

T-2 TOXIN: INCIDENCE AND TOXICITY IN POULTRY

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T-2 toxin is the most toxic type A trichothecene mycotoxin. It is the secondary metabolite of the *Fusarium* fungi, and is common in grain and animal feed. Toxic effects have been shown both in experimental animals and in livestock. It has been implicated in several outbreaks of human mycotoxicoses. Toxic effects in poultry include inhibition of protein, DNA, and RNA synthesis, cytotoxicity, immunomodulation, cell lesions in the digestive tract, organs and skin, neural disturbances and low performance in poultry production (decreased weight gain, egg production, and hatchability). Concentrations of T-2 toxin in feed are usually low, and its immunosuppressive effects and secondary infections often make diagnosis difficult. If at the onset of the disease, a change in diet leads to health and performance improvements in animals, this may point to mycotoxin poisoning. Regular control of grain and feed samples is a valuable preventive measure, and it is accurate only if representative samples are tested. This article reviews the incidence and toxic effects of T-2 toxin in poultry.

KEY WORDS: *cytotoxicity, genotoxicity, trichothecene mycotoxins*

T-2 toxin is a member of a large group of fungal metabolites with the same basic chemical structure, called trichothecene mycotoxins. There are nearly 190 closely related chemical compounds in the group of trichothecene toxins, named after the first isolated trichothecene molecule *trichothecin* (1-6). The basic structure of these molecules is tetracyclic, with a sesquiterpenoid 12,13-epoxytrichothec-9-ene ring system (1, 7, 8). They are divided in four types (A-D), depending on the presence (macrocytic trichothecenes) or absence (non-macrocytic trichothecenes) of macrocyclic ring between C-4 and C-15, and further according to the number of hydroxyl and acetoxy groups attached to a carbon atom.

T-2 toxin was first isolated from the mould *F. tricinctum* (*F. sporotrichoides*) (7, 9). It belongs to non-macrocytic type A trichothecenes. Its chemical structure is characterised by a hydroxyl (OH) group at the C-3 position, acetyloxy (-OCOCH₃) groups at C-4 and C-15 positions, atom of hydrogen at C-7 position and an ester-linked isovaleryl [OCOCH₂CH(CH₃)₂]

group at the C-8 position (Figure 1) (10). It is produced primarily by *Fusarium* species *F. acuminatum*, *F. nivale*, *F. oxysporum*, *F. poae*, *F. sporotrichoides*, and *F. solani*. However, moulds belonging to other genera (*Trichoderma* sp., *Myrothecium* sp.) were also found to produce T-2 toxin (1, 4, 7, 9-14).

Chemical characteristics

T-2 toxin is a non-volatile, low-molecular-weight compound (MW 466.52) insoluble in water and petroleum ether, but highly soluble in acetone, ethyl-acetate, chloroform, dimethyl sulphoxide, ethyl alcohol, methyl alcohol and propylene glycol (15). It is highly resistant to heat and UV light (11, 16). Therefore, it is not inactivated in food production and processing or by autoclaving. T-2 toxin is inactivated by heating at 200 °C to 210 °C for 30 min to 40 min, or by soaking in sodium hypochlorite - sodium hydroxide solution for at least four hours (11). Some bacteria and moulds have the ability to transform and detoxify T-2 toxin (17, 18).

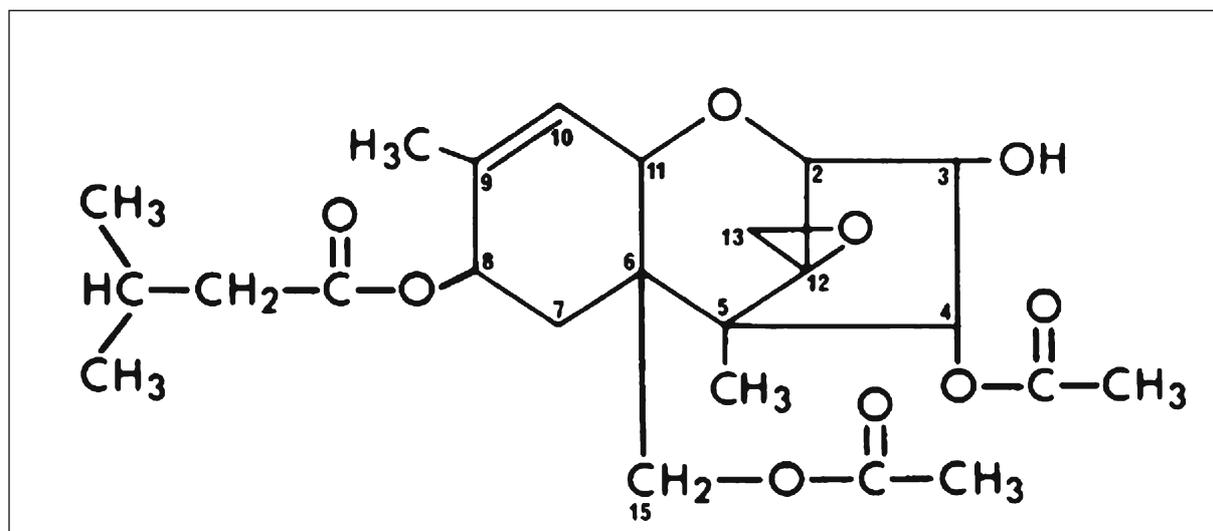


Figure 1 Chemical structure of T-2 toxin. (1)

Natural occurrence in grain and feed

Although reported natural occurrence of T-2 toxin and related mycotoxins shows their worldwide presence, they are predominant in tropical and subtropical regions. Warm and moist weather conditions favour plant infection with *Fusarium* spp., while improper storage and handling of grain with high moisture content can lead to T-2 toxin contamination (19, 20). In short, the most important factors that influence T-2 toxin production are weather conditions, grain defects and moisture content (13 % to 22 %). T-2 toxin is produced at a wide temperature range (0 °C to 32 °C), with maximum production at temperatures below 15 °C (13, 21-23). Namely, *F. sporotrichioides* has a low optimal temperature (6 °C to 12 °C) for T-2 toxin production and can produce this mycotoxin during overwintering under a snow cover in the field and/or during storage (24-26). Among all grains tested so far, corn, wheat, barley, oat, and rye are most frequently contaminated with this mycotoxin (3).

Compared to the related mycotoxin deoxynivalenol, T-2 toxin is less frequent in grain and other agricultural products (3, 8, 27). According to the EU reports, type B trichothecenes such as deoxynivalenol, nivalenol and fusarenon X are more frequent (57 %, 16 % and 10 % of tested grain samples) in European grain samples than type A trichothecenes. However, T-2 toxin is the most common type A mycotoxin (20 % of tested samples), while other toxins of this group are less common: HT-2 toxin (14 %), T-2 tetraol (6 %), neosolaniol (1 %), diacetoxyscirpenol (DAS) (4 %), monoacetoxyscirpenol (MAS) (1 %). Average T-2 toxin mass fraction in grain ranges between 0.03 mg kg⁻¹

and 0.155 mg kg⁻¹ (3, 28-30). A similar incidence of T-2 toxin in grain (13 % to 30 %) with the average mass fraction from 0.01 mg kg⁻¹ to 0.71 mg kg⁻¹ has been reported by other authors (26, 31, 32). In contrast, reports by Haubruge et al. (33) and Rasmussen et al. (20) indicate high incidence of T-2 toxin in grain samples (65 % to 76 %) with average mass fraction from 0.01 mg kg⁻¹ to 0.1 mg kg⁻¹ and maximum value of 3.75 mg kg⁻¹. Sample contamination this high was in a direct relationship with the climate (samples from a warm climate and samples collected after rainy summer period, respectively).

In Croatia in 1988, T-2 toxin and related toxin diacetoxyscirpenol were present in 53.5 % of poultry feed samples in the level from 0.1 mg kg⁻¹ to 0.2 mg kg⁻¹ (34). In a ten-year period (1989-1998) T-2 toxin was found in 18 % of tested samples (35-37). Between 1998 and 2004, T-2 toxin was detected in 16.8 % of tested samples (grain and poultry feed) in mass fractions from 0.1 mg kg⁻¹ to 0.7 mg kg⁻¹. In comparison, other related toxins such as diacetoxyscirpenol and deoxynivalenol were detected in 27.6 % and 41.2 % of the tested samples, respectively, and the levels of these toxins ranged from 0.1 mg kg⁻¹ to 1.2 mg kg⁻¹ and from 0.05 mg kg⁻¹ to 3.44 mg kg⁻¹, respectively (38).

Toxicity of T-2 toxin in poultry

The toxicity of the T-2 toxin and related mycotoxins can be affected by a variety of factors such as administration route, time of exposure, the number of exposures, dose, animal's age, sex and overall health, and presence of other mycotoxins (39-41). The

12,13-epoxide ring of T-2 toxin is responsible for its toxic activity and de-epoxidation results in the loss of any apparent toxicity. Furthermore, toxicity is lowered by deacetylation during which HT-2 toxin is produced as the first metabolite, while the final product of this reaction is scarcely toxic T-2 tetraol (8, 11, 15, 16, 42). A dose T-2 toxin that would kill 50 % of a seven-day-old broiler population (LD_{50}) is 4.97 mg kg^{-1} . In comparison with other mycotoxins in seven-day-old broilers, T-2 toxin is more toxic than aflatoxin ($LD_{50} = 6.8 \text{ mg kg}^{-1}$), HT-2 toxin ($LD_{50} = 7.22 \text{ mg kg}^{-1}$), scirpentriol ($LD_{50} = 9.33 \text{ mg kg}^{-1}$), neosolaniol ($LD_{50} = 24.87 \text{ mg kg}^{-1}$), T-2 tetraol ($LD_{50} = 33.79 \text{ mg kg}^{-1}$), and deoxynivalenol ($LD_{50} = 140 \text{ mg kg}^{-1}$), and is equally or less toxic than ochratoxin ($LD_{50} = 2.1 \text{ mg kg}^{-1}$) and diacetoxyscirpenol ($LD_{50} = 2.0 \text{ mg kg}^{-1}$ to 5.9 mg kg^{-1}) (8, 43). The lethal dose of T-2 toxin in feed during a feeding period of seven days is about 10 mg kg^{-1} of chicken body weight (44).

Mechanism of action

After exposure by the oral, dermal or inhalation route, T-2 toxin can cause severe effects in various animal organs and tissues. So far, toxic effects have been evidenced in the cells of fungi, protozoa, insects, moulds, plants, and different cell cultures (39-41, 45-47). In poultry, the toxic effects of T-2 toxin can be classified as genotoxic and cytotoxic, immunomodulatory effects, effects on the cells of the digestive system and liver, effects on the nervous system and skin and impairment of poultry performance.

Genotoxic and cytotoxic effects

T-2 toxin inhibits DNA, RNA, and protein synthesis in eukaryotic cells, affects the cell cycle, and induces apoptosis both *in vivo* and *in vitro* (2, 3, 5, 48-51). The chemical structure of T-2 toxin molecule (position of chemical groups on the trichothecene ring) again has an essential role that determines the mode and target of action, because it specifies the interaction with protein molecule. Thus T-2 toxin, like HT-2 toxin and diacetoxyscirpenol, inhibits polypeptide chain initiation, while other trichothecenes affect elongation (trichothecin) and termination (deoxynivalenol) (52-54). The mechanism of action resembles the action of certain antibiotics (lincosamides, streptogramins, macrolide antibiotics) on bacterial cells (55).

Cytotoxic effects of toxin have been reported in lymphoid cells (52), while induction of DNA strand

breaks caused impairment of the immune system (56, 57). Highly sensitive to T-2 toxin activity are actively dividing cells (cells of the gastrointestinal tract, bone marrow, lymph nodes, spleen, and liver). Cytotoxic radiomimetic effects of T-2 toxin are considered to be a result of primarily impaired protein synthesis, and consequently of the inhibition of DNA and RNA synthesis (8, 58, 59). Furthermore, *in vivo* T-2 toxin can induce polyploidy in *Allium cepa* (60), sex-linked recessive lethal mutations in *Drosophila melanogaster* (61), DNA single-strand breaks in thymus and spleen of BALB/c mouse (56), chromosomal aberrations in Chinese hamster bone marrow and mice (62, 63), and DNA damage in chicken peripheral lymphocytes (64). According to the results of studies on the genotoxicity of T-2 toxin *in vitro*, T-2 toxin can induce DNA single-strand breaks in primary hematocytes, thymic and spleen lymphocytes of BALB/c mouse (56), gene mutations and sister chromatid exchange in Chinese hamster V79 fibroblasts (65-68), formation of micronucleus in Chinese hamster V79 fibroblasts (67), unscheduled DNA synthesis in human fibroblasts (69), and inhibition of intercellular communication in Chinese hamster V79 cells (70). In addition, T-2 toxin and related mycotoxins can induce apoptosis *in vitro* (49, 71, 72) and *in vivo* in haematopoietic tissue, spleen, liver and intestinal crypts of mice (73-76). In chicken, apoptosis was detected in the thymus, but not in the spleen (57). T-2 toxin induces apoptosis depending on the activation of JNK and p38 MAP kinases, but the precise mechanism has not yet been elucidated.

Effects on the immune system

T-2 toxin is a mycotoxin with immunomodulatory activity, e.g. it can stimulate (immunostimulation) or inhibit (immunosuppression) the activity of the immune system. Its mode of action is time- and dose-dependent. Immunosuppression is the result of action of high doses that cause damage to the bone marrow, lymph nodes, spleen, thymus and intestinal mucosa, leucopenia and consequently increased susceptibility to infection with pathogens (*Listeria monocytogenes* and *Salmonella sp.*) (13, 72, 77). On the other hand, immunostimulation is caused by low doses of the toxin, and is evidenced by increased serum IgA and IgE antibodies because of rapid and transient activation of the genes responsible for the function of the immune system as well as genes important for inflammation response (59, 72, 78, 79).

Immunotoxicity of type A trichothecene (especially of T-2 toxin) is significantly lower than that of type B-trichothecenes (80). However, T-2 toxin can induce necrosis and depletion of lymphoid cells in the thymus, spleen, and lymph nodes of chicken and pullets (50, 81, 82). Exposure of chicken to T-2 toxin caused increased mortality by *Salmonella* infection (44, 83), lower antibody titres against Newcastle disease, and infectious bursal disease (84-85). Although molecular and cellular mechanisms of action of T-2 toxin and other mycotoxins (aflatoxin, ochratoxin and related trichothecenes) are quite different, immunosuppressive effects are the result of direct or indirect inhibition of protein synthesis (59). Since most of the research on T-2 toxin effects on the immune system has been done on laboratory animals, evaluation of possible effects in poultry still needs to be explained.

Effects on the digestive system and liver

T-2 toxin can have toxic effects on almost all cellular processes in the digestive system. Even a small dose of the toxin can damage the mucosa of the digestive tract and impair resorption of nutrients. Necrotic damages have been detected in the mouth (86), gizzard tissue, intestinal mucosa and liver (87, 88). Necrotic lesions in the digestive tract are characterised by white-yellowish mucosal bulge containing caseous-necrotic material (89). Lesions in the mouth and decreased average daily gain in poultry are detected after a single application of the toxin in the dose of 5 mg kg⁻¹, but more frequently after long-term feeding with contaminated feed (1 mg kg⁻¹ to 5 mg kg⁻¹) for at least one week (90, 91). Other type-A trichothecenes such as HT-2 toxin, diacetoxyscirpenol, monoacetoxyscirpenol and scirpentriol (50, 86) can also cause these above mentioned lesions. T-2 toxin and related trichothecenes are quickly absorbed in the intestinal tract, metabolised, and eliminated almost completely (80 % to 90 %) within 48 hours (92-94). However, their toxic effect can be increased by entero-hepatic recirculation (8). Detoxification of T-2 toxin and related trichothecenes by intestinal microflora has not been demonstrated in poultry (95).

The main target of the toxic effects of T-2 toxin *in vivo* is the liver. Inhibition of protein synthesis reduces the activity of the enzymes necessary for the metabolism of toxic substances, induces lipid peroxidation, and increases the activity of glutathione reductase (96). However, these changes in enzyme

activity are usually not dose- and time dependent, and are not useful as biomarkers for the differential diagnosis of T-2 toxicosis and other disorders.

Effects on the nervous system and skin

T-2 toxin and deoxynivalenol act as neurotoxins by damaging the blood-brain barrier (97). Changes such as the loss of appetite, muscular coordination problems, and vomiting (characteristic for deoxynivalenol) have been detected in animals that were eating feed contaminated with trichothecenes (50, 81, 98). These are explained by neuro-chemical changes in the brain and changes in the activity of serotonin. Serotonin synthesis in the brain depends on the amino acid triptophan and enzyme triptophan-hydroxylase. Since the concentration of tryptophan correlates with the concentration of neutral amino acids in the blood, it is assumed that increased blood amino acid concentrations, caused by the inhibition of protein synthesis in the liver and other tissues, increase the concentration of tryptophan in the brain. Consequently, serotonin synthesis and activation of serotonergic neurons are also increased (96). In addition, there is evidence of dopamine concentration increase and a decrease in norepinefrine concentration. All these neural disorders have only been detected in a small number of animals (50, 98).

Dermatotoxic effects of T-2 toxin are characterised as necrohaemorrhagic dermatitis. Some animals showed depigmentation of the skin of the legs and comb cyanosis. Very low feather quality and abnormal position of the wings were found in animals that consumed feed contaminated with high levels of T-2 toxin (4 mg kg⁻¹ to 16 mg kg⁻¹) (50, 99).

Effects on poultry performance

Symptoms of T-2 toxicosis are nearly the same as of the toxicosis caused by other trichothecenes. Usually the differences are only in the extent and severity of changes. First visual signs of the poisoning with T-2 toxin are lower feed intake, reduced weight gain and growth retardation (50, 99, 100). Laying hens had lower egg production (101), lower egg and shell weight and thinner egg shells and decreased hatchability (99, 100). Lower egg production was detected at the dose of 1 mg kg⁻¹ of T-2 toxin (12.5 %), while the dose of 5 mg kg⁻¹ and 10 mg kg⁻¹ led to a decrease of 68 % and 78.9 %, respectively. Changes in the quality of egg shell were detected only at a high toxin level of 20 mg kg⁻¹ (102).

In brief, the following symptoms may indicate the onset of potential poisoning with T-2 toxin: decreased feed intake, growth inhibition with (s)lower weight gain, mouth lesions and depigmentation of the leg skin, changed feather quality, and neural disturbances. In laying hens, poisoning is indicated by reduced egg production (with thinner egg shell), decreased hatchability, reduced feed intake, cyanosis of the comb, leucopenia, and changed feather quality. A conclusive diagnosis is based on anamnesis, clinical and pathological analysis, and detection of characteristic necrotic lesions in the mouth, crop, gizzard, intestinal mucosa and the liver (50).

Regulatory measures and recommendations

In the countries of the European Union, a new monitoring scheme is in use that can detect T-2 toxin and other mycotoxins in cereals and their products. Several guidelines have also been issued which include recommended levels for deoxynivalenol (0.9 mg kg⁻¹ to 12 mg kg⁻¹) and fumonisins (1 mg kg⁻¹ to 50 mg kg⁻¹) for various feed and animal species (103, 104). Since data about the occurrence and toxic effects of T-2 and HT-2 toxin are missing, there are no specific regulations or recommendations of The European Commission about the maximal concentrations in products intended for animal feed. Some other countries have set their guidance values for T-2 toxin in products intended for animal feed. In Ukraine these levels for all trichothecenes are 0.2 mg kg⁻¹ in combined feed for layers and broilers and 0.25 mg kg⁻¹ in feed for calves and older cattle fed for beef) and in Serbia and Montenegro 0.3 mg kg⁻¹ in feed for chickens and pigs and 0.6 mg kg⁻¹ in feed for swine, cattle, and other poultry). In China, T-2 toxin limit in complete feed for all animals is 0.08 mg kg⁻¹. A limit of 0.1 mg kg⁻¹ for T-2 toxin has been set in Israel for all grain and in Iran for complete feed intended for sheep, goats and beef cattle. Complete feed intended for calves, lambs, kids, dairy sheep, goats and cattle in Iran has a limit of only 0.025 mg kg⁻¹ of T-2 toxin. In Canada, feed for swine and poultry can contain up to 1.0 mg kg⁻¹ of T-2 toxin, while feed for cattle and poultry has also a limit of 0.1 mg kg⁻¹ of HT-2 toxin (105). In Croatia, maximal allowed level of T-2 toxin, including its derivative HT-2, in complete and supplemental feed for pigs, poultry and calves is 0.5 mg kg⁻¹ (106).

In conclusion, guidance levels, if there are any, vary a lot between countries, and regulations are

partial. Mycotoxin contamination of grain and feed and the ensuing consumption of these ingredients by animals is an inevitable part of animal production systems. Contamination may be avoided by the use of mould-resistant crops, application of agro-technical measures, and decontamination of food and feed (107-109). It is also recommended that in a case of poisoning, the feed should be completely withdrawn as it can result in fast health improvement. Detection of mycotoxins in feed (representative sample) of sick animals can help the diagnosis. Bear in mind however that detection of moulds in grain, feed, and food is not a proof of mycotoxin contamination, but a sensitive indicator of potential contamination (8). According to the collected data on the occurrence, toxicity, metabolism in animals, and clinical signs of toxicosis in poultry, it has been estimated that the total amount of all of trichothecenes, including T-2 toxin, in poultry feed should not exceed 0.5 mg kg⁻¹ (110). It is therefore advisable to at least monitor the occurrence of T-2 toxin and to use this level as guidance when toxicosis is suspected.

Moreover, mycotoxins represent a public health concern. Several reports have associated outbreaks of human disease with the presence of trichothecenes in food (40, 41). Additionally, animals consuming contaminated feed can indirectly pose a threat for humans because of potentially present residues of these toxins in animal-derived food products. As genotoxicity and cytotoxicity data indicate that T-2 toxin is highly toxic, and as it is widespread in cereals and food, additional research of its toxic potential in animals and in humans is necessary.

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Sažetak

T-2 TOKSIN - POJAVNOST I TOKSIČNOST U PERADI

T-2 toksin je najtoksičniji predstavnik trikotecenskih mikotoksina tipa A. On je sekundarni produkt metabolizma plijesni roda *Fusarium* i često je prisutan u žitaricama i hrani za životinje. Štetni učinci uočeni su u eksperimentalnih životinja i životinja u uzgoju. On se povezuje s pojavom bolesti ljudi od mikotoksikoza. Učinci toksina u peradi su višestruki: inhibicija sinteze proteina, DNA i RNA, citotoksični učinak, imunomodulatorni učinak, oštećenje stanica probavnog sustava, organa i kože, živčani poremećaji te pad proizvodnih karakteristika u uzgoju peradi (slabiji prirast, pad nesivosti i valivosti). Koncentracije T-2 toksina u hrani redovito su vrlo malene, a zbog imunosupresivnog djelovanja toksina te istodobne sekundarne infekcije bolest se često teško dijagnosticira. Pri pojavi bolesti promjenom hrane može doći do poboljšanja zdravstvenog stanja, što također upućuje na moguće trovanje mikotoksinima. Redovita kontrola uzoraka žitarica i hrane za životinje jedna je od preventivnih mjera, a detekcija mikotoksina u žitaricama i hrani pouzdana je samo ako se ispituje reprezentativan uzorak. U radu su opisani učestalost i toksični učinci T-2 toksina u peradi.

KLJUČNE RIJEČI: *citotoksičnost, genotoksičnost, trikotecenski mikotoksini, zakonski propisi*

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