Effect of Addition of a Detoxifying Agent to Laying Hen Diets Containing Uncontaminated or *Fusarium* Toxin-Contaminated Maize on Performance of Hens and on Carryover of Zearalenone

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ABSTRACT A 16-wk experiment with laying hens was carried out to examine the effects of feeding of mycotoxin-contaminated maize (CM) on performance, nutrient digestibility, weight of organs, serum chemical parameters, and antibody titers to Newcastle disease virus (NDV) in serum. Also tested were fimbrien antigen K88 in egg yolk and zearalenone (ZON) residues in eggs and tissues. The *Fusarium*-toxin-contaminated maize contained 17,630 μg deoxynivalenol and 1,580 μg ZON/kg. Moreover, Mycofix Plus (MP), a so-called detoxifying agent, was added to both the uncontaminated control (UCM) and to the CM diet (70% dietary maize inclusion). Each of the four resulting diets (UCM, UCM-MP, CM, CM-MP) was tested on 25 laying hybrids (Lohmann Brown). Feeding of the CM diets significantly depressed feed intake compared to the control groups by approximately 5%. This was mainly due to the effects observed at the beginning of the experiment. Daily egg mass production/hen was 56.6, 58.4, 53.9, and 55.2 g in groups UCM, UCM-MP, CM and CM-MP, respectively. Nutrient digestibility and metabolizability of gross energy were slightly depressed by feeding the CM diets and improved by MP addition. Feeding of the CM diets resulted in a significant decrease in serum titers to NDV and to an increase in yolk titers to antigen K88. No residues of ZON or of its metabolites were found in yolk, albumen, abdominal fat, breast meat, follicles greater than 1 cm in diameter, ovaries including follicles smaller than 1 cm in diameter, magnum, and serum. ZON and α-zearalenol (α-ZOL) were detected in livers of hens fed the CM diets at mean concentrations of 2.1 and 3.7 μg/kg, respectively. It was concluded that feeding maize which was highly contaminated with *Fusarium* mycotoxins adversely influenced performance of hens and modulated immune response. At the given level of zearalenone and at the indicated detection limits, no residues of ZON and its metabolites were found in eggs. The effects of the tested detoxifying agent were quite mycotoxin-independent.

(*Key words*: laying hen, *Fusarium* mycotoxins, immune response, nutrient digestibility, carryover of zearalenone)

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INTRODUCTION

Laying hens and broilers are regarded as very resistant to the *Fusarium* mycotoxins deoxynivalenol (DON) and zearalenone (ZON). For example, feeding of *Fusarium graminearum* inoculated maize containing 82.8 mg DON/kg to laying hens for 27 d did not adversely affect laying performance (Lun et al., 1986). Similarly, Kubena et al. (1987) fed Leghorn chickens with DON-contaminated wheat (18 mg/kg of diet) until the onset of laying and subsequently over 168 d of the laying period. Neither live weight gain during the rearing period nor egg production, egg quality, or fertility was negatively influenced by feeding the DON-contaminated wheat. On the other hand, based on the principal mode of action of trichothecene mycotoxins in inhibiting the protein synthesis by blocking the peptidyl transferase of the 60S-ribosomal subunit (for review see Feinberg and McLaughlin, 1989), these mycotoxins could preferentially affect the rapidly proliferating cells of the immune system. Indeed, Harvey et al. (1991) reported a decrease in serum antibody titer to Newcastle virus (NDV) after feeding a DON-contaminated diet (18 mg/kg) to growing chickens for 18 wk. Moreover, Dänicke et al. (1999) reported a dose-response-related decrease in serum NDV-titers of broilers after feeding diets containing no DON, 7.5 mg and 15 mg

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Abbreviation Key: CM = *Fusarium*-toxin-contaminated maize; DON = deoxynivalenol; MP = Mycofix Plus; NDV = Newcastle disease virus; UCM = uncontaminated maize; α-ZAL = α-zeralanol; β-ZAL = β-zearalanol; ZAN = zearalanone; α-ZOL = α-zearalenol; β-ZOL = β-zearalenol; ZON = zearalenone.

DON/kg of diet. Such changes in immune response might occur without interfering with performance, but should be viewed as an indication for adverse DON-effects on the health of the birds.

ZON, which often co-occurs with DON in naturally contaminated cereals, is even less toxic to laying hens than DON. Allen et al. (1981) did not find any detrimental effects of increasing dietary ZON-concentration up to 800 mg/kg of diet on laying performance or on reproductive performance, including growth of progeny. However, the feeding of ZON-contaminated feed to laying hens might cause residues of the toxin in the yolk as indicated by an experiment by Dailey et al. (1980), who found radioactivity in egg yolks after an oral bolus of ^{14}C-labeled ZON. This could be important to human health because of the estrogenic and genotoxic properties of ZON. Moreover, a possible carryover of DON and ZON to eggs could adversely affect reproductive performance of hens as indicated by the investigations of Bergsj et al. (1993), who reported an increased incidence of chick developmental anomalies when hens were fed these mycotoxins, although fertility hatchability or perinatal mortality was not influenced.

Detoxifying agents used as feed additives are supposed to adsorb mycotoxins under the conditions within the digestive tract, whereby an absorption by the body is avoided. Such effects were proven to be successful in decreasing negative effects of aflatoxins in broilers and turkeys (Kubena et al., 1990; 1991; 1993). In order to cope with the more nonpolar mycotoxins DON and ZON, the adsorbent MP was upgraded with the addition of enzymatic activities that were claimed to degrade these mycotoxins (Pasteiner, 1998).

The aim of the present study was to examine the effects of feeding high levels of maize highly contaminated with DON and ZON to laying hens on performance, immune response, ZON-residues in eggs and several tissues, either in the absence or presence of MP. Moreover, the uncontaminated control diet was also tested with or without MP in order to record possible mycotoxin-unspecific effects of the supplement.

MATERIALS AND METHODS

Experimental Design and Feeding Experiment

Maize (UCM) and mycotoxin-contaminated maize (CM) were included in laying-hen diets at 70% on an air-dry basis. Each of these diet types was tested either in the absence or presence of MP. A total of four diets resulted from this arrangement (Table 1). Each of the diets was tested on 25 Lohmann Brown laying hybrids in a 112-d experiment. Eighteen-week-old hens were placed into single cages in a three-floor cage battery enabling the individual recording of laying performance and feed intake. Hens were fed a commercial layer diet until the commencement of the experiment when the hens were 22 wk old. Hens were weighed and experimental diets were introduced. The mean BW of the flock was 1380 g ± 132 g.

The experiment was subdivided into four 28-d laying periods. Laid eggs were recorded daily. A total of eight eggs was collected from each hen to monitor egg weight for each laying month. Feed was offered in a meal form for ad libitum consumption. Feed not consumed was weighed back every 14 d. Water was supplied by nipple drinkers.

Balance Experiment

An additional 32 hens (eight hens per treatment) were used for the balance experiment that was performed when the hens were 28-wk-old according to the total collection method (Schiemann, 1981). Hens planned for the balance experiment were fed a commercial layer diet until they were placed into single metabolic cages. Hens were acclimatized to the balance cages and to the experimental diets during a 7-d preperiod. Hens were adjusted to a daily feed amount of 100 g/hen in this period. This feed quantity was maintained during the following 5-d collection period. Excreta were collected from the plastic trays beneath the cages in the morning and in the afternoon, and were temporarily frozen. Excreta were then freeze-dried and ground to pass through a 1-mm screen.

Immunological and Clinical-Chemical Parameters

The antibody titers to NDV in blood serum and to fimbrien antigen K88 in egg yolk were determined to evaluate possible immuno-modulating effects of mycotoxins. For this purpose, the hens, which were vaccinated three times during the rearing period with living NDV vaccines, were vaccinated intramuscularly with a killed NDV vaccine (LaSota $10^8 EID_{50}$) at the beginning of the experiment (22 wk of age). Blood was drawn from a wing vein in Week 14 of the experiment to determine the NDV-antibody titers and for the hepatic enzyme activities.

Antigen K88 was injected subcutaneously in Week 3 and 5 of the experiment. Eggs collected during Week 8 of the experiment were prepared for K88-antibody titer analysis (see below).

Organ Weights, Egg Quality, and Zearalenone Residues in Eggs and Tissues

Two eggs were collected from each hen during Week 2, 4, 8, and 16 of the experiment. Eggs were individually weighed; yolk and shell were separated and weighed, whereas the weight of the albumen was calculated by difference. After recording the weight of egg constituents, the yolks and albumen of four to five hens were pooled and homogenized in such a way that five pooled samples were available per group for analysis of ZON and metabolites (each time point) and K88-antibody titer (Week 8 of experiment only). Pooled yolk and albumen samples were

TABLE 1. Composition of experimental diets (g/kg)

	Group			
	UCM	UCM-MP	CM	CM-MP
Components				
Maize (UCM)	700.0	696.5	–	–
Contaminated maize (CM)	–	–	700.0	696.5
Soybean meal	190.0	190.0	190.0	190.0
Fish meal	10.0	10.0	10.0	10.0
L-Lysine HCl	0.8	0.8	0.8	0.8
DL-Methionine	2.5	2.5	2.5	2.5
Dicalcium phosphate	10.2	10.2	10.2	10.2
Calcium carbonate	79.9	79.9	79.9	79.9
Sodium chloride	4.2	4.2	4.2	4.2
Premix[1]	2.4	2.4	2.4	2.4
Mycofix Plus[2]	–	3.5	–	3.5
Composition				
CP				
Calculated	155	155	155	155
Analyzed	154	154	152	152
ME (kcal/kg)				
Calculated	2,703	2,703	2,703	2,703
Determined	2,584	2,656	2,703	2,751
Calculated composition				
Lysine	8.5	8.5	8.5	8.5
Methionine + cystine	7.9	7.9	7.9	7.9
Methionine	5.3	5.3	5.3	5.3
Calcium	34.0	34.0	34.0	34.0
Total phosphorus	5.0	5.0	5.0	5.0
Sodium	1.9	1.9	1.9	1.9

[1]Provided per kilogram diet: Fe, 45 mg; Cu, 5 mg; Zn, 75 mg; Mn, 60 mg; Se, 0.1 mg; I, 0.5 mg; Co, 0.1 mg; vitamin A, 10000 IU; vitamin D_3, 2000 IU; vitamin E, 20 mg; vitamin K, 2.5 mg, vitamin B_1, 1 mg; vitamin B_2, 4 mg; vitamin B_6, 3 mg; vitamin B_{12}, 10 μg; pantothenic acid, 10 mg; nicotinic acid, 25 mg; biotin, 102 μg; folic acid, 0.75 mg; choline chloride, 400 mg; butylated hydroxytoluene, 120 mg.

[2]Biomin GTI, Herzogenburg, Industriestraβe 21, Österreich.

frozen, freeze-dried, and finally ground to pass through a 1-mm screen.

At the end of the experiment, hens were weighed and subsequently killed by cutting the jugular vein. Mixed trunk blood was collected for preparation of serum. Small intestine, liver, spleen, and heart were dissected, emptied (small intestine), and weighed. Moreover, bile fluid, liver, abdominal fat, breast meat, follicles greater than 1 cm in diameter, ovaries including follicles smaller than 1 cm in diameter, magnum (a length of approximately 20 cm from the infundibulum), and serum samples were pooled in a similar manner as described for eggs so that five tissue or fluid samples were available for ZON-residue analysis. Tissues were stored at –20°C. They were homogenized by using an ultra-turrax prior to analysis.

Analyses

Freeze-dried excreta and diets were analyzed for nitrogen and crude fat according to the methods of the Verband Deutscher Landwirtschaftlicher Untersuchungsanstalten (Naumann and Bassler, 1993). Gross energy in feed and excreta was determined using an adiabatic bomb calorimeter[2] for calculation of apparent metabolizable energy that was corrected for a zero N-balance applying the factor of 36.5 kJ/g N-retention (Titus et al., 1959). Apparent CP digestibility was determined as described by Pahle et al. (1983) by analyzing the ninhydrin positive alpha-amino-nitrogen in diets and excreta.

Tissues, bile fluid, blood serum, yolk, and albumen samples were analyzed for ZON, α-zearalenol (α-ZOL), β-zearalenol (β-ZOL), zearalanone (ZAN), α-zearalanol (α-ZAL), β-zearalanol (β-ZAL) by HPLC with fluorescence detection after treatment with 2 U β-glucuronidase (EC 3.2.1.31, Roche No. 127[3]) and 0.9 U aryl-sulfatase (EC 3.1.6.1, Roche No. 698[3]) and cleaning of the extracts by immuno-affinity columns[4] as described by Ueberschär (1999). Detection limits are shown in Table 2. Zearalenone in maize was analyzed accordingly with the exception that samples were incubated with 2 U β-glucosidase (EC 3.2.1.21, Sigma[5] No. G-0395).

Deoxynivalenol in maize was analyzed by HPLC with diode array according to a modified sample preparation procedure as advised by Coring System Diagnostix GmbH.[6] Further trichothecene mycotoxins were determined in contaminated maize by the Institute of Animal Nutrition of the University Hohenheim using a GC-MS-method (Schollenberger et al., 1998). Beauvericin and moniliformin analyses were performed by the Institute for Agrobiotechnology (IFA) (Tulln, Austria) using HPLC-

[2]Model C 4000, IKA-Analysentechnik, Heitersheim, Germany.
[3]Roche-Diagnistics Mannheim, Mannheim, Germany.
[4]Easi-Extract Zearalenone, Rhône-Diagnostics/Coring System Diagnostix GmbH, Gernsheim, Germany.
[5]Sigma-Aldrich Chemistry GmbH, Deisenhofen, Germany.
[6]Mycosep trichothecene, Coring System Diagnostix GmbH, Gernsheim, Germany.

TABLE 2. Detection limits of zearalenone and its metabolites (μg/kg)

	Breast muscle, magnum, liver abdominal fat, follicles, ovaries, yolk	Albumen	Serum	Bile fluid
	5.0 g	3.0 g	2.0 g	1.0 g
Zearalenone	1.0	2.0	2.5	5.0
α-Zearalenol	0.5	1.0	1.5	3.0
β-Zearalenol	3.0	7.0	10.0	20.0
Zearalanone	20	35	50	100
α-Zearalanol	20	35	50	100
β-Zearalanol	40	70	100	200

methods. Fumonisins were analyzed using an ELISA-test kit.[7]

Glutamat dehydrogenase (EC 1.4.1.3) and γ-glutamyl transferase (EC 2.3.2.2) in serum were analyzed using test-kits.[8] Antibody titers to NDV in serum were determined by a hemagglutination-inhibition test (micromethod). K88-antibody titers in yolk samples were analyzed by a double-sandwich ELISA.[9]

Statistics

With the exception of laying performance and egg-quality parameters, all other measures were analyzed by two-factorial design of ANOVA:

$$y_{ijk} = \mu + a_i + b_j + (axb)_{ij} + e_{ijk}$$

where y_{ijk} = parameter of an observation k, subjected to maize i and detoxifying agent j; a_i = maize (uncontaminated, mycotoxin-contaminated); b_j = detoxifying agent (without, with MP); $(axb)_{ij}$ = interactions; e_{ijk} = error term. Laying-performance parameters were analyzed according to a three-factorial design of ANOVA with repeated measurements:

$$y_{ijkl} = \mu + a_i + b_j + c_k + (axb)_{ij} + (axc)_{ik} + (bxc)_{jk} + (axbxc)_{ijk} + d_{l(axb)} + e_{ijkl}$$

where y_{ijkl} = parameter of an observation l, subjected to maize i, detoxifying agent j, and laying month k; a_i = maize (uncontaminated, mycotoxin-contaminated); b_j = detoxifying agent (without, with MP); c_k = laying month (1...4); $(axb)_{ij}$, $(axc)_{ik}$, $(bxc)_{jk}$, $(axbxc)_{ijk}$ = interactions; $d_{l(axb)}$ = effect of repeated measurements (consecutive laying months) within the same hen l; e_{ijkl} = error term. All statistics were carried out using the Statistica for the Windows™ operating system (STATSOFT Inc., 1994).

[7]RIDASCREEN Fast Fumonisin, art. no. R 5603, R-Biopharm GmbH, Darmstadt, Germany.
[8]Kits 1.03373 and 1.12189.0001, Merck, Darmstadt, Germany.
[9]Lohmann Animal Health GmbH & Co. KG, Cuxhaven, Germany.

RESULTS

Performance Experiment

The contaminated maize contained high amounts of DON, 15-Acetyl-DON, nivalenol, and ZON. The ratio between ZON and DON was approximately 1:11 (Table 3). No hen died during the performance experiment. Laying intensity, egg weight, and consequently, daily egg mass increased significantly in the course of the experiment. This increase occurred at a significantly lower level for the groups fed the mycotoxin-contaminated maize (Table 4). Egg weight was significantly increased due to MP addition. This effect was observed both for the UCM- and the CM-groups. No significant interactions were detected between maize and MP-supplementation which suggests a mycotoxin-independent effect of MP. Similar effects were observed for daily egg mass production. The adverse effects of CM on performance were mainly due to a depression in feed intake. However, this effect was especially pronounced at the beginning of the experiment. Hens of these groups adjusted to the mycotoxin-contaminated maize over the course of the experiment. This is also indicated by the significant interactions between maize and laying month. As a result of this adaptation of feed intake, interactions between maize and laying month were significant for the other performance parameters. Feed conversion was only affected by laying month, whereas maize or MP addition was without influence.

TABLE 3. Mycotoxin composition of contaminated and uncontaminated maize (μg/kg)[1]

	CM	UCM
Deoxynivalenol (DON)	17,630	212
15-Acetyl-DON	5,500	NA
3-Acetyl-DON	90	NA
T-2 Toxin	<4	NA
HT-2 Toxin	<10	NA
Nivalenol	1,600	NA
Zearalenon	1,580	43
Fumonisins	<100	NA
Beauvericin	<46	NA
Moniliformin	<89	NA

[1]UCM = uncontaminated maize; CM = *Fusarium* toxin-contaminated maize; NA = not analyzed.

TABLE 4. Performance of laying hens (Week 22 to 38 of age) fed noncontaminated or mycotoxin-contaminated maize, either in absence or presence of Mycofix Plus[1]

Experimental week	Maize	MP (g/kg)	Laying intensity (%)	Daily egg mass (g/hen)	Egg weight (g/egg)	Daily feed intake (g/hen)	Feed conversion (g/g egg mass)
1 to 4	UCM	. . .	95.6	52.2	54.6	100.1	1.93
	UCM	3.5	96.0	52.5	54.7	101.1	1.93
	CM	. . .	91.3	45.4	49.7	85.7	1.93
	CM	3.5	93.1	48.6	52.2	89.3	1.83
5 to 8	UCM	. . .	96.3	55.6	57.7	115.0	2.08
	UCM	3.5	98.6	57.5	58.3	114.2	1.99
	CM	. . .	95.9	52.6	54.7	108.1	2.08
	CM	3.5	93.1	53.0	56.6	110.5	2.15
9 to 12	UCM	. . .	96.1	57.5	59.8	120.4	2.11
	UCM	3.5	99.1	61.1	61.6	119.1	1.95
	CM	. . .	98.9	56.5	57.2	118.1	2.09
	CM	3.5	95.7	57.9	60.3	119.2	2.10
13 to 16	UCM	. . .	98.4	60.9	61.9	118.9	1.96
	UCM	3.5	99.7	62.5	62.7	116.3	1.87
	CM	. . .	100.6	60.9	60.6	113.7	1.87
	CM	3.5	98.9	61.2	61.9	118.3	1.94
1 to 16	UCM	. . .	96.6	56.6	58.5	113.6	2.02
	UCM	3.5	98.4	58.4	59.3	112.7	1.94
	CM	. . .	96.6	53.9	55.6	106.4	1.99
	CM	3.5	95.2	55.2	57.7	109.3	2.01
ANOVA (probability)							
Maize			0.121	0.001	<0.001	<0.001	0.445
MP			0.872	0.062	0.009	0.435	0.221
Period			<0.001	<0.001	<0.001	<0.001	<0.001
Maize × MP			0.112	0.766	0.234	0.137	0.082
Maize × period			0.031	0.001	0.001	<0.001	0.104
MP × period			0.811	0.563	0.155	0.632	0.657
Maize × MP × period			0.106	0.140	0.534	0.524	0.072
Pooled SEM			1.4	1.1	0.7	1.7	0.1

[1]Data are means of 25 hens for each treatment. UCM = uncontaminated maize; CM = *Fusarium* toxin-contaminated maize; MP = Mycofix Plus (3.5 g/kg diet).

Balance Study

Protein digestibility was significantly depressed by feeding of CM diets and improved by MP supplementation, an effect that was independent of mycotoxin contamination, i.e., there were no significant interactions between maize and MP addition (Table 5). Utilization of organic matter was not affected by dietary treatments. Crude fat utilization was slightly increased after MP addition to both maize-type diets ($P = 0.082$), whereas carbohydrate digestibility tended to be lower in the CM groups ($P = 0.072$). ME concentrations were significantly increased in both maize-type diets after MP supplementation. Metabolizability of gross energy was also slightly increased due to MP-supplementation ($P = 0.062$).

Serum Activity of Hepatic Enzymes, Serum NDV-Antibody Titers, and Yolk K88-Antibody Titers

Serum activity of glutamat dehydrogenase and γ-glutamyl transferase was not altered due to dietary treatments (Table 6). Serum antibody titers to NDV were significantly lower in CM-fed hens, whereas yolk antibody titers to K88 were significantly increased at the same time (Table 6).

Weight Change of Hens and Organ Weights

Hens fed the CM diets gained slightly less weight than UCM-fed hens ($P = 0.134$) (Table 7). Neither liver-, spleen-, or heart-weights were affected by dietary treatments. In contrast, weight of emptied small intestine was decreased significantly due to MP addition and slightly decreased in hens fed the contaminated maize ($P = 0.056$) (Table 7). No interactions between maize and MP addition were found.

Egg Components

Egg weights as recorded during Week 2, 4, 8, and 16 of experiment are shown in Table 8. Significant relations were the same as reported as for the regular recording of egg weight (Table 4). Proportion of yolk decreased and that of albumen increased significantly due to MP addition. Feeding of CM resulted in a decrease in the percentage of albumen, whereas shell proportion increased at the same time.

Residues of Zearalenone and Its Metabolites

Neither ZON or its metabolites were detected in egg yolk and albumen at either time point in abdominal fat, in breast meat, in follicles, in the ovaries, in magnum, or in serum. ZON, β-, and α-ZOL were detected in the bile fluid of hens fed the CM diets (Table 9). Bile fluids of hens fed the UCM diets also contained α-ZOL and ZON. The α-ZOL and ZON were detected only in livers of hens fed the CM diets.

TABLE 5. Nutrient digestibility and metabolizable energy of diets containing noncontaminated or mycotoxin-contaminated maize fed to laying hens (Week 28 of age), either in absence or presence of Mycofix Plus[1]

Maize	MP (g/kg)	Apparent digestibility/utilization (%)				ME	
		Protein	Organic matter	Fat	Carbohydrates[2]	Dry matter (kcal/kg)	Gross energy (%)
UCM	. . .	94.8	75.4	71.7	84.2	2,986	74.0
CM	. . .	94.0	75.2	69.2	83.2	2,981	73.8
. . .	. . .	94.0	74.8	68.5	83.9	2,955	73.4
. . .	3.5	94.8	75.7	72.3	83.5	3,014	74.4
UCM	. . .	94.5	74.8	70.5	84.2	2,943	73.4
UCM	3.5	95.1	76.0	72.8	84.2	3,029	74.6
CM	. . .	93.6	74.8	66.5	83.6	2,964	73.3
CM	3.5	94.5	75.5	71.8	82.8	3,000	74.2
ANOVA (probability)							
Maize		0.014	0.702	0.248	0.072	0.825	0.685
MP		0.013	0.149	0.082	0.467	0.011	0.062
Maize × MP		0.549	0.682	0.48	0.523	0.273	0.757
Pooled SEM		0.3	0.6	2.1	0.5	22	0.5

[1]Data are means of eight hens for each treatment.

[2]Sum of N-free extracts and crude fiber. UCM = uncontaminated maize; CM = *Fusarium* toxin contaminated maize; MP = Mycofix Plus.

It became possible, by sequential treatment of bile fluid and liver samples with β-glucuronidase and aryl-sulfatase and analysis of the so-treated samples for ZON and metabolites, to estimate the proportions of ZON and its metabolites present in these specimen in a free form or conjugated with glucuronic acid or sulfate. Respective data of the groups fed the contaminated maize were pooled for this reason (Table 10).

DISCUSSION

The mycotoxin composition of the contaminated maize suggests that the maize was mainly infected by species of the genus *Fusarium graminearum* because DON and its acetylated derivatives, ZON and nivalenol are the main mycotoxins formed by this fungi (Chelkowski, 1998). This is further supported by the absence of any of the other *Fusarium* toxins shown in Table 3, which are synthesized by other *Fusarium* species.

Feeding of *Fusarium*-contaminated maize to laying hens resulted in a significant decrease in feed intake (Table 3). This effect can not be ascribed solely to the well-known feed intake-depressing effect of DON (for review see D'Mello et al., 1999) since substantial amounts of 15-acetyl-DON and nivalenol were also found in this maize. Therefore, an evaluation of the data of the present study with advised tolerable levels of DON in diets for poultry is problematic. For example, a level of 5 mg DON/kg of complete chicken diet was advised by the U.S. Food and Drug Administration, by the Ontario Ministry of Agricul-

TABLE 6. Mean serum enzyme levels, NDV-antibody titers and yolk K88-antibody titers of hens fed diets containing noncontaminated or mycotoxin-contaminated maize, either in absence or presence of Mycofix Plus[1]

Maize	MP (g/kg)	Serum antibody titer to NDV (TKZ)[2]	Yolk antibody titer to antigen K88[3]	Glutamat-dehydrogenase[2]	γ-glutamyltransferase[2]
UCM	. . .	8.9	265	0.98	10.62
CM	. . .	7.7	402	1.02	8.23
. . .	. . .	8.5	381	0.94	6.89
. . .	3.5	8.1	285	1.07	11.97
UCM	. . .	9.3	315	0.90	6.59
UCM	3.5	8.5	214	1.07	14.66
CM	. . .	7.8	447	0.97	7.20
CM	3.5	7.6	356	1.07	9.27
ANOVA (probability)					
Maize		0.046	0.037	0.769	0.403
MP		0.445	0.13	0.252	0.078
Maize × MP		0.615	0.798	0.938	0.295
Pooled SEM		0.6	59	0.11	2.83

[1]Data are means of 25 hens for each treatment except for yolk K88 titer (five pooled samples for each group).

[2]Week 14 of experiment.

[3]Week 8 of experiment. UCM = uncontaminated maize, CM = *Fusarium* toxin-contaminated maize; MP = Mycofix Plus, TKZ = titer indicative figure, logarithm to the base two.

TABLE 7. Live weight change (Weeks 22 to 38 of age) and organ weights (Weeks 38 of age) of hens fed diets containing noncontaminated or mycotoxin-contaminated maize, either in absence or presence of Mycofix Plus[1]

Maize	MP (g/kg)	Live weight change (g/hen in 16 wk)	Organ weight (g/kg of BW)			
			Liver	Spleen	Heart	Small intestine
UCM	. . .	122	22.3	1.05	4.0	24.0
CM	. . .	84	22.5	0.98	3.9	22.6
. . .	. . .	109	22.6	1.04	3.9	24.1
. . .	3.5	97	22.3	1.00	4.0	22.5
UCM	. . .	132	22.6	1.08	3.9	24.9
UCM	3.5	112	22.0	1.03	4.0	23.1
CM	. . .	85	22.5	0.99	3.9	23.3
CM	3.5	83	22.5	0.97	3.9	21.8
ANOVA (probability)						
Maize		0.134	0.753	0.063	0.835	0.056
MP		0.656	0.557	0.375	0.524	0.03
Maize × MP		0.724	0.631	0.661	0.536	0.849
Pooled SEM		25.1	0.5	0.04	0.1	0.7

[1]Data are means of 25 hens for each treatment. UCM = uncontaminated maize; CM = *Fusarium* toxin-contaminated maize; MP = Mycofix Plus (3.5 g/kg diet).

ture and Food (Tarr, 1996), and by the German Federal Ministry of Consumer Protection, Food, and Agriculture (2000). This DON-value can only be taken as an indicator for a *Fusarium* toxin contamination which implies that variable proportions of other trichothecenes are possible. Consequently, the total toxic potential of such contaminated feedstuffs also will vary. Therefore, the adverse effects of the dietary level of approximately 12.34 mg DON/kg of diet as used in the present study might be amplified by approximately 3.85 mg 15-Acetyl-DON and 1.12 mg nivalenol/kg of diet. The latter two *Fusarium* toxins exert their effects in the same way as DON.

The overall decrease in performance of hens fed the CM diets resulted mainly from the pronounced depression in feed intake at the beginning of the experiment when CM-containing diets were introduced. However, hens of these groups were able to adapt to the CM over the course of the experiment, which also explains the significant

TABLE 8. Egg-quality parameters of hens fed diets containing noncontaminated or mycotoxin-contaminated maize, either in absence or presence of Mycofix Plus

Experiment week	Maize	MP (g/kg)	Egg weight (g/egg)	Yolk (%)	Albumen (%)	Shell (%)
2	UCM	. . .	52.6	22.9	67.2	9.9
	UCM	3.5	53.1	22.7	67.4	9.9
	CM	. . .	50.2	23.3	66.7	10.0
	CM	3.5	50.7	22.2	67.7	10.1
4	UCM	. . .	56.3	23.1	67.4	9.6
	UCM	3.5	57.0	22.8	67.4	9.7
	CM	. . .	54.0	23.4	67.0	9.7
	CM	3.5	55.3	23.3	67.1	9.6
8	UCM	. . .	58.8	24.5	66.0	9.5
	UCM	3.5	60.0	24.1	66.6	9.2
	CM	. . .	56.7	24.7	65.8	9.5
	CM	3.5	58.8	24.2	66.4	9.4
16	UCM	. . .	62.3	26.4	64.5	9.1
	UCM	3.5	63.2	26.1	65.0	8.9
	CM	. . .	60.9	26.5	64.2	9.3
	CM	3.5	63.7	26.2	64.6	9.1
. . .	UCM	. . .	57.5	24.2	66.2	9.5
	UCM	3.5	58.3	23.9	66.6	9.5
	CM	. . .	55.5	24.5	65.9	9.6
	CM	3.5	57.1	24.0	66.4	9.6
ANOVA (probability)						
Maize			<0.001	0.226	0.045	0.010
MP			<0.001	<0.001	<0.001	0.146
Period			<0.001	<0.001	<0.001	<0.001
Maize × MP			0.079	0.426	0.489	0.798
Maize × period			0.019	0.597	0.818	0.485
MP × period			0.138	0.483	0.419	0.054
Maize × MP × period			0.512	0.371	0.627	0.464
Pooled SEM			0.5	0.2	0.2	0.1

[1]UCM = uncontaminated maize; CM = *Fusarium* toxin-contaminated maize; MP = Mycofix Plus.

TABLE 9. Residues of zearalenone, α-zearalenol (α-ZOL), and β-zearalenol (β-ZOL) in livers and bile fluids (μg/kg) of hens fed diets containing noncontaminated or mycotoxin-contaminated maize, either in absence or presence of Mycofix®Plus (Week 16 of experiment) after sequential treatment of the samples with water (without enzyme) or with β-glucuronidase or with β-glucuronidase + arylsulfatase[1]

		β-ZOL	α-ZOL			ZON		
Maize	MP (g/kg)	β-glucuronidase + arylsulfatase	Without enzyme	β-glucuronidase	β-glucuronidase + arylsulfatase	Without enzyme	β-glucuronidase	β-glucuronidase + arylsulphatase
Liver								
UCM	. . .	<3.0	ND	<0.5	<0.5	ND	<1.0	<1.0
UCM	3.5	<3.0	ND	<0.5	<0.5	ND	<1.0	<1.0
CM	. . .	<3.0	1.3	2.5	3.8	<1.0	1.6	<1.0
CM	3.5	<3.0	1.3	2.2	3.5	1.4	2.6	3.2
Bile fluid								
UCM	. . .	<20.0	ND	3.1	16.2[a]	ND	25	88.6[a]
UCM	3.5	<20.0	ND	13.1	16.0[a]	ND	93.2	91.9[a]
CM	. . .	125.0	ND	147	201.8[b]	ND	904	992.5[b]
CM	3.5	131.3	139.0	ND	198.8[b]	702.0	ND	1,026.0[b]

[1]UCM = uncontaminated maize; CM = *Fusarium* toxin-contaminated maize; MP = Mycofix Plus. ND = not determined.

interactions between time of experiment and maize. Therefore, changes in egg weight and daily egg mass were mainly secondary effects. However, the positive effects of MP addition to both maize-type diets can not be explained entirely by the changes in feed intake. These effects can be deduced from the improved digestibility of CP and crude fat and by the increased metabolizability of gross energy (Table 4). It should be stressed that no interactions between maize and MP addition were detected for either performance or for nutrient digestibility or ME. This strongly suggests an existing, but mycotoxin-unspecific effect of this feed additive. According to the manufacturer, MP consists of an adsorbing component that was upgraded by the addition of enzymatic activities in order to cope with the more nonpolar mycotoxins such as DON and ZON (Pasteiner, 1998). However, Karlovsky (1999) was unable to detect either epoxidase- or lactonase activity in this product. Therefore, the observed positive effects in the present study were probably due to an unspecific adsorbing component of MP and confirm the beneficial effects of several adsorbing agents in poultry feeding as reviewed by Ramos et al. (1996).

The effects of CM on nutrient digestibility and ME were rather small. In contrast, Hamilton and Trenholm (1988) reported that the TME_N-concentration of maize was significantly decreased by approximately 7% when 4 mg pure DON were added/kg of maize. However, the authors were unable to reproduce this effect when naturally contaminated maize was tested instead. There was no relationship between DON concentration in maize and TME_N up to a contamination level of approximately 20 mg DON/kg of maize. It might be concluded, therefore, that *Fusarium*-contaminated maize has only minor effects on nutritive value.

Serum activities of glutamat dehydrogenase and γ-glutamyl transferase are frequently used to evaluate liver damage. Kubena et al. (1987) found the γ-glutamyl transferase significantly increased in hens exposed to a diet containing 18 mg DON/kg over a period of 168 d. Therefore, either the mycotoxin composition and level and/or the time of exposure of the hens to the CM diet of the present experiment were insufficient to induce detectable liver damage.

A significant reduction in serum antibody titers to NDV was observed in the present study due to feeding of the CM diets which is in accordance with the findings of Harvey et al. (1991) and Dänicke et al. (1999) for growing chickens and broilers, respectively. In contrast, significantly higher titers to antigen K88 were found in yolks of hens fed the CM diets. These opposite effects might be caused by several factors. Enhanced titers in yolk could indicate a protection mechanism for the developing embryo. Therefore, it seems to be worth analyzing for titers both in serum and in yolk in future experiments. In addition, the effects of DON on the immune system should be described as immuno-modulating rather than as immunosuppressing since it stimulates the Ig A-synthesis of B-lymphocytes via a dys-regulated cytokine mRNA-

TABLE 10. Binding form of zearalenone or α-zearalenol in liver and bile fluid of hens fed mycotoxin-contaminated maize independent of Mycofix Plus addition (expressed as % of total recovered amount)

	Binding form of zearalenone or α-zearalenol		
	Free (not conjugated)	Conjugated with glucuronic acid	Conjugated with sulfate
Liver			
Zearalenone	46	54	(<5)
α-Zearalenol	36	28	36
Bile fluid			
Zearalenone	70	20	10
α-Zearalenol	69	4	27

turnover of T-lymphocytes (for reviews see Corrier, 1991; Pestka and Bondy, 1994; Rotter et al., 1996).

Another aim of the study was to examine the possible carryover of ZON or its metabolites into eggs and edible tissues of the hen since Dailey et al. (1980) reported a ZON-equivalent concentration of approximately 2000 μg/kg of fresh egg yolk 72 h after a bolus of 10 mg of ^{14}C-labeled ZON/kg of BW. This concentration is approximately 4,000 μg/kg of dry yolk and consequently 4,000-fold higher than the detection limit of the method applied in the present study. In contrast to their study, nonlabeled ZON from a naturally contaminated source was continuously fed to hens at a mean rate of approximately 0.8 mg/kg of BW. Despite these differences in experimental design, it would appear that substantial proportions of radioactivity detected by Dailey et al. (1980) can be ascribed to degradation products of ZON other than the metabolites analyzed in the present experiment.

Dailey et al. (1980) not only detected radioactivity in egg yolks but also in up to approximately 1% in the expired air and ascribed this to possible impurities of the radioactively labeled ZON. However, a possible microbial involvement can not be excluded since microbes within the digestive tract are able to transform ZON (Kollarczik et al., 1994) and possibly degrade it completely. Such residues from completely degraded ZON could also explain, at least in part, the discrepancy between the findings of Dailey et al. (1980) and the results of the present experiment.

Liver ZON-equivalents decreased from approximately 4,000 μg/kg 2-h post-dosing to 500 μg/kg 72 h after the bolus in the study of Dailey et al. (1980). Steady-state conditions in the present study resulted in a mean ZON plus α-ZOL concentration in livers of hens fed the CM diets of approximately 6 μg/kg. Under such steady-state conditions, a carryover factor can be calculated from the ratio between ZON plus α-ZOL concentration in liver and ZON concentration in diet that amounted to 0.005. Comparable carryover factors of approximately 0.004 can be calculated from the studies published by Olsen et al. (1986) for growing turkeys and by Maryamma et al. (1992) for growing Leghorn chicks in spite of much higher doses used in these experiments.

By sequential treatment of liver samples with β-glucuronidase and arylsulfatase prior to ZON analysis, it could be shown by Olsen et al. (1986) that the proportion of sulfated conjugates of the total conjugates ranged from 3 to 40% and 12 to 52% for ZON and α-ZOL, respectively, in blood plasma of turkeys fed diets supplemented with 800 mg ZON/kg of diet. Neither ZON nor α-ZOL was detected in serum of the hens of the present study, probably because of the 800-fold lower dietary ZON-concentration compared to the experiment of Olsen et al. (1986). In livers, the proportions of free ZON and of α-ZOL were 46 and 36%, respectively, those of gucuronidated were 54 and 28%, and sulfated proportions were <5 and 36%, respectively. ZON and α-ZOL were concentrated approximately 10-fold in the bile fluid when compared to the liver tissue. Moreover, by this concentration process it became also possible to detect β-ZOL in bile fluid. After high oral doses of 50 and 800 mg ZON/kg of diet, Mirocha et al. (1982) and Olsen et al. (1986) succeeded in proving traces of β-ZOL in livers. Therefore, the failure to detect β-ZOL in livers in the present experiment was probably dose-related. Moreover, in contrast to the liver, both ZON and α-ZOL were also detected in the bile fluid of hens fed the UCM diets, which might indicate a cumulative effect from the continuously supplied small dietary amounts of ZON originating from the "uncontaminated" diet.

The main metabolite in liver was α-ZOL, which agrees with literature findings (Olsen et al., 1986), whereas ZON was 10-times higher concentrated than α-ZOL in bile fluid. Summing up, it can be concluded from the experiment that maize which was highly contaminated with DON and ZON adversely affected laying performance, mainly due to a negative effect on feed intake at the beginning of the experiment, and modulated immune response of the hens. The practical importance of these findings needs to be evaluated by using diets that contain more realistic concentrations of DON and ZON, i.e., less than 2 mg and 0.5 mg/kg of diet, respectively.

A dietary level of 1.1 mg ZON/kg does not result in detectable residues of ZON or its metabolites in eggs. Low concentrations can be expected in the liver (carryover factor of 0.005). However, from the view that practical diets normally contain ZON levels that are much lower than those in the present experiment, the possible impact to human health arising from slightly contaminated livers is negligible. It can be easily calculated that a 70-kg human would have to consume approximately 2.8 kg/d of such slightly contaminated livers (assuming a dietary ZON-concentration of 1,000 μg/kg and a carryover factor of 0.005) in order to reach or exceed the temporary tolerable daily intake of 0.2 μg ZON/kg BW as established by the Scientific Committee on Food (European Commission, Health & Consumer Protection Directorate, 2000).

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