communication network between cellular and humoral components.

#### Aflatoxin and immune response

Aflatoxin B<sub>1</sub> has the most potent biological effects. The primary actions of aflatoxins are their ability to bind with both DNA and RNA and inhibit macromolecular synthesis by interfering with transcription and to other aspects of protein formation. Impairment of protein formation and ability to cause involution or hypoplasia of the thymus are the two important general effects of aflatoxin B<sub>1</sub>.

##### a) Effects on antibody production

High doses of aflatoxins (e.g., 0.3 to 6 mg/kg) cause significant histopathological effects in the bursa of Fabricius. Analyses of immune globulins of poultry show that normal levels of immunoglobulins IgG and IgA may show variable amounts of reduction, although IgM levels are generally unaffected.

##### b) Effects on non-specific humoral substances

Complement is a complex nonspecific humoral substance which is antigenically nonspecific in its action but which is an integral part and mediator of many immunological actions, clearance mechanisms and inflammatory reactions. Among the important actions of aflatoxin B<sub>1</sub> is the suppression of the fourth component of complement (C4), which is necessary for the classical activation of the membrane attack complex for hemolytic activity, bacterial lysis, etc. This diminution appears to be the result of aflatoxin effects on liver function, as well as on macrophage function, both of which are involved in formation of C4.

##### c) Effects on cell mediated immune response

Aflatoxin B<sub>1</sub> exerts part of its immunosuppressive activities through lymphokines. Presumably, the action is a quantitative suppression of lymphokine production by T cells. Although aflatoxin consumption causes dramatic hypoplasia of the thymic cortex, no detectable diminution of T-cells in the peripheral blood-lymphocyte pool has been observed.

##### d) Altered resistance to specific infectious processes

Aflatoxin consumption causes a substantial diminution in phagocytic activity by macrophages. The effects of aflatoxin consumption on resistance to infections are highly variable depending on the specific infectious processes involved, host's susceptibility to aflatoxin and host interaction. Resistance to pasteurellosis in poultry is decidedly affected by aflatoxins, while Newcastle disease does not appear to be so.

##### e) Apparent mechanisms for aflatoxic immunosuppression

Inhibition of protein formation: Complement and lymphokines that are produced by highly aflatoxin sensitive tissues,

namely liver and T-lymphocytes, respectively, are noticeably depressed. Both of these substances are associated with the regulation and mediation of immune reaction and cellular clearance.

Suppression of phagocytosis and subsequent diminution of antigen presentation by affected macrophages to the lymphocyte pool appears to be one of the major keys to immunosuppression by aflatoxin. Certain classes of immunoglobulins appear to be more affected than others (e.g. IgG and IgA) making the birds more susceptible to various pathogens.

**Table 1: Effects of Aflatoxin B<sub>1</sub> on the immune system of poultry**

Aflatoxin B <sub>1</sub>
<ul style="list-style-type: none"> <li>Immune system is compromised; detrimental effects on cell</li> </ul>
<ul style="list-style-type: none"> <li>Mediated immunity and impaired phagocytic and bacterial activity of heterophils including decreased peripheral T-lymphocyte counts</li> </ul>
<ul style="list-style-type: none"> <li>Decreased antibody formation against SRBC (injected sheep red blood cells) and decreased weight of the Bursa of Fabricius and Thymus</li> </ul>
<ul style="list-style-type: none"> <li>Aflatoxin carry over via the egg to the embryo, compromises immune system of the progeny, including cell mediated, humoral immunity and phagocytic functions. Progeny are more susceptible to various pathogens</li> </ul>
<ul style="list-style-type: none"> <li>Reduced phagocytic RES (reticuloendothelial system) cells</li> </ul>
<ul style="list-style-type: none"> <li>Increased mortality with <i>Salmonella</i></li> </ul>

### Trichothecenes and immune response

Over 180 trichothecenes are identified so far and most of them can bind to eukaryotic ribosomes and inhibit protein synthesis. The rapidly proliferating tissues such as skin and mucosa, as well as lymphocytic and hematopoietic tissues are primarily affected by these mycotoxins. T-2 toxin apparently binds to receptors on the cell membrane, decreases both RNA and DNA production and interferes with protein synthesis by blocking the initiation of translation.

#### a) Effects on lymphoid tissues and antibody production

Oral administration of T-2 toxin causes thymic atrophy and also necrosis of Gut Associated Lymphoid Tissue (GALT) have been reported, as they show reductions of both T- and B-cells in the circulating lymphocyte pool.

Reduction in number and activity of phagocytes, and reduced chemotaxis in trichothecene-treated birds are commonly observed effects. Both granulocytes and macrophages are also affected to some extent. In addition, there is a depression in complement (C3) formation and reduced production of IgA and IgM. All these changes result in reduction in both T-cell dependent and T-cell independent antibody production. T-2 toxin has also been shown to cause diminution in several non-specific humoral substances that mediate immune responses.

#### b) Effects on cell-mediated immune responses

Besides causing thymus atrophy and decline in circulating T-cells, T-2 toxin also diminishes the mediators of the cell mediated immune (CMI) response (the lymphokines) either

in amount or effectiveness. Interleukin-2, one of the major lymphokines that is responsible for T-cell growth, cytotoxic and killer cell activity, is also reduced by T-2 toxin consumption. Other lymphokines, responsible for lymphoblastogenic responses, graft rejection, and other CMI responses are also affected by T-2 toxin or diacetoxyscirpenol (DAS) consumption. All these changes result in diminishing antigen presentation to the available T- and B- cell population, all combine to suppress CMI responses. Most investigators of the effects of T-2 and DAS have reported significant reduction in delayed cutaneous hypersensitivity to specific antigens.

#### c) Altered resistance to specific infectious processes

The research data on the effect of trichothecene toxins on the number of infectious processes indicates that these toxins are immunosuppressive and reduce resistance to infections like *Salmonella*, *Staphylococci*, *Listeria*, *Mycobacterium* and many others.

#### d) Apparent mechanisms for immune suppression by trichothecenes

Trichothecenes clearly involve both in their effects - the T and B cell portions of the lymphocyte population. They also have a very pronounced effect on protein formation, leucopoiesis (production of white blood cells), complement formation and mucosal integrity.

**Table 2: Effects of Trichothecenes on the immune system of poultry**

TRICHOHECENES (DON, T-2 toxin)
<ul style="list-style-type: none"> <li>Inhibition of lymphocyte proliferation</li> </ul>
<ul style="list-style-type: none"> <li>Alteration in interleukin metabolism</li> </ul>
<ul style="list-style-type: none"> <li>Increased mortality to pathogenic bacterial challenge</li> </ul>
<ul style="list-style-type: none"> <li>Resulted lymphopenia and lymphatic necrosis</li> </ul>

### Ochratoxins and immune response

Major effects of ochratoxins are the inhibition of protein synthesis (translation) through blocking phenylalanine tRNA synthetase. Ochratoxins have major effects on kidney and GALT of affected individuals. Necrosis of proximal tubular epithelium of the kidney is accompanied by the usual signs of nephritis, including polydipsia, polyuria and urinary casts. Lymphoid necrosis of the GALT system extends to bursa of Fabricius with perhaps some very limited effect on the thymus at higher doses of the toxin. There is some influence of ochratoxins on the central nervous system in poultry, where loss of the righting reflex is seen in clinically affected birds. The major effects of ochratoxins on the immune response appear to be on antibody production and to a lesser extent on phagocytosis.

#### a) Effects on gut associated lymphoid tissue and antibody production

The major source of antibody production is from GALT derived lymphocytes, including those from the bursal tissue of poultry. Ochratoxin consumption causes bursal hypotrophy, diminutions of IgG and IgM, as well as significant reduction in antibody titer.

#### b) Effects on phagocytosis and cell-mediated immunity

The most common finding is a reduction in macrophage

motility and a reduction in phagocytosis of foreign particles by granulocytic phagocytes.

**c) Apparent mechanisms for immune suppression by ochratoxin A**

Toxic effects are seen when approximately 0.1 mg/kg/d of ochratoxins are ingested. The major mechanism for immune suppression by ochratoxins is directly related to antibody formation. Phagocytic impairment through reduced motility and phagocytosis reduces both clearance and resultant antigen presentation; thereby no B-cells remain available to produce the necessary antibodies.

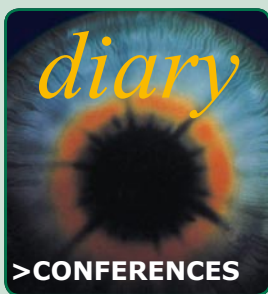
**Table 3: Effects of Ochratoxin A on the immune system of poultry**

**Ochratoxin A**

- Inhibition of humoral, cellular and innate immune responses
- Cellular depletion of lymphoid organs
- Depressed delayed hypersensitivity responses
- Depressed blood monocyte phagocytic activity
- Increased susceptibility to infectious agents
- Leucopenia and impaired phagocytosis by heterophils

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| <b>IPE Atlanta</b><br>Atlanta, Georgia, USA, 24 <sup>th</sup> – 26 <sup>th</sup> January 2007                             | <b>AASV Annual Meeting</b><br>Orlando, Florida, USA, 3 <sup>th</sup> – 6 <sup>th</sup> March 2007 |
| <b>Australian Poultry Science Symposium</b><br>Sydney, Australia, 12 <sup>th</sup> – 14 <sup>th</sup> February 2007       | <b>VIV Asia</b><br>Bangkok, Thailand, 7 <sup>th</sup> – 9 <sup>th</sup> March 2007                |
| <b>8<sup>th</sup> Asian Pacific Poultry Conference</b><br>Bangkok, Thailand, 5 <sup>th</sup> – 6 <sup>th</sup> March 2007 | <b>AFMA Forum 2007</b><br>Sun City, South Africa, 13 <sup>th</sup> – 16 <sup>th</sup> March 2007  |

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**> IMPRESSUM**

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