https://doi.org/10.17221/189/2020-CJAS

Examination of high-resolution feed intake data of growing-finishing pigs confronted with high deoxynivalenol contents present in their feed

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Citation: Loibl P., Windisch W., Preißinger W. (2020): Examination of high-resolution feed intake data of growing-finishing pigs confronted with high deoxynivalenol contents present in their feed. Czech J. Anim. Sci., 65: 411–423.

Abstract: Modern single space feeding systems for fattening pigs allow the detailed assessment of an individual animal's feeding behaviour. In an experiment involving 96 fattening pigs, the influence of deoxynivalenol (DON) contaminated feed (> 4 500 µg/kg DON) on the zootechnical performance and feeding behaviour was compared with a feed with low DON concentration (< 900 µg/kg DON), this served as the control group. Additionally, in separate treatments, two commercial mycotoxin binders were added to the DON-contaminated feeds to assess if an expected DON effect could be attenuated. The high DON content significantly ($P < 0.03$) reduced daily feed intake (500–600 g/day). The DON group showed 240 g less daily gain compared to the control with 728 g/day. Both mycotoxin binders were seen to additionally depress weight gain by approximately 65 g/day ($P < 0.01$). The treatment did not affect the individual feeding behaviour as assessed by daily visits to the feeder, feed intake per visit and the highest feed intake per visit per day. These were influenced only by the pig and its pen, indicating that the animals developed a distinct behaviour within their respective groups. Behaviour analyses of persistency and day-to-day variation showed that the persistency was reduced and variation was increased when DON without or with binder was present. The DON contents therefore seemed to lead to a more erratic and less consistent behaviour that remained dependent on the animal group.

Keywords: behaviour; DON; fattening pig; mycotoxin binder

In modern pig husbandry, mycotoxins are a common feed-borne threat (Escriva et al. 2015). The *Fusarium* family represents the most relevant source of the most hazardous mycotoxins, namely the trichothecenes. They include two of the most commonly observed mycotoxins, deoxynivalenol (DON) and zearalenone (ZEN) (Doll and Danicke 2011). *Fusarium* strains mainly infect the com-

Funded by the Bayerisches Staatsministerium für Ernährung, Landwirtschaft und Forsten (Bavarian State Ministry of Food, Agriculture and Forestry) [Project No. A/13/12 "Schweinefütterung – Beiträge zur Tiergesundheit und zum Tierwohl (Versuche, Beratungsoptimierung)" ("Pig Feeding – Contributions to Animal Health and Animal Welfare (Experiments, Consulting Optimization)"].

https://doi.org/10.17221/189/2020-CJAS

monly consumed feed cereals and produce either one specific toxin or several different toxins (Doll and Danicke 2011). In feed for pigs, depending on the dose and duration of exposure, these toxins can increase the susceptibility to infectious diseases and impair the reproductive performance (Antonissen et al. 2014). DON, however, primarily leads to feed refusal in pigs (Doll and Danicke 2011). DON contamination can cause vomiting resulting in its colloquial name "vomitoxin" (Young et al. 1983). The Commission of the European Communities (2006) defined a guidance value for fattening pigs as 900 µg/kg DON above which feed intake, performance and animal health will deteriorate.

Next to cultivation, physical and chemical procedures such as washing, cleaning and the addition of e.g. ammonium hydroxide are effective ways of reducing the mycotoxin contamination, but usually these measures are too costly to reduce the mycotoxin levels (Ramos and Hernandez 1997; McCormick 2013). Therefore, supplementing the feed with additives is the most common practice towards the prevention of mycotoxin intoxication. These additives utilise different modes of action. Adhesion, being able to bind many toxins, shows no *in vivo* efficacy against deoxynivalenol. (Danicke et al. 2004). However, some *Coriobacteriaceae* strains can metabolise DON to non-toxic metabolites, thereby reducing its hazard (Fuchs et al. 2002).

Since DON causes feed refusal in pigs, modern feeding systems could support the understanding of the mode of action regarding intoxication in its highest detail. Many state-of-the-art feeding systems allow the assessment of feed intake in the utmost detail. Automated single-space feeders record every single feeding action of each animal within a group of pigs.

Therefore, such a system enables the examination of possible alterations of the individual animal's feeding behaviour that will in turn decrease feed intake.

In this study, deoxynivalenol was used to examine the effect on both the zootechnical performance and feeding behaviour of group-housed growing-finishing-pigs fed from automated single-space feeders. Since probable alteration of performance and behaviour could be caused by either toxicity of DON, negative sensory properties, or other unknown effects, it was also pertinent to include two commercial mycotoxin binders in the study. The examination comprised a low-contaminated feed group which served as a control and three highly DON-contaminated feed groups with or without mycotoxin binders.

MATERIAL AND METHODS

Ethics

The trial was officially approved by the responsible state authorities (Reference No. 2532-2-68, Government of Lower Franconia, Germany).

Experimental design

Animal material and stable. A feeding trial was conducted using 96 fattening pigs [(German Large White × German Landrace) × Piétrain] at the experimental site of the Bavarian State Research Centre for Agriculture. For adaptation, pigs (27.7 ± 2.3 kg) were placed one week before the trial commencement in 8 separate pens (12 animals each, 5.0 × 2.6 m) with fully slatted floors. Each pen contained one automatic single-space feeding system (Schauer Compident MLP). The animals were distributed over eight pens in such a way that four pens each were filled with animals either above or below the median body weight, respectively, considering the sex ratio (female to male-castrated 1 : 1) and the litters were equally distributed over all pens.

In each pen one feeding station recorded the time when an animal entered the feeder and the consumed amount of feed. The duration of a single visit could not be obtained due to technical reasons.

Feeds. The feeding procedure followed practical standards and included a starter diet from days 1 to 48, a grower diet from days 49 to 90, and a finisher diet from day 91 until the end of the experiment (day 153).

Table 1 shows the detailed diet formulations as well as the analysed nutrient contents of all experimental diets. The diets were fed as a dry coarse meal and consisted of maize (50.0%), barley (28.0%, 34.5%, 39.0%) and soybean meal (48% CP, 19.0%, 13.0%, 9.0%). A standard macro-premix, which contained synthetic amino acids, was supplemented by 3.0%, 2.5% and 2.0% inclusion rates

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Table 1. Composition of the experimental diets and the respective analysed nutrient contents (standardised on 88% dry matter) of all treatments in the start, grower, and finisher phase

Fattening period	Starter				Grower				Finisher			
Treatment	control	DON	DON + Binder I	DON + Binder II	control	DON	DON + Binder I	DON + Binder II	control	DON	DON + Binder I	DON + Binder II
Diet compostition (%)												
Barley	28	28	27.6	27.72	34.5	34.5	34.1	34.22	39	39	38.6	38.72
Maize (~1 000 µg/kg DON)	50	–	–	–	50	–	–	–	50	–	–	–
Maize (~12 000 µg/kg DON)	–	50	50	50	–	50	50	50	–	50	50	50
Soybean meal (48% CP)	19	19	19	19	13	13	13	13	9	9	9	9
Macropremix[1]	3	3	3	3	2.5	2.5	2.5	2.5	2	2	2	2
Binder I	–	–	0.4	–	–	–	0.4	–	–	–	0.4	–
Binder II	–	–	–	0.28	–	–	–	0.28	–	–	–	0.28
Analysed nutrient contents standardised on 88% DM												
ME (MJ)	13.7	13.8	13.7	13.8	13.6	13.6	13.6	13.7	14.1	13.4	13.6	13.7
Crude ash (g/kg)	40	41	40	44	40	42	42	40	30	36	35	36
Crude fat (g/kg)	27	29	29	27	28	29	29	26	35	30	30	33
Crude starch (g/kg)	505	502	500	499	512	502	515	516	553	509	524	514
CF (g/kg)	29	26	26	27	30	28	28	25	21	29	30	26
CP (g/kg)	148	148	153	150	142	146	142	142	126	140	138	136
Lys (g/kg)	9.7	11.1	9.9	10.7	8.5	8.3	8.1	8.9	7.6	7.2	6.7	7.5
Met + Cys (g/kg)	5.5	6.1	5.7	5.7	5.4	5.3	5.4	5.9	5	4.6	4.5	5.1
Thr (g/kg)	6.9	7.3	–7.1	7.1	6.4	6.3	6.2	6.5	5.6	5.7	5.5	5.8
Trp (g/kg)	1.4	1.7	1.5	1.8	1.8	1.8	1.7	1.7	1.5	1.6	1.7	1.4
Ca (g/kg)	6.6	6.5	7.1	6.0	6.2	6.7	5.6	6.2	4.4	5.6	4.8	4.6
P (g/kg)	4.0	3.9	4.0	4.2	4.0	4.2	3.8	3.7	2.8	4.1	3.9	3.7

Ca = calcium; CF = crude fibre; CP = crude protein; Cys = cysteine; DM = dry matter; DON = deoxynivalenol; Lys = lysine; ME = metabolisable energy; Met = methionine; P = phosphorus; Thr = threonine; Trp = tryptophane

[1]Mineral feed contained the following amino acids: 10% Lys, 3% Met, 4% Thr, 0.5% Trp

through the starter, grower and finisher period, respectively. These adaptations to maturation led to an analysed energy content of 13.7–13.6 MJ ME standardised on 88% DM and a crude protein content of 15.0–13.5%/kg feed during the fattening process. In total, all diets met nutritional requirements (GfE 2006). The differences in nutrient content ranged within analytical tolerances.

The starter, grower and finisher diets were modified at four levels by using maize with low or high contamination (on average 1.0 and 12.1 mg/kg, respectively) of DON (deoxynivalenol) and dietary inclusion of mycotoxin binders (at the expense of barley) according to the following experimental design. Two pens each were assigned to the following treatments:

1. CONTROL (pen 1 and 2): Minimal content of DON, no addition of mycotoxin binders.
2. DON (pen 3 and 4): > 4 500 µg/kg DON, no addition of mycotoxin binders.
3. DON + Binder I (pen 5 and 6): > 4 500 µg/kg DON, addition of 4.0 g/kg Mycofix PLUS 3. EG (Biomin Holding GmbH, Getzersdorf, Austria).
4. DON + Binder II (pen 7 and 8): > 4 500 µg/kg DON, addition of 2.8 g/kg Mycofix PLUS BBSH (Biomin Holding GmbH, Getzersdorf, Austria).

https://doi.org/10.17221/189/2020-CJAS

Binder I primarily consisted of adhesive substances to bind mycotoxins, whereas Binder II also comprised a microorganism capable of producing a non-toxic DON metabolite. The detailed diet formulas are shown in Table 1.

The feed was produced on site. The feed was sampled daily and merged weekly. These pooled samples were analysed for deoxynivalenol using HPLC-MS/MS at Romer Labs (Protocol No. AT-SOP31, Romer Labs Diagnostic GmbH–Europe, Tulln, Austria).

The inclusion of the additives was examined by the supplier. Nutrient contents were analysed at the institution's own laboratory following the respective VDLUFA (2012) methods.

The four experimental diets were distributed among the four pens with light as well as with heavy animals and were fed *ad libitum* throughout the entire study. From day 91 onwards animals reaching 115–120 kg live weight were slaughtered in the institute's slaughterhouse.

The experiment was concluded on day 153 and all remaining animals were slaughtered irrespective of their live weight.

Experimental parameters. All parameters were measured individually for each animal. The feeding stations recorded the number of visits at the feeder and the respective feed intake (g) within each visit. Daily feed intake (DFI) was calculated by summing up the feed consumption of daily visits. Feeding actions with less than 5 g were excluded from the analyses.

The pigs were weighed every second week to assess weight development. Thereof, daily weight gain (DWG, g) was calculated. The feed conversion ratio (FCR, kg feed per kg gain) was calculated as the quotient of DFI and daily gain.

Slaughter started on experimental day 91. Since the elimination of individuals from animal groups might affect social behaviour and hence also feeding behaviour, feeding actions at the feeders were assessed only until experimental day 91. Measurements included daily means of feed intake per visit, numbers of daily feeder visits and the maximum feed quantity consumed within one visit in a day.

At slaughter, the muscle and fat area of the chops (cm^2) and the lean meat content (%) were measured according to the guidelines for assessment of fattening performance of the "Central Association of German Pig Production" (Zentral Verband der Deutschen Schweineproduktion 2007).

Statistical analyses

Zootechnical performance data were analysed according to the following hierarchical model:

$$y_{ijkr} = \mu + treatment_i + pen_j(treatment)_i + \\ + sex_k(pen, treatment)_{ij} + e_{ijkr} \quad (1)$$

where:

y_{ijkr} – dependent performance variable;
μ – grand mean of the observations;
e – random error.

Factors in brackets indicate nested parameters. The factor *sex* (*pen, treatment*) was tested against total deviation. *Pen* (*treatment*) was tested against *sex* (*pen, treatment*) and *treatment* against *pen* (*treatment*).

The following model was used for feeding behavioural measures:

$$y_{ijklr} = \mu + treatment_i + pen_j(treatment)_i + \\ + sex_k\,(pen, treatment)_{ij} + animal_l \\ (sex, pen, treatment)_{ijk} + e_{ijklr} \quad (2)$$

where:

y_{ijklr} – dependent behaviour variable;
μ – grand mean of the observations;
e – random error.

Animal (*sex, pen, treatment*) was tested against residual deviation, and the factor *sex* (*pen, treatment*) was tested against *animal* (*sex, pen, treatment*), and so on.

The persistency of feed intake parameters (DFI, feed intake per visit to the feeder, number of visits to the feeder and highest feed intake within one feeding event per day) from the starter to the grower period was assessed by calculating the Pearson correlation coefficients. Therefore, means for the respective parameters and periods were calculated for each animal.

The correlation was then analysed for each pen. Persistency was expected to result in a statistically significant correlation.

The coefficients of variation (CV) of feed intake parameters were calculated for each animal and averaged per fattening period. Therefore, a possible influence of DON levels and binder addition on the persistency of feeding behaviour was assessed. Possible statistical effects were identified using the Model 1.

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SAS v9.4 (SAS Institute, Cary, USA) was used for all statistical analyses and graphs. An examination for homoscedasticity of the data was routinely conducted before statistical testing. In case of heteroscedasticity no test was conducted. A P-value < 0.05 was considered statistically significant. Significant differences between pens were identified using the Student-Newman-Keuls test.

RESULTS

Five animals were excluded from the trial and statistical analysis. The exclusion was not treatment related.

Deoxynivalenol contents

Figure 1 represents the time course of analysed DON concentrations in feed samples. The control feed contained on average 904 µg/kg DON (min.: 624 µg/kg, max.: 1 316 µg/kg).

The DON levels of treatment groups DON, DON + Binder I, and DON + Binder II ranged around 5 609 µg/kg, 6 370 µg/kg, and 6 446 µg/kg, respectively, with the lowest levels of approximately 4 000 µg/kg between experimental days 71 and 91.

In total, dietary DON concentrations of DON groups exceeded the control level by factor 7 on average (P < 0.01). The recovery of the binder products was on target in all feed samples (94–107%).

Zootechnical performance

Table 2 summarises the zootechnical performance of the four treatment groups. Treatments DON, DON + Binder I, and DON + Binder II led to a reduction in DWG, DFI, meat and fat area in the chops and decreased the FCR in the grower period. In comparison with DON, the addition of Binder I and Binder II further depressed the animal weight at the end of the grower and finisher phase as well as final weight and overall DWG.

The factor *pen* (*treatment*) was found to be statistically relevant only for the FCR in the grower phase. As expected, the pens' starting weight differed significantly since half of the pens were preselected for either light-weight and or heavy-weight animals. The factor *sex* (*treatment, pen*) was statistically relevant in parameters exhibiting sex dimorphism (weight, DWG, total feed intake).

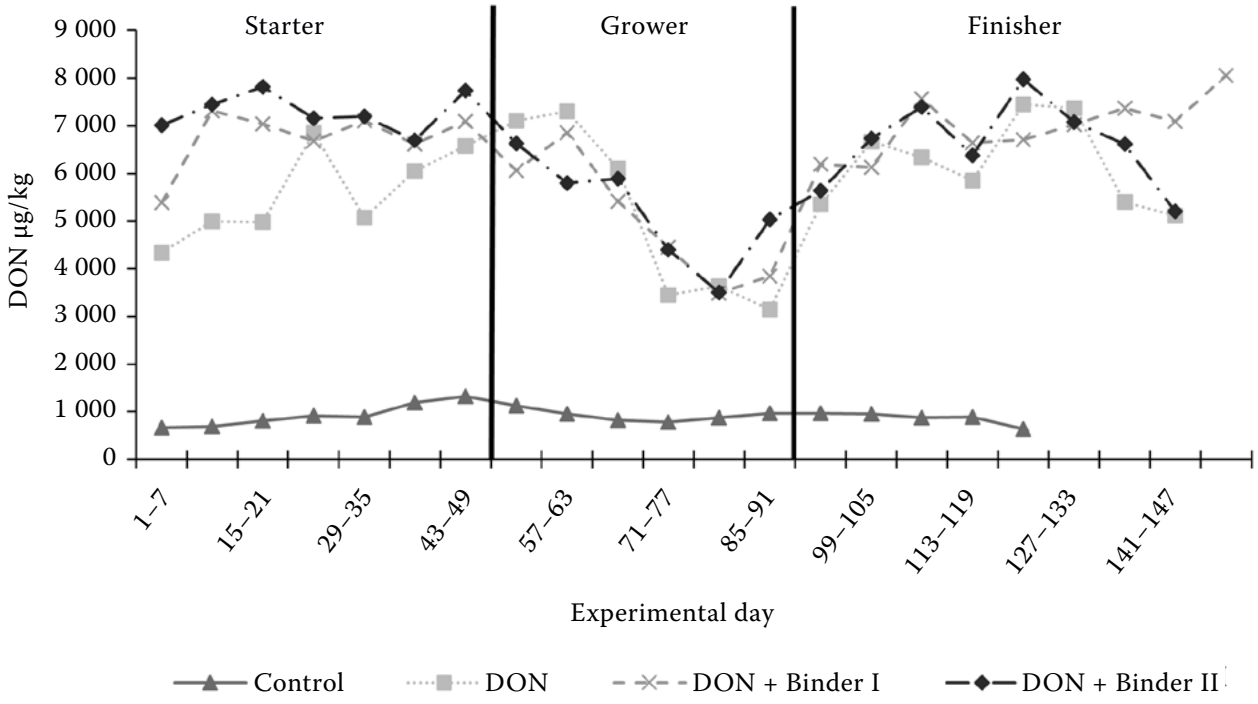

Figure 1. Time course of deoxynivalenol (DON) concentrations in the control group, DON, DON + Binder I, and DON + Binder II diets

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Table 2. Zootechnical performance results (weight, daily weight gain, overall feed intake, feed conversion ratio, meat quality) for the low DON-contaminated feed control, and the highly DON-contaminated feed DON groups with respective mycotoxin binders

Treatment	Control	DON	DON + Binder I	DON + Binder II	Overall	SEM	*P*-value		
							treatment	*pen*	*sex*
Number of animals, individual fattening period									
Animals (*n*)	24	22	22	23	91	–	–	–	–
Duration (days)	115^{b}	148^{a}	149^{a}	145^{a}	137	0.75	< 0.01	0.95	< 0.01
Weight (kg)									
Day 1	33.0	33.0	32.6	32.6	32.8	0.22	1.00	< 0.01	0.21
Day 49	67.9^{a}	54.2^{b}	47.5^{c}	46.9^{c}	54.3	0.63	< 0.01	0.79	< 0.01
Day 91	100.9^{a}	79.8^{b}	74.3^{c}	73.5^{c}	82.4	0.91	< 0.01	0.86	< 0.01
Final	114.6^{a}	103.8^{b}	94.7^{c}	93.8^{c}	102.0	1.09	< 0.01	0.91	< 0.01
Daily weight gain (g/day)									
Starter	712^{a}	441^{b}	302^{c}	282^{c}	439	12.19	< 0.01	1.00	< 0.01
Grower	785^{a}	608^{b}	639^{b}	634^{b}	669	11.14	0.04	0.87	< 0.01
Finisher	595^{a}	431^{b}	360^{b}	378^{b}	443	12.73	< 0.01	0.78	0.08
Overall	728^{a}	488^{b}	424^{c}	423^{c}	519	8.77	< 0.01	1.00	< 0.01
Overall feed intake (kg)									
Starter	77.9^{a}	50.9^{b}	40.4^{c}	41.2^{c}	53.0	1.11	< 0.01	0.60	0.02
Grower	90.1	69.1	69.9	76.2	76.6	1.26	0.13	0.69	< 0.01
Finisher	48.7^{b}	92.6^{a}	82.1^{a}	83.3^{a}	75.3	1.64	< 0.01	0.93	< 0.01
Overall	213.7	212.6	192.4	200.7	205.0	3.01	0.36	0.38	0.15
Feed conversion ratio (kg/kg)									
Starter	2.3	2.5	3.1	3.5	2.8	0.09	0.27	0.07	0.19
Grower	2.6^{a}	2.0^{b}	1.9^{b}	2.1^{b}	2.2	0.05	0.04	< 0.01	0.99
Finisher	3.8	4.2	5.7	5.1	4.7	0.19	0.11	0.68	0.03
Overall	2.7	3.0	3.3	3.5	3.1	0.05	0.10	0.28	0.14
Meat quality measures									
Muscle area (cm^2)	55^{a}	51^{b}	45^{c}	45^{c}	49	0.62	0.01	0.75	0.17
Fatty area (cm^2)	15^{a}	13^{b}	12^{b}	12^{b}	13	0.30	< 0.01	1.00	< 0.01
Lean meat (%)	60	60	60	59	60	0.19	0.69	0.82	< 0.01

DON = deoxynivalenol; FCR = feed conversion ration; SEM = standard error of the mean

$^{a–c}$Superscripted letters indicate significant differences in *treatment* means ($P < 0.05$)

P-values are from hierarchical ANOVA where each source of variance was tested against the column on the right; *sex* was tested against the overall data variation

Grouping factors within feeding behavioural data

Table 3 presents the feeding behavioural patterns at a pen level. DFI was depressed by DON, DON + Binder I and DON + Binder II.

Within treatments, the pens showed no difference. For the other feeding behavioural parameters, however, the pen showed prominent, significant effects ($P < 0.05$).

For example, pen 5 showed the lowest feed intake per visit (86 g/visit from day 1 to 91) whereas pen 7 showed the highest (202 g/visit).

Subsequently, these two pens also showed the highest (15 visits per day, pen 5) and the lowest number of daily feeder visits (8 visits per day, pen 7).

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Table 3. Overall results of feeding behavioural measures (daily feed intake, number of daily visits to the feeder per animal, the overall amount of consumed feed per visit and highest consumed amount feed per visit per day) for the low DON-contaminated control group and the highly DON-contaminated feed DON groups with respective mycotoxin binders

Treatment	Control		DON		Binder I		Binder II		Overall	SEM	*P*-value			
Pen	1	2	3	4	5	6	7	8			*treatment*	*pen*	*sex*	*animal*
Daily feed intake (kg/day)														
Starter	1.6^{A}	1.7^{A}	1.1^{B}	1.0^{B}	0.9^{D}	0.8^{D}	1.0^{C}	0.8^{C}	1.1	0.006	< 0.01	0.41	0.03	< 0.01
Grower	2.2	2.2	1.7	1.7	1.8	1.7	2.1	1.6	1.9	0.008	0.13	0.70	< 0.01	< 0.01
Days 1–91	1.9^{A}	1.9^{A}	1.4^{B}	1.3^{B}	1.3^{C}	1.2^{C}	1.5^{B}	1.2^{B}	1.5	0.007	0.03	0.51	< 0.01	< 0.01
Feed intake per visit (g)														
Starter	168^{a}	125^{b}	87^{d}	91^{c}	69^{e}	94^{c}	121^{b}	70^{e}	103	0.47	0.14	< 0.01	0.70	< 0.01
Grower	182^{c}	157^{d}	127^{f}	140^{e}	100^{g}	190^{b}	312^{a}	156^{d}	160	0.79	0.48	0.01	0.12	< 0.01
Days 1–91	175^{b}	140^{c}	105^{e}	115^{d}	86^{f}	139^{c}	202^{a}	108^{e}	130	0.47	0.44	0.01	0.08	< 0.01
Count of visits to the feeder per animal and day (*n*/day)														
Starter	10^{c}	13^{a}	13^{a}	11^{b}	13^{a}	9^{d}	8^{d}	11^{b}	11	0.06	0.79	< 0.01	0.69	< 0.01
Grower	12^{c}	14^{b}	13^{b}	12^{c}	18^{a}	9^{e}	7^{f}	10^{d}	12	0.06	0.51	< 0.01	0.63	< 0.01
Days 1–91	11^{d}	14^{b}	13^{b}	11^{c}	15^{a}	9^{e}	8^{f}	11^{d}	11	0.05	0.64	< 0.01	0.68	< 0.01
Feed intake of the most extreme feeding action per animal and day (g)														
Starter	392^{a}	353^{b}	285^{c}	260^{d}	202^{f}	225^{e}	341^{b}	168^{g}	281	1.82	0.22	< 0.01	0.44	< 0.01
Grower	528^{b}	494^{c}	460^{d}	442^{d}	371^{f}	462^{d}	707^{a}	393^{e}	488	2.50	0.71	0.03	< 0.01	< 0.01
Days 1–91	455^{b}	418^{c}	365^{d}	344^{e}	280^{f}	334^{e}	516^{a}	272^{f}	376	2.00	0.58	< 0.01	0.05	< 0.01

DON = deoxynivalenol; SEM = standard error of the mean

$^{A–D}$Superscripted capital letters indicate significant *treatment* differences; $^{a–g}$superscripted letters indicate significant *pen* differences

P-values are from hierarchical ANOVA where each source of variance was tested against the column on the right; the animal was tested against the overall data variation

This indication observed for feed intake per visit was also found for the highest feed intake within one daily visit. Pen 5 averaged at the lowest value (280 g per visit, day 1–91) whereas pen 7 reached the highest value (516 g per visit, day 1–91).

Sex (*treatment, pen*) did not affect behavioural parameters except DFI. *Animal* (*treatment, pen, sex*) demonstrated a highly significant influence on all parameters presented in Table 3.

Correlation analyses and examination of CVs of feeding parameters

Figures S1 to S4 in electronic supplementary material (ESM) show means and standard deviations of the assessed feeding behavioural measures, DFI, feed intake per visit to the feeder, number of daily feeder visits and the highest amount of feed consumed in one visit per day for each pig. Standard deviations ranged on average around 29% for DFI, 98% for feed intake per visit to the feeder, 33% for the number of daily feeder visits and 38% for the highest amount of feed consumed in one visit per day, relative to the respective means.

Table 4 presents the persistency assessment of feeding parameters from the starter to the grower period as performed via correlation analyses within pens.

The Pearson correlation coefficients ranged between –0.18 and 0.91. The control and DON pens 1 through 4 showed a correlation higher than 0.58 in nearly all parameters almost all the time. For the DON + Binder I and DON + Binder II pens the situation was more diverse. Pen 6 (Binder I), 7, and 8 (Binder II) did not show any significant cor-

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Table 4. Pearson correlation coefficients for the individual animal's means of feeding behavioural parameters (daily feed intake, feed intake per visit, number of daily visits, and maximum amount of feed consumed per visit) from the starter to the grower period averaged over each pen

Treatment	Pen	Pearson correlation coefficient	Pen	Pearson correlation coefficient
		daily feed intake (g)		feed intake per visit to feeder (g)
Control	1	0.77*	1	0.68*
	2	0.91*	2	0.85*
DON	3	0.64*	3	0.87*
	4	0.67*	4	0.4
DON + Binder I	5	0.87*	5	0.79*
	6	0.70*	6	0.08
DON + Binder II	7	0.47	7	0.72*
	8	–0.18	8	0.65*
Overall		0.68*		0.54*
		count of visits to feeder (*n*/d)		highest feed intake in one visit (g)
Control	1	0.75*	1	0.58*
	2	0.81*	2	0.69*
DON	3	0.80*	3	0.69*
	4	0.61*	4	0.60*
DON + Binder I	5	0.68*	5	0.73*
	6	0.19	6	0.18
DON + Binder II	7	0.38	7	0.44
	8	0.35	8	0.3
Overall		0.70*		0.59*

DON = deoxynivalenol

*Significant correlation coefficient $P < 0.05$

relation for either of them, numbers of daily visits at the feeder and the highest feed intake within one visit per day. The DFI of animals of pen 7 and 8 did not correlate, neither did feed intake per visit for pen 4 and 6.

Table 5 summarises the average CVs of feeding behavioural parameters of the individuals per pen. Overall, the coefficients ranged from ~17% to ~55%.

For DFI, feed intake per visit and highest feed intake per feeding event per day the CVs of the control were always lowest.

For the number of daily feeder visits the observation was more diverse. The control, DON and DON + Binder I group were on a similar level (~31%) while DON + Binder II group was approximately 7% higher. Pen 7 showed the highest variation (24.3–68.1%) in nearly all parameters throughout the experimental time and pen 1 the lowest (17.7–34.4%).

DISCUSSION

This study investigated the effects of high deoxynivalenol contents in diets on the zootechnical performance as well as feed intake behaviour of fattening pigs.

Three feeding groups with either high deoxynivalenol contents (> 4 500 µg/kg) or additionally supplemented with two commercial mycotoxin binders were designed and compared with a low-contaminated control group (< 900 µg/kg).

High resolution feeding data of group-housed grower-finishers were used to assess the feeding behaviour of each pig.

The data were analysed using nested ANOVA models to determine the main sources of deviation.

The nutrient composition was on similar levels, therefore, the effect of the diet other than the DON-contamination can be excluded (Table 1).

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Table 5. Means of coefficients in the variation of the feeding behavioural traits (daily feed intake, feed intake per visit, number of daily visits and maximum amount of feed consumed per visit) of the low DON-contaminated feed control and the highly DON-contaminated feed DON groups with added respective mycotoxin binders

Treatment	Control		DON		Binder I		Binder II		Overall	*P*-value		
Pen	1	2	3	4	5	6	7	8		*treatment*	*pen*	*sex*
Daily feed intake (kg/day)												
Starter (%)	25.1^{C}	23.7^{C}	25.3^{C}	25.0^{C}	37.9^{B}	35.9^{B}	50.5^{A}	42.3^{A}	33.2	< 0.01	0.69	0.02
Grower (%)	18.0^{C}	21.2^{C}	33.4^{A}	33.7^{A}	33.4^{AB}	28.4^{AB}	24.3^{B}	29.5^{B}	27.5	0.02	0.26	0.44
Days 1–91 (%)	21.6^{C}	22.4^{C}	29.3^{B}	29.4^{B}	35.7^{A}	32.2^{A}	37.4^{A}	35.9^{A}	30.3	< 0.02	0.90	0.11
Feed intake per visit (g)												
Starter (%)	34.4^{c}	37.3^{c}	37.6^{c}	35.1^{c}	40.1^{bc}	44.8^{bc}	68.1^{a}	48.8^{b}	43.4	0.10	< 0.01	0.99
Grower (%)	27.1^{C}	27.5^{C}	39.2^{B}	35.7^{B}	39.7^{B}	38.5^{B}	47.4^{A}	46.5^{A}	37.5	< 0.01	0.87	0.67
Days 1–91 (%)	30.7^{fD}	32.4^{efD}	38.4^{cdC}	35.4^{deC}	39.9^{cdB}	41.6^{cB}	57.8^{aA}	47.7^{bA}	40.5	0.02	< 0.01	0.89
Count of visits to the feeder per animal and day (*n*/day)												
Starter (%)	31.3^{b}	36.9^{b}	33.1^{b}	29.2^{b}	31.9^{b}	33.6^{b}	59.3^{a}	30.6^{b}	35.9	0.60	< 0.01	0.93
Grower (%)	29.4	29.9	31.5	29.4	27.4	28.9	35.9	32.4	30.6	0.08	0.36	0.64
Days 1–91 (%)	30.4^{b}	33.4^{b}	32.3^{b}	29.3^{b}	29.7^{b}	31.2^{b}	47.6^{a}	31.5^{b}	33.3	0.48	< 0.01	0.93
Maximum feed intake on one visit per animal and day (g)												
Starter (%)	27.2^{e}	32.0^{de}	35.2^{cd}	34.6^{cd}	40.9^{bc}	40.7^{bc}	62.8^{a}	45.6^{b}	39.9	0.06	< 0.01	0.33
Grower (%)	14.7^{dD}	19.4^{cdD}	24.8^{bcC}	22.5^{bcC}	28.1^{bB}	37.6^{aB}	37.5^{aA}	38.2^{aA}	27.8	0.02	0.04	0.60
Days 1–91 (%)	20.9^{fD}	25.7^{efD}	30.0^{deC}	28.5^{deC}	34.5^{cdB}	39.2^{bcB}	50.1^{aA}	41.9^{bA}	33.8	0.01	0.04	0.61

DON = deoxynivalenol; SEM = standard error of the mean

$^{A–D}$Superscripted capital letters indicate significant *treatment* differences; $^{a–f}$superscripted letters indicate significant *pen* differences

P-values are from hierarchical ANOVA where each source of variance was tested against the column on the right; the animal was tested against the overall data variation

Zootechnical performance

The overall zootechnical performance of the control group was quite low (728 g/day). Loibl et al. (2020) using the same stable and genetics reached higher gains (overall ~860 g/day). The still high DON levels could be one reason for this reduction. They ranged around ~900 µg/kg, which is exactly the threshold of possible alterations regarding performance (The Commission of the European Communities 2006).

Sex (*pen, treatment*) was seen to significantly influence nearly all parameters except FCR. Male castrates and females differed in feed intake, which resulted in different DWG, whereas feed conversion remained unchanged (Hale and Southwell 1967; Pichler et al. 2020). Also, the carcass parameters differed between the sexes (Hale and Southwell 1967). This was the case for all carcass traits except the muscular area.

A significant effect of *pen* (*treatment*) became visible regarding FCR in the grower period. Pen 6 showed the significantly lowest FCR in that period (1.79 kg/kg, data not shown in detail) probably caused by compensatory growth (Kirchgeßner et al. 2014) due to reduced DON levels following day 71. Starting weights of pens were significantly different since the animals of each treatment were divided into a light-weight and a heavy-weight group. This is common practice in feeding trials to increase the homogeneity and decrease the initial variation of the animal material to emphasise the recovery of possible treatment effects (Kohler et al. 2007). After day 35, no statistical difference was recognisable, probably due to increasing weight variation within the respective animal groups.

Besides the expected effect of *sex* (*pen, treatment*), the presence of DON and mycotoxin binders (statistical factor *treatment*) seemed to have a prominent effect on nearly all performance parameters. The high DON contents significantly impaired DWG by > 200 g/day throughout the examination period. This led to significantly longer fattening pe-

https://doi.org/10.17221/189/2020-CJAS

riods as well as reduced slaughter weights. DON typically causes feed refusal as well as immunological problems, vomiting, or dermatitis (Pestka 2007).

Goyarts and Danicke (2005) postulated that the reduced feed consumption caused by DON contamination of feed subsequently reduced daily gains. This was also observed in the present study. More severe reactions, however, were not noted. The higher total feed intake of the control led to increased DWG throughout the trial. The control pigs were slaughtered ~30 days earlier than the rest explaining the reduced feed intake in the finisher period. Overall, all animals consumed nearly the same amount of feed.

Treatment affected FCR only in the grower period. The DON groups showed low ratios around 2.00 kg/kg, whereas the control group ranged about usual 2.57 kg/kg. This seems to contradict previous observations that DON impairs FCR (it leads to higher ratios) (Doll and Danicke 2011). However, dietary DON contents decreased during the last three weeks of the grower period below 4 000 µg/kg in all three DON groups (Figure 1). As a consequence of relief from the exposure to DON, animals seemed to exert compensatory growth, which also entails reduced FCR (Kirchgeßner et al. 2014). Additionally, the FCR increases with maturation and with the change in the composition of accreted body mass (Kirchgeßner et al. 2014). Since the animals fed DON-contaminated feed were significantly lighter, this also might be a reason for the reduced FCR in the grower phase.

The faster growth of the control animals led to differences in maturity (at slaughter, DON animals weighed ~97.4 kg, whereas control pigs reached 114.6 kg) and thereby it altered carcass composition, especially the reduced fat area in the chops. During growth, protein accretion switches toward fat accretion in pigs (Kirchgeßner et al. 2014).

The addition of mycotoxin binders further decreased DWG, overall and in the starter period. Although the products differ in composition, their effect was statistically identical. The DON-detoxifying potential of most commercial adhesive mycotoxin binder products (like Binder I) is described to be somewhat limited (Danicke et al. 2004). *Coriobacterium* BBSH 797 (included in Binder II) metabolises DON to non-toxic de-epoxy-deoxynivalenol (Schatzmayr et al. 2006). However, compared with Binder I, Binder II did not result in any improvement.

One could hypothesise that DON and the detoxifying additives affect taste with the same effect on feed intake. In a review of previous studies by Doll and Danicke (2004), no significant additional negative effects on feed intake due to the addition of similar mycotoxin binders to DON-contaminated pig diets were noted. Alternatively, as another possible explanation, the high DON levels in the DON groups might have exceeded the detoxifying potential of the applied dose of *Coriobacteria*.

Time patterns and the examination of grouping factors within feeding behavioural parameters

The observed behavioural traits except DFI ranged on similar levels as previously published (Kallabis and Kaufmann 2012). Loibl et al. (2020) examined the feeding behaviour of fattening pigs confronted with short-term deviations in stable routine and observed similar behavioural traits in the same stable with identical genetics.

As observed by Loibl et al. (2020), *animal* (*sex, pen, treatment*) was found to be the predominant cause of variation in all feeding behavioural parameters. *Sex* (*treatment, pen*) influenced DFI significantly and feeding actions with the highest feed intake per day during the grower period and overall. This was expected as female pigs show slower growth and reduced feeding capacity than male castrates (Cole et al. 1968). A very similar situation was recently found by Pichler et al. (2020).

The DON levels of > 4 500 µg/kg of feed with or without inclusion of binders (the factor *treatment*) clearly influenced DFI in the starter period and over the first 91 days. Despite its clear effects on zootechnical performance, no treatment effect was found for all other behavioural parameters. Doll and Danicke (2011) reviewed several dose response studies and found that per 1 000 µg/kg the feed intake was reduced by 5.4%. Levels below the European Commission's guidance value of 900 µg DON per kg feed (The Commission of the European Communities 2006) did not alter feed intake. The reduction in feed intake during the starter period and overall met this correlation. The average weekly pooled contents above 7 000 µg/kg DON significantly decreased feed intake by ~43% in the three DON groups. From day 1 to 91, feed intake was reduced by 30%. In the second half of the grower phase the mycotoxin contents

https://doi.org/10.17221/189/2020-CJAS

in the DON groups dropped to ~4 000 µg/kg, which seemed to immediately lead to higher feed intake and subsequently the depression of feed intake was insignificant 18%.

The addition of mycotoxin binders seemed ineffective. In fact, the Binder I product was seen to even further cause a reduction in feed intake. Since the product was added according to the supplier's recommendation, no decrease in feed intake was expected. The causes of this observation therefore remain uncertain although Danicke et al. (2004) using a precursor of the Binder I product found an insignificant but similar effect on DFI. The different modes of action of both binders again showed no improvement in comparison with the DON group.

Pen (*treatment*) was a significant source of variation of all other behavioural traits, such as feed intake per feeder visit, number of daily feeder visits per animal and highest feed consumption within one visit per day. Loibl et al. (2020) observed a remarkably similar situation. In both studies the pen equalled the feeder and comprised a group of 12 animals. Pigs quickly develop a clear and constant hierarchy within a group (Ewbank 1976). Even when confronted with chronic Vomitoxin intoxication this trend seems to remain the same. The factors *animal* (*sex, pen, treatment*) and *pen* (*treatment*) were therefore the most prominent sources of variation. Probably, the individual animal developed a distinct feeding behaviour according to its rank in its respective group. The recently published study of Pichler et al. (2020) seems to confirm this finding. In their study, on average only five animals were allotted to each feeder. The numbers of daily visits were much higher and, correspondingly, feed intake per visit was lower than in our study. This suggests again that the group feeding behaviour depends on (hardly predictable) somewhat persistent behavioural patterns of individual animals.

Correlation analyses and examination of the CV of feeding parameters

The individual animals showed wide deviations in the mean values of the assessed behavioural traits (Figures S1–S4 in ESM). To examine persistency of feeding behaviour from the starter to the grower period the Pearson correlation coefficients were calculated for each pen (Table 4). They ranged between –0.18 and 0.91 and 28% of the correlations were insignificant. The two control pens showed significant and highest correlations in almost all parameters, whereas pen 7 and pen 8 showed the lowest, often insignificant, correlations. The exposure to diets with high DON contents therefore might have led to more erratic and less persistent feeding behaviour of the individual animal, probably due to the increased variation.

Looking at detailed mean values including standard deviations (Figures S1–S4 in ESM) all parameters showed comparably high day-to-day-variation. The addition of DON alone or combined with a binder might have led to increased day-to-day variation. Consequently, the CV of DFI, feed intake per visit, and highest consumed amount of feed per feeder visit was increased (Table 5). However, the number of daily visits was influenced only by the pen. We observed prominent effects of DON and binders on zootechnical performance and DFI. This leads to the assumption that the increased levels of deoxynivalenol with or without binder caused directed alteration of the animals' behaviour that could be identified only by examining the means of the observed parameters. This combined with the distinct difference between the CVs of the treatment groups (the control mostly lowest, DON + Binder II highest) raises the conclusion that the animals' feeding behaviour became more erratic due to the contamination by DON or the addition of a mycotoxin binder.

CONCLUSION

This study examined the influence of DON-contaminated maize based diets (either without or with two binders) on the zootechnical performance and feeding behaviour of fattening pigs. It was shown that chronic DON intoxication (irrespective of the added mycotoxin binders) reduced the zootechnical performance significantly but it did not influence feeding behavioural traits such as daily feeder visits, feed intake per feeder visit and highest feed consumption per visit.

However, the DON contamination and binder addition significantly increased the day-to-day variation in these parameters. This led to lower persistency of the animals' behaviour as well as increased day-to-day variation. The behaviour of the fatteners therefore became more erratic due to the addition of DON irrespective of the addition of the mycotoxin binder.

https://doi.org/10.17221/189/2020-CJAS

Acknowledgement

The authors wish to thank the technical stuff of the LVFZ Schwarzenau research station for all their practical on-site support during the trial.

Conflict of interest

The authors declare no conflict of interest.

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Received: July 9, 2020
Accepted: September 30, 2020