The effect of feeding a diet naturally contaminated with deoxynivalenol on production traits and selected biochemical indicators of broiler chickens

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Abstract

The effect of feeding a diet naturally contaminated with deoxynivalenol (DON) (0-21 days: 1.50 mg·kg^{-1}; 22-39 days: 1.54 mg·kg^{-1}) was studied in 40 broiler chickens. Birds were divided into two groups fed a control diet and a diet contaminated with DON (n = 20 in each). Feed intake was measured daily and individual live weight weekly; daily weight gain and feed to gain ratio were calculated. Five animals from each group were euthanized on days 21 and 39 when blood (blood plasma and red blood cell haemolysates) and liver samples were collected. Concentration of triglyceride, uric acid and glucose and activities of ALT, AST and LDH were measured in blood plasma. Indicators of lipid peroxide and glutathione redox status, malondialdehyde and reduced glutathione concentration and glutathione-peroxidase activity were measured in blood plasma, red blood cell haemolysates and liver homogenates. The low dose of DON did not cause difference in the production traits, but caused significantly lower concentration of uric acid and glucose, and significantly higher concentration of triglyceride in blood plasma on day 21. Enzyme activities in blood plasma did not differ significantly between the treatment groups. Among the markers of lipid peroxide and glutathione redox status, malondialdehyde content was significantly higher in liver homogenate on day 21 in the group fed with DON contaminated diet, but reduced glutathione content and glutathione peroxidase activity did not differ significantly between the treatment groups. The results showed that diet contaminated even with a low content of deoxynivalenol caused alterations in selected biochemical indicators of blood and liver of broiler chicken.

Biochemical markers, malondialdehyde, glutathione, glutathione peroxidase

Moulds produce different mycotoxins that have importance in farm animal nutrition because of their widespread occurrence and diversity (Leeson et al. 1995). Among various mycotoxins, those produced by *Fusarium* moulds, such as deoxynivalenol (DON) are often found in feed ingredients even at high concentrations in different parts of the world under different environmental conditions (Jelinek et al. 1989).

The official recommendation in the European Union for the maximum DON content in the poultry feeds is 5 mg·kg^{-1} (EU 2006). Additionally, Eriksen and Pettersson (2004) found that levels from 9 mg DON·kg^{-1} feed had negative effects in chickens, while no effect was found in chicken fed 5 mg DON·kg^{-1} feed, therefore they proposed a guideline value of 2.5 mg DON·kg^{-1} feed. However, in early experiments of Kubena et al. (1987, 1988) only moderate effects were found in production traits even at extremely high contamination levels of 16 or 18 mg DON·kg^{-1} feed. No significant effect was found on leukocytes using dietary level of up to 3 mg·kg^{-1} DON, except for the attenuated phagocytic activity of granulocytes, and increased number of heterophils (Levkut et al. 2009). The same level of DON contamination resulted in alterations of clinical biochemical indicators in blood plasma, namely lower total protein and triglyceride content and higher alanine-aminotransferase activity (Faixova et al. 2006). Feeding diet artificially contaminated with a low dose of DON (3.4 mg·kg^{-1}) together with zearalenone demonstrated that this

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diet induces oxidative stress (Borutova et al. 2008). The biochemical mode of action of trichothecene mycotoxins on lipid peroxidation and antioxidant defence is not completely understood (Surai 2002; Weber et al. 2010), but marked changes were found even at low levels of contamination (Dersjant-Li et al. 2003).

The aim of the present study was to evaluate the effect of naturally contaminated feed with a low level of DON on some production traits, clinical biochemical indicators of blood, and on lipid peroxide and glutathione redox indicators in broiler chickens.

Materials and Methods

Animals and diets

A total of 40 one-day-old Hubbard cockerels were divided into two groups. One was fed a control diet (C, n = 20) and the other group a diet composed of 40% of maize naturally contaminated with deoxynivalenol and 60% of control diet (DON, n = 20). Nutrient content of the diets met the requirements for broiler chicken according to the Hungarian standards (Hungarian Feed Code 2004a) and was determined according to standard methods (Hungarian Feed Code 2004b). The feeding trial lasted for 39 days. Complete feeds were given in mash form in the first phase (days 1 to 21). The control diet contained 0.05 mg·kg^{-1} DON; the contaminated diet contained 1.50 mg·kg^{-1} DON. In the second phase (days 22 to 39) diets in mash form were fed with the same nutrient content, and had 0.06 mg·kg^{-1} and 1.54 mg·kg^{-1} DON, respectively (Table 1).

The content of DON in diets was measured by HPLC technique (Central Veterinary Institute, Budapest) according to the European Union directive (EU 2005).

Table 1. Nutrition and deoxynivalenol content of diet used for two groups (control and DON) of broiler chicken during two phases of feeding

Nutrient	Phase 1 (days 1 to 21)		Phase 2 (days 22 to 39)	
	Control	DON	Control	DON
Dry matter (g·kg^{-1})	898.80	897.40	897.10	895.60
Crude protein (g·kg^{-1} d.m.*)	196.37	199.13	197.03	198.23
Crude fat (g·kg^{-1} d.m.)	30.15	31.09	31.11	30.84
Crude fibre (g·kg^{-1} d.m.)	23.59	23.07	24.02	23.88
Crude ash (g·kg^{-1} d.m.)	62.97	68.98	63.92	67.45
Nitrogen free extract (g·kg^{-1} d.m.)	686.92	677.74	683.92	679.60
Deoxynivalenol (mg·kg^{-1})	0.05	1.50	0.06	1.54

* d.m. - dry matter; DON - deoxynivalenol

Production traits

Body weight measurements were made individually during the trial on a weekly basis. Feed intake was measured daily in each group and calculated per day and animal. Average daily weight gain and feed to gain ratio were calculated.

Sampling and measurement of biochemical indicators

Five animals from each group were euthanized on day 21 and 39, when dissection was carried out, and blood and liver samples were collected. Blood samples were stored at + 4 °C, then the plasma was separated from the blood cells by centrifugation (2500 *g*, 20 min). Red blood cells were lysed with deionised water (ratio 1:9) and by freezing and thawing. Liver samples were homogenized in nine-fold volume of 0.65% (w/v) NaCl. The samples were stored at -20 °C until analysis.

Clinical biochemical indicators, such as concentration of triglyceride, uric acid and glucose and activities of alanine-aminotransferase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) were determined by commercial enzymatic colorimetric diagnostic kits (Diagnosticum Ltd., Budapest).

For determination of malondialdehyde (MDA) concentration in the blood samples, the method of Placer et al. (1966) and in liver homogenates the method of Mihara et al. (1980) was used. Reduced glutathione (GSH) concentration was measured with the method of Sedlak and Lindsay (1968). Glutathione peroxidase (GSHPx) activity was measured with the method of Lawrence and Burk (1976). For determination of protein content in blood plasma and red blood cell haemolysates the biuret method was used (Weichselbaum 1948). In the 10,000 g supernatant fraction of liver homogenates total protein concentrations were determined according to the method of Lowry et al. (1951).

Statistical methods

Statistical evaluation of the results was carried out by Student's 2-sample *t*-test after calculating the means and standard deviations (SD) with Statistic™ 4.0 (Statsoft Inc. 1993) software.

The experiment was approved by the Animal Experimental Committee of the Faculty of Agricultural and Environmental Sciences of the Szent István University (2/2005).

Results

Production traits

There was no significant difference in the average body weight of birds during the trial between the control group and the group fed DON contaminated diet (Table 2). Feed intake was measured in each group daily and there was no significant difference between the groups (Table 3). Feed to gain ratio was calculated based on the average body weight and feed intake of the groups weekly (Table 4). Feeding DON contaminated diet did not cause differences in the average daily weight gain and feed to gain ratio during the growing period.

Table 2. Live weight of two groups of broiler chickens (g; mean ± SD)

Day	0	7	14	21	28	35	39
Control	41.64 ± 1.04	125.00 ± 19.52	319.77 ± 48.19	665.23 ± 103.86	1028.33 ± 200.72	1508.75 ± 292.36	1795.42 ± 341.32
DON	41.59 ± 0.72	135.91 ± 17.64	342.14 ± 59.99	688.81 ± 104.75	1111.36 ± 203.75	1636.36 ± 227.63	1906.91 ± 233.09

DON-deoxynivalenol

Table 3. Average daily feed consumption ($g \cdot day^{-1}$ per animal) in two groups of broiler chickens

Week	Control	DON
1	16.75	18.05
2	49.81	64.95
3	95.91	90.88
4	175.71	186.75
5	192.26	212.47
6	222.71	201.14

DON-deoxynivalenol

Biochemical indicators

Significant changes were found in some blood plasma biochemistry indicators at samplings on days 21 and 39 (Table 5). The uric acid content, which reflects the protein metabolism, and glucose content in blood plasma was significantly lower in the DON group compared to the control on day 21, but this difference was not observed on day 39. In contrast, triglyceride content of blood plasma was significantly higher in DON group on day 21 compared to the control. Enzymes, such as LDH, AST and ALT, which reflect tissue damage did not show significant differences between the two groups either on day 21 or day 39.

Table 4. Average daily weight gain and feed conversion ratio in the different phases of growing period in two groups of broiler chickens

Week	1	2	3	4	5	6
	Average daily weight gain (g/bird)					
Control	11.91	27.82	49.35	51.87	68.63	71.67
DON	13.47	29.46	49.52	60.36	75.00	67.64
	Feed to gain ratio (g feed consumption/g weight gain)					
Control	1.41	1.79	1.94	3.39	2.80	3.11
DON	1.34	2.20	1.84	3.09	2.83	2.97

DON-deoxynivalenol

Table 5. Effect of diet contaminated with deoxynivalenol on selected blood plasma biochemical indicators of broiler chicken (mean ± SD)

	Protein ($g \cdot l^{-1}$)	TG ($mmol \cdot l^{-1}$)	Uric acid ($mmol \cdot l^{-1}$)	Glucose ($mmol \cdot l^{-1}$)	LDH ($\mu kat \cdot l^{-1}$)	AST ($\mu kat \cdot l^{-1}$)	ALT ($\mu kat \cdot l^{-1}$)
	Day 21						
Control	28.28 ± 6.12	0.69[a] ± 0.19	140.11[b] ± 48.10	35.44[b] ± 12.17	16.08 ± 6.07	2.22 ± 0.50	0.49 ± 0.18
DON	31.22 ± 4.90	0.89[b] ± 0.23	82.20[a] ± 20.32	15.96[a] ± 3.94	18.06 ± 5.55	2.12 ± 1.00	0.39 ± 0.16
	Day 39						
Control	38.52 ± 4.67	2.28[b] ± 1.06	67.26 ± 23.68	15.82 ± 6.63	11.96 ± 8.14	2.42 ± 0.78	0.37 ± 0.18
DON	40.12 ± 6.83	0.57[a] ± 0.29	68.90 ± 25.22	16.21 ± 5.64	19.91 ± 7.53	3.36 ± 1.52	0.38 ± 0.16

[a,b] Different superscripts in the same column mean significant difference at $P < 0.05$ level
DON-deoxynivalenol; TG-triglyceride; LDH-lactate dehydrogenase; AST-aspartate aminotransferase; ALT-alanine aminotransferase

Lipid peroxide and glutathione redox status of blood and liver

Table 6. Effect of feeding DON contaminated diet on malondialdehyde and reduced glutathione content and glutathione peroxidase activity of blood plasma (mean ± SD)

	MDA ($\mu mol \cdot l^{-1}$)	GSH ($mmol \cdot g^{-1}$ protein)	GSHPx ($\mu kat \cdot l^{-1}$)
	Day 21		
Control	6.03 ± 2.88	6.29 ± 1.28	8.63 ± 1.50
DON	6.97 ± 1.49	5.72 ± 1.02	7.64 ± 1.25
	Day 39		
Control	7.12 ± 1.94	4.80 ± 0.69	6.96 ± 1.63
DON	7.05 ± 1.25	4.74 ± 0.68	6.34 ± 1.92

DON-deoxynivalenol; MDA-malondialdehyde; GSH-reduced glutathione; GSHPx-glutathione peroxidase

Table 7. Effect of diet contaminated with deoxynivalenol on malondialdehyde and reduced glutathione content and glutathione peroxidase activity of red blood cell haemolysate (mean ± SD) in broiler chicken

	MDA ($\mu mol \cdot l-1$)	GSH ($mmol \cdot g-1$ protein)	GSHPx ($\mu kat \cdot l-1$)
	Day 21		
Control	7.92 ± 0.78[b]	9.36 ± 4.18	6.26 ± 2.10
DON	6.60 ± 0.73[a]	12.36 ± 3.30	6.59 ± 1.85
	Day 39		
Control	18.52 ± 4.80[b]	10.33 ± 1.81	6.76 ± 1.63
DON	12.33 ± 3.76[a]	10.75 ± 2.54	7.60 ± 1.45

[a,b] Different superscripts in the same column means significant difference at $P < 0.05$ level
DON-deoxynivalenol; MDA-malondialdehyde; GSH-reduced glutathione; GSHPx-glutathione peroxidase

Malondialdehyde content which reflects lipid peroxidation did not change significantly in blood plasma (Table 6), in red blood cell haemolysates it was lower in the DON group compared to control on days 21 and 39 (Table 7), and it was significantly higher in liver homogenate on day 21 but not on day 39 (Table 8).

Reduced glutathione content and glutathione peroxidase activity did not differ significantly as effect of feeding DON contaminated diet either in blood (blood plasma, red blood haemolysates) or in liver homogenate (Tables 6-8).

Discussion

The results of present trial showed that DON at approximately 1 $mg \cdot kg^{-1}$ diet did not cause a decrease in the production traits and only slightly modified the concentration of some blood plasma metabolites, mainly during the first phase (day 1 to 21) of the growing period.

This result is in line with previous studies when feeding diets containing feedstuffs naturally contaminated with DON (5 $mg \cdot kg^{-1}$ feed) did not

Table 8. Effect of diet contaminated with deoxynivalenol on malondialdehyde and reduced glutathione content and glutathione peroxidase activity of liver homogenate (mean ± SD) of broiler chickens

	MDA ($\mu mol \cdot g^{-1}$)	GSH ($mmol \cdot g^{-1}$ 10,000 g supernatant protein)	GSHPx ($nkat \cdot g^{-1}$ 10,000 g supernatant protein)
	Day 21		
Control	3.46 ± 0.85 [a]	2.56 ± 0.60	47.26 ± 8.68
DON	7.43 ± 2.70 [b]	2.34 ± 0.75	48.76 ± 6.68
	Day 39		
Control	5.26 ± 1.80	1.82 ± 0.61	31.90 ± 6.51
DON	4.70 ± 1.44	1.90 ± 0.42	28.65 ± 7.52

[a,b] Different superscripts in the same column mean significant difference at $P < 0.05$ level
DON-deoxynivalenol; MDA-malondialdehyde; GSH-reduced glutathione; GSHPx-glutathione peroxidase

have a significant effect on feed consumption, feed conversion, body-weight gain, and live body weight (Hamilton et al. 1985; Awad et al. 2006).

Blood plasma protein concentration did not differ significantly and triglyceride concentration was higher in the DON group on day 21, but lower on day 39, and the uric acid content was lower on day 21, which differs from the findings by Kubena et al. (1988). However, in that trial much higher DON contamination (16 $mg \cdot kg^{-1}$ feed) was used. Blood plasma glucose content was also significantly lower on day 21 which might be caused by impaired intestinal glucose transport (Awad et al. 2004).

Changes of the malondialdehyde content in blood plasma and red blood cell haemolysate and liver homogenate suggest that the potential pro-oxidant effect of DON did not manifest in blood and the antioxidant system effectively scavenged the free radicals generated by DON in liver homogenate. These results are in agreement with the findings of Borutova et al. (2008) who found that feeding a diet contaminated with DON together with zearalenone at higher concentrations (3.4 $mg \cdot kg^{-1}$ feed both for DON and zearalenone) resulted in significantly decreased activity of GSHPx and increased MDA in liver.

The significant effect of DON on day 21 was eliminated by day 39 in the case of some biochemical indicators which, according to previous findings, may be caused by the development of intestinal microflora of growing chickens which has detoxifying ability of deoxynivalenol to less toxic de-epoxy metabolites (Young et al. 2007). The amount of absorbed amount of DON therefore possibly gradually decreased with ageing.

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