

The effect of ochratoxin A on egg quality, development of embryos and the level of toxin in eggs and tissues of hens and chicks

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ABSTRACT

Six flocks, each numbering 11 hens and 1 rooster, of Rhode Island Red strain, at the age of 54 weeks were divided into three groups, and fed on diets containing different amounts of ochratoxin A. The control group (I) was fed a standard mixture without ochratoxin A, groups II and III were given the same diet, with 2.1 and 4.1 ppm of ochratoxin A, respectively, introduced with moulded wheat.

The daily feed intake decreased as the ochratoxin A content increased and was 156, 135 and 105 g/day, respectively. The toxin negatively affected the quality of the eggs, especially thickness and crushing strength of the shell. Deterioration of egg-shell quality led to greater loss of egg weight during incubation and lower hatchability. As the ochratoxin A content in the feed increased, the percentage of hatched eggs decreased and survival time of embryos dying during incubation declined. The mass of embryos weighted on 6 and 8 day of incubation and the hatched chicks were lower in experimental than in control group. Ochratoxin A was found in the eggs and also in the blood serum, liver and kidneys of hens, roosters and one-day-old chicks.

KEY WORDS: ochratoxin A, laying productivity, egg quality, reproduction, laying hens

INTRODUCTION

Feeds contaminated with mycotoxins adversely affect health and productivity of hens, egg and meat quality and hatchability indices. It is now considered that

aflatoxins and ochratoxin A are the mycotoxins the most harmful to human health and may cause the greatest losses in animal production. According to some authors (Huff and Hamilton, 1974; Huff et al., 1974) ochratoxin A may be more toxic to poultry than aflatoxins. Many reports have been published on the acute and subacute toxicity of ochratoxin A, obtained from cultures of the toxin-producing strain of *Aspergillus ochraceus*. There is an evidence that after ochratoxin A poisoning laying productivity decline or was completely stopped (Scholtyssek et al., 1987; Niemiec and Scholtyssek, 1987; Choudhury et al., 1971; Prior and Sisodia, 1978; Page et al., 1980), egg-shell damage increased, hatchability deteriorated (Page et al., 1980, Niemiec et al., 1993a,b) and sexual maturation of hens was delayed (Choudhury et al., 1971). These findings motivated us to carry out our previous studies on the effect of this toxin on laying performance and reproduction indices in hens (Bauer et al., 1988; Niemiec and Świerczewska, 1988; Niemiec et al., 1988; Niemiec et al., 1990). The present study is a continuation of earlier experiments.

MATERIAL AND METHODS

The experiment was carried out on 72 Rhode Island Red hens at the age of 54 weeks. Six flocks (11 hens and 1 rooster) having similar laying productivity and percentage of fertilized eggs were selected for the experiment. Three groups of two flocks each were fed for 5 weeks with feed differing in ochratoxin A content. The control group (I) was given a standard diet with no ochratoxin A, groups II and III received the same diet to which 2.1 or 4.1 ppm of the toxin was introduced.

Ochratoxin A was obtained by incubation of wheat with the fungus *Aspergillus ochraceus* KA 10 (Schindler and Nesheim, 1970). Fermentation was carried out in sterilized Erlenmeyer flasks, each containing 100 g of moist wheat (70 % DM). Wheat was incubated for 21 days at 25°C. After incubation the grains with the mould were dried at room temperature, autoclaved for 1 min in order to destroy the fungus spores and finely ground. Ochratoxin A content in fermented grain was determined by thin-layer chromatography by the method of Nesheim (1971) modified by Goliński and Szczęśna (1984) and was about 195 ppm per kg.

An appropriate amount of wheat flour containing ochratoxin A was added to the standard mixture to obtain the required concentration of mycotoxin in the experimental diets, while an equal amount of uncontaminated wheat flour was added to the control diet. After 14 days of feeding with the respective diets collection of eggs for hatching and quality assessment began. The quality of the egg-shell was determined by scanning electron microscopy. Three sets of eggs were hatched and the biological quality of each hatched brood was determined.

After 4 weeks of feeding with the experimental diets, blood was sampled from the wing vein of 4 hens and 2 roosters from each group and pooled. After completion of the experiment the birds were killed and their liver, kidneys and heart were sampled and pooled. Three newly hatched chicks were killed from each of the hatching sets and pooled samples of their blood plasma, kidneys and liver were prepared.

Ochratoxin A was extracted from tissues and determined according to Nesheim (1973). The ochratoxin A content in the eggs was determined by the method described by Piskorska-Pliszczynska (1984); in blood plasma, according to Mortensen et al. (1983).

RESULTS

Daily feed intake decreased as the ochratoxin A content increased from 0 to 2.1 and 4.1 ppm and averaged 156, 135 and 105 g per hen, respectively. The laying performance dropped dramatically, to as low as 16.8% in group III given the feed containing 4.1 ppm ochratoxin A. After 5 weeks of the experiment the weight of the hens in control group, increased by 33 g, in group II and III decreased by 243 and 340 g, respectively. As the ochratoxin A content in the feed increased, the weight of the eggs and the percentage of egg-shell in the egg decreased (Table 1).

The quality of the eggs deteriorated as the concentration of ochratoxin A in the feed increased. The largest differences were found in egg-shell quality (Table 1). The shells were thinner, so the eggs showed significantly greater elastic

TABLE 1
Influence of ochratoxin A on egg quality

Indices	Ochratoxin A content in diet, ppm		
	I (0)	II (2.1)	III (4.1)
Number of eggs	40	40	40
Average egg weight, g	62.4 ^A ± 0.56	60.3 ^A ± 1.32	58.6 ^B ± 1.06
Share in egg, %			
- yolk	29.6 ^a ± 0.58	28.7 ^b ± 0.91	28.5 ^b ± 0.82
- albumen	62.3 ^B ± 0.85	64.0 ^A ± 0.96	64.3 ^A ± 1.12
- shell	8.1 ^A ± 0.31	7.2 ^B ± 0.59	7.2 ^B ± 1.05
Egg-shell			
- thickness, mm	0.34 ^A ± 0.03	0.3 ^B ± 0.07	0.28 ^B ± 0.05
- flexible distortion, µm	22.0 ^C ± 0.05	46.0 ^B ± 0.07	81.0 ^A ± 0.03
- crushing strength, kg/cm ²	3.16 ^a ± 0.27	3.07 ^a ± 0.53	3.01 ^b ± 0.42

Means with different superscripts within a row are significantly different
a, b - P ≤ 0.05; A, B - P ≤ 0.01

TABLE 2

Results of hatching

Group	Ochratoxin A in diet ppm	Number of incubated eggs*	Hatchability as % of eggs		Mean survival time of nouviel embryos, days
			incubated	fertile	
I	0	313	83.6 ^A	87.5 ^A	13 ^a ± 0.64
II	2.1	270	79.7 ^A	82.1 ^A	12 ^a ± 0.97
III	4.1	68	32.3 ^B	33.3 ^B	10 ^b ± 1.11

* number of incubated eggs was not equalized, due to lowered laying rate in groups fed diets containing ochratoxin A

Means within a column with different superscripts are significantly different

a, b - $P \leq 0.05$; A, B - $P \leq 0.01$

deformations and lowered resistance to crushing. The worsening of egg quality, especially of the egg-shell, had a large effect on hatching. Ochratoxin A lowered the number of eggs suitable for hatching and worsened their hatchability. The hatching percentage of fertilized eggs also declined as the ochratoxin A concentration in the feed increased. The greatest decrease was observed in group III fed with a mixture containing 4.1 ppm ochratoxin A. This decline in hatchability was concomitant with a decline in laying output and a shortening of the survival time of the embryos that later died (Table 2).

Biological analysis of the hatch showed very marked effects of ochratoxin A on some of the studied parameters. The thinner shell led to increased egg weight loss during incubation (Table 3). The loss of weight by day 6 in eggs from group III fed 4.1 ppm ochratoxin A in their feed was almost threefold that in the control group. On days 18 and 20 of incubation, the loss of egg weight from hens given the toxin continued to be greater than that in the control eggs.

Feed contaminated with ochratoxin A inhibited the development of embryos (Table 4). The weight of the embryos on 6 and 8 day of incubation, as well as the weight of newly hatched chicks decreased, as the toxin concentration increased.

TABLE 3

Effect of ochratoxin A content in diet on the loss of egg weight during incubation

Group	Ochratoxin A in diet, ppm	Number of incubated eggs*	Initial egg weight, g	Loss of egg weight as % of initial egg weight at day		
				6	18	20
I	0	313	59.3 ^a ± 0.25	2.2 ^A ± 0.01	8.8 ^A ± 0.03	21.1 ^A ± 0.18
II	2.1	270	57.3 ^b ± 0.35	3.8 ^B ± 0.03	11.5 ^B ± 0.16	25.5 ^B ± 0.21
III	4.1	68	54.7 ^c ± 0.39	5.1 ^C ± 0.07	13.7 ^C ± 0.18	29.6 ^C ± 0.22

*, a, b; A, B - as in Table 2

TABLE 4

Effect of ochratoxin A content in diet of hens on the weight of embryos and newly hatched chicks

Group	Ochratoxin A in diet, ppm	Number of examined eggs	Initial egg weight, g	Weight of embryos, g at day		Weight of newly hatched chicks, g
				6	18	
I	0	20	58.9 ^{aA} ± 1.59	1.15 ^A ± 0.38	32.86 ^A ± 0.17	45.2 ^A ± 0.37
II	2.1	20	57.3 ^{bA} ± 0.64	0.89 ^{aB} ± 0.51	30.22 ^{aB} ± 0.46	41.4 ^B ± 0.65
III	4.1	20	54.6 ^B ± 0.97	0.77 ^{bB} ± 0.53	28.95 ^{bB} ± 0.26	38.6 ^C ± 0.27

a, b; A, B – as in Table 2

TABLE 5

Content of ochratoxin A in blood plasma, kidneys, livers and hearts of cockerels, hens, newly hatched chicks and in eggs (ppb), determined in pooled samples

Item	Group								
	I			II			III		
	♂	♀	CH	♂	♀	CH	♂	♀	CH
Blood plasma	ND ^{b)}	ND	ND	5.7	13.3	2.7	16.0	37.0	5.5
Kidneys	ND	ND	ND	2.9	8.1	60.6	5.9	9.0	189.0
Liver	ND	ND	ND	1.3	3.3	26.0	1.7	11.9	83.0
Heart	ND	ND	— ^{c)}	0.07	2.1	—	ND	18.6	—
Eggs	—	—	—	—	4.1	—	—	7.9	—

ND – non-detected

– not determined

♂ – cockerels, ♀ – hens, CH – newly hatched chicks of both sexes

Ochratoxin A was found in the blood plasma, kidneys, livers, hearts and eggs of birds fed mixtures contaminated with this toxin, as well as in the chicks hatched from these eggs (Table 5).

The ochratoxin A concentration was the highest in the kidneys and livers of hens and in the plasma of newly hatched chickens. This confirms earlier information that ochratoxin A permeates into the egg.

DISCUSSION

Feeding hens diets containing ochratoxin A caused decrease in feed consumption and resulted in a decline or complete stoppage of laying and degradation of egg quality.

The presented findings are in agreement with the results of Niemiec and Scholtyssek (1987) and Scholtyssek et al. (1987). Also Choudhury et al. (1981) demonstrated the harmful effect of this toxin on productivity, feed consumption and body weight of laying hens. A concentration even as low as 0.5 ppm ochratoxin A in the diet was shown to be toxic.

Ochratoxin A was found to affect negatively egg quality, mainly the egg shell. A thin shell, less resistant to crushing, had a negative effect on hatching. In an earlier study changes in the ultrastructure of the shell were found, as well as changes in the external and internal shell membranes (Niemiec et al., 1993). This suggests that ochratoxin A caused disturbances in mineral metabolism, which are manifested as a thinner egg-shell. The effect of ochratoxin A on mineral metabolism was demonstrated by Huff et al. (1980) in broiler chicks. In their experiments, the tibia bones of broilers fed a mixture containing 2 ppm ochratoxin A were more prone to breakage, while when 4 ppm of this toxin was fed, also bone diameter decreased.

The harmful effect of ochratoxin A on hatching indices and mean time of survival of nonviable embryos is in agreement with the results of our earlier experiments (Niemiec et al., 1990) and generally accepted views (Choudhury et al., 1971; Prior and Sisodia, 1978; Page et al., 1980). We could nowhere find a detailed description of the extent of embryos damage. However, in the previous study by Niemiec et al. (1990) attention was focused on morphological deformities, improper positioning and damage to soft tissues and organs of embryos. These results indicate, that the opinion that such disorders may be of genetic origin only, should be revised.

The inhibition of embryo development by the increasing dietary concentrations of ochratoxin A confirms the results of Vesely et al. (1982) and Vesela et al. (1983) who introduced ochratoxin A into developing embryos.

It can be concluded from the few studies on ochratoxin A presence in the tissues of birds that, similarly as in large farm animals, this toxin is quickly absorbed from the digestive tract of the hens and distributed via the blood throughout their entire body. The presence of ochratoxin A in the blood plasma, tissues and eggs of hens fed a contaminated feed agree well with the results of Bauer et al. (1988) and Niemiec et al. (1988). Plant and animal products contaminated with ochratoxin A can therefore be dangerous to humans consuming such food.

CONCLUSIONS

Feeding hens a diet contaminated with ochratoxin A reduces their laying and breeding performance, as well as exerts a negative effect on the quality of eggs.

A very strong negative effect of this toxin on the quality of the egg shell was found. The fact that ochratoxin A is transferred to the body of the bird and to the eggs indicates that systematic tests for the presence of mycotoxins in feeds are necessary. Due to the very strong negative effects of ochratoxin A on reproduction and hatching indices, eggs obtained from flocks fed mixtures contaminated with mycotoxins should not be taken for hatching.

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STRESZCZENIE

Wpływ ochratoksyny A w mieszance kur niosek na jakość jaj i wyniki wylęgu

Sześć stadek liczących każde po 11 kur i 1 koguta rasy Rhode Island Red w wieku 54 tygodni podzielono na 3 grupy, które żywiono mieszanką o różnej zawartości ochratoksyny A. Grupa kontrolna (I) żywiona była standardową mieszanką nie zawierającą ochratoksyny A, a grupy II i III mieszankami, które zawierały odpowiednio 2,1 i 4,1 ppm tej toksyny.

Dzienne spożycie mieszanki zmniejszało się wraz ze wzrostem zawartości ochratoksyny A w paszy (0; 2,1; 4,1 ppm) i wynosiło odpowiednio 156, 135 i 105 g/dzień. Toksyna ta pogarszała jakość jaj, przy czym największe różnice dotyczyły jakości skorupy. Wskutek cienszej skorupy jaja te charakteryzowały się większym odkształceniem elastycznym oraz zmniejszoną wytrzymałością na zgniatanie.

Pogorszenie jakości skorupy miało wpływ na straty masy jaj podczas inkubacji oraz zdolność wylęgową piskląt. Wraz ze wzrostem skażenia mieszanki ochratoksyną A obniżał się procent wylęgu z jaj zapłodnionych oraz skracał się czas przeżycia zarodków. Kontrolne ważenie zarodków w 6 i 18 dobie inkubacji a także wylutych piskląt wykazało zahamowanie rozwoju zarodków pod wpływem tej toksyny. Stwierdzono obecność ochratoksyny A w jajach, a także w osoczu krwi oraz w wątrobie i nerkach kur, kogutów i jednodniowych piskląt.