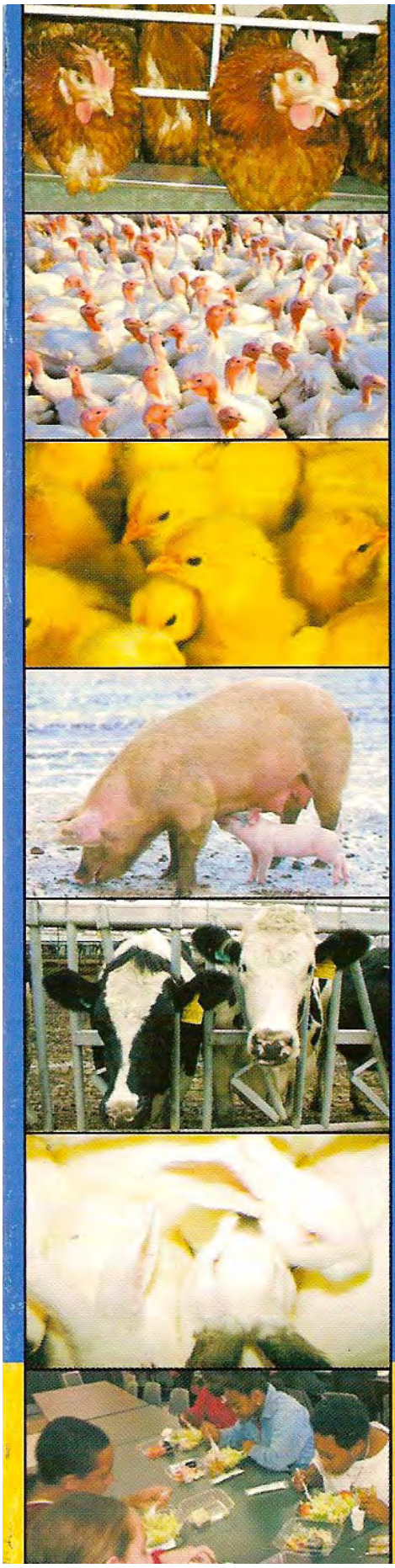

Mycotoxins and Mycotoxicosis in Animals and Humans

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The mycotoxins specialist

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PRESENTATION

Special Nutrients, a U.S. based company, as a sponsor of this manual and as a world pioneer in the applied investigation of detoxification of mycotoxins, has the pleasure of briefly presenting the authors of the following manual.

Alberto Gimeno, a licensed industrial chemical engineer, worked as Technical Director (in various companies in Spain and Portugal), a specialist in quality control of raw materials and animal feeds, specializing in mycotoxins and related field work on their toxicological effects relating to animal nutrition.

Gimeno has been adviser of various doctoral theses focused on mycotoxins. He is an expert in conducting applied research on mold inhibitors and antioxidants.

Gimeno was for five years a Technical Advisor of the Commission for Normalization of Analytical Methods for the Spanish Agricultural Ministry in Madrid. His multi-detection method for analysis of mycotoxins (published in J. AOAC of USA) applied to the analysis of aflatoxins was Spain's official method for 6 years.

Gimeno collaborated with the International Agency for Research on Cancer (World Health Organization) on Mycotoxin Check Sample Survey for 14 years.

Gimeno gave 140 lectures on mycotoxins in Spain, Portugal, Egypt, Thailand, Malaysia, Indonesia, Austria, Brazil, Chile, Peru, Italy, Czech Republic, Cuba, Mexico, Ecuador, Costa Rica, Guatemala, Dominican Republic, and the United States of America.

Gimeno has also carried out a total of 15 courses on feed quality control and food mycotoxicology, and 11 conferences on animal nutrition in Spain and Portugal.

Gimeno has published 55 articles on the analytical procedure control of mycotoxins and food/feed mycotoxicology in various scientific and technical journals, symposium books, web books and web pages such as, J. AOAC (Journal Association of Official Analytical Chemists) (USA), Food Additives and Contaminants (UK), Revista Portuguesa de Ciencias Veterinárias (Portugal), Cunicultura (Spain), Selecciones Avícolas (Spain and Portugal), Albéitar (Spain), Albéitar (Portugal), Suis (Spain), Nuestra Cabaña (Spain), Proceedings Book of IUPAC (International Union of Pure and Applied Chemistry) (Austria), Proceedings Book of FDA (Food and Drug Administration) and NRC

(National Research Centre of Cairo) (Egypt), Sociedad Ibérica de Nutrición Animal (Spain), WPSA (World Poultry Science Association) (Spain and Portugal) and Foodborne Illness (Portugal), Revista Portuguesa de Alimentación Animal of IACA (Portugal). He has an article in the book entitled «Enfermedades del Conejo» (Spain). He has also published a manual in Portuguese entitled «Micotoxicoses en Avicultura – Controlo e Prevenção» and a manual in spanish entitled «Micotoxinas y Micotoxicosis en Animales y Humanos».

He regularly publishes in the web pages www.mycotoxin.com and www.engormix.com (See: mycotoxins for a complete list of technical articles, sections in both English and Spanish.)

Gimeno has been the Technical Consultant of several companies in the area of mycotoxicology and animal nutrition.

Maria Ligia Martins, licensed in Veterinary Medicine, Principal Researcher. Specialist in the quality control of fungi and mycotoxins in both animal feed and human food.

Martins worked in the National Laboratory of Veterinary Research in Lisbon, Portugal for 30 years.

During 14 years, Martins collaborated with the International Agency for Research on Cancer (World Health Organization) on Mycotoxin Check Sample Survey. She also collaborated with the European Program for Monitoring and Assessment of Dietary Exposure for Potentially Hazardous Substances (FEMS / Food – EURO). Martins participated as vocal in the technical commissions of Accredited Laboratories Association (RELACRE) and referee in various scientific works.

Martins has taught 15 courses on the practice of the analysis of fungi and mycotoxins in animal feed and human food. Martins has participated in 42 presentations of conferences and papers on mycology, mycotoxicology, and fungi and mycotoxin analyses in International Congresses in Portugal, Spain, Czech Republic, Hungary, Poland, Italy, Germany, England, Finland, and Norway.

Martins has taught 17 Graduate courses in medical mycology and in the master program in animal production in Portugal. Also she has participated in 4 communitarian projects on mycotoxins.

Martins has published 61 articles on mycology, mycotoxicosis, and fungi and mycotoxin analysis in various scientific and technical journals, symposiums books, and web book and web pages such as,

J. AOAC (Journal Association of Official Analytical Chemists) (USA), Food Additives and Contaminants (UK), Revista Portuguesa de Ciencias Veterinárias (Portugal), Food Protection, Brazilian Journal of Veterinary Research and Animal Science (Brasil), Revista Iberoamericana de Micología, International Journal of Food Microbiology, Proceedings Book of IUPAC (International Union of Pure and Applied Chemistry) (Austria). She has an article in the book entitled «Enfermedades del Conejo» (Spain). She regularly publishes on the following web pages, www.mycotoxin.com and www.engormix.com (See mycotoxins, section in Spanish.)

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DEDICATION

The authors dedicate this manual to their beloved granddaughter, Beatriz, and their children, Carla and son in law Carlos.

INDEX

1. INTRODUCTION

Mycology is a branch of biology that studies fungi (mold and yeast). Mycosis is the name given to the illnesses produced by fungi in humans and animals.

Mycotoxicosis is the name given to the group of illnesses and disorders in humans and animals resulting from toxic secondary metabolites (mycotoxins) produced by some fungi species.

Fungi are vegetables lacking in chlorophyll belonging to the thallophytic group. This lack of chlorophyll not only distinguishes fungi from other vegetables, but it is also an important characteristic of the biological activity of this vegetable. The lack of chlorophyll does not allow fungi to use sunlight as an energy source in order to synthesize organic material. Thus, fungi must develop on a substratum which contains organic material. This factor conditions the site of fungi growth. In such a way, each food source is a special ecological system where the interaction of chemical, physical, and biological factors has a fundamental role in the deterioration of the food source due to fungus development and proliferation.

The most modern classifications group fungi in the protist kingdom (in between plants and animals). Fungi are neither considered plants nor animals due to their alimentary method through absorption; and are classified in their own kingdom called «Fungi.»

Fungi have the capacity to infect live vegetable tissue, leading to great invasion, dissemination, and deterioration of stored products. It should be added, that fungi have the power to produce problems of mycosis and the genetic capacity that some fungi have to produce toxic secondary metabolites, called mycotoxins, consequently producing mycotoxicosis in animals and humans that consume the contaminated food source. These factors contribute to the importance of fungi within the field of microbiological feed.

The most important genus of mold and yeast from the mycosis and mycotoxins point of view are the following:

MOLD: *Alternaria, Aspergillus, Botrytis, Cephalosporium, Cladosporium, Fusarium, Helminthosporium, Monilia, Geotrichum, Gleosporium, Mucor, Penicillium, Rhizopus, Sporotrichum, Trichotecium, Absidía, Thamnidium.*

YEAST: *Candida, Rhodotorula, Mycoderma, Torulopsis.*

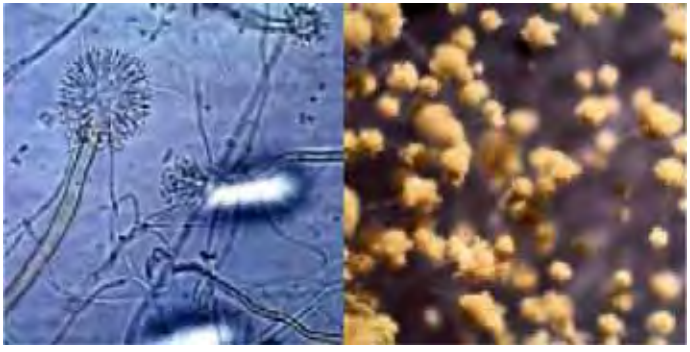


Fig.0. Left, Aspergillus flavus. *Right,* Aspergillus ochraceus.

As previously mentioned, mycotoxins are toxic secondary metabolites produced by some fungi species. Mycotoxins are polyketones compounds resulting from condensation reactions produced under specific physical, chemical, and biological conditions that occur when the reduction of the ketone groups in the biosynthesis of the fatty acids, carried out by the mold, is interrupted. These fatty acids are primary metabolites used by mold as an energy source. Mycotoxins tend to be formed at the end of the exponential phase or at the beginning of the stationary phase of the mold's growth.

More than 200 mycotoxins have been identified until now, nonetheless those most frequently found naturally contaminating human food and animal feed are the following: aflatoxins, ochratoxins, zearalenone, fumonisins, trichothecenes toxins (T-2 toxin, diacetoxyscirpenol, deoxynivalenol or vomitoxin, nivalenol, monoacetoxyscirpenol, triacetoxyscirpenol, scirpentriol), patulin, penicillic acid, sterigmatocystin, alternaria toxins (alternariol, alternariol

monomethyl ether, altenuene, altenuisol, etc), rye ergot alkaloids (ergotamine, ergotoxin, ergometrine), tremorgenic toxins (penitrem A y B), rubratoxins A y B, luteoskyrin, islanditoxin, rugulosin y citreoviridin. Since all these mycotoxins report, to a greater or lesser degree, a series of clinical pathological symptoms, disorders, or toxic effects in animals and humans, they became important in the world of food and feed supply.

Three fundamental factors exist for the development of fungi and the production of mycotoxins. These factors are: a) Physical (humidity or free water and water activity (aw), temperature, microflora zones, physical integrity of the grains). b) Chemical (pH, substratum composition, mineral nutrients, oxy-reduction potential, O_2/CO_2). c) Biological (presence of invertebrates, specific strains). A detailed description of these three factors can be found in (Gimeno, 1999; Gimeno, 2000).

The quantity of water which is contained in the atmosphere and in the substrate, is one of the most important factors for the development of fungi and the production of mycotoxins. However, not only does the quantity of water present influence the development of these organisms, but also the state in which the water is present, either as free water or combined water. Free water exists within and around vegetable tissue or their cells and can be eliminated without seriously interfering with vital processes. Combined water is present in vegetable and animal tissue bound with proteins and carbohydrates, forming and integral part of the cells that compose said tissues.

Free water is necessary for the germination of fungi spores. Free water is often referred to as humidity. There are two units related to free water (humidity), which are the following:

a) Relative humidity of equilibrium (RHE), this is the quantity of water available to the microorganisms, once the equilibrium between free water in the substrate and water vapor in the environment which surrounds the microorganism is reached. The RHE is expressed in percentage which varies between different feeds and foods sources depending on their carbohydrate and lipid content.

b) Water activity (aw), this is the relationship that exists between free water (humidity) in feeds and foods sources and the capacity of

microorganisms to proliferate in these substrates. The aw indicates the quantity of water available for the development of microorganisms once the hydric equilibrium in the food-environment system has been reached.

If the humidity of the food source is in equilibrium with the relative humidity of equilibrium (RHE) of the environment which surrounds the food source, the water activity in the food source is numerically equivalent to the following: aw = RHE/100. RHE refers to the equilibrium between the environment and the product. The aw refers to the product itself. Pure water has an aw of 1 and it is at equilibrium with an environment of 100% RHE. The aw of any food or feedstuff source is always less than 1.

The HRE varies depending on carbohydrate and lipid content of the feeds and foods (amylaceous or oleaginous substrates), as previously mentioned. For instance, the corn (amylaceous) containing 13.5% free water (humidity) will be at equilibrium with a RHE of 70% (inside a vertical silo), therefore the aw would be 0.70. At the same temperature, full fat soya (oleaginous containing 19-20% of fat) containing 13.5% free water (humidity) will be at equilibrium with a RHE of 75% (inside a vertical silo), thus, the aw would be 0.75. At the same temperature (25ºC), sunflower seeds (oleaginous containing 41% of fat) containing 13.5% free water (humidity) will be at equilibrium with a RHE of 85% (inside a vertical silo), therefore, the aw would be 0.85. It is more difficult to preserve oleaginous seeds even with relatively low humidity (free water) due to the lipid content which facilitates the evaporation of water and therefore its inclusion into the atmosphere that surrounds the substrate particles. Hence, the hydric equilibrium is reached more rapid and efficiently.

Generally, with water activity of 0.85 at 25ºC, which corresponds to approximately 15-16% humidity or free water in the substrate, fungi spores germinate within 5 to 12 days. On the other hand, at the same temperature with water activity of 0.75 (which corresponds to approximately 13-14% humidity in the substrate), fungi spores take 4 to 12 weeks to germinate. Nonetheless, things can vary significantly depending on the substrate (amylaceous or oleaginous) as previously mentioned.

Hence, with temperatures around 25ºC, cereal grains such as corn, wheat and sorghum, maintaining at a state of equilibrium at 12.5%

humidity or less (which corresponds to a state of equilibrium with a RHE of 65%, or water activity of 0.65) can be stored safely during a long period of time. The same can not be said for the full fat soya or sunflower seeds, because under these conditions, a 12.5% humidity in state of equilibrium corresponds to a RHE of almost 82%.

Any feed stored at a state of equilibrium with a RHE below 65% (aw = 0.65) has a remote possibility of mold growth and proliferation.

Mold growth also occurs inside horizontal silos. In this case, however, the problem occurs within the feedstuffs mass, given that the air that surrounds the substrate particles remains enclosed in the interior of that mass. This has given rise to the recommendation of moving the feedstuffs in order to liberate and renovate the air that surrounds the substrate particles; or the suggestion of pumping cold, dry air (from the bottom-up) in vertical or horizontal silos; which also reduce the temperature of the feedstuffs.

Although many mycotoxins have been mentioned, we will examine those that present the most significant risks of mycotoxicosis in different animals (chickens, hens, ducks, turkeys, pigs, dairy cows, and rabbits) and humans. Also, we shall refer to *Aspergillus* mycotoxins (aflatoxins and ochratoxin A), *Fusarium* mycotoxins (zearalenone, vomitoxin or deoxynivalenol, diacetoxyscirpenol, fumonisins, T-2 toxin, monoacetoxyscirpenol, triacetoxyscirpenol, and escirpentriol), and *Penicillium* mycotoxins (patulin).

2. ASPERGILLUS MYCOTOXINS

Aspergillus are molds that belong to storage flora. Generally, the necessary minimum temperature and water activity (aw) for developing and produce mycotoxins is 10 to 12ºC and 0.75 to 0.83, respectively. The optimal conditions for the growth of *Aspergillus* and the production of mycotoxins are 25ºC with water activity of 0.95. Nonetheless, there are strains of *Aspergillus flavus*, which grow on substrates such as rice, at temperatures between 6 and 45ºC with an optimal temperature of 37ºC, and the production of mycotoxins between 11 and 36ºC with a maximum production at 30 ºC (Hesseltine, 1976).

In substrates such as peanuts, rice, sorghum, wheat, and corn, strains of *Aspergillus parasiticus* NRRL 3000 and NRRL 2999 can produce 107, 107, 72, 72, 53 mg/Kg (ppm) and 104, 185, 88, 19, 47 mg/Kg of aflatoxins, respectively. However, the production of aflatoxins in soya meal is only 19 and 2.8 mg/Kg, respectively. The strain NRRL 3145 produces 8.50, 10.60, 57.60, 7.10, and 5.50 mg/Kg of aflatoxins in peanuts, rice, sorghum, wheat, and corn, respectively; but, the production of aflatoxins in soya meal is significantly lower, around 0.06 mg/Kg.

Therefore, under optimal temperature and water activity (aw) conditions, the mycotoxin production depends on the genetic strain and the substrate composition. The *Aspergillus flavus* strains NRRL 3251, 3357, 3517, and 3353 produce aflatoxins. However, the strain NRRL 1957 does not produce aflatoxins (Hesseltine, 1976).

The main mycotoxins produced by *Aspergillus* are aflatoxins and ochratoxins. Other mycotoxins such as patulin and penicillic acid can also be produce by some *Aspergillus*, however, manual will focus on the first two (aflatoxins and ochratoxins), which have the risk of producing mycotoxicosis in animals such as: chickens, hens, ducks, turkeys, pigs, dairy cows, rabbits, and humans.

Patulin must be taken into account given that it presents a risk for humans. However, there are still many studies to be done in order to classify this mycotoxin as a high risk, although the European Union (EU) already has a rigorous legislation towards this mycotoxin, mainly in fruit juices. Patulin belongs more to the *Penicillium* mycotoxins.

2.1. Aflatoxins

Aflatoxins are produced mainly by *Aspergillus flavus* and *Aspergillus parasiticus*. Currently there are 18 known types of Aflatoxins, of which the most toxic are Aflatoxin B1 (**AFB1**) and Aflatoxin M1 (**AFM1**). Aflatoxin M1 is the hydroxilated metabolite of Aflatoxin B1, which is originate by the metabolism of some animals, normally found in milk and urine.

Other aflatoxins listed in order of toxicity, from most toxic to least toxic, are G1 (**AFG1**), M2 (**AFM2**), B2 (**AFB2**), and G2 (**AFG2**) (M2 being a metabolic derivative of aflatoxin B2 produced by animals and found in milk and urine).

Aflatoxins are found as natural contaminants in cereals (specifically in corn, wheat, and rice) and cereal by-products, oilseed meals (cottonseed meal, peanut meal, rapeseed meal, coconut meal, sunflower seed meal, and others), cassava, and a series of other human food sources, mainly, cereals, dry fruits, sausage products, spices, wines, coffee, legumes, fruits and their juices, milk, and dairy products.

Aflatoxins have high carcinogenic, teratogenic, and mutagenic activity. The major effect produced by aflatoxins is hepatotoxicosis (Fig. 1 and 2), but also they can produce kidney problems. The most affected organs are: the liver, the kidneys, and the brain (Hesseltine, 1976; Edds, 1979).

Aflatoxins are immunosuppressant, since they inhibit both phagocytosis and protein synthesis (antibodies are proteins), interrupting DNA and RNA synthesis, as well as ribosome protein synthesis. Amino acid absorption is altered leading to the rise of amino acid hepatic retention. (Sharma, 1993).

Fig. 1. Hepatotoxicosis caused by Aflatoxin B1. (Alberto Gimeno).

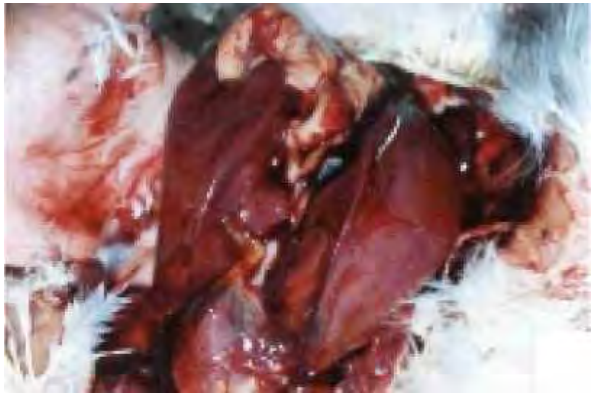


Fig. 2. Liver lesions produced by aflatoxin B1. (Dr. Horacio López Bonilla. AVIMEX - Mexico)

Aflatoxins can impair reproduction indirectly, as a result of the immunosuppression. The immunosuppressant effect predisposes the animal to be infected by pathological microorganisms, some of which can cause mammitis, agalaxy, and metritis problems. These mycotoxins seem to cause a diminished sperm count in boars, as well as higher levels of weak spermatozoids, and an increase in abnormal spermatozoids (Picha et al., 1986).

2.2. Ochratoxins

Ochratoxins are produced by *Aspergillus ochraceus, Penicillium viridicatum, and Penicillium cyclopium*. There are 7 types of ochratoxins of which ochratoxin A (**OTA**) is the most toxic.

Ochratoxin A is found as a natural contaminant in cereals (mainly in barley and rice) and cereal by-products, flour and peanut meal, and in a series of human foods such as: Green and roasted coffee beans, grape and grape juice, dried vine fruit, legumes, cheeses, smoked meats (ham, bacon, and sausages), wines, and others.

The main effect produced by ochratoxin is nephrotoxic (Fig. 3), but also can produce a liver disorder which produces an accumulation of glycogen in hepatic and muscular tissue. The main organs affected are: the liver and kidneys (Carlton, 1979; Gimeno and Martins, 1982). Ochratoxins are also immunosuppressant (Sharma, 1993).

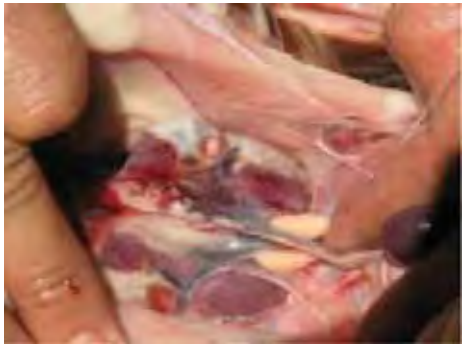


Fig.. 3. Renal lesions due to ochratoxin A contamination (Dr. Douglas Zaviezo, Special Nutrients, U.S.A.).

Ochratoxin A can affect spermatozoid quality and production in boars. This mycotoxin alters the stability of the spermatic membrane during spermatogenesis, due to an inhibiting factor during protein synthesis (Solti et al., 1999). Other studies revealed that OTA can diminish spermatozoid volume during ejaculation as well as an alteration in mobility and viability of the spermatozoid (Biró et al., 2003).

3. FUSARIUM MYCOTOXINS

Fusarium is a genus of mold that forms part of the field flora (phytopathogenic substrates, living plants) and the intermediate flora (substrate of freshly harvested, moist cereals). This mold grows in temperatures between 6 and 40ºC. Its optimal growing temperature is between 18 and 30ºC. This mold is aerobic and generally need water activity (aw) to be greater than 0.88 in order to grow and proliferate, and water activity of 0.91 in order to produce mycotoxins. There are cases, such as *Fusarium roseum*, which needs a minimum of 15ºC in order to develop, with an optimal growth temperature between 24 and 27ºC, and is able to produce the mycotoxin zearalenone at temperatures of 10 to 12ºC. Nonetheless, there are varieties of *Fusarium roseum*, such as *Fusarium roseum* «gibbosum» and *Fusarium roseum* «semitectum» that are capable of producing zearalenone on a sorghum substrate at a temperature of 25ºC, equivalent to the quantity produced at 10ºC. The *Fusarium* is one of the mold groups with the greatest genetic capacity for producing mycotoxins when the adequate physical, chemical, and biological conditions are present.

Fusarium contaminates cereals in the field. The mold dies once this contaminated cereal is dried or processed; but the mycotoxin remains in the cereal. It is no wonder that mycological and mycotoxin analyses often find mycotoxins but not the *Fusarium* in stored cereal. This is not to say that *Fusarium* is never present in stored cereals. *Fusarium* is present in stored cereals when the treatments were insufficient to completely kill the organism or due to post-contamination often attributed to spores transport vectors such as air and insects.

This manual will review the most prevalent mycotoxins produced by *Fusarium* which can produce mycotoxicosis i.e., zearalenone (**ZEN**), vomitoxin or deoxynivalenol (**DON**), fumonisin B1 (**FB_1**), T-2 toxin, diacetoxyscirpenol (**DAS**) monoacetoxyscirenol (**MAS**), ,

triacetoxyscirpenol (**TAS**), and escirpentriol (**STO**), in animals such as: chickens, hens, ducks, turkeys, pigs, dairy cows, and rabbits. Humans are mostly affected by vomitoxin or deoxynivalenol, and fumonisin B1 which onset mycotoxicosis when foods consumed are contaminated by said mycotoxins.

3.1. Zearalenone (F-2)

Zearalenone (**ZEN**) is mainly produced by *Fusarium roseum, F. tricinctum, F. roseum «Culmorum,» F. roseum «Graminearum,» F. oxysporum*, and *F. moniliforme*. *F. roseum* produces the greatest concentration of zearalenone (3000-15000 mg/Kg of substrate) while *F. moniliforme* synthesizes small quantities (1-19 mg/Kg).

There are 16 derivatives of zearalenone; being the most important zearalenone, alpha and beta zearalenol, respectively.

Zearalenone is found as a natural contaminant in corn and corn by-products, barley, wheat, oatmeal, sorghum, sesame seed, hay, and silages.

The main sign of zearalenone toxicity is estrogenic, producing cases of significant hyper-estrogenism which entails dilated and reddened vulvas (vulvovaginitis and vulva edema) (Mirocha and Christensen, 1974; Mirocha, 1977; Christensen, 1979). Sows, especially young and pre-pubescent sows, are very sensitive to zearalenone (Fig. 4 and 5).

Fig. 4. Adult sow with dilated and reddened vulva due to zearalenone contamination
(Dr. Pedro Barreiros. PROVIMI, Portugal).

Fig. 5. Young sows with dilated vulva produced by zearalenone contamination (Alberto Gimeno).

The active factor in zearalenone is similar to that of estrogen. Zearalenone inhibits the follicle development and ovulation by reducing the FSH (follicle stimulating hormone) concentration, given that this mycotoxin (although structurally different) can adopt a similar configuration to that of 17-Beta-estradiol and other natural

estrogens that bind with estrogen receptors. This is especially true in pre-pubescent sows, causing hyper-estrogenism and tumefaction and hypertrophy of the vulva, uterus, mammary glands, and nipples, as well as a significant atrophy of the ovaries (Gbodi and Nwude, 1988). Vaginal and rectal prolapses can also occur. As microscopic disturbances, the myometrium and endometrium undergo a hyperplasia and hypertrophy producing an engrossment and edema of the uterus. If contaminated, cyclic females experience failed conceptions, pseudo-conception, and abortions. Also, the function of the corpus luteum is affected and intervals between consecutive heats are prolonged (Roy et al., 2005). Young boars can have a reduction in spermatozoid production, testicular weight, and libido (McEvoy et al., 2001).

3.2. Fumonisins

Fumonisins are predominantly produced by *Fusarium moniliforme*. There are 6 types of fumonisins: B1, B2, B3, B4, A1, and A2 (Marasas, 1995; Visconti et al., 1995). However, fumonisin B1 (**FB1**) and fumonisin B2 (**FB2**) are the most prominent and most toxic.

FB1 and FB2 can be found as natural contaminants in cereals (mainly in corn and corn by-products). The most significant signs of these fumonisins are the following: neurotoxic (leucoencephalomalacia), nephrotoxic, pulmonary and cerebral edema, hepatotoxic and cardiac lesions. The following organs are affected: the brain, the lungs, the liver, the kidneys, and the heart. These mycotoxins inhibit the synthesis of lipoproteins called sphingolipids (sphinganine and sphingosine) (Prelusky et al., 1974; Marasas, 1995; Visconti et al., 1995).

3.3. Trichothecenes mycotoxins

Fusarium tricinctum, F. nivale, F. roseum, F. graminearum, F. solani, F. oxysporum, F. lateritium, F. sporotrichioides, F. rigidiusculum, F. episphaeria, and F. poae are the main molds producing trichothecenes mycotoxins. Other fungi also produce trichothecenes toxins, more notably, *Cephalosporium crotocigenum, Myrotecium verrucaria, Stachybotrys atra, Calonectria nivalis, Trichoderma viride, Tricotecium roseum, and*

Gibberella saubinetti. There are 40 different trichothecenes derivatives. However, the most significant natural contaminates are: T-2 toxin, diacetoxyscirpenol (DAS), vomitoxin or deoxynivalenol (DON), and nivalenol (Abdelhamid et al., 1992; Marasas, 1995). Other derivatives mentioned in this article are: monoacetoxiscirpenol (MAS), triacetoxyscirpenol (TAS), and escirpentriol (STO).

Trichothecenes receive their name from the skeletal tetracycline in their molecule, 12, 13-epoxyitricotec-9-eno.

Trichothecenes toxins are found as natural contaminants in cereals (corn, barley, sorghum, oatmeal, wheat, rice, rye, millet, and cereal products).

The main effect of trichothecenes toxins is gastro-enteric, but also affecting the digestive, the nerves, and the circulatory systems, as well as the skin. It is characteristic of vomitoxin to induce vomiting and rejection of food in some animals species. In a general way the toxicological characteristics of these mycotoxins (it depends on the animal species) (Smalley and Strong, 1974; Bamburg, 1976; Sato and Ueno, 1977; Ueno, 1977; Mirocha 1979; and Betina, 1989) include:

a) Vomiting, irregular heartbeats, and diarrhea; b) hemorrhaging, edemas, cutaneous tissue necrosis; c) hemorrhaging of the stomach and intestinal epithelial mucosa; d) destruction of hematopoietic tissues; e) decrease of white blood cells and circulating platelets; f) hemorrhaging meninges (cerebral); g) alteration of the central nervous system; h) rejection of food; i) necrosis lesions on different parts of the mouth; j) pathological deterioration of bone marrow, lymphatic nodules, and intestinal cells.

Trichothecenes mycotoxins have powerful immunosuppressive activity (Sharma, 1993). These are divided into two groups, macro cyclical and non-macro cyclical. There have been few studies focused on the toxicology of macro cyclical mycotoxins (roridins, verrucarins, satratoxins, and others) in animals. On the other hand there have been many studies on the toxicology of non-macro cyclical mycotoxins.

Trichothecenes non-macro cyclical mycotoxins are divided into two groups, A and B. The A group mycotoxins are more toxic to poultry

than B group mycotoxins. Some mycotoxins included in group A are the following: T-2 toxin, diacetoxyscirpenol (DAS), triacetoxyscirpenol (TAS), escirpentriol (STO), and HT-2 toxin. Mycotoxins included in group B are the following: fusarenone-X, vomitoxin or deoxynivalenol (DON), and nivalenol (NIV).

The main toxic effects of trichothecenes mycotoxins are produced at a cellular level. These mycotoxins inhibit the protein synthesis followed by an interruption of DNA and RNA synthesis. Also there is a cell division in the gastrointestinal tract membrane, the skin, lymphoid cells and erythrocytes (Leeson, 1995).

Contact with trichothecenes mycotoxins (extremely alkalines, specially T-2 toxin and diacetoxyscirpenol) produces a toxic effects of which consist of an extensive necrosis of the skin and mouth mucosa. Contact can also result in acute problems in the gastrointestinal tract, deterioration of bone marrow, and a significant inhibition of the immune system. There can also be hemorrhaging of the stomach and intestinal mucosa and a destruction of the hematopoietic tissue. They can also produce serious lesions in the gizzard of poultry. The typical oral lesions in poultry consist of a proliferation of caseous white-yellowish plates (albuminoidal material). These oral lesions appear most frequently in the superior and inferior areas of the beak, palatal, mouth, and tongue mucosa. Oral erosions are characteristic (Fig. 6 and 7) of mycotoxins (specially with T-2 toxin and diacetoxyscirpenol) exposure. Evidently, the severity of the lesions becomes more serious the longer the animals are exposed to the mycotoxin (Leeson, 1995).

Affected chickens can suffer from delayed growth, abnormal feathering, regression of the bursa of Fabricius, and anemia. Laying hens exposed to this mycotoxin suffer oral lesions, reduced feed intake, egg production, and deterioration of the eggshell quality of the eggs, with a significant increase in soft-shell eggs (Leeson, 1995).

Fig.6. Oral lesions resulting from a T-2 toxin contamination. Lesions produced by diacetoxyscirpenol are similar. (AVIMEX Laboratory, Mexico).

Fig.7. Gizzard lesions and ulcers resulting from a T-2 toxin contamination. Lesions produced by diacetoxyscirpenol are similar (AVIMEX laboratory, Mexico).

There are two differents arguments that explain oral lesions produced by T-2 toxin and diacetoxyscirpenol. The first argues that the contaminated feed adheres primarily to the oral region due to the high moisture in this area. Given that these mycotoxins are extremely alkaline, this alkalinity provokes the oral lesions. The second argues

that after absorption of these mycotoxins through the gastrointestinal tract, they are eliminated through the animal's saliva. Again, the alkalinity of said mycotoxins produces the oral lesions (Ademoyero and Hamilton, 1991a).

The feathering problems produced by these mycotoxins can be explained by epidermis necrosis and feathering follicle necrosis, as well as protein synthesis inhibition (Hoerr et al., 1981 and 1982).

4. MOST SIGNIFICANT MYCOTOXINS AND MYCOTOXICOSIS AFFECTING VARIOUS ANIMALS.

There are a number of factors that can influence (increase or decrease) the toxicity of mycotoxins in animals. Some of these factors are: a) The animal species and breed; b) The mycotoxin concentration and the duration of the contamination period (extent of time in which the animals are ingesting the contaminated feed); c) The animal's nutrition and overall health; d) The animal's age and sex; e) Bacterial, viral, or parasitical infections; f. inadequate conditions of the animal's habitat (temperature, moisture, ventilation, handling, and others); g) Medication administered; h) Presence of other mycotoxins and synergism or association amongst them.

Most of the toxicity cases presented in the manual correspond to experiments where animals were under optimal conditions, and where the previously mentioned factors were controlled (so as to not have an influence over the outcome). In other words, the cases presented in this manual are different from the cases that are found daily in the field or on the farm. Hence, there could be lower concentrations of contamination in everyday conditions than the ones presented in this manual. However these low concentrations can cause severe problems if exacerbated by one of the factors previously mentioned or any stress that the animal suffers due to poor handling conditions or environmental conditions. Therefore, it is risky to say that there are levels of mycotoxin contamination that will not cause problems. It is safe to say, however, that there are relatively «safer» levels of contamination.

4.1. Aflatoxin B1 (AFB1)

4.1.1. Chickens

An aflatoxin B1 (AFB1) contamination of 75 to 800 ppb (micrograms/Kg) in mixed feed given to 1 day-old chicks for a period of 3 to 10 weeks, impeded the development of the chicks given the lower doses, and resulted in hepatic lesions and death in chicks given the higher doses (Allcroft, 1965; Doerr et al., 1983). After 3 weeks at a dose of 500 ppb, observations showed problems of fatty liver and an increase of liver size (Asplin and Carnaghan, 1961).

Doses with a concentration of 308 and 610 ppb resulted in 8 and 11% mortality rates, respectively, between 0 to 9 weeks (Gimeno and Martins, 2000). However, when feed contaminated with 2500 and 5000 ppb of AFB1 were given to 23 day old chickens for a period of 32 days, observations determined no major complications other than slightly friable livers and a reduction of calcium concentration in the serum. Histological lesions were vacuolization of the hepatocytes with infiltration of fat (Fernandez et al., 1994). These results are in agreement with the results obtained by other researchers (Lanza et al., 1980). With age, chickens become more resistant to the toxic effects of aflatoxins (Gimeno et al., 2003).

Day-old chicks given a mixed feed containing 20% crude protein and 5000 ppb of AFB1 for 3 weeks, suffered a weight loss of 20% compared to the control group. However, when the crude protein of the feed was 30%, with the same level of AFB1 contamination, the weight loss was only 5.4% (Gimeno and Martins, 2000). As previously stated, aflatoxicosis alters the ability of the animal to digest protein and the ability to absorb amino acids, the hepatic amino acid retention increases and the ability to synthesis DNA, RNA, and ribosome protein decreases. All these factors produce an increase in the protein needs in poultry and therefore, there is a delay in development. Apparently, increasing the protein level in the diet to 30% helped to ameliorate the growth retardation (Gimeno and Martins 2000; Gimeno et al., 2003).

Day-old chicks given a mixed feed contaminated with 250 to 500 ppb of AFB1 for a total of 3 weeks experienced a resistance to the immunization against *Pasteurella multocida* (Edds, 1979; Gimeno et al.,

2003). Feed contaminated with 200 ppb, administered for a total of 29 days produced an increase in the chicks' susceptibility to coccidiosis by *Eimeria tenella*, and a decrease in the effectiveness of the anticoccidial used (Edds, 1976; Gimeno et al., 2003). Chickens became more susceptive to salmonelosis and candidiasis when fed diets contaminated with different levels of aflatoxins (Hamilton and Harris, 1971; Pier et al., 1978). AFB_1's interference with normal hepatic function possibly reduces serum-immunoglobulin synthesis which has a great influence in pathogenesis and morbidity (Gimeno and Martins, 2000).

4.1.2. Layers and Breeders

Concentration of 100 ppb of AFB1 in the mixed feed of breeder hens for 6 months resulted in chicks with birth problems and soft-shell eggs (Gimeno, 1999; Gimeno, 2000).

Higher concentrations of AFB1 (610 ppb) fed for 33 weeks to laying hens, resulted in decreased egg production, hepatotoxicosis, and deaths (Edds, 1979).

4.1.3. Ducks

Observations showed that one to seven day old ducklings given feed with a concentration of 300 to 600 ppb of AFB1 for 7 to 14 days had serious hepatic lesions, as well as a significant death rate (Gimeno, 1999; Gimeno, 2000).

Specifically, in a group of 30 one-day-old ducklings given feed with a contamination of 305 ppb of AFB1 and 20 ppb of AFG1 for 42 days, there were 43.33% and 90% deaths at 19 and 42 days of age, respectively (Gimeno, 1988).

Affected ducks displayed a delay in development, hyperkeratosis of the cornea and the oral mucosa, malformations and bone fragility, leg paralysis, inflammatory edema of the eyelids, dermatitis, and scarce feathering. The ducks suffered massive avitaminosis and a deficiency in calcium, phosphorus, and manganese absorption (Figs. 8).

Figs. 8. Ducks with serious aflatoxicosis problems (Alberto Gimeno).

Necropsies of ducks revealed an enlarged, fatty, and friable liver, pale-yellowish in color, massive necrosis, jaundice, and cirrhosis. There was also atrophy of the bursa of Fabricius and of the thymus. The liver and some muscles had petechial hemorrhages and necrotic focus. There was a deterioration of the hepatic cells, as well as fibrosis and hyperplasia of the bile duct. The surface of some of the livers was granulated and there were scattered lymphatic nodules (Fig. 9 and 10).

Fig .9. Hepatotoxicosis produced by Aflatoxin B1 contamination (Alberto Gimeno).

Fig.. 10. Livers with hepatotoxicosis problems induced by Aflatoxin B1 (Alberto Gimeno).

4.1.4. Turkeys

Observations showed that 14 day-old turkeys given feed containing a concentration of 100 to 800 ppb of AFB1 for a period of 35 days had a significant reduction in body weight gain and had hepatic lesions. A 500 ppb concentration of AFB1 significantly reduced the efficiency of the vaccine used against Marek's disease (Gimeno, 1999; Gimeno, 2000).

4.1.5. Pigs

Observations showed that new born piglets given feed containing a concentration of 230 ppb of AFB1 for a period of 4 days had friable livers, anemia, and delay in growth. Giving a mixed feed containing a concentration of 400 to 800 ppb of AFB1 to 15 to 20 kg pigs for a period of 3 to 9 months, produced a significant growth reduction, hepatotoxicosis, and were distinctly susceptive to salmonelosis (Edds, 1979).

Contaminated feed containing different concentrations of mycotoxins were given to three different groups of sows during the periods of gestation and lactation. The first group was given feed with a concentration of 800 ppb of AFB1, the second group was given feed with a concentration of 800 ppb of AFG1, and the third group was given feed with a concentration of 400 ppb of AFB1 and 400 ppb of AFG1. On the day 5 and 25 after farrowing, there were traces of AFM1 and AFB1 in the milk of group 1, AFG1 in the milk of group 2, and AFM1, AFB_1, and AFG1 in the milk of group 3. Although the concentration of aflatoxins in the milk was 1000 times lower than in the complete feed, there was an increase of these concentrations after day 25 after farrowing. The piglets born from these sows had serious immune-toxicological problems (Silvotti et al., 1997).

4.1.6. Dairy Cows

Holstein dairy cows (mid-lactating period) were given doses corresponding to 13 mg of AFB1/cow/day, during a period of 7 days. This corresponds to a final contaminated ration of 433 ppb of AFB1, considering a consumption of 30 Kg of final ration/cow/day. Some cows received AFB1 in its pure form and others in its impure form i.e.

harvested from *Aspergillus parasiticus* that contained other aflatoxins and their metabolites. Feed consumption and milk production decreased significantly in these cows. The somatic cell count was not apparently affected, and the concentration of aflatoxin M1 in milk fluctuated between 1.05 and 10.58 ppb (micrograms/Liter). No aflatoxin M1 was found in the milk after 4 days of ceasing the supply of AFB1. However, the deleterious effects seemed more serious in the cows that received impure aflatoxin in comparison to those which received pure aflatoxin (Applebaum et al., 1982; Gimeno and Martins, 2002).

Dairy cows were induced with a mammalian infection using *Streptococcus agalactiae, Staphylococcus aureus, and Staphylococcus hyicus,* during the lactating period. Subsequently they received and oral dose of AFB_1 corresponding to 0.3 mg/Kg of body weight/day during periods of 12 to 14 days. Considering a 550 Kg body weight cow, with a consumption of 30 Kg of final ration/day, this corresponds to an AFB1 contamination in the final ration of 5500 ppb. Clinical signs of mycotoxicosis and mastitis were studied before, during, and after the mycotoxin administration period. The cows suffered from a lack of appetite, weight loss, decreased milk production and significant enzymatic variation during 1 to 3 weeks after ingesting AFB1. There were no signs of acute mastitis, nonetheless, the bacterial count in the milk increased during the consumption of the mycotoxin. There was an increase in the number of test positive to mastitis during the period following the last administration of the mycotoxin. Aflatoxin M1 was found in the milk within 3 to 6 hours after the consumption of AFB1 and persisted for a period of 72 hours after giving the last doses of mycotoxin. Aflatoxins B1 and M1 were found in the cow's urine 6 hours after the consumption of AFB1 and persisted 72 to 120 hours after giving the last dose of the mycotoxin (Brown et al., 1981; Gimeno and Martins, 2002).

AFB1 concentrations of 2000 to 2400 ppb in final ration given to 2 year old cows during a period of 7 months caused serious hepatotoxicosis problems, as well as a significant reduction of milk production (Mirocha et al., 1977; Brown et al., 1981; Applebaum et al., 1982; Gimeno and Martins 2002).

4.1.7. Rabbits

One of the main consequences that characterize mycotoxicosis in rabbits is a significant reduction of feed intake. Therefore, when given feed containing between 100 and 150 ppb of AFB1, feed intake diminished between 20 and 60%. The decrease in feed intake causes significant growth delay and a decrease in production. This also diminishes unspecific defense mechanisms of the animal, which are affected by some mycotoxins. For example, AFB1 at low doses of 5 to 8.5 micrograms/Kg of body weight/day interfere with the metabolism of the amino acids and the vitamin B complex. 50 micrograms of AFB1/ Kg of body weight/ day interfere with the vaccine against *Bordetella bronchiseptica* (Gimeno and Martins, 2000a).

Typical clinical signs of mycotoxicosis resulting from the intoxications can be observed, as well as intercurrent signs such as enteritis-diarrhea in young lactating rabbits, mucoidal enteritis in weaned young rabbits, consuming feed containing 50 ppb of AFB1. It has been suggested that AFB1 can act as a predisposing factor toward mucoidal enteritis, allowing the proliferation of microorganisms such as *Clostridium perfringens* and *Escherichia coli.* Mycotoxicosis produces chronic and acute pathological symptoms, depending on the mycotoxin, its concentration, exposure time, accumulative effect, synergism, and physiological state of the rabbit. The most serious cases resulted in death, especially of young rabbits. There were also incidences of abortion in breeding rabbits, and deaths resulting from mycotoxicosis (Gimeno and Martins, 2000a).

There have been reported cases of spontaneous aflatoxicosis in rabbits, with different levels of aflatoxin B1 in feed: 33 ppb, 44 ppb, 90 ppb, 110 ppb, 540 ppb, and 10400 ppb. The affected rabbits showed signs of anorexia, lack of coordination, weight loss, and they had a pronounced jaundice before dieing. Trichophagia (hair eating) is an observed clinical sign of micotoxicosis. Pathological symptoms are the following: the liver is congested and has a structure similar to cork. There can also be renal, spleen, and pulmonary congestion. Deaths occurred 3 to 4 days after clinical signs appeared. In some cases there was an elevated mortality rate of 58.6% (Gimeno and Martins, 2000a).

A concentration as low as 15 ppb of aflatoxin B1 in the feed was enough to cause sickness. Consumption of feed contaminated with 300 ppb of AFB1 can produce hypertrophy of the liver and spleen, and a tendency for the rabbits to eat their own fur (Gimeno and Martins, 2000a).

The LD50 is 0.300 mg/Kg body weight. This means that rabbits are slightly more sensitive than one day old ducklings (0.335 mg/Kg body weight.) (Butler, 1974).

Giving rabbits 0.050 and 0.625 mg of AFB1/Kg body weight/day during a period of 24 days, the following alterations were found: anorexia, a reduction of body weight gain, lethargy, dehydration, jaundice, and death. Total plasma protein decreased and the coagulation time increased. Bilirubin, alanine and aspartate amino transferase also increased. Subsequent studies (Clark et al., 1986; Sahoo et al., 1993) presented similar results. These studies also showed an increase in glucose levels, serum cholesterol, platelets count, prothrombin and thromboplastin time, and a decrease in fibrinogen, factor IX, VIII, and V activity.

A concentration of 0.050 mg of AFB1/Kg body weight/day during a period of 30 days given to female rabbits resulted in anorexia, apathy, weight loss, pronounced atrophies, and deaths of new born rabbits, who's livers presented the following histopathological alterations; changes in the area centrolobulilar of the hepatocytes, with hydropic and fatty changes, and focal zones of necrosis (Gimeno and Martins, 2000a).

Rabbit feed contaminated with 100 ppb of each of the following aflatoxins, B1, B2, G1, and G2 produced a significant increase in liver, kidney, heart and suprarenal glands weight. There was also a decrease of hemoglobin content, percent hematocrit, sedimentation velocity, nitrogen, and oxalacetic glutamic transaminase. The levels of calcium, inorganic phosphorus, cholesterol, phospholipids, and piruvic glutamic transaminase were elevated. A high percentage of residual aflatoxins was found in muscles, serum, liver, heart, and kidneys. Only 1.42% of the total ingested aflatoxin was excreted through feces. These effects were substantially more moderate in rabbits that consumed the same contaminated feed but with the addition of 0.25% activated charcoal. These leads to the conclusion that the absorbing effect of the activated charcoal, resulted in an antidote against aflatoxins (Gimeno and Martins, 2000a).

After 17 days of ingesting contaminated feed with a similar contamination as the previous one (100 ppb) of only AFB1, resulted in increased glucose, urea, cholesterol and bilirubin levels in the blood, and producing degenerative changes and necrosis of the liver, kidneys, and the heart (Gimeno and Martins, 2000a).

4.2. Ochratoxin A (OTA)

4.2.1. Chickens

Observations showed that one-day-old chicks given feed containing 200, 500, 800, and 1600 ppb of OTA during a period of 2 to 3 week had delays in development and a reduction of live weight (all levels of mycotoxin concentration). Chicks receiving feed with higher levels of this mycotoxin had an edematous crop, pancreas, liver and kidney, an increase in the size of the mentioned organs, as well as a decrease in the size of the bursa of Fabricius. Mortality was increased with presence of bone fragility and delayed prothrombin and blood re-calcification times. Nephropathy was commonly observed and pigmentation was deficient. Chicks given feed containing 500 ppb of OTA presented already lymphopenia and immunosuppressive problems (Tucker and Hamilton, 1971; Doerr et al., 1974; Huff et al., 1974; Huff and Hamilton, 1975; Huff et al., 1975; Chang et al., 1979; Hamilton et al., 1982).

A contamination of 140 ppb of OTA associated with a mold microflora (85% Scopulariopsis spp) in the feed resulted in a significant reduction of body weight gain, severe nephritis and necrotic enteritis problems, and increased mortality in 8,800 chicks. The main cause is believed to be the fungi and not the mycotoxin concentration. (Abramson et al., 1983).

Nephrotoxicosis problems due to OTA are often associated with a hemorrhaging syndrome characterized by typical muscular petechiaes. However, during aflatoxicosis or during and after infectious bursal disease these petechial problems can also be seen (Gimeno et al., 2003).

4.2.2. Laying Hens

One-day-old White Leghorn chicks and 26 week old White Leghorn laying hens were given feed containing 300 to 1000 ppb of OTA and 500 to 4000 ppb of OTA, respectively, for a period of 341 and 42 days, respectively. The chicks had serious renal lesions, microscopic changes and histopathological alterations in the liver. Laying hens had a decrease in egg production, egg weight, feed intake, and body weight. Prothrombin time increased and total serum protein decreased (Carlton and Krogh, 1979; Gimeno et al., 2003).

White Leghorn laying hens given mixed feed containing a concentration between 500 and 1000 ppb of OTA had a significant decrease in egg production, stained egg shells, and increased levels of uric acid in their serum (Page et al., 1980; Gimeno et al., 2003).

4.2.3. Ducks

Khaki Campbell ducks were given a diet containing 2000 ppb of OTA from birth until 18 days of age. The ducks suffered from delayed development, enlarged livers and kidneys, and regression of the thymus. Microscopic observations showed an accumulation of glycogen in the liver, and an infiltration of lymphoid cells in the kidneys (Burns and Maswell, 1987).

4.2.4. Turkeys

Turkey poults were fed with diets containing 0, 1000, 2000, 4000, and 8000 ppb of OTA from birth until 3 weeks of age. They suffered from delayed development, enlargement of the proventriculus and grizzard, and a regression of the thymus. At the highest level of mycotoxin concentration , the feed conversion ratio deteriorated from 1.63 (control) to 2.07. Deaths were numerous. Turkeys fed with 4000 and 8000 ppb of OTA had a reduction in water intake and an increase of uric acid in plasma. They also showed a leukopenia that was initially a lymphopenia (Chang et al., 1981).

Turkeys given feed containing 4000 ppb of OTA from birth until 10 weeks of age experienced serious osteoporosis problems (Duff et al., 1987).

Other studies have shown that OTA in turkeys can produce feed rejection, an effect that does not occur in chickens (Burditt et al., 1984).

4.2.5. Pigs

Pigs provided with feed containing 200 to 400 ppb of OTA during 3 to 4 months during the period of 20 to 90 kg of body weight, had delayed growth, increase in water consumption, and microscopically detectable renal lesions (Carlton and Krogh, 1979).

Immunosuppressive problems were observed in 20 kg gilts fed during 35 days with a diet containing 2500 ppb of OTA (Harvey et al., 1992).

4.2.6. Dairy Cows

There is a lack of information on the toxic effects of OTA in dairy cows. This lack of data is probably due to the different capacities of the rumen protozoa microflora to easily metabolize OTA and hydrolyze it into ochratoxin-alpha, which is not toxic and does not degrade. These capacities vary because this microflora is affected by the feed consumed by the cow.

There are studies in sheep demonstrating that the type of diet has a large influence on the metabolizing process of certain mycotoxins. Hence, a diet based on 100% hay takes the ruminal fluid to a pH of 7.1. Mycotoxins, such as ochratoxin A (OTA) are hydrolyzed to ochratoxin-alpha (non toxic) in only 0.6 hours. If the percentage of hay is reduced (70% hay) and the percentage of grain or concentrated feed is increased (30%), the pH of the ruminal fluid changes to 6.5, the hydrolysis of OTA takes more time (1.3 hours). If the final ration contains 100% grain or is a concentrated feed, the hydrolysis takes up to 3.6 hours at a ruminal fluid pH of 5.7 (Xiao et al., 1991; Hohler et al., 1999; Gimeno and Martins, 2002). This could be applied to dairy cows, as some authors suggest (Muller et al., 1998; Gimeno and Martins 2002a).

Ruminal fluid is considered the first defense against certain mycotoxins, such as: zearalenone, ochratoxin A, T-2 toxin, and diacetoxyscirpenol. However, this fluid has no affect against aflatoxin B1, or vomitoxin or deoxynivalenol (Kiessling et al., 1984; Gimeno and Martins, 2002a). Still, there are some authors that argue that an anaerobic incubation of vomitoxin in contact with cow ruminal fluid produces the metabolite diepoxy-deoxynivalenol, which is not toxic (Hedman and Pettersson, 1997).

4.2.7. Rabbits

Young rabbits given a feed containing 10000 ppb of OTA for a period of 90 days had total and differential alterations of white blood cell counts, with the consequent immunosuppressive problems (Verma and Mathew, 1998).

4.3. – Zearalenone (ZEN)

4.3.1. Chickens

Chicks receiving contaminated feed containing 1 to 30000 ppb of (micrograms/kg) ZEN for a period of 7 to 8 weeks had no problems (Bacon and Marks, 1976). Feed containing 300000 to 600000 ppb of ZEN given to chicks for 4 days provoked an enlargement of the bursa of Fabricius and an increase of cysts in the genital tract (Christensen, 1979). LD50 (administered orally as a single dose) is too elevated for chicks and is situated in 15 gr/kg of body weight. (Mirocha et al., 1978; Christensen, 1979).

4.3.2. Laying Hens

Hens are also quite resistant to zearalenone since there were no complications after administering feed containing 25000 and 100000 ppb of ZEN to 20 and 42 week-old White Leghorn hens for a period of 17 and 7 weeks, respectively. Furthermore, the percentage of egg production/hen/day was greater in hens that consumed the ZEN contaminated feed (5.9% and 7.9% more, respectively) (Marks and Bacon, 1976).

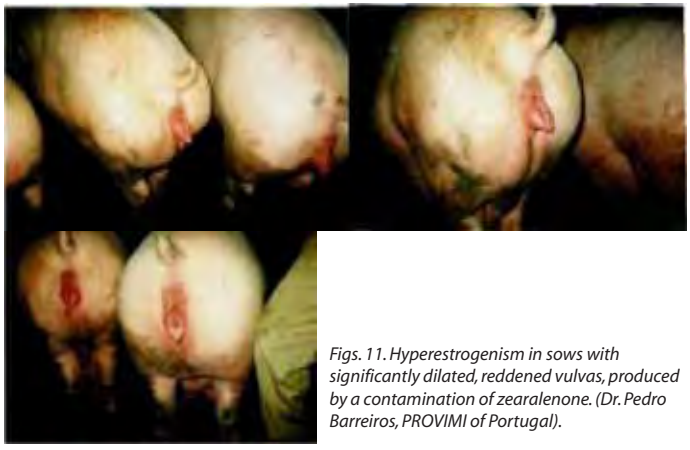
4.3.3. Pigs

10 and 12-week old gilts weighing 27 to 31 kg were fed a diet containing 1000 and 5000 ppb of ZEN for a period of 4 days or more. The lowest concentration of ZEN, 1000 ppb, caused vulvovaginitis on the 4th day of consumption (Mirocha and Christensen, 1974).

After seven days of consuming a contaminated diet with ZEN concentrations between 1500 and 2000 ppb, 70 day old gilts presented dilated and reddened vulvas (Rainer et al, 1990).

It seems that the daily consumption of zearalenone which can cause estrogenic problems in young gilts is 1000 micrograms of ZEN/sow/day. (Mirocha and Christensen, 1974; Gimeno and Martins, 2003). According to this data, feed containing 335 and 500 ppb of ZEN could cause estrogenic problems in sows during gestation, given that during this period they consume 2 to 3 kg of feed per day. On the other hand, lactating sows which consume 5 and 6 kg of feed per day, a feed contamination of 167 and 250 ppb of ZEN could cause problems.

This is evidently all related to the duration of the contaminated feed ingestion and the sensitivity of each individual sow. (Figs. 11). There could be cases where some of these concentrations will not affect the sow in any significant way. (Gimeno, 1987; Gimeno, 2000; Gimeno and Martins, 2003).



Figs. 11. Hyperestrogenism in sows with significantly dilated, reddened vulvas, produced by a contamination of zearalenone. (Dr. Pedro Barreiros, PROVIMI of Portugal).

There have been cases of piglet deaths during farrowing (Fig. 12) and rectal prolapses in sows that have consumed feed contaminated with ZEN (Fig. 13).

Fig. 12. Piglet death due to zearalenone contaminated feed consumed by the sow (Dr. Pedro Barreiros. PROVIMI of Portugal).

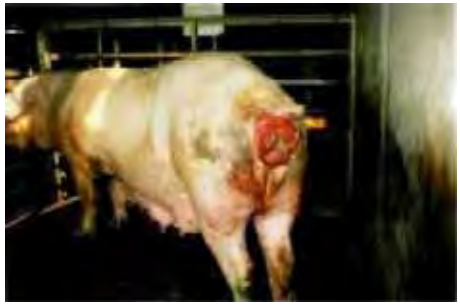


Fig. 13. Rectal prolapse produced by zearalenone contamination (Dr. Pedro Barreiro. PROVIMI of Portugal).

Other than the characteristic vulva edema, breeding sows also had mammary gland edema, which provokes the retention or absence of milk, causing death of newborn piglets (Eich, 1990). Fertility and conception rates diminished and consequently there was a decrease of the size and weight of the litter. The length increase in returning to heat is characteristic.

New born piglets experience dilated and reddened vulvas when the sow has ingested feed contaminated with ZEN. In many instances there are no typical clinical symptoms produced by zearalenone in sows. This can be attributed to a low concentration of mycotoxin contamination below the concentration amount that causes problems. It is thought that the cause of problems in piglets is due to a transferal of zearalenone or some of its metabolites (alpha and/or beta zearalenol) from sow to newborn through the placenta and/or milk. However, the cause and effect relationship is not sufficiently clear (Veterinary News, 1996) (Fig. 14).

Fig. 14. New born piglets born with dilated and reddened vulvas due to transferal of zearalenone from the sow. (Dr. Pedro Barreiros. PROVIMI of Portugal).

4.3.4. Dairy Cows

Field studies suggest that dairy cows given a final ration with more than 250 ppb of ZEN can have estrogenic problems, abortion, a decrease of feed consumption, a decrease in milk production, vaginitis, vaginal secretions, reproductive deficiencies, and heifers experienced an increase of the mammary glands (Gimeno 1999; Gimeno, 2000).

Also, it has been observed vaginal prolapses problems in cows consuming feed contaminated with ZEN. (Fig. 15)

Fig.15. Vaginal prolapse in cow caused by consumption of forage contaminated with zearalenone. (Dr. Rubén Rivera. AVIMEX Laboratory, Mexico)

4.3.5. Rabbits

Rabbits given 1 mg of zearalenone/kg of body weight/day during a period of 12 days, which corresponds to a consumption of feed contaminated with 7000 ppb of zearalenone, had a 50% increase in the size of the uterus (Lebas and Perez, 1998; Gimeno and Martins, 2000). Hence, this mycotoxin alters fertility and embryonic viability, even though pre-ovulation follicle rate increases, meaning the acceptance is good (estrogenic effect).

Uterus secretion analysis showed the influence zearalenone has on reproduction. Besides detecting the mycotoxin, changes were observed on the amino acid and micro-elements contents with an ingestion of 11.5 mg of zearalenone/kg of body weight/day during a period of 10 days (Osborn et al., 1988; Gimeno and Martins, 2000).

An anabolic effect was observed when 4 month-old rabbits started to ingest contaminated feed. The percentage of hemoglobin, hematocrit, calcium, phosphorus, and vitamin C in the serum, and liver fat and bone density increased. Histopathological changes in the liver, kidneys, lungs, heart, adrenal gland, spleen, and uterus were observed after the rabbits ate feed contaminated with 500 and 1000 ppb of zearalenone during a period of 18 days (Abelhamid et al., 1992; Gimeno and Martins, 2000; Gimeno et al., 2001).

In a group of 350 doe rabbits that consumed a feed contaminated with a concentration of 200 ppb of ZEN, there was a decrease in productivity and an increase in mortality. The doe rabbits accepted the male rabbits, but there was a decrease in the gestation rate. Furthermore, there were abortions, and yellow diarrhea in young lactating rabbits. Consistency of female feces was not normal and it could be observed a large number of dung under the cages. Mortality rate increased with diarrhea and enteritis symptoms. Feed intake diminished to one half of normal consumption. (Gimeno and Martins, 2000).

4.4. Fumonisin B1 (FB1)

4.4.1. Chickens

Two-day-old chicks given feed containing 10000, 30000, 75000, 300000, and 525000 ppb of FB1 for a period of 6 to 21 days, presented a decrease in body weight and a decrease in the absolute weight of liver, spleen, and bursa of Fabricius, with alterations of the enzymatic system and hematological parameters. There were variations in the levels of free sphinganine and the relationship between sphinganine/sphingosine (Weibking et al., 1993; Espada et al., 1994).

4.4.3. Laying Hens

Concentrations producing diarrheas and a decrease in egg production were in the order of 8000 to 16000 ppb (Espada et al., 1994).

4.4.3. Ducks

Very high levels of FB1 in the diet, 100000, 200000, and 400000 ppb, where fed to 1 day old white Pekin ducks for 21 days. Feed intake and body weight gain decreased significantly. Liver, heart, kidney, pancreas, and proventriculus absolute weights increased. The relationship between sphinganine/sphingosine increased significantly. It was found a moderate hepatocellular hyperplasia in the liver and in the gallbladder (Bermudez et al., 1995).

4.4.4. Turkeys

One week-old turkeys were given feed containing 25000 and 50000 ppb of FB1. The higher level produced a significant decrease in feed intake and clearly increased the relationship between sphinganine/sphingosine. The lower level did not produce any relevant problems (Broomhead et al., 2002).

A very high concentration of FB1 (200000 ppb) fed to one-day-old turkeys for 2 to 3 weeks produced lack of effectiveness of the vaccine against Newcastle disease (Li et al., 2002). This same concentration of FB_1 increased the relative weight of the liver and the

levels of aspartate aminotransferase and lactic dehydrogenase enzymes. At 21 days there was a moderate hepatocellular hyperplasia (Bermudez et al., 1997).

Also, with elevated contamination of 100000 and 200000 ppb of FB1 in feed given to day old turkeys for a period of 21 days, produced a decrease in body weight gain, an increase in liver, kidney, and pancreas weight, and a decrease of heart and spleen weight. There were important variations in the levels of some enzymes and a significant gallbladder hyperplasia (Weibking, 1993).

4.4.5. Pigs

Gilts and barrows consumed feed contaminated with 100 to 10000 ppb of FB1 for a period of 8 weeks. Generally, the toxicity of FB1 was more severe in barrows than in gilts. Barrows that consumed 1000 and 10000 ppb had a decrease in body weight gain of 8 and 11%, respectively. The lowest level, 100 ppb, produced a reduced growth of the barrows during the first 5 weeks. The feed consumed was slightly higher than the control group during the first 4 weeks, but decreased 6 to 7% each week afterwards.

Barrows fed 1000 and 10000 ppb of FB1 had an increase in cholesterol levels two weeks into the experiment. Gilts given 1000 ppb of FB1 had elevated cholesterol levels at the end of the trial. Barrows had an alteration of pancreas and suprarenal gland weight. There was an increase in sphinganine levels, as well as an increase in the relationship of sphinganine/sphingosine. The highest concentrations also produced pulmonary edemas (Rotter et al., 1996).

4.4.6. Dairy Cows

During mid-lactation period, Jersey cows were given a final ration contaminated with 75000 ppb of fumonisins (FB1 + FB2 + FB3) in order to have an ingestion of 3mg of fumonisins/kg of body weight/day for a period of 14 days.

Observations showed slight diarrhea during initial ingestion of the contaminated feed, and increased cholesterol levels in the serum. Nonetheless, there were no further anomalies in the animals (Richard et al., 1996).

4.4.7. Rabbits

Rabbits were given levels of 0.15 to 1 mg of FB1/kg of body weight/day during a period of 4 to 5 days, producing multi-organs alterations: foremost in the kidneys, followed by liver, lung, heart, brain, SNC (leucoencephalomalacia) and decrease of fetal weight. For a 2.4 kg body weight rabbit consuming a daily amount of 150 g of feed, the approximate FB1 contamination level should be between 2400 and 16000 ppb (Gimeno and Martins, 2000a; Gimeno et al., 2001).

4.5. Vomitoxin or deoxynivalenol (DON)

4.5.1. Chickens

Chickens are very resistant to the toxic action of DON. Feeds contaminated with 15000 and 50000 ppb of DON were given to 6 day-old chicks for a period of 42 and 6 days, respectively. The highest concentration only produced few oral erosions (Romer, 1983; Halloran, 1983).

4.5.2. Laying Hens and Breeders

Hens are significantly more sensitive to DON than chickens, since feed contaminations of 350 to 700 ppb of DON consumed by hens for a period of 10 weeks resulted in a decrease in egg weight and in an increment of soft-shell eggs (Hamilton et al., 1981). Higher doses of DON, 2500 and 4900 ppb, given to breeders for a period of 10 weeks resulted in significant anomalies in the progenies development (weak chicks) (Bergsjo, 1993a).

4.5.3. Ducks

Wild ducks kept in captivity and given wheat containing 5800 ppb of DON for a period of 14 days did not refuse the wheat, and there were no detectable differences in serum protein levels, calcium, glucose, creatininekinase, aspartate aminotransferase, or uric acid, compared to the control group. Body weigh, as well as certain organ weight was the same as ducks feeding the non-contaminated feed. Moderate concentrations of DON given during short periods of time did not have any adverse effects on the ducks (Boston, 1996).

4.5.4. Turkeys

Day-old turkey poults given feed containing 20000 ppb of DON during a period of 21 days did not have any variations in daily feed intake, or in body weight gain, compared to the control group. There were no histological lesions or significant adverse effects (Morris, 1999).

4.5.5. Pigs

Pigs consuming feed containing 300 to 700 ppb of DON resulted in rejection of the feed, vomiting and decrease of body weight gain (Trenholm et al., 1983). Higher concentrations of DON, 700 to 3500 ppb, resulted in a decrease of daily feed intake, a decrease in body weight gain, rejection of the feed, and vomiting. The highest levels of mycotoxin resulted in an enlargement of the liver and a decrease in the concentrations of protein and albumin in the serum (Bergsjo et al., 1993b).

4.5.6. Dairy Cows

Although some researchers reported that only high concentrations of DON in the feed, 6000 to 12000 ppb, given to dairy cows for a period of 10 weeks produce a significant decrease in milk production and milk fat (Charmley et al., 1993), others (Jones et al., 1994) have suggested, that according to field study statistics, the presence of DON in concentrations higher than 300 ppb in the feed can produce a reduction in feed intake, a decrease of milk production, a significant increase of somatic cell count, and a significant decrease of reproductive efficiency. A decrease in milk production of about 12.5 liters/cow/day can occur with contamination levels of 500 ppb or more of this mycotoxin.

4.5.7. Rabbits

Experimentally, it has been observed significant reproductive damages (reabsorption, abortions, birthing of weak offspring) when rabbits ingested 1.8 and 2.0 mg of DON/kg of body weight/day; which corresponds to a feed contamination of 120000 and 240000 ppb (extremely difficult to find in the field). These doses of mycotoxin

decreased feed consumption from a normal 135 g to 75-50 g/day (Khera et al., 1986; Gimeno and Martins, 2000).

Levels of 1 to 1.6 mg of DON/kg of body weight/day corresponding to 30000 and 60000 ppb DON in the feed, respectively, caused a decrease in fetal weight. Doses of 0.3 and 0.6 mg/kg of body weight/day which correspond to 7500 and 15000 ppb, respectively, were not toxic for the does and did not cause adverse effects in the fetus (Khera et al., 1986).

Other authors (Lebas and Perez, 1998; Gimeno et al., 2001) mention that feed with a concentration of 120000 ppb of DON resulted in embryonic problems. However, a concentration of 10000 ppb had no visible effects.

4.6. T-2 Toxin

4.6.1. Chickens

Day-old chicks were given feed containing 400 ppb of T-2 toxin for a period of 49 to 63 days. This dose produced oral lesions and a decrease of body weight gain. These effects were comparable to a contamination of 1000 ppb administered for a period of 21 days (Chi et al., 1997a; Chi et al., 1977b).

Higher doses of contamination, 4000, 8000, and 16000 ppb, given to day old chicks for a period of 21 days resulted in oral lesions, high mortality rate (evident on the 7th day of ingestion), and an increase rate of liver hematomas. Relative spleen and pancreas weight increased and the weight of the bursa of Fabricius decreased, when given T-2 toxin concentrations of 8000 and 16000 ppb (Wyatt et al., 1973).

Day-old chicks given a concentration of T-2 toxin of 200 and 4000 ppb for a period of 9 weeks had no alterations in the serum levels of glutamic oxalacetic transaminase, glutamic piruvic transaminase, lactic dehydrogenase or creatinine phosphokinase (Chi et al., 1977a).

Day-old chicks given feed containing 1000 to 16000 ppb of T-2 toxin for a period of 7 days had lesions on their palates and tongues. Feeding these levels for 21 days resulted in neurological disturbances,

delayed growth, feathering alterations, increase oral lesions size and necrotic lesions in the gizzard. Some chicks were unable to close their mouths and had difficulties in eating.

Oral lesions were characterized by a proliferation of caseous yellowish-white plaques around the beak, palate mucosa, mouth and tongue; tissue inflammation and localized necrosis. Externally the oral lesions were fibrinous and soft, while in the interior had an infiltration of granular leukocyte. Areas with erosion had a large number of «coccus» bacteria disperse throughout the affected tissue (Wyatt et al., 1972; Gimeno and Martins, 2001).

Based on contaminated feed consumption of broiler chicks from 1 to 21 days of age, the maximum concentrations of T-2 toxin without affecting different parameters are as follows: up to 2000 ppb did not affect growth rate; up to 2000 ppb did not have an affect on pancreas weight, up to 2000 ppb had no effect on spleen weight, up to 4000 ppb had no effect on bursa of Fabricius weight, less than 1000 ppb did not produce oral lesions (Wyatt et al., 1973).

On the other hand, Chi et al., 1977a and Chi et al., 1977b had different results. The levels of T-2 toxin that did not affect certain health parameters were the following: up to 200 ppb had no effect on body weight gain, up to 2000 ppb had no effect on serum cholesterol, up to 200 ppb did not produce oral lesions and less than 200 ppb did not affect serum uric acid.

4.6.1.1. Interactions

When broiler chickens were fed diets containing a concentration of 2000 to 4000 ppb of T-2 toxin, the mycotoxin reduced the efficacy of monensin sodium, an ionophor coccidiostat, which was working effectively against *Eimeria tenella*. Similar concentrations of T-2 toxin reduced narasin LD50 (from 176 mg/kg of body weight to 12 mg/kg of body weight) (Vániyi et al., 1989). Feed containing 500, 1250, and 6000 ppb of T-2 toxin significantly affected the efficacy of lasalocid sodium, another ionophor coccidiostat, to prevent damages produced by *Eimeria tenella* and *Eimeria mitis* in young roosters (Vara and Ványi, 1992).

4.6.2. Laying Hens and Breeders

Feed containing 1000, 5000, and 10000 ppb of T-2 toxin given to hens for a period of 28 days resulted in a decrease in egg production (reductions of 12.5, 68.0, and 78.9%, respectively), and a decrease in hatchability of these same eggs (Tobias et al., 1992).

Just 24 hours after consuming a feed containing 2000 ppb of T-2 toxin, laying hens experienced harmful effects including, oral lesions affecting the palate, tongue, and beak; a reduction in feed intake; and a decrease in egg production (Leeson et al., 1995).

With a concentration of 500 ppb breeder hens already developed oral lesions, after consuming the feed for a period of 3 weeks. Levels of 2000 and 8000 ppb negatively affected fertility and hatchability of the fertile eggs. This was in addition to a decrease in feed intake, egg production, and egg-shell thickness (Chi et al., 1977b). Fig 16 shows an example of oral lesions.

4.6.3. Ducks

Feed containing 250, 500, and 1000 ppb of T-2 toxin was given to day old Muscovy duckings (*Cairina moshata*) for a period of 7 days. After 16 hours of consuming the feed, ducks suffered from oral lesions produced by all levels of T-2 toxin contamination (Shlosberg, 1986).

Feed containing T-2 toxin levels at 2000 ppb was given to 6 week-old ducks for a total of 9 days. Ducks developed significant ulceration and erosion of the esophagus and the oral cavity. There was also a decrease in body weight, thymus weight, spleen weight, and bursa weight when compared to those from the control group (Neiger, 1994).

Fig. 16. Oral lesions due to T-2 toxin contamination. These lesions are similar to those caused by diacetoxyscirpenol. (AVIMEX Laboratory, Mexico).

4.6.4. Turkeys

Young turkeys given feed containing 1000 ppb of T-2 toxin for a period of 32 or more days did not have any alterations in their development, weight gain, small intestinal morphology, or antibody production, when compared with the control group. However, when the 1000 ppb T-2 toxin contamination was in combination with 1000 ppb diacetoxyscirpenol contamination, young turkeys had serious and evident oral lesions 7 to 15 days after ingesting the contaminated feed. There were moderate morphological changes of the small intestine, without pathological and/or histopathological lesions (Sklan, 2003).

4.6.5. Pigs

Feed containing a concentration of 1000 to 8000 ppb T-2 toxin given to pigs for a period of 8 weeks produced a decrease in feed consumption and a decrease in body weight gain. Pigs also developed oral lesions (Mirocha, 1979).

Concentrations of 500, 1000, 2000, and 3000 ppb of T-2 toxin fed to 49-day old piglets weighing 9 kg for a period of 21 days produced a marked debilitation of the immune system (Rafai et al., 1995a). There was a decrease in feed intake and body weight gain even with the lowest mycotoxin level. It was also observed rejection of the feed (Rafai et al., 1995b).

4.6.6. Dairy Cows

Dairy cows died after consuming for several months a ration naturally contaminated with 1200 ppb of T-2 toxin, although the researchers believe T-2 toxin levels could have been higher than 1200 ppb (Hsu et al., 1972).

Another study (Jones et al., 1994) indicated that the presence of T-2 toxin can cause dairy cows to reject the feed, have decreased milk production, gastroenteritis, intestinal hemorrhaging, and even mortality. T-2 toxin also is associated with a decreased immune response in calves (Mann et al., 1982; Mann et al., 1984). Statistical data from field observations indicate that the maximum tolerable T-2 toxin contamination should not exceed 100 ppb in the animal's total diet (Jones et al., 1994).

4.6.7. Rabbits

Feed contaminated with 190 and 284 ppb of T-2 toxin (the higher dose was given for a period of 4 to 7 weeks) (Szilagyi et al., 1994; Lebas and Perez, 1998; Gimeno and Martins, 2000) resulted hepatotoxic and nephrotoxic in rabbits, causing also alterations in the digestive, respiratory, and reproductive systems. The LD50 obtained in an experimental test was 1.1 mg of T-2 toxin/kg of body weight (Chan and Gentry, 1984).

4.7. Diacetoxyscirpenol (DAS)

4.7.1. Chickens

Feed containing 1000 to 2000 ppb of DAS caused oral lesions and delayed growth in day-old chicks which consumed the feed for 3 weeks (Ademoyero and Hamilton, 1991a).

Day-old chicks consuming feed containing 5000 ppb of DAS for a period of 3 weeks had more pronounced oral lesions than chicks fed feed containing 5000 ppb of T-2 toxin. Oral lesions, caused by feed containing 5000 ppb of DAS, were evident after 5 days of consuming the contaminated feed (Chi and Mirocha, 1978) Fig. 17 shows an example of these types of lesions.

Fig. 17. Oral lesions due to diacetoxyscirpenol contamination. These lesions were similar to those produced by T-2 toxin (AVIMEX laboratory, Mexico).

4.7.1.1. Interactions

Feed containing 12% fat and contaminated with 4000 and 8000 ppb of diacetoxyscirpenol given to chickens for a period of 3 weeks caused a greater decrease in body weight than feed containing the same mycotoxin levels but only containing 6% fat. This could be explained by an increase in micelle lipid absorption of DAS when administered in diets with higher fat content (Ademoyero and Hamilton, 1991b).

4.7.2. Laying Hens

Layer feed containing 2000 ppb of DAS resulted in oral lesions that affected the palate, tongue, and beak, a decrease in feed intake and a decrease of egg production, which was manifested 24 hours after initial feed intake (Leeson et al., 1995). An example of these types of lesions can be seen in Fig. 18 and 19.

Feed containing 500 ppb of DAS given to 50 week-old hens for a period of 4 weeks decreased the hatchability of fertile eggs (Allen et al., 1982).

Fig. 18. Oral lesions caused by diacetoxyscirpenol contamination. T-2 toxin produces similar lesions (AVIMEX laboratory, Mexico).

Fig. 19. Oral lesions due to diacetoxyscirpenol contamination. T-2 toxin produces similar lesions (AVIMEX laboratory, Mexico).

4.7.3 Ducks

Feed contaminated with 250, 500, and 1000 ppb of DAS was given to day-old Muscovy duckings (*Cairina moshata*) for a total of 7 days. All levels of DAS induced oral lesions 16 hours after they start eating (Shlosberg, 1986).

4.7.4. Turkeys

Young turkeys were given feed containing 1000 ppb of DAS during 32 days or more. There were no effects on growth, body weight gain, small intestine morphology, and antibody production, when compared to the control group. However, when 1000 ppb of DAS was combined with 1000 ppb of T-2 toxin, oral lesions were evident 7 to 15 days after initial intake of feed containing both mycotoxins. Morphological changes in the small intestine were moderate, without any pathological and/or histopathological lesions (Sklan, 2003).

4.7.5. Pigs

Feed containing low concentrations of DAS, 380 and 500 ppb, caused intestinal hemorrhages in pigs (Mirocha, 1979).

Young pigs given feed containing 2000 to 10000 ppb of DAS for a period of 9 weeks, had serious oral lesions, intestinal disorders, and a significant decrease in body live weight (Mirocha, 1979).

4.7.6. Dairy Cows and Rabbits

The authors have no data to which they can refer to concerning the effects of this mycotoxin in dairy cows or rabbits.

4.8. Monoacetoxiscirpenol (MAS), Triacetoxyscirpenol (TAS) and Escirpentriol (STO)

4.8.1. Chickens

The LD50 values for these three mycotoxins in day-old chicks (one oral dose), resulted in the following toxicity: 3.4 mg/kg of body weight for 15-monoacetoxiscirpenol; 7.2 mg/kg of body weight for triacetoxyscirpenol, and 9.3 mg/kg of body weight for escirpentriol. 15-MAS was more toxic than 3- and 4- MAS whose LD50 were 8.1 and 9.6 mg/kg of body weight, respectively (Leeson et al., 1995).

Feed containing individual concentrations of 500 ppb of MAS, 4000 ppb of TAS, and 2000 ppb of STO was given to day old chicks for 21 days. Each mycotoxin generated oral lesions within 7 days of initial

ingestion. The number of lesions triples after 14 days of feed intake. MAS and STO, at the mentioned concentrations, produced feather abnormalities (frayed feathers and lack of plumage framework) and STO produced a significant reduction in growth. Higher concentrations of MAS (2000 ppb) and TAS (8000 ppb) also produced a significant growth depression (Ademoyero and Hamilton, 1991a; Ademoyero et al., 1991).

4.8.2. Laying Hens

Very high contamination levels (25000 and 50000 ppb) of monoacetoxyscirpenol in feed given to hens for 28 days resulted in a decrease of feed intake, a decrease of body weight, and an interruption of egg production (Leeson et al., 1995).

4.8.3. Ducks, turkeys, pigs, dairy cows, and rabbits

The authors have no data to present regarding the toxicity of these mycotoxins in ducks, turkeys, pigs, dairy cows, or rabbis.

5. SYNERGISMS AND/OR ASSOCIATIONS OF MYCOTOXINS

Synergism among mycotoxins is understood as meaning, toxicity problems produced by the presence of several mycotoxins in the feed at specific concentrations that would not result from individual mycotoxin contamination at the same concentrations.

«Associations» is defined as the presence of several mycotoxins in the same feed at certain concentrations producing toxicity problems equal to those seen when the same mycotoxins exist individually in a feed at the same concentrations. However, with associations' cases, there is an increase in the severity of the toxicity problems as well as an increase in the variety of symptoms.

This is a difficult and complicated subject, presenting great variability. Studies done up to date are scarce and do not allow to formulate definitive conclusions; even sometimes create confusion, because it is taken as a given that low concentrations of mycotoxins that individually would not produce any problems, should automatically induce problems when found combined in any given feed.

The following are published or documented experiences of different cases of mycotoxin synergism and/or associations.

5.1. DON, AFB1, and DON+AFB1

5.1.1. Chickens

Day-old Hubbard chicks were fed diets contaminated with 16000 ppb of DON (individual contamination), 2500 ppb of AFB1 (individual contamination), 16000 ppb of DON+2500 ppb of AFB1 (combined contamination) for 3 weeks. The following problems were observed:

AFB1 decreased body weight gain and increased the relative weight of the spleen, liver, and kidneys. Chickens suffered from hepatic hyperlipidemia, and the levels of protein, albumin and phosphorus in the serum decreased, as well as the lactic dehydrogenase activity.

DON decreased growth rate, increased feed conversion and increased gizzard relative weight. Also produced anemia, and decreased lactic dehydrogenase activity and triglycerides in the serum.

AFB1+DON combination caused the same problems previously cited. However, these symptoms were more severe. Yet, this increase in severity was not sufficiently significant to conclude that this combination of mycotoxins represented a synergic toxicity (Huff et al., 1986).

Regarding individual contamination, Romer, 1983 and Halloran, 1983 (mentioned in 4.5.1) cited that 15000 to 50000 ppb of DON given to 6 day old chicks for a period of 42 and 6 days, respectively, the highest concentration only produced few oral erosions; which one not agree with the symptoms observed by Huff et al., 1986.

5.1.2. Pigs

Six-week old pigs were given feed containing 3000 ppb of DON (individual contamination), 3000 ppb of AFB1 (individual contamination), 3000 ppb of DON + 3000 ppb of AFB1 (combined contamination) for 28 days. Listed below are the effects of these mycotoxins:

AFB_1 and AFB1+DON resulted in a significant decrease in body weight gain. DON and AFB1+DON resulted in vomiting and rejection of feed.

The enzymatic alterations were significant only with AFB1 and AFB1+DON (Harvey et al., 1989).

5.2. T-2 Toxin, AFB1, and T-2 Toxin+AFB1

5.2.1. Chickens

Day-old Hubbard chicks given feed contaminated with 4000 ppb of T-2 toxin (individual contamination), 2500 ppb of AFB1 (individual contamination), and 4000 ppb of T-2 toxin + 2500 ppb of AFB1 (combined contamination) for 3 weeks had the following consequences:

T-2 toxin generated oral lesions, a decrease in protein, albumin, potassium, and magnesium levels in the serum, as well as a decrease of certain enzyme activity in the serum.

AFB_1 resulted in a decrease in body weight gain, and an alteration of protein, albumin, glucose, cholesterol, calcium, magnesium, and enzymatic levels in the serum. Chicks also had an increase of relative liver, kidney, spleen, pancreas, proventriculus, and heart weight.

The combination of AFB1+T-2 toxin had a serious impact on the severity of all previously mentioned conditions (Huff et al., 1988).

5.3. T-2 Toxin, OTA, and T-2 Toxin+OTA

5.3.1. Chickens

Day-old chicks given feed containing 4000 ppb of T-2 toxin (individual contamination), 2000 ppb OTA (individual contamination), 4000 ppb T-2 toxin + 2000 ppb OTA (combined contamination) for 3 months, had the following responses:

OTA and OTA+T-2 toxin decreased the nutritional efficiency of the feed. OTA individual contamination significantly increased the relative liver, kidney, gizzard, and pancreas weight. OTA+T-2 toxin increased the severity of previously mentioned effects and also decreased body weight gain, protein levels, and lactic dehydrogenase activity in the serum.

The interaction between these two mycotoxins resulted in an increase of triglycerides levels in the serum and a decrease in gamma-glutamic transferase activity, and calcium in the serum (Kubena et al., 1989).

5.3.2. Pigs

Growing pigs were given feed containing 8000 ppb of T-2 toxin (individual contamination), 2500 ppb of OTA (individual contamination), and 8000 ppb of T-2 toxin + 2500 ppb of OTA (combined contamination) for a period of 30 days. The following deleterious effects were observed:

There was a decrease in body weight and in body weight gain with all contaminations, however the decrease was more pronounced in the pigs consuming feed with combined contamination. The relative

liver weight decreased with the combined contamination. There was an increase in relative kidney weight due to the individual contamination of OTA. This contamination also reduced cholesterol, inorganic phosphorus, alkaline phosphatase, and hemoglobin levels in the serum. On the other hand, creatinine and protein levels increased.

T-2 toxin decreased hemoglobin and alkaline phosphate levels in the serum. The combination of T-2 toxin and OTA significantly affected the severity of the effects previously mentioned (Harvey et al., 1994),

5.4. T-2 Toxin, DON, and T-2 Toxin+DON

5.4.1. Chickens

Day-old chicks were given feed containing 4000 ppb of T-2 toxin (individual contamination), 16000 ppb of DON (individual contamination), and 4000 ppb of T-2 toxin+16000 ppb of DON (combined contamination) for 3 weeks. The following problems were observed:

T-2 toxin+DON contamination decreased body weight gain and body weight at the end of the test. However, these effects were not significant when individual contamination of T-2 toxin, and DON were given to chicks. Oral lesions were evident with T-2 toxin individual contamination, but these lesions were more severe with the combined contamination.

Other parameters that remained unaffected with individual contamination, were significantly affected when both mycotoxins were present (combined contamination) (Kubena et al., 1998a).

5.5. DAS, OTA, and DAS+OTA

5.5.1. Chickens

Day-old chicks given feed containing 6000 ppb of DAS (individual contamination), 2000 ppb of OTA (individual contamination), and 6000 ppb of DAS + 2000 ppb of OTA (combined contamination) for 19 days, had the following adverse effects:

All contamination decreased body weight. DAS and DAS+OTA decreased the nutritional efficiency of the feed. There was a significant

antagonistic interaction between OTA and DAS in uric acid and cholesterol.

DAS+OTA increased the relative liver, kidney, and gizzard weight and decreased the total protein concentration and hemoglobin levels in the serum.

Ninety percent of the chicks suffered oral lesions after consuming the different contaminants. OTA and OTA + DAS resulted in severe nephropathy (Kubena, et al., 1994)

5.6. DAS, AFB1, and DAS+AFB1

5.6.1. Pigs

Growing pigs from 10 to 14 week-old consumed feed containing 2000 ppb of DAS (individual contamination), 2500 ppb of AFB1 (individual contamination), and 2000 ppb of DAS+2500 ppb of AFB1 (combined contamination) for 28 days. All doses resulted in a decrease of body weight and body weight gain. The most pronounced decrease was produced by the combined contamination. AFB1 and AFB1+DAS increased relative kidney and spleen weight.

AFB1 increased the levels of some enzymes and hemoglobin in the serum, and decreased urea nitrogen levels and iron binding capacity. DAS decreased iron binding capacity.

Combined contamination significantly increased the severity of all the disorders previously mentioned (Harvey et al., 1991).

5.7. FB1, T-2 toxin, DON, FB1+T-2toxin, and FB_1+DON

5.7.1. Chickens

Newborn chicks given feed containing 300000 ppb of FB_1(individual contamination), 5000 ppb of T-2 toxin (individual contamination), 15000 ppb of DON (individual contamination), 300000 ppb of FB1 + 5000 ppb of T-2 toxin (combined contamination), and 300000 ppb of FB1 + 15000 ppb of DON (combined contamination) for 19 to 21 days had the following problems:

FB1 decreased body weight by 18 to 20%, T-2 toxin decreased body weight by 18%, DON reduced body weight by 2%, FB_1+T-2 toxin

decreased body weight by 32%, and FB1+DON decreased body weight by 19%. Nutritional feed efficiency was especially affected by FB1, when present individually or in combination with other mycotoxins.

There was 15% mortality in chicks fed with the combination of FB1+T-2 toxin. Diets contaminated with FB1, with or without other mycotoxins, increased the relative weights of liver and kidneys, and the levels of cholesterol in serum.

FB1 individual contamination and combined contamination with T-2 toxin or DON increased the activity level of certain enzymes (Kubena et al., 1997).

5.8. DON+ZEN

5.8.1. Pigs

Association of vomitoxin and zearalenone at a rate of 1800 ppb + 250 ppb, 1000 ppb + 175 ppb, 60 ppb + 3600 ppb, 1000 ppb + traces of zearalenone, resulted in feed rejection, vomit, and bloody feces (Mirocha, 1979).

5.9. FB1, AFB1, and FB1+AFB1

5.9.1. Turkeys

Day-old turkey poults were fed diets contaminated with 75000 ppb of FB1 (individual contamination), 200 ppb of AFB1 (individual contamination), and 75000 ppb of FB1+200 ppb of AFB1 (combined contamination) for 21 days. Poults showed a decrease in body weight gain and significantly poorer feed efficiency when given AFB1 and AFB1+FB1.

The diet containing FB1 as an individual contaminant increased the relative liver and spleen weight, while the diet containing AFB1+FB1 in combination increased only the spleen weight.

Feed containing AFB1 as an individual contaminant and feed combining both mycotoxins decreased albumin, total protein, and cholesterol levels in the serum.

The sphinganine/sphingosine relationship in the serum increased when poults were given feed containing FB1 as an individual contaminant, and feed containing combined mycotoxins (Weibking, 1994).

5.10. FB1, DAS, OTA, FB1+DAS, and FB_1+OTA

5.10.1. Turkeys

New-born Large White Nicholas female turkey poults given feed containing 300000 ppb of FB1 (individual contamination), 4000 ppb of DAS (individual contamination), 3000 ppb of OTA (individual contamination), 300000 ppb of FB1+4000 ppb of DAS (combined contamination), and 300000 ppb of FB1+3000 ppb of OTA (combined contamination) for 3 weeks, presented the following problems:

FB_1 decreased body weight gain by 30 and 24%, DAS decreased body weight gain by 30%, OTA decreased body weight gain by 8%, FB1+DAS decreased body weight gain by 46%, and FB1+OTA decreased body weight gain by 37%.

Feed efficiency was negatively affected by all contaminated diets, except FB1 as an individual contaminant, which decreased body weight by 24%.

All contaminations resulted in a significant increase of the relative liver weight, except feed containing DAS as an individual contaminant.

Feed containing FB1 as an individual contaminant and as a combined contaminant (FB1+DAS and FB1+ OTA) decreased cholesterol levels in the serum, increased certain enzyme activity, and altered some hematological values (Kubena, 1977a).

6. MOST SIGNIFICANT MYCOTOXINS AND

MYCOTOXICOSIS IN HUMANS

Humans can be affected by mycotoxins found in food by indirect contact; through mycotoxins ingested by animals in their feed that can leave residues in animal products humans consume, such as meat, eggs, and milk, or through direct contact, consuming food such as cereals, cereal products, dried fruit, fruit, etc. contaminated with toxicogenic molds able to produce mycotoxins.

The main factors that influence the toxicity of mycotoxins in humans are: the bioavailability and toxicity of the mycotoxin, the synergisms among mycotoxins, the amount of mycotoxin ingested daily in relation to the concentration of the mycotoxin and the food consumed, the continuity or intermittency of food ingestion, the individual body weight and his or her physiological and health state, and the age of the individual.

Children and youngsters are more susceptible to mycotoxins toxicity due to a greater variation in their basal metabolism. They could not have yet enough biochemical mechanisms for detoxification. Children's brains continue to develop many years after birth. This can cause a greater susceptibility to mycotoxins that affect the central nervous system (Kuiper-Goodman, 1994).

The conjunction of all the factors previously mentioned, which have an influence on the toxicity of mycotoxins, makes the risk analysis in relation to human health problems (immunosuppressive, hepatotoxic, nephrotoxic, neurotoxic, gastro-enteric, and carcinogenic) to be complex and for the most part, difficult to understand and correlate (Smith et al., 1994).

The situation is still more complicated because for the interpretation of epidemiological data, which can be related to

mycotoxins, it is necessary to keep in mind the possibility of influences from other factors such as, the individual's nutritional state, endemic infections, and ingestion of other toxic substances (Kuiper-Goodman, 1994).

Studies of mycotoxin toxicity in humans are normally done with laboratory animals. For a complete evaluation, it is necessary to obtain acute toxicity data, toxicity at 30-90 days after starting the test, metabolic changes, reproductive effects, teratogenicity, mutagenicity, and chronic toxicity or carcinogenetic.

With the information collected, interpolations and parameters can be established, of which the following will be cited in the article: NOAEL (No Observed Adverse Effect Levels). TD50, the dosage of mycotoxin with which 50% of individuals can develop malignant tumors, establishing that way its estimated carcinogenic potential. NEL (No Effect Level), LOAEL (Lowest Observed Adverse Effect Level), and a commonly used parameter, TDI (Tolerable Daily Intake)

The previously mentioned parameters will be expressed in the following way: micrograms of mycotoxin/kg of body weight (bw)/day; or in the case of TDI can be expressed in nanograms (ng) of mycotoxin/kg of body weight (bw)/day. Normally, the value of TDI is obtained dividing the value of NOAEL, or NEL, or the value of TD50 by a safety factor which can oscillate between 50 and 50000, depending on the extrapolation method used. TD50 is most often used with carcinogenic mycotoxins. TDI normally has a risk factor of 1/100000 (Kuiper-Goodman, 1990; Kuiper-Goodman, 1994). When the TDI value is still being studied and it is only provisional, then the PTDI (Provisional Tolerable Daily Intake) is used.

This manual will focus on the mycotoxins Aflatoxin B1 (AFB1), Aflatoxin M1 (AFM1), Ochratoxin A (OTA), Fumonisin B1 (FB1), Vomitoxin or Deoxynivalenol (DON) and Patulin because these are some of the mycotoxins that can commonly affect food safety and in relative terms (depending on the mycotoxin) public health (Kuiper-Goodman, 1990; Kuiper-Goodman, 1994; JECFA, 2001; WHO, 2002; CAST, 2003). Almost all these mycotoxins are immunosuppressive given that they inhibit protein synthesis and interrupt DNA and RNA synthesis, also inhibiting phagocytosis (Sharma, 1993).

7. MYCOTOXINS AND MYCOTOXICOSIS

7.1. Aflatoxins

Aflatoxins can be found as natural contaminants in a variety of food ingredients such as, cereals and cereal products, peanuts, nuts, almonds, pistachios, hazelnut, and other dried fruits, coconut, cocoa, sweet potato, peanut butter, lentil, bananas, cheeses, wines, spices, milk and milk products (essentially AFM1), and other foods.

The main aflatoxicosis in humans are: hepatotoxicity, liver cancer, and probably «Reye's Syndrome».

7.1.1. Relation between quantity of AFB1 ingested and concentration of AFM1 excreted in milk

AFM1 (4 hydroxy-aflatoxin B1) is the hydroxilated metabolite of AFB1. It can be found in milk and milk products coming from dairy cattle that have consumed feed contaminated with AFB1. AFM1 residues in milk can be found 6-24 hours after the cow has ingested the AFB1 contaminated feed. In dairy cows, the relationship between concentration of AFB1 (ppb or micrograms/kg) in the final ration and AFM1 (ppb or micrograms/liter) secreted in the milk could be 300:1. However, this ratio is too much of an approximation, given the range variation between 34:1 and 1600:1 (Rodricks and Stoloff, 1977; Gimeno and Martins, 2000b; Gimeno and Martins, 2000c).

With an ingestion of 2 to 60 milligrams (mg) of AFB1/cow/day the AFM1 residues in crude milk can fluctuate from 1 to 50 micrograms/liter (Edds, 1979).

The level of AFM1 residue/day (mg) in crude milk could be approximately 2.2% of the daily ingested AFB1 (mg) with a CV (coefficient of variation) between 42 and 59%. Dividing the result obtained by the number of liters of milk produced/cow/day and multiplying that by 1000, would give the concentration (micrograms/liter) of AFM1 in crude milk (Patterson et al., 1980; Van Egmond, 1989). Even an equation has been proposed: $y = - 2.5 + 0.84x$ ($r^2 = 0.73$; n= 43) where x = mg AFB1/cow/day and y = micrograms of AFM1/liter of milk (considering an average output of 20 liters of milk /cow/day) (Sieber y Blanc, 1978; Van Egmond, 1989).

The concentration of AFM1 in milk varies according to cow breed, AFB1 concentration in the ration, quantity and duration of the ingestion of contaminated feed, and animal's health status. Moreover, lack of agreement in the results of different laboratories, could be due to the ruminant metabolic system, which can create that the AFM1 concentration in milk varies from animal to animal, from one day to the next, and from milking to milking.

7.1.2. Distribution of AFM1 in some milk products.

The distribution of AFM1 in some foods manufactured with contaminated milk is the following: 40-60% in cheeses, 10% in cream, and less than 2% in butter.

Since AFM1 is very soluble in water, it is not understood why a greatest percentage goes to cheese and not whey. One explanation could be the association of AFM_1 with casein when it precipitates (Yousef and Marth, 1989).

7.1.3. TD50, TDI, and NOAEL for AFM1 and AFB1

AFM1 and AFB1 have a TD50 of 10.38 and 1.15 micrograms/kg of body weight (bw)/day, respectively, which makes AFM1 approximately nine times less carcinogenic than AFB1. TDI for AFB_1 is between 0.11 and 0.19 ng (nanograms)/kg bw/day, with a safety factor of 5000 and a risk factor of 1/100000. NOAEL values for AFM_1 and AFB_1 are < 2.5 and 0.75 micrograms/kg bw/day, respectively (Kuiper-Goodman, 1990;

Kuiper-Goodman, 1994). Dividing the TD50 value of AFM1 by the safety factor of 5000, the TDI value for AFM1 should be hipothetically 2 ng/Kg bw/day, which approximately represents, ten times more tolerance than AFB1 compared with the greatest value of TDI for AFB1 (Gimeno and Martins 2003b).

7.1.4. Legislation

The European Union (EU) has legislation (Official Journal of the European Communities, 2002a and 2002b; Official Journal of the European Union, 2003; Mycotoxins, 2003) for these mycotoxins in food for human consumption. The current maximum level admissible are established at 0.05 micrograms/kg (0.05 ppb) of AFM1 in milk (crude milk, milk for manufacturing milk derivatives and thermally treated milk); and vary between 2 and 8 micrograms/kg for AFB1 and between 4 and 15 micrograms/kg for AFB1+AFB2+AFG1+AFG2, depending on the different foods (peanuts, fruits, dried fruit and derived products, cereal and derived products) used for direct human consumption or as ingredients in other food products. The legislation includes in these foods those that are part of the ingredients of another food or submitted to a selection process or other physical treatments before direct human consumption; being aware that such processes can reduce the original AFB1 concentration. It is also specified that these maximum admissible concentrations refer to the edible parts, excluding peels or shells in those foods that contain them.

EU legislation also establishes maximum permissible levels of 5 micrograms/kg for AFB1 and 10 micrograms/kg for AFB1+AFB2+AFG1+AFG2, in some spices. In the case of baby food and cereal based food for nursing babies, children, or dietetic foods for special medical use specifically made for nursing children, the maximum permitted concentration of AFB1 is 0.10 micrograms/kg. In the case of formula for nursing babies, continuing formula (including milk for nursing babies and modified or continuing milk), and dietetic foods destined for special medical use specifically made for nursing children, the maximum permitted concentration of AFM1 is 0.025 micrograms/kg (Official Journal of the European Union, 2004).

In other countries (Australia, Brazil, Holland, Romania, Switzerland, USA) the maximum admitted concentration of AFM1 in milk and milk products vary from 0.01 and 0.50 micrograms/kg, depending on the country and the type of milk product. In Switzerland, the maximum contamination of AFM_1 in milk products destined for children is 0.01 micrograms/kg. In Australia, the United States of America (USA) and the countries forming part of MERCOSUR (Brazil, Argentina, Paraguay, and Uruguay), the maximum concentration of AFM1 permitted in milk is 0.50 micrograms/kg.

There is no legislation for AFM1 in cheese or butter. However, Holland has established a maximum tolerance of 0.20 and 0.02 micrograms /kg, respectively. Some countries such as Austria and Switzerland have a limit for AFM1 in cheese of 0.25 micrograms/kg (Smith et al., 1994; EHSO; FDA, 2000; CAST, 2003).

As previously mentioned, the regulation adopted by the USA for AFM1 in milk, has also been adopted by some Latin American countries, among them Argentina, Brazil, Paraguay, and Uruguay (Mycotoxins on line). This means that in the case of the USA and those other countries, the tolerance level for AFM1 in milk is 10 times higher than the one in the EU.

Countries such as Australia, Canada, Colombia, Hungary, India, Japan, Mexico, Cuba, Thailand, and the USA have a maximum tolerance level for other aflatoxins that oscillate between 5 and 30 micrograms/kg for AFB1 and for the sum of the four aflatoxins, depending on the country and the type of food (fruits with peels and their products, peanuts, and all food ingredients). India has the highest level of tolerance (30 micrograms of AFB1/kg for all foods). Mexico and the USA have the highest level of tolerance for the sum of all four aflatoxins (20 micrograms/kg in all foods) (Smith et al., 1994).

7.1.5. Occurrences of Aflatoxin M1 and Ingestion.

Contamination levels found in food in some European countries since 1995 (France, Italy, Germany, Holland, Portugal) fluctuate between < 0.001 and 0.108 micrograms/kg or liter (ppb) in commercial milk (mean of 0.009 ppb); between < 0.001 and 0.103 micrograms/kg in

powdered milk (mean of 0.027 ppb); between < 0.001 and 0.98 micrograms/kg in yogurt (mean of 0.010 ppb); and between < 0.005 and 0.500 micrograms/kg in cheese (mean of 0.150 ppb). The previous values correspond to a total of 604, 189, 134, and 311 analyzed samples, respectively (Galvano et al., 1998; Jonker et al., 1999; COST, 2002a; Martins and Martins, 2000; Martins and Martins, 2004).

Data corresponding to the analyses of 7573 samples of milk during the year 1999 from various EU members showed that all the samples contained < 0.05 ppb of AFM1. Results corresponding to 81 samples of milk from Canada between 1997 and 1998 indicated that all the samples contained < 0.015 ppb of AFM1 (JECFA, 2001; WHO, 2002).

Studies done around the world indicate that mean contamination of AFM1 in milk corresponding to diets from Europe, Latin America, Asia, Middle East, and Africa are the following: 0.023, 0.022, 0.36, 0.005, 0.002 micrograms/kg, respectively and which correspond to the analysis of 10778, 893, 1191, 231, and 15 samples, respectively. Considering this data, as well as the average consumption of milk in those countries, the ingestion of AFM1 is estimated to be: 6.8, 3.5, 12, 0.7, and 0.1 ng/person/day in Europe, Latin America, Asia, Middle East, and Africa, respectively (JECFA, 2001; WHO, 2002). A youngster weighing 50 kg would consume 0.14, 0.07, 0.24, 0.01, and 0.002 ng/kg of bw/day in Europe, Latin America, Asia, Middle East, and Africa, respectively. All these values are under the TDI (2 ng/kg bw/day) previously mentioned.

Assuming that all the milk consumed had a contamination of AFM1 of 0.05 micrograms/kg (maximum level permitted in the EU) or of 0.50 micrograms/kg (maximum level permitted in the USA and other countries), the ingestion of AFM1 in Europe and the other countries would be 15 and 150 ng/person/day, respectively (JECFA, 2001; WHO, 2002). Therefore, considering youngsters weighing 50 kg, the ingestion of AFM1/kg of bw/day would be 0.3 ng and 3 ng for the two maximum levels indicated, respectively. The first value is under the TD1 for AFM1, hypothetically considered to be 2 ng/kg of bw/day, but the second value is above this level. Applying this calculation to children weighing 10 kg, the ingestion would be of 1.5 and 15 ng/kg bw/day, for the two maximum levels permitted, as previously mentioned, respectively. The first value continues to be below the mentioned TDI, but not the second value. Applying these calculations to a child weighing 20 kg

whom consumes 0.50 liters of milk a day contaminated with 0.05 ppb or 0.50 ppb of AFM1, the daily ingestion of the mycotoxin would be 1.25 or 12.5 ng/kg bw/day, respectively. The first value is less than the TDI, while the second value is significantly above the TDI. The maximum level of AFM1 permitted in the USA and other countries is not accepted in the EU (Gimeno and Martins, 2003b; Gimeno, 2004; Gimeno 2004a).

It is important, however, to take into account that the values of TDI depend on the security factor that is applied, which can fluctuate between 50 and 50000 (one of the way of obtaining TDI, when dealing with carcinogenic mycotoxin, is dividing the TD50 by the safety factor, which depends on the criteria or extrapolation method used) (Kuiper-Goodman, 1990; Kuiper-Goodman, 1994; Gimeno and Martins, 2000c; Gimeno and Martins, 2003b). Since this factor appears as a denominator, it is evident that the higher it is, the lower the TDI value will be, and therefore the more rigorous and safe; and vice versa, the lower it is, the higher the TDI value will be, and therefore the less rigorous and safe.

7.1.6. Occurrences of AFB1 and Ingestion.

There is a large variation in the levels of AFB1 contamination found lately in different countries and depends on the food type, the country, and the availability of public records. It ranges between 0.05 and 789 micrograms/kg of AFB1 and between 0.05 and 1870 micrograms/kg for the sum of all four aflatoxins in peanuts, pistachios, other dry fruits, spices, and other foods (COST, 2001; Martins et al., 2001).

The FDA (Food and Drug Administration) estimates that the ingestion of AFB1 across food types is between 2.73 ng/kg of bw/day in the USA and 3.5 to 22.4 ng/kg of bw/day in Thailand and Eastern Africa. Levels of 500 micrograms of AFB1/kg have been found in kidney and other tissue of individuals in Europe and North America (Smith et al., 1994). Considering the maximum value of TDI for AFB1 of 0.19 ng/kg of bw/day, previously mentioned, these values are above this TDI value, but below the NOAEL value of 750 ng/kg of bw/day (0.75 micrograms/kg of bw/day), also mentioned earlier.

Considering the lowest maximum concentration of AFB1 (2 micrograms/kg) permitted by the EU en food such as cereals and certain dry fruits, and remembering the TDI level of 0.19 ng/kg of bw/

day, then a youngster weighing 50 kg could ingest 9.5 ng of AFB1/day. Hence, the daily maximum ingestion of foods uniformly containing 2 micrograms of AFB1/kg can not be greater than 5 g, approximately. It must be considered that these calculations are being made with a value of TDI which is approximately 4000 times inferior to the NOAEL value (Gimeno and Martins, 2003a).

7.1.7. Mycotoxicosis (aflatoxins M1 and B1)

Although it is assumed that AFM1 induces liver cancer in rodents by means of a similar mechanism of AFB1, there are no suitable epidemiological studies that relate the dose-response between AFM_1 ingestion, exposition to viral hepatitis B or C and liver cancer. The additional risks for the prediction of liver cancer using levels of AFM1 comparable to 0.05 micrograms/kg (maximum permitted level by the EU) and 0.50 (maximum permitted level by the USA and other countries) are very small. In a population like the USA and Western Europe where the prevalence of viral hepatitis B is 1%, the additional prevalence of liver cancer cases associated to the contamination of milk with 0.50 micrograms/kg versus 0.05 micrograms/kg would be 29 cancer cases / 1000 million individuals / year (JECFA, 2001; WHO, 2002). The debate between the EU and countries that defend the maximum level of contamination of AFM1 in milk to be 0.50 micrograms/kg instead of 0.05 micrograms/kg continues (CCFAC, 1999; CCFAC, 2000; CCFAC, 2001; CODEX, 2002).

Concerning other aflatoxins, specifically AFB1, hepatotoxic problems with significant incidence of liver cancer were found in 1971 and some years earlier. Acute aflatoxicosis in India, Africa, and Thailand were produced by the consumption of foods, essentially corn, cassava, rice, peanuts, sweet potatoes, and bananas contaminated with AFB_1 levels that fluctuated between 10 and 144000 micrograms/kg.

Reye's syndrome, which is characterized by an anatomicopathological association of an acute cerebral edema with a fatty liver degeneration in children, was also attributed to the consumption of foods contaminated with AFB1. However, etiology of this syndrome is very problematic and its direct relation with AFB1 is not clear.

Many studies have been done in Thailand, China, and Africa regarding chronic aflatoxicosis, concluding that there is enough evidence to consider AFB1 as a risk factor responsible for carcinogenic problems (Gimeno and Martins, 1987; Smith et al., 1994; CAST, 2003).

7.2. Ochratoxin A (OTA)

OTA can be found as a natural contaminant in cereal and cereal products, cocoa beans, legumes, cheese, peanuts, raw and toasted coffee beans, smoked meat (ham, bacon, and sausage), wine, beer, and other foods.

The main mycotoxicosis affecting humans is nephrotoxicity. There are not enough studies or evidences to say that OTA is a carcinogenic in humans.

7.2.1. TDI and PTDI for Ochratoxin A

TDI for OTA is not yet clearly established. Based on carcinogenic studies, the level of TDI oscillates between 1.5 and 5.7 ng/kg of bw/day with safety factors of 50000 and 5000, respectively, and a risk factor of 1/100000. The value of TDI corresponding to 5.7 was obtained by dividing the value of NEL by 5000 and the value of TDI = 1.5 was obtained by dividing the TD50 value by 50000.

Canada proposed a TDI of 4 ng/kg of bw/day, while Nordic countries propose a value of 5 ng/kg of bw/day. The FAO/WHO Joint Expert Committee on Food Additives, proposed provisional TDI values (PTDI) between 10 and 16 ng/kg of bw/day with a safety factor of 500. These values were based on studies done on pigs presenting renal function alterations. The greatest value of TDI corresponds to the LOAEL value divided by 500. It is evident that this ample range of PTDI, which goes from 1.5 to 16 ng/kg of bw/day, derived from the toxic effects on which the studies are based, the different methods of extrapolation, and the different safety factors applied. More studies are necessary on this subject.

Probably, the tendency is to establish a TDI value equal to or less than 5 ng/kg of bw/day, based on carcinogenic studies. However, there is not enough evidence that OTA is carcinogenic in humans; only it

has been demonstrated in animals (Kuiper-Goodman, 1994; JECFA, 1995; Smith et al., 1994; JECFA, 2001; WHO, 2002).

7.2.2. Legislation

The EU has legislation (Official Journal of the European Communities, 2002; Mycotoxins, 2003; Official Journal of the European Union, 2005) that establish maximum levels of OTA permitted in foods for human consumption to be 5 and 3 micrograms/kg for whole grain cereals and products derived from cereals, respectively. This last group includes transformed cereal products and cereal grains for direct consumption. The maximum permitted level for raisins is 10 micrograms/kg.

Toasted coffee beans and ground toasted coffee, with exception of soluble coffee (instant coffee), the maximum permitted level is 5 micrograms/kg and for instant coffee the maximum permitted level is 10 micrograms/kg.

Wine (red, white, rosé) and other beverages based on wine and/or grape must have a maximum permitted level of 2 micrograms/kg. This includes sparkling wine, aromatic wine, aromatic beverages with a wine base, and aromatic cocktails derived from wine. Excluded from this group are wine liqueurs, and wines with alcohol content by volume greater to 15% vol. A maximum content of 2 ppb applies to all products derived from the harvest of 2005 and those following that harvest.

Grape juice, beverages containing grape juice as an ingredient, including fruit nectar, and grape juice concentrate, grape must, and grape must concentrate, fruit juices, fruit juice concentrate, and fruit nectars destined for human consumption has a maximum permitted level of 2 micrograms/kg. This level is applied to products derived from the harvest of 2005 and those following that harvest.

Foods for children and foods elaborated from cereal for nursing children and young children, and dietetic foods for special medical use specifically directed to nursing babies, have a maximum permitted concentration of OTA of 0.50 micrograms/kg (based on dry matter for children food and foods elaborated from cereal) (Official Journal of the European Union, 2004).

In other countries, such as Hungary and Romania, the maximum permitted levels are 20 and 5 micrograms/kg, respectively, for all foods. Brazil has a maximum of 50 micrograms/kg in cereals. Denmark has a maximum permitted level in pig carcass and viscera that range between 10 and 25 micrograms of OTA/kg (Smith et al., 1994).

7.2.3. Occurrences of Ochratoxin A and Ingestion

The range in levels of contamination in foods is ample and depends on type of food, geographic area, and available published data. Thus, in cereal there is contamination levels that range from 10 to 2400 micrograms/kg, toasted coffee ranges between 0.2 and 1.7 micrograms/kg, raw coffee beans range from 0.4 to 23 micrograms/kg, wine contamination range from 1 to 7.63 micrograms/kg, being greater in red wine. OTA analysis done in pig kidneys and human biological fluids ranged in contamination levels from 0.1 to 240 micrograms/kg and 0.05 to 14.4 micrograms/kg, respectively (Smith et al., 1994; Leoni et al., 2000; Otteneder and Majerus, 2000; COST, 2001; Pietri et al., 2001).

The presence of OTA is frequent in the European diet. Studies showed an average ingestion of 6.42 ng/kg of bw/day of OTA, considering a corporal weight of 60 Kg. Cereals and wine contribute approximately with 3.57 and 1.43 ng of OTA/kg of bw/day (55% and 22%), respectively. Other foods, such as grape juice and coffee contribute 0.29 and 0.43 ng of OTA/kg of bw/day (4.51% and 6.7%), respectively, and a variety of foods such as dried fruit, beer, tea, milk, cocoa, legumes, and other contribute less than 0.14 ng of OTA/kg of bw/day (2.18%). The consumption of edible pig tissue contributed 0.21 ng of OTA/kg of bw/day (3.27%).

Given those levels of contamination, the risk for individuals that consume large quantities of cereal is higher. Studies reveal that those individuals can have OTA ingestions of 13.14 ng/kg of bw/day (JECFA, 2001; WHO, 2002).

Taking into account studies done considering carcinogenic problems and considering a value of TDI of 5.7 ng/kg of bw/day, the average ingestion of OTA in the European diet would be 112% of TDI.

However, considering the criteria of alterations of renal function and previous values of TDI, for example 16 ng/kg of bw/day, the average ingestion would be 40% of TDI. Remembering that the maximum admissible concentration of OTA in cereals established by the EU is 5 micrograms/kg, an individual weighing 60 kg would have an ingestion limit of OTA of 324 ng/day (TDI = 5.7 ng/kg of bw/day) or 960 ng/day (TDI = 13 ng/kg of bw/day) which represents a maximum permissible daily intake of 68.5 or 192 g of cereal uniformly contaminated with 5 micrograms of OTA/kg, respectively. However, these values of TDI are 5000 and 500 times lower than the value for NOAEL and LOAEL, respectively (Gimeno and Martins, 2003a). If this were applied to children, the limits of ingestion would be lower, given the rigorousness imposed by the legislation which established a maximum permitted concentration of 0.50 ppb OTA (Official Journal of the European Union, 2004).

7.2.4. Mycotoxicosis

The first problems caused by OTA in humans date from 1956 with the appearance of endemic nephropathy in the Balkan region (Bulgaria, Romania, and Yugoslavia). These problems were attributed to smoked meats contaminated with OTA in concentrations ranging between 10 and 920 micrograms/kg. The main source of contamination was due to lack on hygienic conditions in the storage of these smoke meats. The analysis of OTA in the serum of affected individuals ranged between 1 and 40 micrograms/kg. Epidemiological studies reveal that approximately half of the European population is exposed to OTA. However, the direct relation between OTA exposure and nephropathy is not clear since levels of OTA ranging from 0.1 and 14.4 micrograms/liter of blood or maternal milk have been found in healthy individuals in Germany, France, Italy, Denmark, Sweden, Czechoslovakia, Poland, and Canada. The presence of OTA in biological fluids is considered as an indirect evaluation of mycotoxin exposure. In some areas of Africa, 95% of individuals that suffer from nephropathy problems are OTA positive, with blood concentrations of 90 micrograms/liter, and the prevalence of ochratoxicosis is 55 to 80% greater than that of Europe (Gimeno and Martins, 1987; Smith et al., 1994; CAST, 2003).

7.3. Fumonisins (FB1, FB2, FB3)

Fumonisins can be found mainly in cereals (specially in corn) and cereals products. The most important mycotoxicosis in humans are: gastrointestinal complications and probably stomach and esophagus cancer.

7.3.1. PTDI and NOAEL for Fumonisins

PTDI (provisional TDI) for FB1, FB2, and FB3, individually or in combination is 2 micrograms/kg of bw/day. This value was obtained by dividing the value of NOAEL of 200 micrograms/kg of bw/day by a safety factor of 100 (JECFA, 2001; WHO, 2002).

7.3.2. Legislation

The EU does not have legislation for fumonisins and the FDA (Food and Drug Administration) suggests maximum contamination levels for fumonisins (FB1+FB2+FB3) of 2000 micrograms/kg for degerminated corn products, 4000 micrograms/kg for partially degerminated corn products, corn bran and clean corn destined for pasta production, and 3000 micrograms/kg for clean corn destined for popcorn production. In Switzerland, the maximum admissible contamination proposed for fumonisins (FB1+FB2) was 1000 micrograms/kg in corn products (Zoller et al., 1994; FDA, 2000a; CAST, 2003).

7.3.3. Occurrences of Fumonisins and Ingestion

The principal incidences of contamination by FB1 and FB2 occur in corn and corn products (corn bread, popcorn, corn flakes, corn flour, corn pasta, polenta, and other corn products); the range of contamination found is very ample and can fluctuate for FB1 between 0.15 and 7900 micrograms/kg, and for FB2 between 0.10 to 2250 micrograms/kg. In moldy corn used for manufacturing beer the levels of FB1 ranged between 110 and 117520 micrograms/kg (average of 53740 micrograms/kg), and the levels of FB_2 between 0 to 22960 micrograms/kg (average of 13680 micrograms/kg) (COST, 2001; Marasas, 1995).

Statistical studies have shown that the average ingestion values of FB1 for different regions in micrograms/kg of body weight/day are the following: Europe, 0.2; Latin America, 1.0; Africa, 2.4; Middle East, 1.1; Asia, 0.7; Canada, 0.02; and the USA, 0.08. It is important to mention that the average intake of the United Kingdom (UK) and Switzerland is 0.03 micrograms/kg of bw/day; and in Holland, 0.06 and 1.0 microgram/kg of bw/day for the total population and for regular consumers of corn, respectively. Considering the previously mentioned PTDI for FB1 of 2 micrograms/kg of bw/day, it is clear that the average consumption for all these regions, except in Africa, are below the PTDI value, even when ingestion of FB_1 is incremented by 40%, taking into account the presence of fumonisins FB2 and FB3 (JECFA, 2001; WHO, 2002).

7.3.4. Mycotoxicosis

The toxic effects of fumonisins are essentially the result of inhibiting sphingolipids synthesis (lipoprotein such as sphinganine and sphingosine), which control cellular communication.

There is no direct evidence to asseverate that fumonisins cause health problems in humans. However, stomach and esophagus cancers have been associated to the frequent consumption of foods contaminated with fumonisins, especially corn products in countries such as South Africa, China, and Italy. Gastrointestinal problems such as diarrhea and spasm pains occurred in India were associated to the consumption of moldy sorghum and corn contaminated with high levels of fumonisins.

However, the International Agency for Cancer Research (IARC) concluded that available studies and quantitative data are not significantly conclusive and are not enough to suggest that oral intake of fumonisins are carcinogenic for humans, therefore, they are only considered as «possibly carcinogenic» (IARC, 1993; EC, 2000; FDA, 2000a; CAST, 2003).

7.4. Vomitoxin or deoxynivalenol (DON)

Vomitoxin or deoxynivalenol (DON) belongs to the trichothecenes mycotoxin family. It can be found as a natural contaminant in cereal

and cereal products. The main mycotoxicosis in humans are: gastrointestinal complications and a reduction in the growth development of children.

7.4.1. TDI, PTDI, NOAEL for Vomitoxin or deoxynivalenol

TDI for DON varies between 0.04 and 0.375 micrograms/kg of bw/day depending on the orientation given to the studies on the critical effect of these mycotoxins. Currently, TDI has been established according to the critical effect produced on the growth decrease in mice during a period of two years, and thus a NOAEL value of 100 micrograms/kg of bw/day was established, dividing by the safety factor of 100, resulting in a provisional TDI (PTDI) of 1.0 microgram/kg of bw/day (Iverson et al., 1995; EC, 1999; Pieters et al., 1999).

7.4.2. Legislation

Currently, the EU does not have legislation for DON. In 1999, Holland proposed maximum concentration limits for this mycotoxin of 120 micrograms/kg for clean wheat; 60 micrograms/kg for bread, and 120 micrograms/kg for foods containing more than 33% of wheat. It was suggested that foods containing less than 33% of wheat should control the clean wheat used as an ingredient and apply the established criteria for clean wheat (Pieters et al., 1999).

7.4.3. Occurrences of Vomitoxin or Deoxynivalenol and Ingestion

The main incidences of contamination occur from cereal (essentially wheat, corn, and barley) and cereal products (bread, cookies, cakes, pasta, breakfast cereal, croissant, and others). The range of contamination can fluctuate between 1 and 5700 micrograms/kg in wheat; from 3 to 3700 micrograms/kg in corn; from 4 to 9000 micrograms/kg in barley; from 4 to 760 micrograms/kg in oatmeal; from 6 to 5100 micrograms/kg in rice; and from 13 to 240 micrograms/kg in rye. In wheat based breakfast cereals have been found contamination levels between 103 and 6040 micrograms/kg with an average of 754 micrograms/kg (COST, 2001; Martins and Martins, 2001; JECFA, 2001; WHO, 2002).

The ingestion of DON in African and Middle Eastern diets is estimated to be 0.77 and 2.4 micrograms/kg of bw/day, respectively.

The authors do not have data indicating average DON ingestion in European, Latin American, and Asian diets. However, the main consumption of wheat (64 to 88% of the total diet) is found in Europe, Latin America, and the Middle East; while there is more variety in the consumption of cereals in Africa and Asia (JECFA, 2001; WHO, 2002).

7.4.4. Vomitoxin or deoxynivalenol in children

Holland is greatly concern about the ingestion of DON. They considers that children ages 1 to 4 (with an average body weight of 10 kg) are at greater risk of ingesting this mycotoxin, given that they calculate consumptions of wheat is in the order of 4.5 to 8.5 g/kg of bw/day. The children rapid rate of growth can decrease due to the adverse effects of DON.

Dutch children's diet is based on bread and wheat products containing more than 33% wheat. The country considers that children ages 1 to 4 with a corporal weight of 10 kg can ingest an average of 51 g of bread + 72 g of wheat products, and 46 g of bread + 46 g of wheat products daily, respectively.

Dutch scientists assume that if the bread and wheat products are uniformly contaminated with the maximum permitted concentration of DON allowed in Holland, 60 micrograms/kg in bread and 120 micrograms/kg in wheat products, the calculations according to these numbers suggest that the total daily ingestion of DON for boys would be 11.7 micrograms and 8.3 micrograms for girls. If we divide this by 10 (average corporal weight of the children), it would give us the daily ingestion per kg of body weight (Pieters et al., 1999; Gimeno, 2003) and if we compare those with the TDI for DON previously mentioned of 1 microgram/kg of bw/day, we would conclude that boys under these conditions ingest 117% of the TDI and girls ingest 83% of the TDI. It is evident that these contamination, the higher they are, the greater the risk for boys and girls.

The maximum permitted concentration of DON proposed by Holland in 1999, would not lead one to believe that there would be

adverse effects threatening the health of children or the general population. Those concentration limits were calculated considering that children consumed the greatest amount of wheat, 8.5 g/kg of bw/day. If we assume that the consumption of wheat is 4.5 g/kg of bw/day, which is the lowest quantity, the maximum tolerable concentration of DON could be twice as large as the previously mentioned concentration. Given that the adverse effect of delayed growth is reversible, it could be said that current concentration limits of this mycotoxin could be even double the previous concentration limits (Pieters et al., 1999). On the other hand, those values are still significantly lower than the maximum concentrations of DON of 2000 and 1000 micrograms/kg, established by the USA and Canada, respectively, for wheat and wheat based products for human consumption (FAO, 1997).

7.4.5. Mycotoxicosis

In Asia (between 1961 and 1985) there were acute cases of gastrointestinal disorders with nausea, vomiting, bloody diarrhea, dizziness, headaches, and fever that were related to the consumption of cereals, selected for human consumption, contaminated with 3000 and 93000 micrograms of DON/kg. Symptoms appeared 5 to 30 minutes after ingesting the contaminated food and they affected a total of 7818 people. There were no deaths. Similar problems affected 50000 people (150 families) in India in 1987. These problems were related to the consumption of bread elaborated from moldy wheat and contaminated with 340 to 8400 micrograms of DON/kg. Analyses also showed other trichothecenes mycotoxins such as acetyl-dioxynivalenol (600 to 2400 micrograms/kg), nivalenol (30 to 100 micrograms/kg), and T-2 toxin (550 to 4000 micrograms/kg). It is evident that these other mycotoxins, which can sometimes be associated with DON, could have exacerbated the previously mentioned problems (Kuiper-Goodman, 1994; JECFA, 2001). As it was mentioned, vomitoxin or deoxynivalenol can produce a decrease in the normal rate of growth of a child.

7.5. Patulin

Patulin is a mycotoxin produced by various species of *Penicillium*, but mainly *Penicillium espansum. Aspergillus and Byssochylamys* species

can also produce patulin. It can be found as a natural contaminant in apples, pears, apricots, peaches, grapes, and other fruits containing rotten spots, juices and fruit jams, and cheese. The main syndrome is neurotoxic, affecting the nervous system. It can also produce hepatotoxicosis, nephrotoxicosis, and cancer. There have been cases of vomiting and nausea. It is immunosuppressive. However, there are many studies to be done in order to prove that these adverse effects are risks affecting humans.

7.5.1. NOAEL and PTDI for Patulin

The NOAEL value for patulin is derived from studies done during 109 weeks with different patulin doses (0.0, 100, 500, and 1500 micrograms/kg of bw) administered to male and female mice three times a week through gastric intubations. The highest concentration caused elevated deaths in both sexes. There were no adverse affects resulting from 100 micrograms/kg of bw dose, which corresponds to 300 micrograms/kg of bw/week, and 43 micrograms/kg of bw/day. This was established as the NOAEL value. If this value is divided by a safety factor of 100, the resulting number is the provisional TDI (PTDI) value = 0.43 micrograms/kg of bw/day (FDA, 2000b).

7.5.2. Legislation

The EU has legislation for patulin (Official Journal of the European Union, 2003a). The maximum levels of permitted contamination vary from 10 to 50 micrograms/kg. These limits are confined to fruit juices, fruit juices from concentrate, preserves and products derived from apples. The lowest permitted contamination of 10 micrograms per kg is for the same mentioned products but specifically designated for children consumption.

Switzerland have a maximum permitted concentration of 50 micrograms patulin/liter of apple juice. Poland, on the other hand, has a maximum permitted limit of 20 micrograms/liter (Smith et al., 1994).

7.5.3. – Occurrences of Patulin

Incidences of patulin contamination in fruit juices from 2 to 60 micrograms/liter have been found. In apple juice concentrates have

been detected contaminations between 460 to 1450 micrograms/liter; and in apple juice between 5 to 960 micrograms/liter. Moldy pears and apples with rotten spots have had patulin contaminations of 972 to 25760 micrograms/kg and 372 to 20634 micrograms/kg, respectively. The majority of the contaminated apples and pears were also contaminated with citrinin levels fluctuating from 11.84 to 28.44 micrograms/kg in apples and from 51.6 to 139.8 micrograms/kg in pears (COST, 2001; Martins et al., 2002).

7.5.4. Mycotoxicosis

Data concerning patulin toxicity is scarce. There are no epidemiological data or conclusive data that this mycotoxin is carcinogenic for humans. It is thought that exposure to patulin represents a higher risk for children ages 2 to 5 having an average body weight of 20 kg, since their average consumption of apple juice is 350 ml/week, whereas adults with an average body weight of 60 kg consume 430 ml of apple juice/week. Other studies assign consumptions of 216 ml of apple juice per day for children ages 1 to 2 with 12 kg average body weight and 200 ml/day for a 64 kg body weight person.

Assuming that the apple juice were contaminated uniformly with 10 micrograms of patulin/kg (maximum permitted level in the EU for children) and considering the apple juice consumption data, children ages 1 to 2 would be ingesting 0.18 micrograms of patulin/kg of bw/day (less than the PTDI value). Adults would be ingesting 0.16 micrograms of patulin/kg of bw/day (less than the PTDI value) if the apple juice is uniformly contaminated with 50 micrograms/kg (maximum permitted level by the EU for youngsters and adults).

The adverse gastrointestinal, immunotoxic, and neurotoxic effects produced by this mycotoxin in studies conducted with mice have not yet been extrapolated in humans. Studies resulting in immunotoxicity have been induced with patulin levels that are substantially higher than those normally ingested by humans (Smith et al., 1994; FDA, 2000).

8. SEVERAL RECOMMENDATIONS TO REDUCE MYCOTOXICOSIS

The following are general recommendations that can attenuate the effects of a mycotoxicosis in animals once these effects have already started. (Gimeno, 1999; Gimeno, 2000):

a. Increase the level of protein and energy in the diet, as well as the levels of some vitamins, specially riboflavin and D3, given that these vitamins help animals, especially poultry, to detoxify mycotoxins such as AFB1. On the contrary, a deficiency in thiamin has a protecting effect against aflatoxicosis since its deficiency mobilizes the lipid reserves, interfering with the hepatic metabolism of aflatoxins (Gimeno et al., 2003).

b. Provide the contaminated feed to adult animal, except breeding animals. The susceptibility to mycotoxins decreases with age.

c. Use low levels of broad spectrum antibiotics with vitamins and electrolytes in drinking water.

d. Increase the levels of methionine and cystine in the diet. These amino acids are the precursors of glutathione, which forms conjugated complexes with AFB1 inside the animal and especially in the liver. These complexes are then eliminated through feces and urine.

e. Maintain animals, especially poultry, at relatively low temperatures. Poultry are more susceptible to aflatoxicosis at high temperatures.

f. Reduce or eliminate factors that could produce stress in the animals such as sudden changes in temperature and moisture, vaccination, lack of water, inadequate ventilation or high levels of ammonia.

g. Reformulate the feed using a lower concentration of contaminated ingredients.

h. If the contaminated ingredient(s) can not be eliminated, give the feed containing the ingredient(s) to animals that are less sensitive, or not sensitive to the mycotoxin that is contaminating the feed.

9. PREVENTION, DECONTAMINATION, DETOXIFICATION, AND INACTIVATION STRATEGIES

The best prevention starts in the fields were much of the basic ingredients, such as cereal, are harvested. It is in these fields were contamination begins. The interest in developing genetically modified cereals or varieties resistant to toxicogenic mold growth and proliferation, and insect attack is greater every day. However, the potential adverse effects on humans who consume these cereals have limited the studies on these prevention methods.

Adequate use of fungistats, fungicides, and insecticides are recommended (caution should be exercised when using the last two, making sure that the residue levels are not harmful and within permitted parameters). These products not only reduce the possibility of fungi growth and proliferation, but they also maintain the physical integrity of the grain (concerning insect attack). Insects not only attack and deteriorate the grain, but also serve as transporting vectors that act as disseminators of microflora, contributing to fungi contamination (Fig. 20 and Fig 21). The grain intact integument protects fungi access into the endospermic starch. However, the insects break the grain's pericarp and the insect's metabolism elevates the moisture in the grain allows the fungi to grow in the interior of the grain. The internal part of the grain is more vulnerable to fungi than the external part, cuticle, of the grain.

Fig.20. Corn contaminated with Fusarium *spp, possible mycotoxin contamination (Alberto Gimeno).*

Figs.21. Deteriorated corn due to fungi contamination, and possible mycotoxin contamination (Alberto Gimeno

Birds also contribute to the deterioration of the grain. This should be avoided using adequate methods to scare them away from the fields, such as the classic scarecrow.

One of the first steps to have in consideration for eliminating or reducing mycotoxicosis is the implementation of a rigorous quality control system for purchasing and utilization of raw materials in the manufacturing of animal feed and food for humans. Other factors to be aware of are, constant hygiene and periodic disinfection of the storage of ingredients and of the food/feed manufacturing plants; and quality control analysis of finished products should be put in place in order to prevent mycotoxicosis risk.

Ingredients should be stored at 9-12% humidity, 0.65 water activity. These levels will drastically decrease the growth and proliferation of fungi that produce mycotoxin in stored ingredients.

Temperature in the interior of storage silos should be kept relatively low. The use of systems which forcibly introduce dry and cold air in these silos greatly helps avoid microflora proliferation zones and reduce moisture and temperature in the ingredient.

Generally, fungi grow and proliferate at a temperature greater than 20ºC and water activity greater than 0.70, which approximately corresponds to 12.5-13% humidity in the substrate (depending if the substrate is amylaceous or oleaginous).

The production of mycotoxins can take place starting at 0.85 water activity (production of mycotoxins, in general, are slim to none in less water activity). Bacterial growth takes place starting at 0.90 water activity.

Physical, chemical, and biological methods for the prevention, decontamination, detoxification, and inactivation of mycotoxins have been studied. Some of these studies are still in a preliminary phase of pilot plant or laboratory testing. Other studies are not practical due to their high cost or because of detoxification ineffectiveness. Other studies that are both effective and economical leave residues in food/feed that could be hazardous to the consumer's health.

These methods should be effective to work in facilities that are able to treat large quantities of food/feed or ingredients. Its application should be able to decontaminate, detoxify or inactivate high concentrations of mycotoxins. These methods should take into account that mycotoxins can be protected inside the substrate, bound to protein structures or other components. Additionally, these methods must take into account the fact that due to microflora zones, mycotoxins are not uniformly distributed throughout the ingredient. They must be efficient, affordable, and should not significantly modify the nutritional value of the food/feed ingredient. The treatment should not leave residues which could later have adverse effects on the health of humans or animals.

We will briefly mention several methods that can be applied, with some degree of efficacy, to prevent, decontaminate, detoxify, and inactivate mycotoxin. Some of these methods are common in the food/feed manufacturing industry.

9.1. Physical Methods

The ideal conditions when storing ingredients used in feed manufacturing are the following: a moisture level no greater than 12%, a water activity less than 0.70 and a temperature of 20-22ºC. Silos containing primary ingredients should be treated with cold, dry air or even carbonic dioxide since most fungi are anaerobic, therefore, a lack of oxygen will affect their growth and the total absence of oxygen can kill them. Cleaning and disinfection of all ingredient/feed circulating areas in the feed plant help reduce the growth of these toxicogenic molds, as well as the possibility of mycotoxin production. The greater problem originates when the ingredients have been contaminated with mycotoxins before storage.

Grain selection and the mechanical hulling of grains and the extraction of cereal powder is a useful technique of decontamination, given that the greatest concentration on mycotoxins is found in the pericarp of grains and in the cereal dust.

Thermal treatments can also have good results. During food/feed manufacturing, the extrusion and expanded processes or even pelleting temperatures of 70 - 80ºC are effective in reducing or eliminating the presence of fungi. However, mycotoxins are in general resistant to relatively high temperatures.

Thermal detoxification effectiveness is limited to the amount of pressure used during these treatments, and the exposure time to certain temperature. Greater exposure can detoxify feed/food ingredients more effectively, but can also deteriorate them, making them not suitable for consumption. Furthermore, thermal treatments can be ineffective in treating some contaminations. Some mycotoxins, such as aflatoxins, ochratoxin A and fumonisins are resistant to temperatures of 120, 100, and 150ºC, respectively. Patulin resists pasteurization and temperature of up to 100ºC. Vomitoxin or deoxynivalenol resists temperatures exceeding 150ºC, as well as those

reached during the production of bread, cookies and other wheat products. T-2 toxin, diacetoxyscirpenol and zearalenone resist temperatures of 120, 120 and 110ºC, respectively.

Stored pelleted feed submitted to deficient cooling conditions, can lead to unwanted condensation, creating optimal conditions for the growth and proliferation of fungi (Fig. 22), given that short exposure to a temperature of 70-80ºC is not enough to completely eliminate fungi. In some instances the pelleting process is performed even with temperatures below 70ºC.

Fig.22. Feed contaminated with high levels of fungi and possibly contaminated with mycotoxins (Alberto Gimeno).

Toasting or frying peanuts, nuts, corn and other foods at 150-200ºC for a period of 30 minutes can reduce AFB1 by 40 to 80%. The same process done to coffee beans, for a period of 5 minutes, reduces OTA by 80 to 90%. A temperature of 120ºC in autoclaves for a period of 30 to 40 minutes can reduce AFB1 contamination in corn meal, peanut meal, rice, fruits, and spices by 29 to 95%. Autoclaves at 120ºC for 3 hours can reduce the contamination of OTA in cereal based products by 70%. The manufacturing process of corn tortillas can reduce AFB1 contamination by 70%. Decaffeination of coffee reduces OTA contamination by 90% (Smith et al., 1994; EC, 1999; JECFA, 2001; WHO, 2002).

There are different pasteurization methods for milk, 64ºC for 20 minutes, 64-100ºC for 15-20 minutes and direct heating for 3-4 hours; all of them ineffective in reducing the AFM1 concentration in milk.

However, in pasteurization methods of 71-120ºC for 30 minutes it is possible to reduce the presence of AFM1 by 35%. Other sterilization and pasteurization methods as well as the Roller and Spray drying have reduced AFM1 concentrations by 32 to 86%. Observations showed that contaminated milk kept for 0, 6. 12, and 18 months at 4 and -20ºC had no significant reductions in mycotoxin content (Josephs et al., 2005).

There was not a significant decrease of AFM1 in cheese processed at 82-100ºC during 5-30 minutes, only a 9% reduction at 90ºC for 30 minutes. Cheese made from AFM1 contaminated milk, contained 40-60% of the total AFM1 present in that milk. Although AFM1 is very soluble in water, it is quite possible that during casein precipitation, it associates with AFM1 in such a way that it results in high levels of AFM1 in cheese rather than in serum (Yousef and Marth, 1989).

The simplest and most efficient technique to eradicate patulin contamination in manufactured fruit juices is by eliminating fruits containing mold (rotten spots) before processing said fruit. Traditional fruit juice production techniques reduce patulin concentrations by only 20%. However, filtration, centrifugation, and enzyme treatments can reduce patulin contamination by 70 to 77%. The greater part of patulin contamination in fruit juices is found in the pulp (Martins et al., 2002).

9.2. Chemical Methods

Chemical detoxification methods consistently reduce aflatoxin concentrations. Some of these methods are: ammonia treatments, calcium hydroxide treatments, and monomethylamine. These treatments can reduce aflatoxin concentrations by 98% in peanut meal, cottonseed meal and oilseeds by transforming these aflatoxins into non-toxic metabolites (aflatoxins B2a and G2a). Ammonia treatments decreased cysteine in ingredients by 15 to 30%; this treatment also caused discoloration of corn and the ammonia residue left an odd smell in the treated cereals. Calcium hydroxide and monomethylamine treatments did not reduce protein digestibility, net protein utilization, or interfere with the organoleptic characteristics of the primary ingredients (Giddey, 1977; Jemmali, 1983; CAST, 2003).

Boiling corn kernels in an aqueous solution of calcium hydroxide, followed by cooling and cleaning of the grains to remove the pericarp and excess calcium hydroxide can significantly reduce fumonisins

contamination by converting these fumonisins to their hydrolyzed form. Cleaned grains are used to manufacture corn products such as corn tortillas and other corn foods. However, the hydrolyzed products could also be toxic (FDA, 2000; JECFA, 2001; WHO, 2002).

9.3. Fungistats Utilization

Fungistats have been used for years in animal feed and human food as inhibitors of the metabolism, growth, and proliferation of fungi (mold and yeast). Fungi need to synthesize a series of enzymes in order to grow and proliferate. These enzymes are able to biodegrade the fungi substrate, and the degraded substrate becomes the source of nutrients for the fungi.

Fungistats act as inhibitors of the synthesis of various enzymes at a cellular level, stopping fungi metabolism, growth, and proliferation. Some fungistats modify intracellular pH, interfering with fungi metabolism, altering cell membrane permeability, making it difficult or inhibiting the transport of the bio-transformed substrate. These fungistats also inhibit the NADH (reduced form of nicotinamide adenine dinucleotide) oxidation, which is an important coenzyme in fungi metabolism. After using these fungistats, the risk of mycotoxin contamination will be reduced or eliminated. However, if mycotoxins are already present in the food, feed or ingredients, fungistats will have no effect on them.

The most common fungistats used in animal feed are: propionic acid and its calcium and sodium salts, ammonium propionate, sorbic acid and its potassium salt, formic acid and its calcium and sodium salts. The most common fungistats used in human food are: propionic acid and its calcium and sodium salts, sorbic acid and its potassium salt, which are used in cheese, wheat products, pastry, dry fruit, fruit juices, margarine, mayonnaise, wine, marmalade and jelly, and others.

The fungistats previously mentioned are often erroneously called «fungicides». Fungicides, however, destroy the cellular membrane of the fungi thus killing the fungi, as is the case of formaldehydes, pesticides, herbicides, and others. Fungicides are not authorized to be use directly on food/feed products, due to their toxic and pugnacious effects can cause serious consequences on animals and humans.

The improper use of fungistats is a risk since sub-inhibiting dosages can be metabolized by certain toxicogenic molds, favoring the production of mycotoxins (Smith *et al*, 1994).

9.4. Use of Mycotoxin Adsorbents and Bio-transforming Enzymes Additives.

There is currently a lot of interest in using adsorbing additives such as hydrated sodium and calcium aluminosilicates (HSCAS), which form part of the phyllosilicate clay family, and the use of sterified glucomannans in animal feeds. There are other types of clays that also have been used as adsorbents. The binding process occurs in the gastrointestinal tract of the animal, where mycotoxins are adsorbed, forming stable and irreversible inert compounds that are eliminated through the animal feces. Some of these clays have a limited action spectrum and limited adsorption efficiency. Some even could present the risk of absorbing nutrients. Currently, there are modified and purified phyllosilicates which appear to have better adsorption efficiency with a broader action spectrum.

The use of enzymes that transform mycotoxins is also prevalent. These enzymes are incorporated into the feed and biotransform mycotoxins into derived compounds within the animal, which the animal later eliminates through urine and feces. In some cases, these derived compounds can be less toxic or not toxic at all, compared to the original mycotoxin. However, this is not so for some mycotoxins. Compounds as or more toxic than the original mycotoxin can sometimes form during the intermediate reactions of the biotransformation.

Humans indirectly benefit from the incorporation of these additives in the feed. For example, in dairy cows, these additives can detoxify aflatoxin B1; eliminating the risk that AFB1 would be transformed into AFM1. If the detoxification is effective for other mycotoxins, this would prevent toxic residue from accumulating in edible tissues consumed by humans.

9.5. Biological Methods

There have been studies conducted with some microorganisms such as *Sacharomices cerevisiae, Flavobacterium aurantiacum, Rhizopus spp, Neurospora sitophila*, and rumen microorganisms that degrade mycotoxins under certain conditions. Effective results in the degradation of aflatoxins, ochratoxin A, patulin, zearalenone, T-2 toxin,

diacetoxyscirpenol, and rubratoxin A have been obtained in the laboratory. However, the practical application of these biological methods is still under development or studies.

9.6. Silage Preparation

Finally, we would like to highlight the care that must be put into silage preparation for dairy cow consumption. The humidity level of silage is very high and ideal for mold growth and the possibility of mycotoxin production.

Oxygen or the ratio between oxygen and carbon dioxide is vital for fungi growth and its potential mycotoxins production, since the majority of fungi are aerobic. Therefore, it is vital to ensure that silage preparation is done in a completely anaerobic atmosphere.

The following are some practical recommendation for silage preparation that will help avoid fungi and mycotoxin contamination (Gotlieb, 2002):

1.- The raw material being used for silage should be resistant to insect attacks and plant diseases which cause rotting stem, ear or tassel.

2.- Harvest and silage the cereal at a free water (humidity) level adequate for the storage facility. Do not leave the cereal in the field after maturity.

3.- Cut stems homogeneously and sharply. These should have the right length to be properly packed.

4.- Do not delay the harvest of forage. Package in compact form so as to leave as little air space as possible.

5.- Use acidifying additives and fungistatic mixes, incorporating them to the silage in order to increase fermentation, assuring a better, safer storage.

6.- Make sure that the silo is securely closed in order to maintain an anaerobic atmosphere. Cover forage with plastic and place heavy weights on top. The use of tires is practical and gives good results.

7.- Eliminate spoiled forage.

8.- Make sure water does not enter the silo when it rains.

9.- Take out all forage residues from the feeders and clean them before putting in fresh feed.

10. COMMENTS

All animal species tested were sensitive to the toxic effects of aflatoxin B_1, especially younger animals. Rabbits are particularly sensitive to the toxicity of aflatoxin B1, having adverse reactions to concentrations as low as 15 ppb. On the other hand, older chickens are more resistant to problems associated with aflatoxin contamination.

The European Union legislation (Official Journal of the European Union, 2003b) establishes the following maximum permitted aflatoxin B1 concentrations in feedingstuffs and feed materials with a moisture of 12%:

a.20 ppb (micrograms/Kg) of AFB1 in cattle, sheep, and goat complete feedingstuffs (except complete feedingstuffs destined for dairy animals, calf, or lamb).

b. 5 ppb of AFB1 in complete feedingstuffs for dairy animals.

c. 10 ppb of AFB1 in calf and lamb complete feedingstuffs.

d. 20 ppb of AFB1 in swine and poultry complete feedingstuffs (except young animals).

e. 10 ppb of AFB_1 in complete feedingstuffs for other animals (including young birds, young pigs and rabbits).

f. 20 ppb of AFB1 in complementary feedingstuffs for cattle, sheep, and goats (except complementary feedingstuffs for dairy animals , calves and lambs).

g. 20 ppb of AFB1 in complementary feedingstuffs for pigs and poultry (except young animals).

h. 5 ppb of AFB1 in other complementary feedingstuffs.

i. 20 ppb of AFB1 in all feed materials.

This rigorous maximum contamination limits in dairy animals are due to legislation establishing a maximum permitted concentration of 0.05 ppb AFM1 residue in milk and milk products destined for human (Official Journal of the European Communities, 2002a; Official Journal of the European Communities, 2002b; Official Journal of the European Union, 2003; Mycotoxins, 2003) and 0.025 ppb of AFM1 in milk and milk products destined for children (Official Journal of the European Union, 2004).

Apart from the EU legislation criterion, the following maximum tolerable concentrations for AFB1 in feed for different species can be established from field observations ((Jones *et al.,* 1994):

- Young poultry, 20 ppb
- Laying hens, 50 ppb
- Pigs weighing less than 34 kg body weight, 20 ppb
- Pigs weighing 34 to 57 kg body weight, 50 ppb
- Pigs weighing more than 57 kg body weight, 100 ppb
- Reproductive sows and boars, 50 ppb
- Dairy cattle, 24 ppb
- Rabbits, 8 ppb

These maximum contamination levels are simply practical recommendations, and have nothing to do with European Union legislation.

Chickens, hens, ducks, turkeys, and pigs are very sensitive to the toxicity of ochratoxin A. This sensitivity is not true for dairy cows or rabbits. The detoxifying action of the ruminal fluid in dairy cows could be responsible for this. The type of complete feed given to dairy cows has a large influence on the effectiveness of the ruminal fluid to detoxify ochratoxin A. The authors have no data regarding levels of ochratoxin A and its toxic effects when inadequate feed impairs the detoxifying effects of the ruminal fluids.

A Commission Recommendation of 17 August 2006 (Official Journal of the European Union, 2006) was published with a Guidance Values for ochratoxin A relative to a feedingstuff with a moisture

content of 12%. The Guidance Values are the following :

a. 250 ppb (micrograms/Kg) in cereals and cereals products (cereal forages and roughages are also included).

b. 50 ppb and 100 ppb in complementary and complete feedingstuffs for pigs and poultry, respectively.

From the data collected, we can conclude that chickens and hens are very resistant to the toxic action of zearalenone, which is not the case for swine, dairy cows, and rabbits. The following maximum tolerable concentrations for zearalenone in complete feedingstuffs for different species can be established from field observations (Jones *et al.,* 1994): Piglets and fattening pigs, 200 ppb; reproductive sows and boars, 100 ppb; and dairy cows, 250 ppb.

The Guidance Values EU Commission (Official Journal of the European Union, 2006) for zearalenone relative to a feedingstuff with a moisture content of 12%, are the following:

a. 2000 ppb (micrograms/Kg) in cereals and cereals products (cereal forages and roughages are also included) except corn by-products.

b. 3000 ppb in corn by-products.

c. 100 ppb in complementary and complete feedingstuffs for piglets and young sows.

d. 250 ppb in complementary and complete feedingstuffs for sows and fattening pigs.

e. 500 ppb in complementary and complete feedingstuffs for calves, dairy cattle, sheep (including lamb) and goats (including kids).

Rabbits and pigs proved to be more sensitive to the toxicity of fumonisin B_1 than dairy cows, chickens, hens, ducks and turkeys. Nonetheless the Guidance Values EU Commission (Official Journal of the European Union, 2006) for fumonisins B1+B2 relative to a

feedingstuff with a moisture content of 12% (these Guidance Values which will be applied from 1 October 2007), are the following :

a. 60000 ppb in corn and corn products (corn forages and roughages are also included).
In complementary and complete feedingstuffs:

b. 5000 ppb (micrograms/Kg) for pigs, horses (*Equidae*), rabbits and pet animals.

c. 10000 ppb for fish.

d. 20000 ppb for poultry, calves (< 4 months), lambs and kids.

e. 50000 ppb for adult ruminants (> 4 months) and mink.

Chickens, rabbits, and turkeys are very resistant to the toxic action of DON, whereas hens, pigs, and dairy cows proved to be very sensitive to this mycotoxin. Relatively moderate concentrations of DON, 5800 ppb given to ducks during short periods of time did not have adverse effects.

Based on statistical data gathered from field observations, the following maximum tolerable concentrations of DON were established: piglets and fattening pigs, breeding sows and boars, and dairy cows, less than 300 ppb (Jones et al., 1994).

The Guidance Values EU Commission (Official Journal of the European Union, 2006) for deoxynivalenol (DON) relative to a feedingstuff with a moisture content of 12%, are the following :

a. 8000 ppb in cereals and cereal products (cereal forages and roughages are also included) with the exception of corn by-products

b. 12000 ppb in corn by-products.

c. 5000 ppb in complementary and complete feedingstuffs with the exception of:

d. 900 ppb in complementary and complete feedingstuffs for pigs.

e. 2000 ppb in complementary and complete feedingstuffs for calves (< 4 months), lambs and kids

The data collected and reviewed shows that all the animal species tested were sensitive to the toxic action of T-2 toxin. Turkeys experienced problems when T-2 toxin concentrations were higher than 1000 ppb. However, when T-2 toxin levels were accompanied in concentrations up to 1000 ppb with diacetoxyscirpenol, serious oral lesions appeared.

Both poultry and pigs are sensitive to the toxicity of diacetoxyscirpenol. The effects of this mycotoxin on dairy cows and rabbits are unknown to us given the lack of information. Concentrations higher than 1000 ppb can cause problems in turkeys. Diacetoxyscirpenol contamination accompanied by T-2 toxin contamination of 1000 ppb induced serious oral lesions.

There are not guidance values for T-2 toxin from the Commision of the European Union because according to the scientific opinions and the lack of reliable data on T-2 and HT-2 toxins, together with the large year to year variation in occurrence of these mycotoxins, it is appropiate collect more data on these mycotoxins in the different feed materials and feedingstuffs, in addition to the data already available fron the coordinated control programmes for 2002, 2004 and 2005 years (Official Journal of the European Union, 2006).

It is also important for reading the «whereas and hereby recommends published in the Official Journal of the European Union, 2006

We only have data relevant to chickens and hens concerning the trichothecenes monoacetoxyscirpenol, triacetoxyscirpenol and escirpentriol. Chickens are sensitive to all these mycotoxins. We do not have data for hens concerning triacetoxyscirpenol or escirpentriol. However, we can conclude from our data that the concentration levels of monoacetoxyscirpenol needed to cause problems are higher than those found as natural contaminants.

It is very difficult to have significant conclusions in a complex subject such as mycotoxicosis in human, because many other important contaminations risks (heavy metals, hydrocarbons, pesticides, dioxins or bacterial contaminations) can occur at the same time or when mycotoxin toxicity symptoms are complicated with individual health problems.

Legislating mycotoxins is always a difficult process given that there are certain basic factors that need to be in place before creating legislation:

a) availability of toxicological data;

b) availability of data concerning mycotoxin incidence in differents feeds and foods;

c) homogeneousness of mycotoxins in food (hot spots);

d) availability of analytical methods for control of minimum detectable mycotoxin levels;

e) legislation in countries importing or exporting feed, food or ingredients and the need for a sufficient food supply (Van Egmond, H.P., 1999).

Legislation and regulations concerning mycotoxins in 88 countries can be found in the reference CAST, 2003.

AFB1 is the mycotoxin with the highest risk for humans, due to its carcinogenetic risk all over the world concerning liver cancer.

Previously mentioned references (CCFAC, 1999; CCFAC, 2000; CCFAC, 2001; CODEX, 2002) have the results of the arguments concerning maximum contamination levels of AFM1 being 0.05 micrograms / kg (UE) versus 0.50 micrograms / kg (USA and other countries).

Taking into account the concerns about public health, the EU maintains a maximum contamination level of 0.05 micrograms/kg in milk and 0.025 micrograms/kg in dairy products for nursing children.

The maximum concentration level should follow the ALARA (As Low As Reasonably Achievable) principle, contrary to the opinion of countries that defend the 0.50 micrograms/kg level.

Even though AFM1 has a 9 to 10 times lower carcinogenic potential than AFB1, and the previously mentioned studies (JECFA, 2001; WHO, 2002) conclude that the estimated additional risk of developing liver

cancer when the contamination level change from 0.05 micrograms/kg to 0.50 micrograms/kg is insignificant, any exposure to a genotoxic carcinogenic, as is the case of AFM1, can present a sanitary risk for the consumer, especially children. This reinforces the use of the ALARA principle, which states that there is no dose, of these types of carcinogenic, which does not produce malignant tumors. Hence, the exposure should be 0 in order to completely avoid liver cancer caused by aflatoxins in general. The EU Scientific Committee suggests that risks derived from exposure to aflatoxins must be carefully evaluated, since the intake of milk and dairy products is considerable among children.

There is not enough evidence suggesting that OTA is a carcinogenic for humans. However, there is an awareness that exposure to mycotoxins must be reduced in order to decrease nephropathy problems attributed, in some countries, to the intake of food contaminated with OTA. The EU has established a rigorous legislation for OTA in a variety of foods.

Although fumonisins have been considered possible carcinogenic, countries where incidences of esophagus cancer and gastrointestinal problems are significant, have not regulated the contamination of food with fumonisins, especially FB_1. Nonetheless, some of these countries relate those cancer problems with de fumonisins occurrence in foods. The FDA is the only organization that has established regulations for fumonisins in relation to some foods. We consider that more studies should be done concerning the risks posed by exposure to these mycotoxins.

Vomitoxin or deoxynivalenol seems to receive special attention in countries were children consume large quantities of wheat products, because there is a concern that exposure to this mycotoxin increases the risk of growth retardation.

Vomitoxin can contaminate foods in combination with other trichothecenes mycotoxins, such as T-2 toxin, complicating its evaluation of risks.

Perhaps patulin is, at the moment, the least relevant mycotoxin concerning mycotoxicosis in humans. However, studies on its toxicity

continue since the potential risk for children, whom consume large quantities of fruit juices. As previously mentioned, the EU has legislation for this mycotoxin.

The data we have concerning mycotoxins residue in meat and eggs has been collected from published experimental tests where the mycotoxin contamination in the animal feed had to be extremely high in order to create significant residue concentrations. We must also keep in mind that cooking these foods before being consumed can reduce contamination levels. Therefore, residue in edible tissues is not relevant to the topics addressed in this manual (Gimeno and martins, 2002a and 2002b).

This manual is not intended to cause alarm, but rather to communicate the current state of mycotoxins. The consumption of a variety of feeds and foods helps to reduce the risk of mycotoxicosis. However, there are countries or populations within a country that do not have the economic means to vary their dietary intake not only for humans but also for animals. For this reason, the efforts to create better and safer prevention methods of detoxification and inactivation, and the rigorousness with which mycotoxin levels are controlled in food and feed, should continue.

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