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Non Ruminant Nutrition

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Effect of long-term feeding of graded levels of deoxynivalenol (DON) on growth performance, nutrient utilization, and organ health in finishing pigs and DON content in biological samples

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Abstract

The prevalence of deoxynivalenol (**DON**) is a concern for swine producers, and although there has been extensive research into the effects of DON in pigs, focus has been in young pigs and/or in short-term studies. The objective of the study was to determine the effect of long-term exposure to DON-contaminated diets in finisher pigs. A total of 200 pigs (76.6 ± 3.9 kg initial weight) were group housed (five pigs per pen; n = 10 pens/treatment) in a 6-wk trial. Pigs were fed a wheat-barley-soybean meal-based control (**CONT**) diet with no DON or the basal diet in which clean wheat was replaced by DON-contaminated wheat and wheat screenings to provide DON content of 1, 3, or 5 ppm (**DON1**, **DON3**, and **DON5**, respectively). Individual BW and pen feed intake were recorded weekly to calculate average daily gain (**ADG**), average daily feed intake (**ADFI**), and gain to feed ratio (**G:F**). Blood was collected on days 0, 14, and 43 and analyzed for indicators of liver and kidney health. Nitrogen (**N**)-balance was conducted immediately following the growth performance period to determine the effect of DON on nutrient utilization. Blood and urine samples collected during N balance were analyzed for DON content. Feeding DON reduced ($P < 0.05$) ADFI and ADG from days 0 to 28 compared with CONT, after which there was no effect of diet on ADFI and ADG. The G:F was lower ($P < 0.05$) in DON5 fed pigs compared with all treatments during days 0 to 7; however, no treatment effects on G:F was observed from days 8 to 42. Nitrogen retention was lower ($P < 0.05$) in DON3 and DON5 compared with DON1-fed pigs. Nitrogen retention efficiency was higher ($P < 0.05$) in DON1 compared with DON3 and DON5 and protein deposition for DON1 pigs was higher ($P < 0.05$) than all treatments. There were no treatment effects on indicators of liver and kidney health. As dietary DON intake increased, concentration of DON in blood and urine increased. Overall, although there was an initial decrease in ADG and ADFI in pigs receiving diets containing >1 ppm DON, pig performance recovered after a period of time, whereas nutrient utilization continued to be affected after recovery of performance. Moreover, the lack of DON on G:F indicates that the negative effects of DON on growth performance are largely due to reduced feed intake. Overall, although pigs maybe capable of adapting to intake of DON-contaminated diets, their final body weight will be reduced when fed diets containing >1 ppm DON.

Key words: average daily gain, average daily feed intake, deoxynivalenol, gain:feed, nitrogen balance, swine

Abbreviations

ATTD	apparent total tract digestibility
AST	aspartate aminotransferase
BW	body weight
CCK	cholecystokinin
CTK	creatine kinase
DON	deoxynivalenol
G:F	gain:feed
GGT	gamma-glutamyl transferase
GLDH	glutamate dehydrogenase
HPLC	high-pressure liquid chromatography
PBS	phosphate-buffered sodium
PD	protein deposition

Introduction

Mycotoxins are secondary metabolites of fungi that cause adverse physiological effects in humans and animals when ingested. Although many mycotoxins have been identified, the mycotoxin deoxynivalenol (**DON**) is one of the most significant mycotoxins in agriculture, as it contaminates common feed grains, such as corn, wheat, oats, and barley (Rotter et al., 1996; Chaytor et al., 2011). In North America, it was reported that 85% of all grain samples analyzed and about 90% of complete feed samples contained DON (Gruber-Dorninger et al., 2019). There is evidence that DON consumption in animals results in reduced feed intake, digestive dysfunction (e.g., gastroenteritis, gastrointestinal tract lesions, reduced nutrient absorption), immune suppression, and reduced growth performance (Pinton et al., 2008; Serviento et al., 2018). Although all species respond to DON exposure, pigs are particularly susceptible (Wu et al., 2010). Previous studies have reported that DON ingestion in pigs causes a reduction in average daily feed intake (**ADFI**) and body weight gain (Goyarts et al., 2005; Ghareeb et al., 2015). This has also been shown to cause intestinal damage, which may lead to reduced intestinal barrier function and increased susceptibility to enteric pathogens, further resulting in reduced nutrient absorption and utilization (Pinton et al., 2009; Ghareeb et al., 2015). To date, most studies on the effects of DON in swine have been completed in weaned pigs with the assumption that the physiological effects of consuming DON are highest in the young animal (Dersjant-Li et al., 2003). As reported by Andretta et al. (2012), the average initial age of pigs used in DON challenge studies was 44 d, with approximately 64% of these studies completed in the nursery phase, 18% in the grower phase, and only 3% in the finisher phase. Moreover, the majority of studies have examined the impact of DON over a relatively short period of time (Dersjant-Li et al., 2003). Therefore, there is a need to evaluate the impact of DON contamination in older pigs (finishing pigs) and over an extended period. It may be possible that due to the overall higher feed intake in the finishing period, the effects of DON may be greater in this production stage. However, it has also been suggested that pigs may adapt to DON-contaminated feed (Rotter et al., 1994).

A necessary step in mitigating the effects of DON in swine production starts with accurately determining DON content in feed ingredients and complete feeds. Unfortunately, there are high discrepancies and variability when it comes to testing for DON as previously reported (Whitaker et al., 2000; Beaulieu et al., 2009; Kong et al., 2015). Sampling methods such sampling time and location, batches sampled, and the type of samples (grain dust vs. actual grains) will all affect the accuracy of test results (Whitaker et al., 2000). Harvesting and storage conditions have with also been reported to play a role in the inconsistencies analysis DON concentration in diet or ingredient samples (Champeil et al., 2004). With these challenges in mind, it is worth considering the potential for determining DON exposure through analysis of biological samples. For example, previous studies have shown that there is a strong correlation between the intake of DON and its presence in excretions (e.g., urine; Dänicke and Brezina, 2013). Therefore, the objectives of the present study were to determine the effect of long-term exposure of finishing pigs to DON-contaminated diets on growth performance, nutrient utilization, organ health, and DON content in biological samples.

Materials and Methods

The experimental protocol used in the present study was reviewed and approved by the Animal Research Ethics Board of the University of Saskatchewan (no. 20130054) and followed the guidelines of Canadian Council on Animal Care (CCAC, 2009).

Animals, housing, diets, and experimental design

A total of 200 mixed-sex finishing pigs (Camborough Plus × C337; PIC, Canada) with initial body weight (**BW**) of 76.6 ± 3.9 kg were used in a 42-d study at the Prairie Swine Centre, Inc. (Saskatoon, SK, Canada). The pigs were group housed in pens (five pigs per pen) in environmentally controlled rooms. The pens were randomly assigned to one of four dietary treatments (n = 10 pens/treatment; Table 1), which consisted of a control diet (**CONT**) containing no (or 0 ppm) DON or a diet containing 1, 3, or 5 ppm DON (**DON1**, **DON3**, or **DON5**, respectively). All diets were wheat-barley-soybean meal-based and formulated to be isonitrogenous and isoenergetic and met or exceeded nutrient requirements according to National Research Council (NRC, 2012). The DON-contaminated diets were formulated by replacing DON-free wheat with appropriate amounts of DON-contaminated wheat and wheat screenings. Prior to diet manufacture, the concentration of DON and other mycotoxins was determined in ingredients by Central Testing Laboratory, Ltd. (Winnipeg, MB, Canada) in composite samples prepared from single batches of the ingredients. All diets were made in separate batches, and diets with the least amount of DON were made first to prevent contamination. Pigs were fed ad libitum and had free access to water for the duration of the study.

Growth performance and nitrogen balance

Individual pig BW were measured at the start of the experiment (initial BW) and weekly for the duration of the study for determination of average daily gain (**ADG**). Each week, feed intake was recorded and the ADFI determined. The gain to feed ratio (gain:feed [**G:F**]) was calculated from the weekly ADG and ADFI.

On day 35 of the growth performance period, one barrow per pen (identified and marked at the start of the experiment for serial blood sampling and nitrogen [**N**]-balance) was individually housed in metabolism crates (56" × 58.5") in a temperature-controlled room (21 ± 2 °C) for N-balance collection (n = 10/treatment). The selected pigs remained on their original dietary treatment during the N-balance period and the same experimental diets were fed except for the inclusion of celite as an indigestible marker at the expense of uncontaminated wheat. Pigs were fed at 2.8 × maintenance energy requirement for metabolizable energy (197 kcal/kg $BW^{0.60}$/d; NRC, 2012) and

Table 1. Ingredient composition and calculated and analyzed nutrient content of experimental diets[1] (as-fed basis)

Ingredient, %	CONT	DON1	DON3	DON5
Barley	44.0	44.0	44.0	44.0
Wheat	40.0	33.3	20.0	6.7
DON wheat[2]	0.0	4.9	14.8	24.7
Wheat screenings[3]	0.0	1.7	5.2	8.6
Soybean meal	10.0	10.0	10.0	10.0
Canola oil	3.5	3.5	3.5	3.5
L-Lysine–HCl	0.30	0.30	0.30	0.30
DL-Methionine	0.07	0.07	0.07	0.07
L-Threonine	0.10	0.10	0.10	0.10
Limestone	0.8	0.8	0.8	0.8
Dicalcium phosphate	0.5	0.5	0.5	0.5
Salt	0.5	0.5	0.5	0.5
Vitamin/mineral premix[4]	0.2	0.2	0.2	0.2
Calculated nutrient content[5]				
ME, kcal/kg	3282	3282	3282	3282
Dry matter, %	86.49	86.51	86.54	86.58
Crude protein, %	15.9	15.9	15.9	16.0
Lysine, % SID[6]	0.76	0.76	0.76	0.76
Calcium, %	0.50	0.50	0.50	0.50
Phosphorus, %	0.48	0.48	0.48	0.48
Analyzed nutrient content				
Growth performance diet				
Dry matter, %	88.9	88.2	88.3	88.8
Crude protein, %	14.6	14.2	13.5	14.8
N-balance diet[7]				
Dry matter, %	88.5	88.1	88.9	88.7
Crude protein, %	14.5	14.6	14.1	14.7

[1]CONT, 0 DON; DON1, 1 ppm DON; DON3, 3 ppm DON; DON5, 5 ppm DON.
[2]DON wheat contains 6.9 ppm DON (Central Testing Laboratory, Winnipeg, MB, Canada).
[3]Wheat screenings contain 32.8 ppm DON (Central Testing Laboratory, Winnipeg MB, Canada).
[4]Supplied per kg of complete diet; vitamin A, 8,000 IU; vitamin D, 1,500 IU; vitamin E, 30 IU; menadione, 2.5 mg; vitamin B12, 0.025 mg; thiamine, 1.00 mg; biotin, 0.10 mg; niacin, 20 mg; riboflavin, 4 mg; pantothenate; 12 mg; folic acid, 0.50 mg; pyridoxine, 2.0 mg; Fe, 100 mg; Zn, 100 mg; Mg, 40 mg; Cu, 15 mg; Se, 0.30 mg; and I, 1 mg.
[5]Nutrient content of diets based on the nutrient content of feed ingredients according to NRC (2012) and analysis of nutrient content of wheat screenings (Central Laboratory Testing, Ltd, Winnipeg, MB, Canada).
[6]SID, standardized ileal digestible.
[7]Nitrogen balance diet contained 0.4% celite as a marker added at the expense of wheat.

fed in two equal meals each day at 0700 and 1500 h. After a 7-d dietary and environmental adaptation, total urine and fresh-fecal samples were collected over a 2-d N-balance period. During the sample collection period, urine was collected quantitatively over two 24-h periods via urine jars placed beneath the urine collection trays containing a sufficient amount of HCl to maintain pH < 3 (Columbus et al., 2014). At the end of each 24-h period, urine was weighed and a 5% aliquot was sampled and stored at –20 °C. Fresh-fecal samples were taken daily by rectal palpation and immediately stored at –20 °C. At the end of the 2-d sample collection period, urine samples were thawed and pooled for each pig, filtered with glass wool to remove any debris, and a 5% subsample was obtained and stored at –20 °C until further analysis. Similarly, fecal samples were thawed, pooled for each pig and homogenized, and a subsample taken and stored at –20 °C until further analysis.

Blood sampling

Blood samples were obtained from the representative pigs selected at the start of the experiment on days 0, 14 (growth performance period), and 43 (on day 1 of N-balance period, 3 to 4 h after the morning meal) via jugular puncture into heparin-coated and additive-free tubes (5 mL; BD vacutainer tubes, Mississauga, ON, Canada). Blood collected into additive-free tubes was allowed to clot, and then blood samples were then centrifuged at 2,500 × *g* for 15 min to harvest plasma and serum samples, after which they were stored at –20 °C until further analyses.

Analytical procedures

Analysis of feed, fecal, and urine samples

Dry matter content of the diet and fecal samples were analyzed according to method 930.15 of AOAC (2007). Nitrogen content in diet, feces, and urine samples were analyzed using an automatic analyzer (LECO FP 528; MI, USA; AOAC, 2007; Method 990.03). The acid-insoluble ash content of both diet and fecal samples were analyzed according to the method described previously (Van Keulen and Young, 1977). The complete experimental diets were analyzed for DON and other mycotoxins at the University of Natural Resources and Life Sciences, Vienna (Tulln, Austria; Table 2) according to Sulyok et al. (2020). All analyses were performed in duplicate.

Serum analysis for kidney and liver metabolites

Serum harvested from blood samples taken on days 0 and 14 of the growth performance period and day 43 (N-balance period)

were analyzed for key indicators of liver and kidney function and health using an automatic blood chemistry analyzer (Prairie Diagnostic Services).

Deoxynivalenol analysis in serum and urine samples

All mycotoxin analysis in biological matrices was performed at the laboratory of the BIOMIN Research Center (Tulln, Austria). Analytical standards for DON were acquired commercially (Romer Labs GmbH, Tulln, Austria). Phosphate-buffered saline (**PBS**) was obtained from Sigma–Aldrich (Vienna, Austria), methanol (**MeOH**) and acetic acid from VWR International (Vienna, Austria), and acetonitrile from Chem-Lab NV (Zedelgem, Belgium). In urine, direct quantification of DON was performed. The determination of the analytes was performed on a QTRAP 6500 using an HPLC–MS/MS-based method described previously (Schwartz-Zimmermann et al., 2017). The samples were measured in duplicate. Serum samples obtained from pigs during the N-balance collection were subjected to indirect quantification of DON via enzymatic pretreatment. To this end, 35 mg of β-glucuronidase (*Escherichia coli*, Type IX-A; Sigma–Aldrich) was dissolved in 2.5-mL PBS and added at 50 µL per 100-µL serum. After incubation for 18 h (37 °C, 80 rpm), 300 µL of MeOH/acetic acid (99.8/0.2, v/v) was added. HPLC–MS/MS analysis was performed as described for urine samples.

Statistical Analyses

All data were verified for normality using the PROC UNIVARIATE (SAS Institute, Cary, NC, Version 9.4), and outliers were tested using the studentized residual analysis. The growth performance data were initially analyzed as a repeated measure with treatment, time, and treatment × time interactions included in the model; however, since there were no significant treatment × time interactions, the data were reanalyzed as a randomized complete block design with the fixed effect of dietary treatment (CONT, DON1, DON3, and DON5) and block (room) as a random variable (PROC MIXED, SAS Institute, Version 9.4). The N-balance data were also analyzed as a randomized complete block design with the fixed effect of dietary treatments (CONT, DON1, DON3, and DON5) and the random variable was the block (room) (PROC MIXED, SAS Institute, Version 9.4). The blood chemistry data were analyzed as a repeated measure with day as the repeated variable. Regression analysis was used to describe relationships between DON intake and ADG, total BW gain relative to CONT, urinary DON output, and DON content in serum (PROC REG, SAS Institute, Version 9.4). The Tukey–Kramer mean separation test was used to separate means, and differences between means were considered significant at $P \leq 0.05$ and a trend toward significance was considered at $0.05 > P < 0.10$.

Results

Calculated vs. analyzed DON levels in diets

Although the majority of diets contained similar DON content compared with formulated values, the high DON content in the CONT diet used in the N balance (Table 2) was unexpected and further indicates the variability in DON content and the difficulty in accurately determining DON content in dietary ingredients, as the same ingredients, including the same clean wheat, DON-contaminated wheat, and DON-contaminated wheat screenings, were used in all diets.

Growth performance

Growth performance data are presented in Table 3. Initial BW (day 0) was not different among the dietary treatments ($P > 0.05$). BW was reduced in DON3 and DON5-fed pigs by day 7 of the study compared with CONT, with the greatest reduction observed with DON5 ($P > 0.05$). This reduction in BW was maintained throughout the study. For the duration of the study, ADG in DON1-fed pigs was not different than pigs receiving the CONT diet ($P > 0.05$). From days 0 to 7, DON3-fed pigs had reduced growth compared with both CONT- and DON1-fed pigs ($P < 0.05$) but were not different from CONT-fed pigs from days 8 to 42 ($P > 0.05$). Pigs fed DON5 had reduced ADG from days 0 to 21 compared with all other dietary treatments ($P < 0.05$). From days 22 to 28, ADG of DON5-fed pigs was not different than DON1- and DON3-fed pigs ($P > 0.05$). From days 29 to 42, there were no differences in ADG among dietary treatments ($P > 0.05$). Overall (days 0 to 42), ADG was reduced in DON3- and DON5-fed pigs compared with both CONT- and DON1-fed pigs, with the greatest reduction observed with DON5 ($P < 0.05$). There was no impact of DON1 on ADFI compared with CONT ($P > 0.05$). From days 0 to 7, DON3-fed pigs had reduced ADFI compared with both CONT- and DON1-fed pigs ($P < 0.05$), after which no difference was observed ($P > 0.05$). In DON5-fed pigs, ADFI was

Table 2. Analyzed mycotoxin contents of experimental diets[1] (as-fed basis)

	Finisher diet							
	Growth performance diets				N-balance diets			
Mycotoxin, ppm	CONT	DON1	DON3	DON5	CONT	DON1	DON3	DON5
Deoxynivalenol	0.11	1.34	3.59	5.72	1.56	1.32	3.09	4.94
3-acetyldeoxynivalenol	ND[2]	ND	ND	ND	ND	ND	ND	ND
15-acetyldeoxynivalenol	ND	ND	ND	ND	ND	ND	ND	ND
HT-2 toxin	ND	ND	ND	0.050	ND	ND	ND	0.03
Nivalenol	0.15	0.18	0.53	0.64	0.12	0.11	0.12	0.08
Ochratoxin A	0.01	0.03	0.01	0.01	0.01	0.03	0.07	0.09
Zearalenone	ND	0.002	0.009	0.014	0.003	0.002	0.009	0.013
Total Ergot alkaloids	0.99	0.57	1.03	1.26	0.24	0.16	0.39	0.67

[1]CONT, 0 DON; DON1, 1 ppm DON; DON3, 3 ppm DON; DON5, 5 ppm DON.
[2]ND, not detected or below limit of detection.

Table 3. Growth performance of finisher pigs fed graded levels of deoxynivalenol[1]

	Dietary treatments[2]					
Item	CONT	DON1	DON3	DON5	SEM	P-value[3]
BW, kg						
Initial	76.9	77.0	76.3	76.0	1.18	NS
Day 7	85.4[a]	84.8[a]	83.0[b]	80.8[c]	0.34	<0.001
Day 14	95.3[a]	95.3[a]	92.4[b]	88.7[c]	0.42	<0.001
Day 21	103.4[a]	103.8[a]	99.8[b]	95.7[c]	0.50	<0.001
Day 28	112.1[a]	111.9[a]	107.8[b]	103.0[c]	0.53	<0.001
Day 35	119.7[a]	119.8[a]	114.9[b]	110.4[c]	0.63	<0.001
Day 42	126.7[a]	126.9[a]	123.6[b]	118.5[c]	0.80	<0.001
ADG, kg/d						
Days 0–7	1.27[a]	1.18[a]	0.93[b]	0.60[c]	0.05	<0.001
Days 8–14	1.40[ab]	1.49[a]	1.33[b]	1.13[c]	0.04	<0.001
Days 15–21	1.17[ab]	1.21[a]	1.06[b]	1.01[c]	0.04	0.004
Days 22–28	1.24[a]	1.17[ab]	1.15[ab]	1.04[b]	0.04	0.033
Days 29–35	1.08	1.12	1.01	1.06	0.04	NS
Days 36–42	1.06	1.00	1.20	1.14	0.06	NS
Overall	1.19[a]	1.20[a]	1.12[b]	1.00[c]	0.02	<0.001
ADFI, kg/d						
Days 0–7	2.59[a]	2.59[a]	2.22[b]	1.70[c]	0.06	<0.001
Days 8–14	2.98[a]	3.07[a]	2.89[a]	2.55[b]	0.07	<0.001
Days 15–21	3.03[a]	3.03[a]	2.88[a]	2.56[b]	0.05	<0.001
Days 22–28	3.25[a]	3.19[a]	3.13[a]	2.85[b]	0.05	<0.001
Days 29–35	3.22	3.20	3.19	3.04	0.06	NS
Days 36–42	3.19	3.11	3.36	3.05	0.08	NS
Overall	2.99[a]	3.06[a]	2.94[a]	2.60[b]	0.05	<0.001
G:F, kg/kg						
Days 0–7	0.49[a]	0.46[a]	0.41[a]	0.34[b]	0.02	<0.001
Days 8–14	0.47	0.49	0.47	0.44	0.01	NS
Days 15–21	0.38	0.40	0.37	0.40	0.01	NS
Days 22–28	0.38	0.36	0.37	0.36	0.02	NS
Days 29–35	0.33	0.35	0.32	0.35	0.01	NS
Days 36–42	0.33	0.32	0.36	0.37	0.01	NS
Overall	0.40	0.39	0.38	0.38	0.01	NS

[1]Values are least squares means (*n*, 10/treatment).
[2]CONT, 0 DON; DON1, 1 ppm DON; DON3, 3 ppm DON; DON5, 5 ppm DON.
[3]NS, not significant.
[a,b,c]Means within a row without a common superscript are significantly different ($P < 0.05$).

reduced from days 0 to 28 compared with all other dietary treatments ($P < 0.05$), after which no difference was observed ($P > 0.05$). Overall (days 0 to 42), ADFI was only reduced in DON5 fed pigs ($P < 0.05$). G:F was reduced in DON5-fed pigs from days 0 to 7 compared with all other dietary treatments ($P < 0.05$), which were not different from each other ($P > 0.05$). There was no effect ($P > 0.05$) of dietary treatment on G:F from days 8 to 42 or overall (days 0 to 42).

Relationship between dietary DON intake and BW gain

A linear regression model was applied to study the relationship between DON intake and ADG (Figure 1). There was a negative relationship between DON intake and ADG, such that as DON intake increased, there was a linear reduction in ADG. This relationship was consistent from days 0 to 35; however, the strength of the impact of DON intake on ADG reduced consistently and was lowest from days 28 to 35 with a slope not different than zero (Figure 1E).

Nitrogen balance

The results for the N-balance study are presented in Table 4. It is important to note that the CONT diet used in the N balance had unexpectedly high DON content and so results should be interpreted appropriately. Average daily N intake for pigs fed DON3 and DON5 was not different but were both lower ($P < 0.05$) than DON1 and CONT diet. Urinary N output from CONT, DON3, and DON5 diets were not different ($P > 0.05$), but the pigs fed DON1 had ($P < 0.05$) lower urinary N output compared with the other dietary treatments. Fecal N output was not different between DON1, DON3, and DON5, but was significantly ($P < 0.05$) higher in the CONT diet. The apparent total tract digestibility of N was significantly ($P < 0.05$) lower in the CONT diet compared with all the other dietary treatments. Nitrogen retention was lower ($P < 0.05$) in pig fed CONT, DON3, and DON5 diets compared with DON1. As such, N retention efficiency was higher ($P < 0.05$) in DON1 compared with CONT, DON3, and DON5, and therefore, protein deposition (**PD**) for DON1 pigs was higher ($P < 0.05$) than pigs fed CONT, DON3, and DON5 diets.

Blood metabolites

Key indicators of kidney and liver health and function are presented in Supplementary Table 1. There was no effect ($P > 0.05$) of dietary DON content on the selected liver and kidney blood parameters. For most of the analyzed metabolites, there was a significant effect of day, except for potassium and creatine kinase that tended to be different ($P = 0.075$ and 0.073, respectively) and gamma-glutamyl transferase that was not affected ($P > 0.05$)

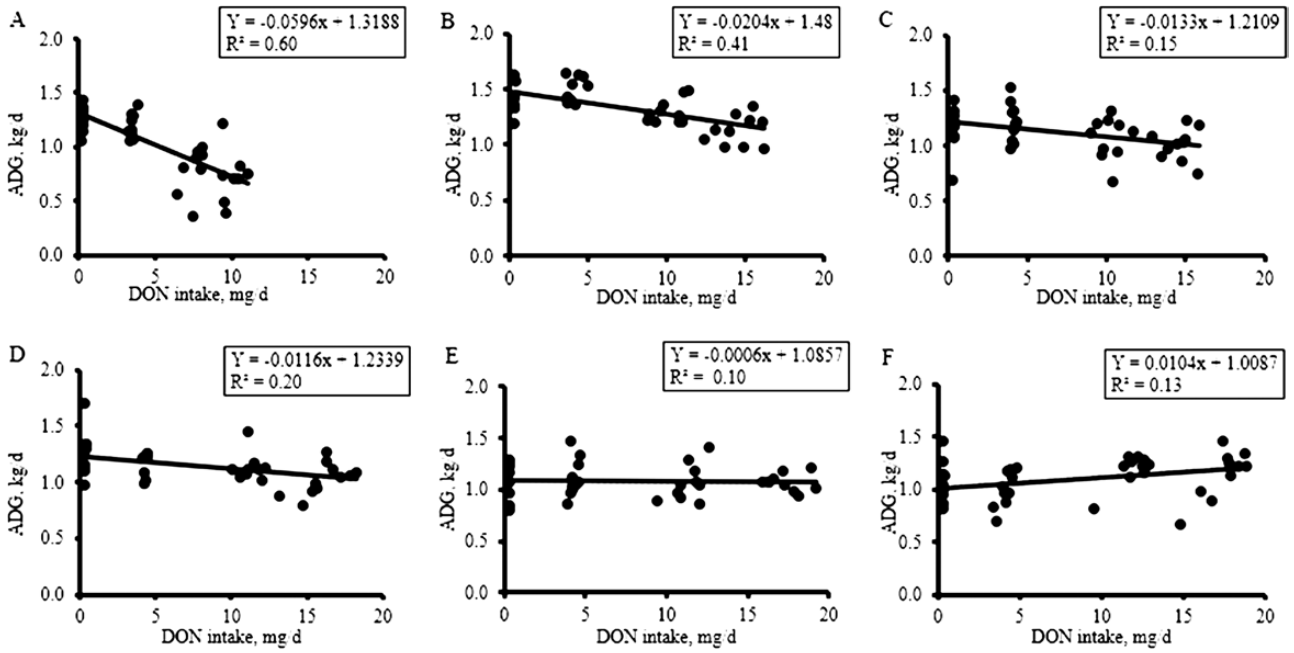

Figure 1. Regression analysis of the relationship between DON intake and weekly ADG. The figures represent days 0 to 7 (A), days 8 to 14 (B), days 15 to 21 (C), days 22 to 28 (D), days 29 to 35 (E), and days 36 to 42 (F). The coefficient of determination (R^2) values range from 0.1 to 0.6, suggesting a high level of variance in the dependent variable (ADG). Points on graph represent an experimental pen (n = 10 pens per treatment).

Table 4. Nitrogen balance in pigs fed diets containing graded levels of deoxynivalenol[1]

	Dietary treatments[2]					
Item, g/d	CONT	DON1	DON3	DON5	SEM	P-value
N intake	67.84[a]	68.45[a]	63.48[b]	63.32[b]	1.27	0.024
Fecal N output	14.10[a]	8.82[b]	9.28[b]	9.26[b]	1.05	0.005
Urinary N output	25.53[a]	15.19[b]	28.38[a]	22.69[ab]	3.59	0.015
ATTD[3] of N, %	79.27[a]	87.12[b]	85.41[b]	85.44[b]	1.56	0.009
N retained	28.30[b]	44.40[a]	25.85[b]	31.34[b]	3.17	<0.001
PD[4]	176.89[b]	277.78[a]	161.56[b]	195.99[b]	19.81	<0.001
N retention efficiency[5],%	41.68[b]	65.12[a]	40.62[b]	49.64[b]	4.75	<0.001

[1]Values are least squares means (n, 10/treatment).
[2]CONT, 0 DON; DON1, 1 ppm DON; DON3, 3 ppm DON; DON5, 5 ppm DON.
[3]ATTD, apparent total tract digestibility.
[4]PD, N retained × 6.25.
[5]Nitrogen retention efficiency calculated as the N retained divided by N intake and expressed as a percentage.
[a,b]Means within a row without a common superscript are significantly different ($P < 0.05$).

by day. These differences are considered unrelated to dietary treatment as there was no significant diet × day interaction.

Concentration of DON in serum and urine

A linear regression model was used to analyze the relationship between DON intake and DON concentration in serum and urine as shown in Figures 2 and 3, respectively. As dietary DON intake increased, the amount of DON in the blood increased ($P < 0.05$; $R^2 = 0.80$). The same relationship was observed for urine, where as dietary DON intake increased, there was an increase in DON excretion in urine ($P < 0.05$; $R^2 = 0.50$).

Discussion

In general, DON intake causes reduced performance and can have potentially negative impacts on animal health. The majority of studies on the effects of mycotoxins in swine are performed in young animals with the assumption that the physiological effects of consuming mycotoxin contaminated feed are highest in the young animal (Dersjant-Li et al., 2003; Chen et al., 2008; Savard et al., 2015). Moreover, previous studies have examined the impact of mycotoxins over a relatively short period of time (Accensi et al., 2006; Alizadeh et al., 2015; Dänicke et al., 2017). The objective of the present study was to examine the effects of feeding graded levels of DON to finisher (75 to 120 kg) pigs on growth performance, nutrient utilization, and overall health status over a 43-d period.

In the present study, the diets were formulated to target dietary DON levels of 0, 1, 3, and 5 ppm. For the most part, the formulated and analyzed levels of the DON in the complete diets were similar. One exception to this is the analyzed DON content (1.56 ppm) versus targeted content (0 ppm) in the CONT diet used in the N-balance study. These inconsistencies in

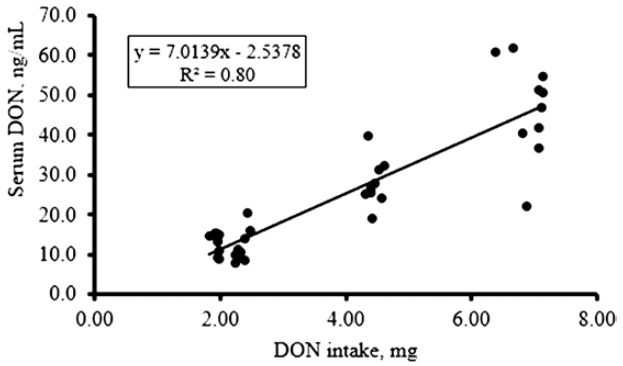

Figure 2. Regression analysis of the relationship between DON intake and serum DON concentration (ng/mL). The blood samples were taken during the nitrogen balance period (3 to 4 h after a single meal) of the experiment and analyzed for DON concentration (n = 10 pigs per treatment). Data are expressed as the DON intake after a single meal and the serum DON concentration (ng/mL) after that meal. The coefficient of determination (R^2) of the regression curve is 0.80 at $P < 0.05$.

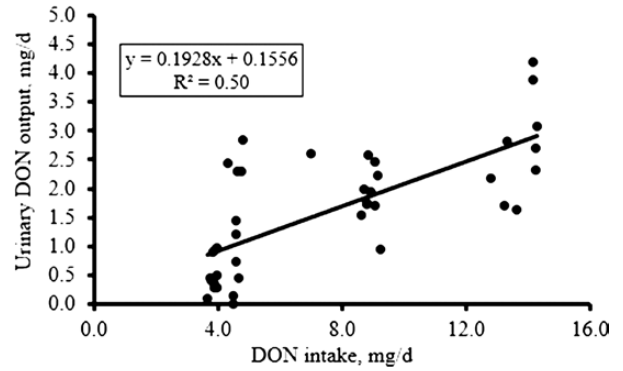

Figure 3. Regression analysis of the relationship between deoxynivalenol (DON) intake and DON output in urine (n = 10 pigs per treatment). The urine samples were collected during the nitrogen balance period of the experiment over 24 h and analyzed for DON content. Data are expressed as the DON intake per day (mg/d) and the urine DON output (mg/d) and the coefficient of determination (R^2) of the regression curve is 0.50 at $P < 0.05$.

accurately determining DON levels in complete diets have been previously reported (Patience et al., 2014; Kong et al., 2016) and could be due to the mechanisms by which *Fusarium* infects grain and proliferates in spots or localized portions of the grain batch during storage, leading to uneven distribution of *Fusarium* growth in samples (Swamy et al., 2002; Kong et al., 2012, 2015).

In the current study, upon initial exposure to DON-contaminated diets, there was a 27% and 53% reduction in ADG in pigs fed the DON3 and DON5 diets, respectively, which agrees with Serviento et al. (2018) who fed pigs between 70 and 95 kg BW with DON-contaminated diets (4.8 ppm) and observed a 40% to 60% reduction in ADG. We also observed a consistent reduction in ADG during the first 28 d of DON exposure, which was expected and in agreement with previous studies examining the effect of DON intake in pigs (Kong et al., 2015; Serviento et al., 2018; Nguyen-Ba et al., 2020). There was limited or no effect of DON intake on G:F, which suggests that the reduced growth performance is largely due to the reduction in feed intake and not due to reduced nutrient utilization. This observation is in agreement with Goyarts et al. (2005) who demonstrated that pigs fed a DON-contaminated diet for 11 wk had a 15% reduction in feed intake and 13% reduction in weight gain, but no difference in G:F. Likewise, Pastorelli et al. (2012) determined that approximately 85% of the reduction in ADG during mycotoxicosis is due to the observed reduction ADFI, with the remainder due to G:F. The present study evaluated a single mycotoxin (DON) and in much older pigs (finisher pigs), whereas Pastorelli et al. (2012) included studies examining different mycotoxins, alone or in combination, as well as studies examining mycotoxicosis in postweaned pigs, indicating there may be a difference in response due to age and physiological stage.

Previous studies have suggested that pigs may be able to adapt to DON intake, with the depression in feed intake and growth being most severe in the first week after exposure (Pollmann et al., 1985; Foster et al., 1986; Rotter et al., 1994; Serviento et al., 2018). Indeed, Serviento et al. (2018) observed an immediate reduction in ADG and ADFI upon initial exposure to a DON-contaminated diet, which gradually recovered over a 7-d period. Likewise, in their meta-analysis, Dersjant-Li et al. (2003) demonstrated a strong linear relationship between DON intake and the reduction in ADG in pigs; however, the strength of this relationship (i.e., R^2 value) was lower when the duration of the study was longer, suggesting adaptation and recovery of performance. We observed a similar response in the current study when examining the linear response of ADG to DON intake, with a strong relationship immediately after exposure which weakened over time. Rotter et al. (1994) fed up to 3 ppm DON to young pigs and observed a decreased weight gain over the initial 7 d; however, growth performance did not differ among groups by the end of the 4-wk study. This is similar to the observed recovery period in the current study, in which ADG had started to recover by 28 d post-DON exposure. Serviento et al. (2018) also observed a rapid and significant decrease in feed intake in both grower and finisher pigs when exposed to DON-contaminated diets, which recovered after approximately 7 d. Overall, it appears that pigs do have the ability to adapt to DON intake. The overall reduction in BW gain observed in a meta-analysis by Dersjant-Li et al. (2003) was approximately 20% with 5 ppm DON. The length of time available to recover postexposure probably plays a role, as in the current study, we only observed a 6% reduction in BW in DON-fed pigs.

N balance can be used as an indicator of the efficiency of nutrient utilization, specifically of dietary protein for PD (i.e., lean gain). It has been suggested that DON intake can interfere with PD (Swamy et al., 2003). Unfortunately, in the current study, we were not aware of DON contamination in the CONT diet used for N balance. We, therefore, lack a true control diet to compare the results of the other diets. If we assume that the DON1 response is similar to a DON-free diet (based on similar growth performance results as CONT-fed pigs), then it would appear that DON intake reduced N retention. The response of pigs fed the CONT diet is similar to DON3 and DON5, despite having similar DON content to the DON1 diet. It is possible that this observation is indicative of the initial response in nutrient utilization to a low-level DON contamination. Overall, N utilization may be impaired with DON exposure; however, this was not evident in measures of G:F in the current study.

There is some evidence suggesting that, once absorbed, DON can cause kidney and liver damage and can suppress immune function, resulting in decreased ability to resist disease challenge (Chaytor et al., 2011). As a reduction in immune function and organ damage may not be evident in growth performance, we evaluated pig health via measurement of key indicators of liver and kidney health. In the current study, we saw no evidence

of kidney and liver damage, as evaluated using a standard veterinary liver/kidney health panel. Similarly, previous studies observed little or no effect on hematological, biochemical, or immune response in pigs fed diets containing DON (Dänicke et al., 2004b; Goyarts et al., 2005; Accensi et al., 2006) or DON combined with aflatoxin (Chaytor et al., 2011). Moreover, many of the negative effects observed in the study by Goyarts et al. (2005) on blood biochemistry were due more to the restricted feeding regime (i.e., pair-fed pigs simulating the effect of DON on feed intake) than to feeding DON-contaminated diets.

Given the difficulties with obtaining consistent and/or reliable measures of DON in feedstuffs and feed, we evaluated whether DON content in biological samples could be used as an indicator of actual DON ingestion in pigs. It is known that levels of DON in plasma increase after intake of DON-contaminated diets, reaching a peak between 1.5 (Goyarts and Dänicke, 2006) and 4 h (Dänicke et al., 2004b) postintake. In the current study, we confirmed that DON ingestion can be determined through analysis of DON in blood serum samples taken between 3 and 4 h postingestion. The level of DON in serum was correlated with DON intake and could, therefore, be used to determine the degree of DON exposure under controlled conditions. The higher than expected DON content in the diets used for the N-balance measures was confirmed by serum DON concentration, with pigs fed CONT diets having detectable levels of DON in serum (Figure 2). The main route of excretion of ingested and absorbed DON is through urine as previously reported by Frobose et al. (2017) who determined urinary DON output in pigs fed DON-contaminated diets with dietary DON-detoxifying agents. In the current study, DON concentration in urine was highly correlated with actual DON ingestion. Dänicke et al. (2004a) reported >50% DON recovery in urine, confirming that urine is a major route of excretion. They also found that DON concentration in urine increased in DON-fed vs. control pigs. With the results of the current study, we provide evidence that urinary DON (24-h period) can also be used to estimate actual DON exposure in pigs under controlled conditions. Still, when interpreting DON levels in biological matrices, emphasis needs to be put on influencing factors such as suitable analytical methods, sampling time point, and individual variations in metabolism (Dänicke and Brezina, 2013).

Conclusion

In summary, we observed an initial reduction in ADFI and ADG upon introduction of diets containing >1 ppm DON to finisher pigs; however, performance (i.e., ADG, ADFI) recovered after 28 d, suggesting that pigs may be able to adapt to consumption of DON-contaminated diets. The lack of negative effect of DON intake on G:F suggests that the effect of DON intake on ADG is largely due to the reductions in feed intake. The consumption of diets containing DON up to 5 ppm appears to have little impact on organ function. Overall, although pigs maybe capable of adapting to intake of DON-contaminated diets, BW remains low compared with pigs fed up to 1 ppm DON.

Supplementary Data

Supplementary data are available at *Journal of Animal Science* online.

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Conflict of interest statement

V.N. is an employee of BIOMIN Holding GmbH. All other authors declare no conflicts of interest.

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