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Effect of *Fusarium* toxin contaminated wheat on health, nutrient digestibility and semen quality of adult cockerels

Einfluss von mit *Fusarium* kontaminiertem Weizenauf die Gesundheit, Nährstoffverdaulichkeit und Spermienqualität von erwachsenen Hähnen

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Introduction

Deoxynivalenol, also called DON or vomitoxin, is a well-known type B trichothecene mycotoxin produced mainly by several species of the genus *Fusarium* such as *F. graminearum* and *F. culmorum*. It contaminates cereals worldwide and it frequently occurs in toxicologically relevant levels in grains such as wheat, barley, oats, and maize (LOGRIECO et al., 2002).

Poultry is considered to be quite resistant to DON compared with swine (DÖLL and DÄNICKE, 2011). However, the high tolerance of poultry to the presence of DON in feedstuffs resulted in possible diversion of the infected and suspected cereal batches from swine feeding into poultry which may probably result in higher exposure of this animal category. Additionally, there is clear evidence of the diversion of contaminated grains and grain products destined for human consumption, suggesting that the poor quality grains are probably diverted to poultry feeding. Moreover, the by-products, such as bran, often serve as animal feed and usually contain even higher concentrations of DON (EFSA, 2004a).

DON was found to impair the synthesis of various proteins in broilers and laying hens (CHOWDHURY and SMITH, 2004). One important consequence of the impaired protein synthesis could be decreased spermatogenesis as protein synthesis is required for seminiferous tubules for the production of semen (GONZALES et al., 2004). In addition, the intestinal tract represents the first barrier to ingested food contaminates and the first line of defence against them. Indeed, GIRGIS and SMITH (2010), in a recent study, demonstrate histopathological and electrophysiological changes in the small intestine of birds fed 3.5 mg/kg DON in diet. Moreover, the morphological modifications in the small intestine during the long term exposure to DON can be characterized by the decrease in villus height and area (AWAD et al., 2006a; GIRGIS and SMITH, 2010). In addition, feeding DON alters the intestinal permeability by repressing the expression of tight junction proteins such as ZO-1 and occludin (PINTON et al., 2010); such effects can probably affect the nutrient digestibility and the semen quality.

There are many published studies on the effects of feeding DON contaminated diets on the health and performance of poultry (CHOWDHURY and SMITH, 2004; CHOWDHURY et al., 2005; DÄNICKE et al., 2003; DÄNICKE et al., 2002; SWAMY et al., 2004) but there is a dearth of literature regarding the effects of DON on cockerel's health, fertility and nutrient digestibility. The objectives of this study were, therefore, to investigate the effect of long term feeding of DON to adult cockerels on health, nutrient digestibility, semen quality and the excretion of DON and its metabolite, de-epoxy DON into excreta.

Material and Methods

Birds, Diets and Housing

Twenty four adult cockerels (40 weeks old) of a commercial strain "New Hampshire hybrids" were individually weighed and randomly assigned to individual cages, serving as 8 replicates for each of the three treatment groups.

Non-contaminated wheat was progressively substituted by DON contaminated wheat to create 3 diets with target DON concentrations of 0, 5, 10 mg/kg (Table 1).

Table 1. Composition of the experimental diets (g/kg as fed)[1]

Zusammensetzung der Versuchsfuttermischungen (g/kg, lufttrocken)

	Group		
Item	**1**	**2**	**3**
Components			
Wheat	287	287	287
Control-wheat	600	400	0
Contaminated-wheat	0	200	600
Soybean meal	60	60	60
Lime stone	18	18	18
Soya oil	15	15	15
Premix[1]	10	10	10
Salt	6	6	6
Dicalcium phosphate	3	3	3
L-lysine HCL	1	1	1
Calculated composition			
CP	128.8	128.8	128.8
AMEn (MJ/kg)	12.3	12.3	12.3
Lysine	5.4	5.4	5.4
Methionine + cystine	4.8	4.8	4.8
Methionine	2.9	2.9	2.9
Ca	8.1	8.1	8.1
P	3.6	3.6	3.6
Na	2.4	2.4	2.4
DON (mg/kg)[2]	0	5	10
Analyzed composition			
Dry matter DM [g/kg]	888	878	880
Organic matter [g/kg DM]	818	818	820
Crude ash [g/kg DM]	60	60	60
Crude protein [g/kg DM]	148	153	154
Ether extract [g/kg DM]	36	37	38
Crude fibre [g/kg DM]	27	27	29
N-free-extractives [g/kg DM]	606	601	599
DON (mg/kg)	0.844	4.72	11.0

[1] Provided per kg diet: Fe, 40 mg; Cu, 10 mg; Zn, 80 mg; Mn, 100 mg; Se, 0.25 mg; I, 1.2 mg; vitamin A, 10000 IU; vitamin D_3, 2500 IU; vitamin E, 20 mg; vitamin K_3, 4 mg; thiamine, 2.5 mg; riboflavin, 7 mg; pyridoxine, 4 mg; nicotinic acid, 40 mg; pantothenic acid, 10 mg; folic acid, 0.6 mg; biotin, 25 µg; choline chloride, 400 mg.

[2] DON = deoxynivalenol.

The contaminated wheat was obtained by artificial inoculation using three isolates of *Fusarium culmorum* at a concentration of 200,000 – 400,000 spores/ml. The inoculum suspension was sprayed onto the wheat spikes at the beginning of full blossom at a rate of 500 l/ha. Immediately before inoculation, Tween 20 was added to the suspension in a final concentration of 0.05% in order to ensure uniform dispersion of conidia (MATTHÄUS et al., 2002).

The feeding trial lasted 10 months; all birds had unlimited access to feed and water whereas feed intake was determined every two weeks during the experiment. No signs of morbidity were noticed on the birds during the trial.

Balance Experiment

The balance experiment was performed according to the total collection methods as described by SCHIEMANN (1981) and lasted for 9-d.

All cockerels were weighed before and after the experiment, birds had an average body weight of 3.4 ± 0.3 kg. Cockerels were adjusted to a daily feed amount of 90 g/cockerel in a pre-collection period of 2 days before excreta were collected quantitatively for 7 days. Excreta were collected twice a day (morning and afternoon) from the plastic trays beneath the cages and kept temporarily frozen between collections. Finally excreta were freeze dried and ground to pass through a 1 mm screen and analyzed.

Feed consumption and excreta weight during the 7-d collection period were used to calculate nutrients intake and excretion.

Semen Collection

Semen was collected from each cockerel by the abdominal massage method (LAKE, 1957) to assess semen quality and characteristics (ejaculate volume, sperm concentration, motility and velocity estimated by computer-assisted sperm analysis, viability and sperm morphology). Within 10 months, from September until July, the semen samples were collected 13 times (at days 7, 16, 23, 30, 36, 57, 79, 98, 190, 243, 264, 285 and 305 after the beginning of the experiment). The intervals between the semen collections were irregular with a 3-month interruption between December and March. The animals were 80 weeks old at the last semen collection.

The ejaculated semen was diluted 1:2 immediately after collection with an extender at room temperature. The extender was prepared as described by HANZAWA et al. (2006): Sodium glutamate (H2O) 1.2 g, potassium acetate (anhydrous) 0.3 g, trehalose 3.8 g, glucose (anhydrous) 0.2 g, N,N-Bis (2-hydroxyethyl)-2-aminoethansulfonic acid (BES) 0.5 g, Bis(2-hydroxyethyl) iminotris (hydroxymethyl) methane (Bis-tris) 0.5 g, gentamicin sulfate 0.001 g per 100 ml distilled water, osmolarity 360 mOsm/kg, pH 6.8. The diluted semen was transported in a box with 4°C during 1 hour to the laboratory.

Slaughtering and Collecting Samples

At the end of the experiment all birds were killed by cutting the jugular vein after electrical stunning, blood was collected into heparinised tubes for haematological evaluation (blood smears and haematocrit) and preparation of the plasma. Organs (breast muscle, heart, liver, spleen, glandular stomach, gizzard, small and large intestine and testes) were excised, emptied (glandular stomach, gizzard, small and large intestine) and weighed. The relative weight of each organ was calculated by dividing the individual absolute weight by the body weight (BW) and expressed as g organ weight/kg BW (relative organ or tissue weight).

Tissues for histological study were taken directly after the slaughter of the cockerels.

Analyses

Nutrients. Diets and freeze-dried excreta were analysed for crude nutrients [Dry matter (DM), crude ash (Ash), crude fibre (CF), crude protein (CP), ether extract (EE)] according to the methods described by NAUMANN and BASSLER (1993), whereas the N-free-extractives and the organic matter were calculated by difference.

Mycotoxins. Deoxynivalenol in wheat and diets was analysed by HPLC with UV detection after a clean-up with immuno-affinity columns (IAC) (DONprepTM, R-Biopharm Rhone, Darmstadt, Germany) as described elsewhere in detail (OLDENBURG et al., 2007), the limit of detection was 30 µg/kg DM. Deoxynivalenol and its metabolite de-epoxy-deoxynivalenol in freeze-dried excreta were analyzed with LC-MS/MS (liquid chromatography/tandem mass spectrometry).

The sample preparation was done according to VALENTA et al. (2003) with modifications. Briefly, freeze-dried excreta were incubated with ß-glucuronidase (type H-2, min. 98 800 U/ml, Sigma, Steinheim, Germany) at pH 5.5 (acetate buffer) and 37°C overnight and extracted with a mixture of acetonitrile and water. Subsequently, the extracts were defatted with petroleum ether, pre-cleaned with a mixture of charcoal, alumina and celite and cleaned-up with IAC (DONprepTM, R-Biopharm Rhone). DON and de-epoxy-DON in the samples were determined with LC-ESI-MS/MS in

negative mode as briefly described by GOYARTS et al. (2010). The detection limits for DON and de-epoxy-DON in excreta were approximately 0.8 and 1.6 ng/g DM, respectively, with mean recoveries of 93% and 92%, respectively.

In wheat samples, further mycotoxins were determined by the Institute of Agrobiotechnology (IFA) (Tulln, Austria) applying a LC-MS/MS method as described by VISHWANATH et al. (2009).

Histopathology. Organ specimens of liver, kidney, spleen and the left testes from Groups1 and 3 (control cockerels and cockerels fed highest DON concentration) were sampled at slaughter for histopathological examination.

Samples were collected directly after slaughtering and then fixed in 10% neutralized formaldehyde solution (Roti®-Histofix 10%, Carl Roth GmbH + Co KG, Karlsruhe, Germany); after 24 hours, the initial fixating solution was replaced with new formaldehyde (10%) and after that samples were embedded in paraffin blocks, microtome sections were stained with haematoxylin and eosin (HE) and then examined for tissue changes by two veterinary pathologists blind to the experimental treatments.

The histological examination was carried out at the Institute of Pathology, University of Veterinary Medicine Hannover.

Haematology. Blood was collected into heparinised tubes from the jugular veins at the slaughtering point. Haematocrit was determined by using heparinised capillaries for blood sampling after 6–8 minutes of centrifugation at 13,000 RPM (RCF: 16,060 × g) in a micro-haematocrit centrifuge.

Differential white blood cell counts were performed using blood smears from each blood sample stained with Wright-Giemsa stain (WGS) according to an established protocol by SAMOUR and PENDL (2009). Two hundred cells were counted to each ratio per light microscope (Carl Zeiss Microscopy GmbH, Jena, Germany) at a magnification of × 100, and heterophils, lymphocytes, monocytes, eosinophils, and basophils were identified. Heterophil/lymphocyte (H/L) ratios were determined by dividing the number of heterophils by the number of lymphocytes. The absolute counts of heterophils, lymphocytes, monocytes, eosinophils, and basophils as well as H/L ratios were determined by methods described by CAMPBELL and DEIN (1984).

Plasma Clinical Chemistry. After collecting blood, samples were centrifuged at 5000 rpm at 15°C for 15 minutes, 1 ml of the separated plasma was removed into 1.5 ml tubes and samples were frozen at-70°C until analysis. Activities of aspartate amino-tranferase (AST), glutamate dehydrogenase (GLDH), γ-glutamyl transferase (GGT) as well as total protein, albumin, glucose, bilirubin, cholesterol, triglycerides and urea concentrations in plasma were determined photometrically by an automatic clinical chemistry analyser (Eurolyser, Qinlab Diagnostic GbR, Martinsried, Germany).

Semen Evaluation. Ejaculate volume was measured visually. Sperm cell concentration was determined by measuring the Spermatocrit (percent packed cell volume) by centrifugation of micro-haematocrit capillary tubes filled with native sperm as described by TANEJA and GOWE (1961). The motility characteristics of spermatozoa were evaluated by means of CASA (computer-assisted sperm analysis) with a Hamilton Thorne Biosciences-IVOS (Beverly, USA) and 4-Leja analysis chambers of a thickness of 20 μm (Minitube, Tiefenbach, Germany).

The instrument's setting was adapted from KLIMOWICZ et al. (2008); only the temperature of analysis was increased from 24°C to 30°C. Semen was diluted 1:200 in HS1 medium before measurement. The parameters measured were: percentage of total motile sperm (MOT%), percentage of progressive motile sperm (ProgMot%), average path velocity (VAP, μm/s), progressive velocity (VSL μm/s), curvilinear velocity (VCL, μm/s), amplitude of lateral head displacement (ALH, μm), beat cross frequency (BCF, Hz), straightness of track (STR %, ratio of VSL/VAP) and linearity of track (LIN %, ratio of VSL/VCL).

For the morphological examination, sperm cells were fixed in formol citrate solution and microscopically evaluated using a 100x oil immersion objective (ALKAN et al., 2002). In each preparation, 100 spermatozoa were counted, and the percentage of defect types was calculated.

Sperm membrane integrity was assessed flow cytometrically using a FACScan® (BD Bioscience, Heidelberg, Germany). The cells were diluted with their respective extender to1x106 sperm/ml and stained with propidium iodide (3 μM final). Measures were always performed as duplicates.

Calculations and Statistics

The Statistica for the WindowsTM operating system (Version 10, Stat Soft Inc. 1984–2011) was used for the data analysis (except for semen data). With the exception of feed intake, pathological changes, values of GLDH and the semen data, all other measures were subjected to analysis of variance (ANOVA) according to a one-factorial design: $y_{ij} = \mu + a_i + e_{ij}$

Where y_{ij} = jth observation related to DON-level i; μ = overall mean; a_i = effect of dietary DON-level; e_{ij} = error term.

Mycotoxin residue concentrations in excreta which were lower than the detection limits were considered with a concentration of zero in evaluating the data. This implies that calculated mean values might be lower than the detection limits.

Feed intake was analysed by 1-way ANOVA with repeated measurements. The values of GLDH were not normally distributed and therefore evaluated using the Kruskal-Wallis test. The differences among the pathological changes were evaluated by the Exact Wilcoxon Two-Sample Test. Significant mean value differences were evaluated by the Tukey HSD test.

For the semen data a repeated measures analysis of variance was performed using the JMP 7 software. The mixed model contained cockerels as random effect and date, treatment group and interaction date* treatment group as fixed effects.

Apparent digestibility of the nutrient contents of the experimental diets were calculated according to the total collection method by the difference between total quantities ingested and excreted by the cockerels.

$$\text{Nutrient digestibility (\%)} = \frac{\text{Nutrient intake} - \text{Nutrient output}}{\text{Nutrient intake}} \times 100$$

Results

Wheat and Diet Analyses

The composition of the contaminated diets differed only slightly from the control diet (Table 1); the analyzed DON concentrations of the three feeding diets were (0.8, 4.7, 11.0 mg/kg).

The mycotoxin compositions of contaminated and uncontaminated wheat are shown in Table 2. The contaminated wheat contained high concentrations of DON (13448 µg/kg), aurofusarin, culmorin, 15- and 5- hydroxy culmorin. In contrast, the control wheat contained trace amounts of DON and nivalenol, whereas ZEN was even lower than detection limits.

Table 2. Mycotoxin composition of contaminated and uncontaminated wheat (µg/kg) corrected for recovery[1]

Mykotoxinzusammensetzung des kontaminierten und des unkontaminierten Weizens (µg/kg, lufttrocken, korrigiert um die Wiederfindung)

	UCW	*CW*
Deoxynivalenol (DON)	54	13448
Deoxynivalenol-3-glucoside	3.81	761
3-Acetyldeoxynivalenol	< 4	223
15-Acetyldeoxynivalenol	63.9	< 8
Nivalenol	4.13	8.33
Zearalenon (ZEN)	< 1.5	20.6
Zearalenon-4-sulfate	0.125	3.91
α-Zearalenol	< 0.5	< 0.5
β-Zearalenol	< 0.8	2.59
Enniatin B	0.675	3.65
Enniatin B1	1.64	3.56
Enniatin A1	0.959	0.827
Enniatin A	0.142	0.059
Beauvericin	0.600	0.387
Butenolid	< 4	600
Moniliformin	< 0.5	0.532
Apicidin	1.53	0.819
Equisetin	1.66	2.37
Fusaproliferin	12.1	< 12
Aurofusarin	79.7	4734
Avenacein Y	6.00	< 4
Chlamydosporol	< 0.8	< 0.8
Culmorin	16.03	2763
15-Hydroxy-Culmorin	15.18	2267
5-Hydroxy-Culmorin	< 10	1065
Alternariol	0.185	< 0.15
Alternariolmethylether	< 0.1	< 0.1
Tentoxin	0.575	0.176
Altertoxin-I	< 0.2	< 0.2
Emodin	1.53	1.16
Chrysophanol	< 1	< 1

[1] UCW = uncontaminated wheat; CW = Fusarium toxin-contaminated wheat.Determined by the Institute of Agrobiotechnology (IFA) (Tulln, Austria) applying a LC-MS/MS method as described by VISHWANATH et al. (2009), except for DON in contaminated wheat which was determined with HPLC/UVas described by OLDENBURG et al. (2007).

Feed Intake, Body and Organ Weights

The dietary treatment had no significant effect on the cockerel's feed intake. However, feed intake was significantly decreased in the course of the study from 97.5 g/cockerel/day at the beginning of the feeding trial to 90.5 g/cockerel/day at the end of the study (Table 3).

Table 3. Effect of dietary DON on feed intake[1)]

Einfluss von DON auf die Futteraufnahme

Experimental month	DON mg/kg	Feed intake g/cockerel
	0.8	105
1–5	4.7	89.0
	11.0	98.1
	0.8	92.9
6–10	4.7	89.6
	11.0	89.0
ANOVA (probability)		
DON		0.449
Time		< 0.001
DON × time		0.488
PSEM		3.511

[1)] Data are reported as means; PSEM = pooled standard error of means.

Cockerels' live body weight at the end of the trial and the relative weight of selected organs are summarized in Table 4.

Table 4. Effect of dietary DON on body weight and the relative weight of selected organs of adult cockerels[1)]

Einfluss von DON auf das relative Körper-und Organgewicht

Organ g/kg of BW	DON in diet (mg/kg)			P-values
	0.8	4.7	11.0	
Body weight	3449 ± 105	3289 ± 112	3394 ± 105	0.580
Breast muscle	39.2^{b} ± 1.8	46.5^{a} ± 1.9	46.9^{a} ± 1.8	0.013
Liver	8.4 ± 0.45	8.0 ± 0.48	7.8 ± 0.45	0.599
Heart	5.9 ± 0.33	5.6 ± 0.35	5.0 ± 0.33	0.136
Spleen	1.3 ± 0.1	1.3 ± 0.1	1.2 ± 0.1	0.696
Glandular stomach	2.7 ± 0.3	2.8 ± 0.3	2.7 ± 0.3	0.969
Gizzard	11.5 ± 0.7	11.0 ± 0.8	10.8 ± 0.7	0.774
Duodenum	1.9 ± 0.1	2.0 ± 0.1	2.2 ± 0.1	0.516
Jejunum	5.5 ± 0.3	5.0 ± 0.3	5.1 ± 0.3	0.526
Ileum	6.2^{a} ± 0.3	3.3^{c} ± 0.3	4.4^{b} ± 0.3	< 0.001
Rectum	1.0 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.719
Caecum	3.6^{a} ± 0.3	2.7^{ab} ± 0.3	2.5^{b} ± 0.3	0.037
Left testis	2.7 ± 0.3	2.5 ± 0.3	2.3 ± 0.3	0.690
Right testis	2.7 ± 0.3	2.6 ± 0.3	2.6 ± 0.3	0.900

[1)] Data are reported as means ± SE; DON = deoxynivalenol, (a-c) within the same row, means with different letters are significantly different (Tukey HSD test).

Cockerels' live body weight was not affected by the dietary treatment, likewise the relative weight of liver, heart, spleen, glandular stomach, gizzard, duodenum, jejunum, rectum and testis (left and right testis) were also not affected by the presence of DON in cockerels' diet. The relative weight of breast muscle was significantly increased in the cockerels fed DON contaminated diets compared with the control group, while relative weights of ileum and caecum were significantly decreased.

Balance Study

Fat digestibility was progressively increased as the concentrations of DON in the diet increased ($p < 0.001$) (Table 5). Utilizations of organic matter, carbohydrates and nitrogen balance were not affected by the dietary treatment.

Table 5. Nutrient digestibility of control and DON contaminated diets fed to adult cockerels[1)]

Nährstoffverdaulichkeit der Kontrolle und DON kontaminierten Diäten bei erwachsenen Hähnen

	Apparent digestibility/utilization (%)			
DON mg/kg	**Nitrogen balance**	**Organic matter**	**Fat**	**Carbohydrates[2]**
0.8	19.5	79.9	64.3^{c}	92.1
4.7	20.4	79.8	74.5^{b}	91.9
11.0	20.0	79.9	77.0^{a}	92.3
ANOVA				
P values	0.848	0.939	< 0.001	0.627
PSEM	1.163	0.257	0.647	0.267

[1)] Data are reported as means, (a-c) within the same row, means with different letters are significantly different (Tukey HSD test), PSEM = pooled standard errors of means.
[2)] Sum of N-free extractives and crude fiber.

Excretion of DON and De-epoxy-DON into Excreta

The daily DON intake by the cockerels was determined by the dietary DON concentration. The contaminated diets resulted in average exposure of 130–291 µg/kg body weight daily, thus exposure was approximately 6–10 times higher than the control group (Table 6).

Table 6. Effect of dietary DON on the fate of DON and metabolites in adult cockerels exposed to control and DON contaminated diets[1)]

Einfluss von DON-Aufnahme auf die Ausscheidung von DON und Metaboliten bei adulten Hähnen

DON in diet mg/kg	**DON intake (µg/kg b.w/d)**	**DON excretion (µg/kg b.w/d)**	**de-epoxy-DON excretion (µg/kg b.w/d)**	**de-epoxy-DON excretion (% of DON + de-epoxy- DON excretion)**	**DON + de-epoxy-DON excretion (% of DON intake)**
0.8	21.2^{c}	0.44^{b}	0.00^{b}	0.0^{b}	2.10^{a}
4.7	130.6^{b}	1.36^{a}	0.02^{ab}	1.75^{ab}	1.07^{b}
11.0	291.0^{a}	1.46^{a}	0.09^{a}	4.97^{a}	0.54^{c}
ANOVA					
P values	< 0.001	< 0.001	0.038	0.025	< 0.001
PSEM	4.9	0.2	0.0	1.2	0.1

[1)] Data are means of 8 cockerels for each treatment; (a-c) within the same row, means with different letters are significantly different (Tukey HSD test), PSEM = pooled standard errors of means.

The excretion of DON of the cockerels fed the DON contaminated diet was significantly higher compared with the control group, while the absolute excretion of de-epoxy-DON and its proportion of the DON plus de-epoxy-DON were significantly higher in the highest DON fed group. Moreover, excretion of DON and de-epoxy-DON, when related to feed intake, was progressively decreased as the concentrations of DON in diets increased.

Haematology

Haematocrit and the concentrations of white blood cells (Differential white blood cell counts) were not significantly affected by the dietary treatment (Table 7).

Table 7. Effect of dietary DON on Haematocrit and differential leukocyte count[1)]

Einfluss von DON auf den Hämatokrit und das Differentialblutbild

DON mg/kg	HCT Vol.%	WBC x10^3/µl	Lymphs %	Het %	H/L Ratio	Eos %
0.8	43.5	15.4	57.3	34.9	0.6	7.0
4.7	42.8	13.6	60.9	33.2	0.6	4.6
11.0	40.3	14.8	63.3	29.8	0.5	6.1
ANOVA						
P values	0.26	0.66	0.58	0.59	0.59	0.38
PSEM	1.45	1.37	4.10	3.65	0.09	1.19

[1)] Data are reported as means; DON = deoxynivalenol; HCT = haematocrit; WBC = white blood cells; Lymphs = lymphocytes; Het = heterophils; H/L = heterophils/lymphocytes ratio; Eos = eosinophils; PSEM = pooled standard errors of means; basophils and monocytes proportion were in the range: 0 –2% with no dietary effect.

Clinical Chemistry

The plasma concentrations of total protein, bilirubin, albumin and cholesterol were not influenced by the dietary treatment. The plasma urea content was significantly increased in the cockerels fed the highest DON concentrations (Table 8). Moreover, triglyceride concentration was significantly lower in plasma of cockerels fed 4.7 mg/kg DON compared with the control group.

Table 8. Effect of dietary DON on plasma biochemistry in adult cockerels[1)]

Einfluss von DON auf klinisch-chemische Parameter im Plasma

DON (mg/kg)	Protein (g/l)	Urea (mg/dl)	Glucose (mg/dl)	Bilirubin (mg/dl)	Triglyceride (mg/dl)	Albumin (g/l)	Cholesterol (mg/dl)
0.8	44.4	5.9^b	262	1.3	47.6^a	22.0	105
4.7	39.5	5.7^b	233	1.3	34.5^b	18.9	91
11.0	47.6	9.5^a	310	1.4	40.7^{ab}	25.1	111
ANOVA							
P values	0.469	0.049	0.053	0.804	0.015	0.077	0.327
PSEM	4.486	1.164	21.04	0.031	2.833	1.790	9.196

[1)] Data are reported as means; DON = deoxynivalenol. (a-b) within the same row, means with different letters are significantly different (Tukey HSD test); PSEM = pooled standard errors of means.

A trend effect of DON (P = 0.053) was noticed on the concentration of glucose as it increased slightly in the in plasma of cockerels fed 11.0 mg/kg DON.

The plasma activity of aspartate amino transferase (AST), γ-glutamyl transferase (GGT) and glutamate dehydrogenase (GLDH) were not altered by the dietary treatment (Table 9).

Table 9. Effect of dietary DON on Plasma enzyme activities of adult cockerels[1)]

Einfluss von DON auf Enzymaktivitäten im Plasma

DON mg/kg	AST (U/l)	GGT (U/l)	GLDH[2)] (U/l)
0.8	296	12.58	1.95 (<d.l. – 7.3)
4.7	254	9.61	0.5 (<d.l. – 3.5)
11.0	372	11.69	2.4 (<d.l. – 5.7)
ANOVA			
P values	0.219	0.634	
PSEM	46.64	2.19	

[1)] Data are reported as means; DON = deoxynivalenol; [2)] Evaluated applying the Kruskal-Wallis test, mean (minimum-maximum); PSEM = pooled standard errors of means

Histopathology

Histological examination of liver, spleen, kidney and testis of both the control and highest DON groups revealed no pathological changes related to the dietary treatment.

It should be stressed that a moderately to severely active spermatogenesis, and slight multifocal exfoliation of a gamete cells in the lumen of sperm canal, was observed in all testis samples, this slight exfoliation of cells into the lumen of sperm canal may indicate an incipient degeneration (not to be assigned to the presence of DON in diet).

Semen Quality and Characteristics

The long lasting feeding of DON had no significant effects on the quality parameters of cockerel semen studied (Table 10).

Table 10. Quality parameters of cockerel's semen (Least Sq Mean ± SEM) in the treatment groups[1)]

Einfluss von DON auf die Spermienqualitätsparameter

Sperm	DON in diet (mg/kg)			P values
Parameters	0.8	4.7	11.0	
Volume (ml)	0.77 ± 0.04	0.77 ± 0.04	0.80 ± 0.04	0.76
Spermatocrit	8.7 ± 0.68	8.4 ± 0.68	8.8 ± 0.68	0.91
% motile	87.0 ± 1.59	89.1 ± 1.59	89.9 ± 1.59	0.43
% ProgMot	76.8 ± 2.78	78.4 ± 2.78	79.0 ± 2.78	0.85
VAP (µm/s)	77.4 ± 2.14	76.4 ± 2.14	74.1 ± 2.14	0.55
VSL (µm/s)	66.7 ± 2.53	65.6 ± 2.53	63.4 ± 2.53	0.64
VCL (µm/s)	116.8 ± 2.22	115.4 ± 2.22	113.6 ± 2.22	0.59
ALH (µm)	4.9 ± 0.08	4.9 ± 0.08	4.9 ± 0.08	0.94
BCF (Hz)	34.8 ± 0.69	35.4 ± 0.70	34.6 ± 0.69	0.67
STR (%)	84.2 ± 1.17	83.9 ± 1.17	83.6 ± 1.17	0.95
LIN (%)	56.9 ± 1.36	56.5 ± 1.37	55.7 ± 1.37	0.82
% abnormal	11.0 ± 1.15	10.0 ± 1.15	8.3 ± 1.15	0.26
% live	99.2 ± 0.15	99.1 ± 0.15	98.8 ± 0.15	0.14

[1)] Volume, ejaculate volume; spermatocrit, percent packed cell volume; % motile, percentage of motile sperm; % ProgMot, percentage of progressive motile sperm; VAP, velocity average path; VSL, velocity straight line; VCL, velocity curve line; ALH, average lateral head displacement; BCF, beat-cross frequency; STR, straightness ((VSL/VAP)x100); LIN, linearity ((VSL/VCL)x100); % abnormal, percentage of morphologically abnormal sperm; % live, percentage of live sperm.

No significant influence of DON on semen parameter had to be proved; the date had well an influence. At some semen parameters there was an inconsistent interaction of date* treatment group ($P < 0.05$).

Discussion

Feed Intake, Cockerel's Body and Organ Weights

The prolonged dietary DON exposure was described to cause anorexia, decreased live weight gain and altered nutrient efficiency (PESTKA and SMOLINSKI, 2005); However, poultry shows obviously low sensitivity towards DON and feed refusal and the reduction of weight gain are only observed when the dietary DON concentrations reach 16 –20 mg/kg feed (HARVEY et al., 1991; KUBENA and HARVEY, 1988).

Feeding DON to the adult cockerels did not affect feed intake; in accordance with that BERGSJO et al. (1993a) and BERGSJO and KALDHUSDAL (1994) also reported no effects of feeding DON on feed intake. Moreover, LUN et al. (1986) noticed that laying hens tolerated the feeding of high levels of DON (82.8 mg/kg) without any effect on body weight or feed consumption. Moreover, poultry seems to be able to adapt to DON. Indeed, DÄNICKE et al. (2002) noticed an overall adverse effect of DON (17.6 mg/kg) on laying hen performance (resultingfrom feed intake reduction); however these hens were able to adapt to the diet over the course of experiment.

The absence of any effect of the dietary treatment on cockerel's feed intake can explain the non-affected body weight. Accordingly, HAMILTON et al. (1985) and KUBENA et al. (1987) also demonstrated no effect of DON on body weight.

In the present study, a decrease was noticed in the relative weight of ileum and caecum due to the presence of DON in the diet. Indeed, AWAD et al. (2006a) found that the weight of small intestine decreased in broilers fed DON contaminated wheat. DÄNICKE et al. (2002) also found a slight decrease in the weight of small intestine in laying hens. AWAD et al. (2006a, b) reported an intestinal change due to the dietary inclusion of DON (slight villus atrophy and irregular crypts) resulting from the cytotoxicity and inhibition of protein synthesis following the exposure of the intestinal epithelial cells to DON.This alteration can explain the decrease in ileum relative weight. All other organ's relative weights (liver, heart, spleen, glandular stomach, gizzard, duodenum, jejunum, left and right testis and rectum) were not significantly affected by the dietary treatment; these findings are in accordance with the findings of DÄNICKE et al. (2002) and SWAMY et al. (2004).

Balance Study

The impact of DON on nutrient utilization in poultry has rarely been studied. The dietary treatment resulted in increased fat utilization. A similar increase in fat digestibility was also noticed in pigs (DÄNICKE et al., 2004; GOYARTS and DÄNICKE, 2005). However, the impact of the *Fusarium* fungus on the wheat kernel might probably result in changes in the feeding value of the infected feedstuff. Indeed, it could be shown by MATTHÄUS et al. (2002) that *Fusarium* infection resulted in changes in the nutrient composition of the contaminated wheat compared to the uncontaminated wheat. Moreover, KANG and BUCHENAUER (2000) reported a series of alterations in host tissues after the colonisation of the wheat spikes indicating that the fungi invade the pericarp and aleurone and penetrate the cell walls quickly to enter the starchy endosperm.

Therefore, it could be hypothesised that the impact of the *Fusarium* infected wheat on the cockerels resulted from both DON toxic effects and the structural changes caused by the fungal growth.

Excretion of DON and De-epoxy-DON into excreta

The total recovery of DON and de-epoxy DON was approximately 0.5 – 2.1%. Similarly low recovery (approximately 2 – 5%) was also reported by DÄNICKE et al. (submitted). On the other hand, experiments with radioactively labeled DON usually show a high recovery range of between 7–69% (LUN et al., 1989) and reach more than 90% of the single radioactively labeled DON (PRELUSKY et al., 1986).

In the both mentioned studies, radioactivity was exclusively analyzed, while we analyzed both DON and de-epoxy-DON directly in the excreta by LC-MS/MS. The relatively low recovery of ingested DON from excreta indicates that it has been absorbed and/or transformed into unidentified metabolites that were not detectable in the used analysis methods and suggests that metabolites might have been generated which need to be identified in further studies.

De-epoxy-DON was only detected in the excreta of cockerels fed DON contaminated diets. This finding is in accordance with study of LAUBER et al. (2000) who demonstrated that the gut microflora adapts to increasing amounts of DON with increasing ability for de-epoxidation.

De-epoxy metabolites of trichothecenes have been detected in the excreta of chickens (LUN et al., 1986). Moreover, ROTTER et al. (1996) and SWANSON and CORLEY (1989) assumed that the de-epoxidation reaction occurs in the gastrointestinal tract of monogastrics before the absorption. This de-epoxidation is the most important step in the detoxification of the trichothecenes. If a significant proportion of the trichothecenes is de-epoxidised before the

absorption or any alteration occurs on the GIT epithelial layer, this ability may significantly reduce the toxicity of trichothecenes (AWAD et al., 2008). This gastrointestinal de-epoxidation in the gut of some species can contribute to species differences in sensitivity towards DON, which may partly explain the relatively high tolerance of poultry.

Haematology

Feeding DON did not affect cockerels' haematology. In accordance with these results, DÄNICKE et al. (2003) and HARVEY et al. (1991) also noticed no effect of feeding DON contaminated diets on the haematological parameters. Thus, DON appeared to be non-hematotoxic in cockerels.

Clinical Chemistry

Urea is formed by the liver and carried by the blood to the kidneys for excretion. Because urea is cleared from the bloodstream by the kidneys, urea concentration in plasma can be used as a test of renal function. In the present study, urea concentration was significantly higher in plasma samples of cockerels fed 11 mg/kg DON (Table 8). However, the increase in plasma urea levels observed suggests possible kidney damage induced by this mycotoxin. Indeed, DINISCHIOTU et al. (2007) also reported an increase in the urea concentrations in piglets after 15 days of DON treatment.

A similar decrease in triglyceride concentrations was reported in a study with white Leghorn chicks and broiler chicks (FAIXOVA et al., 2010; ZUGEL et al., 1998), suggesting that the presence of DON in diets probably affects the metabolism of proteins and lipids in the cockerels (GHAREEB et al., 2012).

Plasma AST, GGT and GLDH activity indicating liver damage and other plasma chemical parameters (concentrations of total protein, bilirubin and cholesterol) remained unchanged in the cockerels fed DON contaminated diets. Indeed, YEGANI et al. (2006), in a study with broiler breeders, noticed that the consumption of 12.6 mg/kg DON for 12 weeks did not affect any of blood biochemical parameters including AST, GGT and GLDH. Moreover, DÄNICKE et al. (2003) and MORRIS et al. (1999) also reported no effect of DON on serum parameters.

It should be stressed that glucose concentrations were slightly increased ($P = 0.053$) in plasma samples from cockerels fed 11 mg/kg DON, a similar increase in blood glucose was reported in growing Wistar rats which received 250 ml/150 g B.W DON (SZKUDELSKA et al., 2002). This finding is in contrast with the suggested decrease of glucose absorption reported by AWAD et al. (2004). However, the effect of DON on insulin levels may also play an important role. Indeed, KOBAYASHI-HATTORI et al. (2011) noticed that the insulin level was significantly decreased in pooled plasma samples from female mice fed DON.

Albumin concentrations tended to be lower ($P = 0.077$) in plasma samples from cockerels fed 4.7 mg/kg DON. However, it has been reported that dietary contamination with *Fusarium* mycotoxins (8.6 mg/kg DON) resulted in a reduction of the albumin levels in serum of growing piglets (DÖLL et al., 2004). Moreover, BERGSJO et al. (1993b) also reported a significant decrease in serum albumin in growing pigs fed a diet containing 3.5 mg/kg DON.

Histopathology

The histological examination revealed no pathological changes in tissues examined to be related to the dietary treatment. These findings are in accordance with the findings of (BERGSJO and KALDHUSDAL, 1994; BOSTON et al., 1996; MORRIS et al., 1999). The absence of DON effects on liver weight and plasma AST, GGT and GLDH activities, in addition to the histopathological findings indicate that feeding DON to cockerels did not induce detectable liver damage.

Semen Quality and Characteristics

There are only a few reports on the effects of feeding *Fusarium* mycotoxin- contaminated feed on the reproductive performance of Cockerels (see review of GIRGIS and SMITH, 2010). YEGANI et al. (2006) fed Cockerels with DON contaminated grains (6.4 mg/kg of feed) for about 12 weeks. It was found that Cockerel semen volume, sperm concentration, viability and motility were not affected by the feeding of contaminated diets.

Conclusion

The outcome of the present study indicates that feeding of cockerels with DON contaminated wheat had no negative impact on semen quality, nutrient digestibility and feed intake.

With the exception of the content of urea and triglyceride in plasma; feeding DON did not affect the blood haematology and plasma clinical chemistry parameters. Moreover, utilizations of organic matter, carbohydrates and nitrogen balance were not affected by the dietary treatment while fat digestibility was even improved.

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Summary

A ten-month-feeding trial was conducted with twenty four adult cockerels of a commercial strain "New Hampshire hybrids" to evaluate the effect of feeding of wheat, mainly contaminated with the *Fusarium* toxin deoxynivalenol, on cockerel's health, nutrient digestibility, semen quality and DON metabolism. Birds were individually weighed and randomly assigned to one of three treatment groups (control, 4.7 mg DON/kg and 11 mg DON/kg).

Feed intake and cockerels' body weight were not affected by the dietary treatment, nor were the relative organ weights affected. On the other hand, breast muscle relative weight was increased in the cockerels fed DON contaminated diets, while the relative weight of ileum and caecum were significantly decreased at the same time.

The content of urea was significantly increased in plasma of cockerels fed the highest DON concentrations, while the triglyceride concentration was significantly lower in plasma of cockerels fed 4.7 mg/kg DON. Haematological and other clinical-chemical parameters remained unaffected. Moreover, crude fat utilization was progressively increased as the concentrations of DON in the diet increased. Utilizations of organic matter, carbohydrates and nitrogen balance were not affected by the dietary treatment. Furthermore, the long term feeding of cockerels with DON contaminated wheat had no negative impact on semen parameters.

Taken together, it might be concluded that cockerels are quite resistant to the effects of DON regarding reproductive traits, nutrient digestibility and feed intake. The dose-dependent alterations in the relative weights of breast muscle and digestive organs require further consideration.

Key words

Deoxynivalenol, adult cockerels, health, digestibility, semen quality

Zusammenfassung

Einfluss von mit *Fusarium* kontaminiertem Weizenauf die Gesundheit, Nährstoffverdaulichkeit und Spermienqualität von erwachsenen Hähnen

Ein 10-monatiger Fütterungsversuch mit 24 erwachsenen Hähnen der Rasse „New Hampshire Hybride" wurde durchgeführt, um den Effekt der Fütterung von mit dem *Fusarium*-Toxin Deoxynivalenol (DON) kontaminierten Weizenauf die Gesundheit, Nährstoffverdaulichkeit, Spermaqualität und den DON Stoffwechsel zu prüfen. Die Hähne wurden einzeln gewogen und zufällig einer von drei Behandlungsgruppen (Kontrolle, 4,7 mg DON/kg und 11 mg DON/kg) zugeordnet.

Es gab keinen signifikanten Effekt von DON auf die Futteraufnahme und auf das Körpergewicht der Hähne. Dagegen waren die relativen Gewichte vom Brustmuskel der Hähne die DON mit dem Futter erhalten haben erhöht, während die relativen Gewichte des Ileums und des Blinddarms deutlich verringert waren.

Die Harnstoff Konzentration war im Plasma der Hähne der höchsten DON-Gruppe (11 mg/kg) signifikant angestiegen, während die Triglycerid Konzentration im Plasma der Hähne der4.7 mg/kg DON-Gruppe signifikant niedriger war. Der Hämatokritwert, das Differentialblutbild und die anderen klinisch-chemischen Parameter waren nicht betroffen. Darüber hinaus war die Verdaulichkeit des Rohfetts schrittweise parallel zu einer steigenden Konzentration von DON in der Diät erhöht. Die Verdaulichkeit der Organischen Substanz, Kohlenhydrate sowie die Stickstoffbilanz und Spermienparameter wurden nicht beeinflusst.

Zusammengefasst könnte gefolgert werden, dass Hähne relativ resistent gegenüber den Auswirkungen von DON auf Reproduktionsmerkmale, Nährstoff-Verdaulichkeit und Futteraufnahme sind. Die dosis-abhängigen Veränderungen der relativen Gewichte des Brustmuskels und der Verdauungsorgane benötigen weitere Klärung.

Stichworte

Deoxynivalenol, Erwachsene Hähne, Gesundheit, Verdaulichkeit, Spermienqualität

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