KRMIVA

NUCLEOTIDE SUPPLEMENTATION ELIMINATES LEUKOCYTE DNA DAMAGE INDUCED BY T-2 TOXIN AND DEOXYNIVALENOL IN BROILER CHICKENS

DODAVANJE NUKLEOTIDA ELIMINIRA OŠTEĆENJE LEUKOCITNE DNA IZAZVANE - T-2 TOKSINOM I DEOXYNIVALENOLOM U BROJLERSKIH PILIĆA

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INTRODUCTION

Fusarium fungi, producing a number of different mycotoxins including the class of trichothecenes, are probably the most characteristic toxin-producing fungi of the northern temperate regions. Regarding the toxicity of trichothecenes, food and feed consisting of cereal grains contaminated with T-2 toxin, HT-2 toxin, deoxynivalenol (DON) and nivalenol represent a health threat to humans and animals in Europe, USA and Asia (Hussein and Brasel, 2001; Creppy, 2002).

The chronic toxicity of trichothecenes is characterised by anorexia, reduced weight gain, diminished nutritional efficiency, neuroendocrine changes and immunological effects (Larsen et al., 2004). The known mechanism of action of *Fusarium* mycotoxins indicates that they can be particularly toxic to rapidly dividing cells of the immune system, hepatic tissue and gastrointestinal mucosa (Larsen et al., 2004). On the cellular level they can induce lipid peroxidation through promoting free radical production, causing damage to cell membranes and DNA. DNA damage in immune cells, caused by oxidative stress or by mycotoxins' direct impact, can decrease proliferation of leukocytes and thus cause impaired immune response in case of infection.

There are many commercial products on the market that are used as detoxifying agents and are said to prevent or decrease toxic effect of mycotoxins by their adsorptive and enzymatic mode of action. On the other hand, some nutrients, including omega-3 polyunsaturated fatty acids (PUFA), certain amino acids, pre- and probiotics, polyphenols and other antioxidants, and nucleotides may have beneficial effects on the suppressed immune system and gastrointestinal function during mycotoxicosis. Nucleotides, nucleosides or nucleic acids may become essential nutrients in pathological conditions that demand intense nucleic acid and protein synthesis, such as rapid growth and repair of certain tissues (Perez et al., 2004).

The objective of present study was to determine the effect of either 10 mg of T-2 toxin or 10 mg deoxynivalenol (DON) per kg feed on DNA fragmentation and oxidative stress in broiler chickens, and furthermore, to evaluate the potential of dietary nucleotides in reduction of toxin-induced DNA damage.

MATERIALS AND METHODS

The experiment was performed on 20 days old male broiler chickens (Ross 308, mean live weight 748 g ± 18g). 50 broiler chickens were randomly divided into 5 groups (N=10): (1) control, (2) 10 mg

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DON/kg feed, (3) 10 mg DON/kg feed + 2 g nucleotides/kg feed, (4) 10 mg T-2 toxin/kg feed, (5) 10 mg T-2 toxin/kg feed + 2 g nucleotides/kg feed. Feed consumption and live weight gain were recorded weekly. After 17 days of treatment blood, spleen and liver were collected for analysis. DNA damage of leukocytes was measured by Comet assay, lipid peroxidation was studied by malondialdehyde (MDA), total antioxidant status (TAS) of plasma and glutathione peroxidase (GPx) assays, and the hepatotoxicity were studied by measuring plasma alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma glutamyl transferase (GGT). Relative organ weights of liver, spleen, brain, kidney, heart, gizzard, small intestine and bursa of Fabricius were also determined.

RESULTS AND DISCUSSION

The administration of 10 mg T-2 toxin per kg feed reduced body weight gain by 47 % in comparison to the control (Table 1). Impaired growth was a consequence of significantly reduced feed consumption in the T-2 toxin fed group. 10 mg DON/kg feed reduced body weight gain and feed consumption by a much smaller extent than T-2 toxin. Nucleotide supplementation, when added to the T-2 toxin and DON contaminated diets, did not increase feed intake and body weight gain. Although, poultry are less sensitive to trichothecenes than other species, it is obvious from this study that T-2 toxin and DON have a negative impact on production parameters when they are present in feed at concentration of 10 mg/kg.

Exposure to trichothecenes results not only in economic losses due to worse performance, but also in alterations on cellular level. T-2 toxin and DON induced DNA fragmentation in chicken leukocytes (Figure 1). It is clearly seen that T-2 toxin is more genotoxic than DON. Results obtained show that dietary nucleotides reduced the degree of DNA damage induced by the action of T-2 toxin. The same trend was observed when nucleotides were added to DON, although the difference was not statistically significant. Our findings on DNA damage can be supported by research of Atroshi et al. (1997) who reported an increase in DNA damage in livers of mice fed T-2 toxin, and of Rizzo et al. (1998) who tested the genotoxic effect of T-2 toxin and DON on rat liver cells. The significant decrease in plasma total antioxidant status (TAS) in the group with T-2 toxin treatment indicates that oxidative stress was induced by T-2 toxin. Other markers of lipid peroxidation were not affected by the mycotoxins and nucleotide supplementation. An increase in liver enzymes in serum during mycotoxicosis is believed to be due to subsequent leakage of enzymes into the circulation because of hepatocyte degeneration. Results from liver enzymes determination suggest that liver was not severely damaged by either T-2 toxin or DON.

Table 1. Body mass, feed intake and live weight gain of chickens fed 10 mg T-2 toxin/kg feed and 10 mg DON/kg feed with (+) and without nucleotide supplementation

Tablica 1. Tjelesna masa, unos hrane i prirast žive vage pilića hranjenih s 10 mg toksina T-2/kg hrane i 10 mg DON—a/kg hrane sa i bez dodavanja nukleotida

Treatment Tretman	Body mass-start Tjelesna masa početak (g)	Body mass-end Tjelesna masa kraj (g)	Feed intake Unos hrane (g/day)	Live weight gain Prirast žive vage (g/day)	Feed/gain ratio Hrana/prirast
Control	746.8	1655^{a}	108.3^{a}	63.6^{a}	1.72^{a}
DON	739.8	1528^{b}	96.8^{b}	52.6^{b}	1.84^{ab}
DON+	747.8	1510^{b}	98.3^{b}	53.4^{b}	1.90^{ab}
T-2	745.4	1158^{c}	70.7^{c}	33.5^{c}	2.20^{ab}
T-2+	748.9	1242^{c}	71.9^{c}	34.1^{c}	2.36^{b}
SEM	5.67	45.5	3.17	3.18	0.15

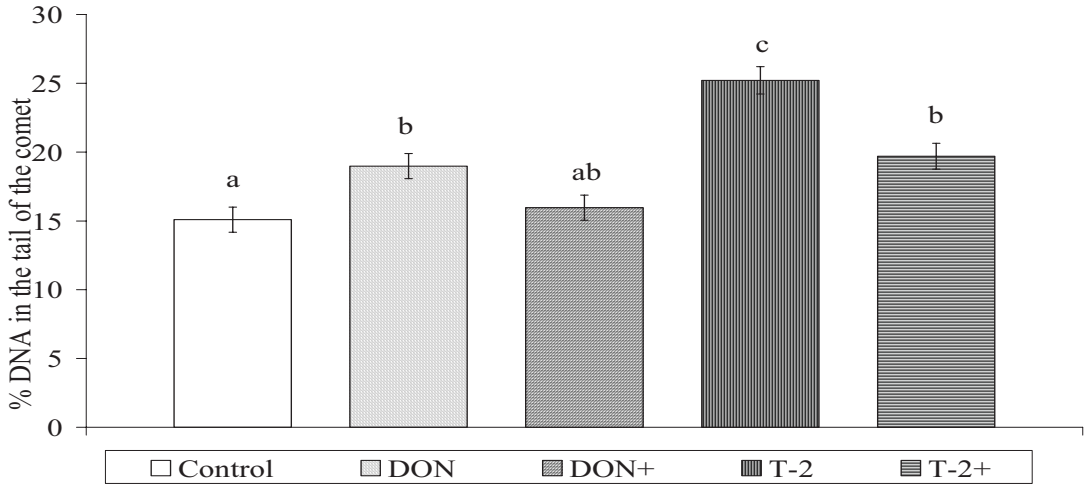

Fig. 1. DNA damage in groups treated with 10 mg DON/kg feed and 10 mg T-2 toxin/kg feed, with (+) and without nucleotide supplementation, presented as % of DNA in the tail of the comet; LS-means ±SE. Groups without the same superscript differ significantly, P<0.05.

Slika 1. Oštećenje DNK u skupinama tretiranim s 10 mg DON-a/kg hrane i 10 mg toksina T-2/kg hrane sa i bez dodavanja nukleotida, prikazano kao % DNK u repu kometa; LS - prosjek ± SE. Skupine bez jednakog natpisa značajno se razlikuju, P<0.05.

Relative weights (g/100g BW) of kidney, gizzard, brain and small intestine increased in groups fed T-2 toxin and T-2 toxin with nucleotides (Table 2). T-2 toxin with nucleotide supplementation increased the relative weight of the large intestine by 17 %, and an 11 % decrease in relative weight of the heart was observed in T-2 and T-2+ group. We expected the relative weights of spleen and bursa of *Fabricius* to alter in the toxin treated groups as a sign of malfunctioning of these immune organs, but there was no difference in relative organ weights of spleen, bursa of Fabricius, liver and pancreas among the groups. However, increased relative weights of gizzard, small intestine, kidney and brain in T-2 treated groups with or without addition of nucleotides can be interpreted as a consequence of an irritation and/or intensified function of these organs. The relative weights of heart ether increased or did not change when DON or T-2 toxin were added in experiments of Chi et al. (1977), Harvey et al. (1997) and Dänicke et al. (2003) which is in contrast to our study where T-2 toxin decreased relative heart weights. DON statistically increased the relative weight of the large intestine. Nucleotide supplementation in the DON treated group tended to reduce the weights of kidney, gizzard and large intestine to the level of the control group (Table 2).

Table 2. Relative weights of organs (g/100g BW) of chickens fed 10 mg T-2 toxin/kg feed and 10 mg DON/kg feed with (+) and without nucleotide supplementation

Tablica 2. Relativne težine organa (g/100g BW) pilića hranjenih s 10 mg DON—a/kg hrane sa i bez dodavanja nukleotida

Treatment Tretman	Liver Jetra	Kidney Bubreg	Heart Srce	Gizzard Želudac	Brain Mozak	Small intestine Tanko crijevo	Large intestine Debelo crijevo
Control - Kontrola	2.12	0.61^{a}	0.45^{ab}	1.59^{a}	0.100^{a}	2.89^{a}	0.58^{a}
DON	2.12	0.65^{ab}	0.46^{a}	1.74^{ab}	0.109^{a}	3.02^{ab}	0.64^{ab}
DON+	2.03	0.63^{a}	0.43^{ab}	1.62^{a}	0.114^{a}	3.03^{ab}	0.60^{a}
T-2	2.00	0.69^{b}	0.40^{b}	2.10^{b}	0.142^{b}	3.32^{b}	0.63^{ab}
T-2+	1.98	0.66^{ab}	0.42^{ab}	2.07^{b}	0.141^{b}	3.35^{b}	0.68^{b}
SE	0.08	0.01	0.01	0.09	0.01	0.09	0.02

CONCLUSION

This study shows that *Fusarium* mycotoxins, T-2 toxin and DON impair the performance of broiler chickens, induce DNA damage in chicken leukocytes and cause oxidative stress in the organism. When considering feed consumption and live weight gain in mycotoxicosis induced with high levels of T-2 toxin and DON present in the feed, dietary nucleotides do not have beneficial effect. The crucial role of nucleotide supplementation in feed is to repair DNA damage in immune cells, which are highly sensitive to mycotoxin action. When broilers are exposed to *Fussarium* mycotoxins their immune function can be depressed, thus they can be more susceptible to virus or bacterial infections. The addition of dietary nucleotides may increase proliferation of immune cells and optimize the function of the immune system in case of infections.

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SAŽETAK

Ovaj rad pokazuje da mikotoksini Fusarium, toksini T-2 i DON kvare proizvodne rezultateU brojlera, izazivaju oštećenje DNK u leukocitima pilića i prouzrokuju oksidacijski stres u organizmu. S obzirom na konzumaciju hrane i prirast žive vage u mikotoksikozi izazvanoj visokim razinama toksina T-2 i DON-a što se nalaze u hrani, dijetalni nukleotidi nemaju blagotvorno djelovanje. Presudna uloga dodavanja nukleotida u hranu je obnoviti gubitak DNK u imunim stanicama koje su vrlo osjetljive na djelovanje mikotoksina. Kad su brojleri izloženi mikotoksinima Fusarium njihova se imuna funkcija može smanjiti pa mogu biti osjetljiviji na virusne ili bakterijske infekcije. Dodavanje dijetalnih nukleotida može povećati razmnožavanje imunih stanica i optimirati funkciju imunog sustava u slučaju infekcije.