FINAL REPORT

Long-term feeding of graded levels of deoxynivalenol in grower-finisher pigs

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Principal Investigator

Dr. Daniel Columbus
Research Scientist – Nutrition
Prairie Swine Centre, Inc.
Box 21057, 2105-8th Street East
Saskatoon, SK, S7H 5N9
306-667-7432
dan.columbus@usask.ca

Collaborators

1. Dr. Denise Beaulieu, Department of Animal and Poultry Science, University of Saskatchewan, denise.beaulieu@usask.ca
2. Dr. Natacha Hogan, Department of Animal and Poultry Science, University of Saskatchewan, natacha.hogan@usask.ca
3. Dr. Veronika Nagl, Biomin Holding GmbH, veronica.nagl@biomin.net
4. Mr. Ken Engele, Prairie Swine Centre, ken.engele@usask.ca

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Abstract/Summary

The mycotoxin deoxynivalanol (DON), is of significant importance to agriculture since it commonly contaminates critical feedstuffs such as corn, wheat, and barley and is one of the most prevalent mycotoxins. DON intake general causes reduced performance and can have potentially negative impact on animal health. In general, 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 is highest in the young animal. Moreover, previous studies have examined the impact of mycotoxins over a relatively short period of time. We conducted two studies to determine the impact of long-term feeding diets containing 0, 1, 3, or 5 ppm DON to either grower-finisher or finisher pigs. While there was an initial reduction in feed intake and average daily gain in both groups of pigs upon initiation of DON intake, performance recovered after a period of time, indicating that pigs can adapt to DON intake. The negative effects of DON were less when the initial introduction of DON-contaminated feed occurred earlier. There appears to be little impact of DON intake on feed efficiency, nutrient utilization, or carcass characteristics. There was no indication of negative effects of DON intake on organ function or immune function. Feeding of DON-contaminated diets containing > 1 ppm DON significantly reduced margin over feed costs. Overall, while pigs are capable of adapting to intake of DON-contaminated diets, adjustment would be needed in order to allow for feeding of diets containing > 1 ppm DON to reduce financial losses.

Introduction

Mycotoxins are secondary metabolites of fungi which, when ingested, exert toxic effects resulting in adverse physiological responses in both humans and animals. While approximately 400 mycotoxins have been identified, of principal concern to agriculture are aflatoxins, zearalenone, and deoxynivalenol (DON) due to their presence in common animal feedstuffs and the known physiological effects on agricultural species (Binder et al., 2007ab; NRC, 2012). The presence and degree of mycotoxin contamination in grains is highly dependent on environmental conditions, such as temperature and humidity (Binder et al., 2007ab), but has been increasing in recent years.

The mycotoxin, DON (vomitoxin), is of significant importance to agriculture since it commonly contaminates corn, wheat, oats, and barley and is one of the most prevalent mycotoxins in temperate regions of the world, such as Europe and North America (Rotter et al., 1996; Chaytor et al., 2011). In the 2019 World Mycotoxin Survey conducted by BIOMIN, DON was reported to be the most prevalent mycotoxin in many ingredients of importance in swine, occurring in 85% of corn and 77% of cereal (wheat, barley, oats, rice) samples. In North America, 85% of all grain samples analyzed and 90% of finished feed samples contained DON (Biomin, 2019). Data for wheat in Saskatchewan shows an increase in the incidence of fusarium, with 80-90% of wheat (CWRS and Durum) downgraded due to DON contamination. Recent articles in 'All About Feed' in 2016 discussed the issues of mycotoxins, stating that with advances in mycotoxin analysis it has become clear that the mycotoxin problem is much larger than once imagined and the costs associated with mycotoxin contamination will worsen with climate change.

Typical negative effects of mycotoxin consumption include reduced feed intake, digestive dysfunction (e.g., gastroenteritis, gastrointestinal tract lesions, reduced nutrient absorption), immune suppression, and reduced growth performance (Sergent et al., 2006; Pestka, 2007; Pinton et al., 2008; Serviento et al., 2018) with the primary physiological effect dependent on the mycotoxin present. Specifically, in addition to reduced feed intake and growth performance, consuming DON contaminated feed results in damage to the intestinal tract epithelial cells through alteration of intestinal growth and barrier function as well as increased susceptibility to enteric pathogen challenge (Pinton et al., 2009; Ghareeb et al., 2015). Damage to the intestine also results in a reduction in nutrient absorption (Ghareeb et al., 2015) and therefore availability for growth. Once absorbed, DON inhibits protein synthesis, causes kidney and liver damage, and can suppress immune function resulting in decreased ability to resist disease challenge (Chaytor et al., 2011). While all species respond to mycotoxin exposure, pigs are particularly susceptible among farm animals (Wu et al., 2010). Because of this, the CFIA currently limits DON content in finished diets for swine to a maximum of 1 ppm.

In general, the majority of studies on the effects of mycotoxins in swine are performed in young (e.g., weaned) animals with the assumption that the physiological effects of consuming mycotoxin contaminated feed is highest in the young animal. Moreover, previous studies have examined the impact of mycotoxins over a relatively short period of time (Dersjant-Li et al., 2003). Indeed, in a review of studies to determine the impact of mycotoxins on performance in swine and poultry, the data was heavily skewed towards the newly-weaned period and based on short-term exposure (Dersjant-Li et al., 2003). In their meta-analysis, Andretta et al. (2012)

reported that the average initial age of pigs used in DON studies was 44 d and 64% of studies were completed in the nursery phase, 18% in the grower phase, 3% in the finisher phase, and 15% overall. It is possible that due to the overall higher feed intake in grower-finisher pigs and longer possible exposure time that the effects of mycotoxins 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; Pollmann et al., 1985), with feed intake and growth performance recovering after a period of exposure.

Contaminated grains are commonly downgraded for use in livestock feed and, while the best strategy for livestock producers is to avoid feeding mycotoxin-contaminated grain altogether, with the increased incidence and level of contamination this is no longer a viable option. Therefore, many strategies have been proposed to eliminate or reduce the negative effect of mycotoxins in animal feeds. Most of these strategies are based on deactivation of the mycotoxin through binding of the mycotoxin using adsorbents, such as silicate clays and activated carbon, which can be included in feed as non-nutrient additives. In general, however, current feed additives are relatively ineffective in mitigating the negative effects of mycotoxins (NRC, 2012; Zhu et al., 2016) and may not be effective for all mycotoxins. For example, some adsorbent agents have proven effective at reducing the negative effects of some mycotoxins, such as aflatoxin (Schell et al., 1993), but have shown little or no impact in pigs fed DON contaminated diets (Chaytor et al., 2010; Olejniczak, 2020). Recent studies have examined the use of additives consisting of different blends of yeast/yeast product, preservatives, antioxidants, amino acids, and probiotics which have shown potential for success in DON-contaminated diets in grower-finisher (Patience et al., 2014) and weaning pigs (Van Le Thanh et al., 2015), however, the results are inconsistent and require further validation. There are currently no additives available in Canada for use to mitigate the effects of DON.

Given the increasing incidence of DON contamination there is an obvious economic impact of mycotoxin contamination for both the grain and pork sectors. In addition, with the lack of effective mitigation strategies specifically for DON-contamination in diets for pigs, further information is required on long-term DON exposure in grower-finisher pigs. This information can be used to develop feeding programs which maximize inclusion of DON-contaminated grains while minimizing the impact on growth performance and profitability of both pork and grain producers.

Therefore, specific objectives of this project were:

1. To determine the effect of long-term exposure to mycotoxin-contaminated diets on growth performance of grower-finisher pigs;
2. To determine the effect of long-term exposure to mycotoxin-contaminated diets on nutrient utilization in grower-finisher pigs;
3. To determine the effect of long-term exposure to mycotoxin-contaminated diets on health status in grower-finisher pigs;
4. To determine estimates of mycotoxin exposure and absorption via biological samples.
5. To determine the effect of long-term exposure to mycotoxin-contaminated diets on carcass characteristics; and,

6. To determine the economic viability of feeding mycotoxin-contaminated diets in the grower-finisher period.

Methodology

All animal studies were approved by the University of Saskatchewan Animal Research Ethics Board under Animal Use Protocols 20130054 and followed Canadian Council on Animal Care guidelines.

Animals, Housing, Diets, and Experimental Design

Study 1 (Objectives 1-4 and 6) – Effect of long-term feeding of graded levels of deoxynivalenol on finisher pig performance, nutrient utilization, and health status

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 experiment at the Prairie Swine Centre Inc. (Saskatoon, SK, Canada). The pigs were grouped housed in pens (5 pigs/pen) in environmentally controlled rooms. The pens were randomly assigned to 1 of 4 dietary treatments (n=10 pens/treatment; **Table 1 & 2**). Dietary treatments consisted of a control diet (CONT) containing no deoxynivalenol (DON) or a diet containing 1, 3, or 5 ppm DON (DON1, DON3, or DON5). The basal diet was wheat-barley-soybean meal-based and formulated to be isonitrogenous and isoenergetic with nutrients meeting or exceeding the recommended requirement for finisher pigs (NRC, 2012). The dietary DON levels were achieved by replacing DON-free wheat with DON-contaminated wheat and wheat screenings proportionally according to the target DON levels. The mycotoxin profile and content of the DON contaminated wheat and wheat screenings used in the present experiment was determined at Central Testing Laboratory (Winnipeg, MB, Canada), whereas the complete diets were analyzed at Biomin Holding GmbH (Tulln, Austria). The pigs had *ad libitum* access to feed and water. At the start of the growth performance trial, 1 barrow in each pen representing the average pen BW was selected for blood sampling and nitrogen (N)-balance. On d 0, 14 and 42, blood samples were collected from the representative pig in each pen via jugular puncture into heparin-coated and additive-free tubes (5mL; BD vacutainer, BD, Mississauga, ON, Canada) which were stored on ice before centrifugation at 2500 × *g* for 15 mins to harvest plasma and serum, respectively. Blood samples on d 42 for DON analysis were obtained 3-4 h after the morning meal. The plasma and serum samples were stored at -20 ℃ until further analyses. Serum samples were analyzed for DON and DON metabolite concentrations and markers of liver and kidney health and function.

On d 35, 1 pig/pen (identified at the start of the experiment) representing the average BW of the pen were individually housed in metabolism crates (56” × 58.5”) in a temperature-controlled room (21 ± 2 ℃) for N-balance collection. The selected pigs from the growth performance trial remained on the same dietary treatment during the N-balance period. During the N balance period, the same basal wheat-barley-soybean- diets were fed except for the inclusion of celite (0.4%) at the expense of uncontaminated wheat. The daily feed allocation was set at 2.8 × maintenance net energy requirement (197 kcal/kg $BW^{0.60}$/d; NRC, 2012) and fed in two equal

meals at 0700 h and 1500 h. Following a 7-d dietary and environmental adaptation period, total daily urine output and fresh-fecal grab samples were collected over a 2-d period.

Study 2 (Objectives 1-6) – Effect of long-term feeding of graded levels of deoxynivalenol on grower-finisher pig performance, nutrient utilization, health status, and carcass quality

A total of 240 grower-finisher pigs (Camborough Plus × C337; PIC Canada) with an initial body weight of 35.9 ± 1.1 kg were used in a 77-d experiment at the Prairie Swine Centre Inc. (Saskatoon, SK, Canada). The pigs were group-housed in pens (6 pigs/pen) in environmentally controlled rooms (21 ± 2 ℃). The pens were randomly assigned to 1 of 4 dietary treatments (n=10/treatment; **Table 3 & 4**). Dietary treatments consisted of a control diet (CONT) containing no DON or a diet containing 1, 3, or 5 ppm DON (DON1, DON3, or DON5). Dietary DON content was achieved by replacing uncontaminated wheat with a proportional amount of DON-contaminated wheat and wheat screenings to reflect the targeted DON levels in the final diet. The mycotoxin content of wheat and wheat screenings used in formulating the experimental diets were analyzed at Central Testing Laboratory (Winnipeg, MB, Canada). The basal diet was wheat-barley-soybean meal-based and formulated to be isonitrogenous and isoenergetic with nutrients meeting or exceeding the recommended requirement for grower (25-75 kg) and finisher pigs (75-120 kg) according to NRC (2012). The diets were fed according to a 2-phase protocol, with grower phase diets fed from d 0 – 42 and finisher phase diets fed from d 43 – 77. The pigs had *ad libitum* access to feed and water. At the beginning of the experiment, 2 pigs/pen were identified as representative pigs for the grower and finisher phases. On d 14 and d 56 of the experiment, the previously selected pigs in each pen were inoculated intramuscularly with a commercial vaccine to elicit a humoral response (Farrowsure® Gold, Serial No.: 316169Zoetis) against Leptospirosis caused by *Leptospira canicola, Leptospira grippotyphosa, Leptospira hardjo, Leptospira icterohaemorrhagiae, and Leptospira pomona* (Wunder Jr et al., 2020). Blood samples were taken before (d 0) and after the vaccine injections on d 14, 42, 56, and 84 via jugular puncture into both heparin-coated and additive-free tubes (5mL; BD vacutainer, BD, Mississauga, ON, Canada), centrifuged at 2500 × *g* for 15 mins for plasma and serum sample collection, respectively which were stored at -20 ℃ until further analyses. Blood samples on d 42 and 84 for DON analysis were obtained 3-4 h after the morning meal.

On d 35 and 77, the selected pigs (identified at the start of the experiment) representing the average BW of the pen were isolated and used for N-balance collection. The pigs were individually housed in metabolism crates (56" × 58.5") in a temperature-controlled room (21 ± 2 ℃) and assigned to the same dietary treatments as in the growth performance study. The same basal wheat-barley-soybean-based diets were used, except for the inclusion of celite (0.4%) at the expense of uncontaminated wheat. The daily feed allowance was 2.8 × maintenance net energy requirement (197 kcal/kg $BW^{0.60}$/d; NRC, 2012) and fed in two equal meals at 0700 h and 1500 h. After a 7-d dietary and environmental adaptation period, total daily urine output and fresh-fecal grab samples were collected over a 2-d period.

Sample Collection and Analyses

Growth Performance (Study 1 and 2)

In both studies, individual pig body weight and per pen feed intake was measured weekly for the duration of the study for determination of average daily gain (**ADG**), average daily feed intake (**ADFI**), and feed efficiency (gain:feed; **GF**).

Nitrogen-Balance (Study 1 and 2)

During the sample collection days, urine jars containing sufficient HCl to maintain pH < 3 were placed underneath the urine collection trays of each metabolism crate to collect urine samples over two 24-h periods. At the end of each 24-h period, urine was weighed, and a 5% aliquot was sampled and stored at -20℃. 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 ºC until further analysis. Fresh fecal grab samples were taken daily by rectal palpation, pooled. and homogenized at the end of the 2-d collection period and a subsample stored at -20 ℃ until further analysis.

Analysis of feed, fecal, and urine samples (Study 1 and 2)

The dry matter content of the diet and fecal samples and were analyzed in duplicate (AOAC, 2007; Method 930.15). The nitrogen content in the diet, feces, and urine was 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). Nitrogen retention was calculated as the difference between nitrogen intake and nitrogen output (urine and fecal). Protein deposition was calculated as nitrogen retention × 6.25.

Deoxynivalenol Analysis in Diet, Serum, and Urine Samples (Study 1 and 2)

All mycotoxin analyses were performed in the laboratory of Biomin Holding GmbH (Tulln, Austria). Analytical standards for deoxynivalenol (DON) and de-epoxy-deoxynivalenol (DOM) were acquired commercially (Romer Labs GmbH, Tulln, Austria), whereas standards for DON-3-glucuronide (DON-3-GlcA, produced by chemical synthesis, Fruhmann et al., 2012), DOM-3-glucuronide (DOM-3-GlcA), DOM-15-glucuronide (DOM-15-GlcA), isoDON and isoDOM were provided by Dr. Schwartz-Zimmermann (University of Natural Resources and Life Sciences, Vienna, Austria). Creatinine, β-glucuronidase (Escherichia coli, Type IX-A) and PBS buffer were obtained from Sigma-Aldrich (Vienna, Austria), methanol (MeOH) and acetic acid from VWR International (Vienna, Austria), and acetonitrile from Chem-Lab NV (Zedelgem, Belgium). For the direct quantification of DON and its metabolites in urine, high-performance liquid chromatography (HPLC) with tandem mass spectrometry (MS/MS) (HPLC-MS/MS) based analysis was performed according to the method described by Schwartz-Zimmermann et al. (2017). Creatinine content was determined according to Warth et al. (2012). Subsequently, samples were diluted to 0.2 mM creatinine to compensate for matrix effects (first to 2 mM creatinine using ultra-pure water, then to 0.2 mM creatinine with MeOH/water, 20/80, v/v).

For serum samples, indirect quantification of DON metabolites was performed. Samples were analyzed both with and without β-glucuronidase pretreatment. 100 µL of serum samples without β-glucuronidase was added to 200 µL of MeOH/acetic acid (99.8/0.2, v/v), shaken for 1 hour on a vortex shaker and centrifuged (19.000 rcf, 20 min). Afterward, 200 µL of the supernatant was transferred into an HPLC vial and subjected to HPLC-MS/MS analysis. For enzymatic hydrolysis, 35 mg of β-glucuronidase was dissolved in 2.5 mL PBS and added at 50 µL per 100 µL serum. After incubation for 18 hours (37° C, 80 rpm), 300 µL of MeOH/acetic acid (99.8/0.2, v/v) were added. Subsequent shaking and centrifugation steps were performed as described above. Determination of analytes was performed on a QTRAP 6500 using an HPLC-MS/MS-based method described previously (Schwartz-Zimmermann et al., 2017). Samples were measured in duplicate and quantified using matrix-matched samples. To this end, urine samples from the control group were spiked with standard compounds at six spiking levels (0.5 – 100 ppb for DON, DOM, DON-3-GlcA, DOM-3-GlcA, DOM-15-GlcA; 0.25 – 50 ppb for isoDON and isoDOM). DON-15-glucuronide (DON-15-GlcA) was quantified via the DON-3-GlcA standard.

Kidney and Liver Blood Metabolite Analysis (Study 1 and 2)

Serum samples were analyzed for key indicators of liver and kidney function and health using an automatic blood chemistry analyzer according to established methods (Prairie Diagnostic Services, Saskatoon, SK, Canada).

Analysis of Serum IgG Titer (Study 2)

Blood serum was analyzed for *Leptospira* antibody ELISA according to Wilson-Welder et al. (2020) at the Vaccine and Infectious Disease Organization - International Vaccine Centre (Saskatoon, SK, Canada). Briefly, the detection of the antibody Immunoglobulin G (IgG) against *Leptospira spp* in pig serum was completed in a reconstituted *Leptospira* vaccine (Vanguard® L4, Zoetis Canada Inc., Kirkland, QC). Following that, a protein assay was completed to determine the protein concentration. A vaccine antigen was diluted to 10 µg/mL in a carbonate-bicarbonate buffer (pH 9.6), applied to Immulon plates (Thermo Fisher Scientific, Mississauga, ON, Canada) at 100 µL per well, covered and incubated overnight at 4 ℃. The plates were subsequently washed with 300 µL per well four times with Tris-buffered saline containing 0.05% Tween 20 (TBST). The serum samples were diluted 1/10 in the first well (13 µL + 120 µL TBST) and four-fold serial dilutions across the plate for 6 wells, including a negative and positive control for each plate and incubated for 2 h at room temperature. Plates were washed again with TBST and 100 µL of KPL Goat anti-Swine IgG (H+L) phosphatase labeled affinity purified antibody (catalogue KP-15-14-06, Invitrogen, Life Technologies Inc., Burlington, ON, Canada) added at a dilution of 1/5000 in TBST and allowed to incubate at room temperature for an hour. Plates were further washed and p-nitrophenyl phosphate di(tris) salt crystalline (PNPP) (Sigma N3254, St. Louis, Missouri, United States) substrate was added at 100 µL per well and incubated for 2.5 h. Finally, the reaction was stopped by adding 30 µL 2N H_2SO_4 per well and the plate read at 405 nm, reference 490 nm on a SpectraMaxplus microplate ELISA reader (Molecular Devices, Sunnyvale, CA, USA).

Carcass Quality (Study 2)

On d 77 of the study, pigs were transported to a commercial abattoir (Maple Leaf Foods, Brandon, MB, Canada) for slaughter and collection of carcass characteristic data, including slaughter weight, backfat thickness, loin depth, and overall yield for further analysis to assess the impact of DON on carcass yield and quality. Only slaughter data from pigs accurately identified based on tattoo number were included in the final analysis.

Statistical Analysis

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 and nitrogen balance data were analyzed as a randomized complete block design with the fixed effect of dietary treatments (CONT, DON1, DON3, and DON5) and the random variable as the block (room) (PROC MIXED, SAS Institute, Cary, NC, Version 9.4). The immune response data were analyzed as a repeated measure with day as a repeated variable. Regression analysis was used to describe relationships between DON intake and ADG, total BW gain, urinary, and blood DON metabolites (PROC REG, SAS Institute, Cary, NC, Version 9.4). Blood chemistry analyses for samples collected over time were analyzed as a repeated measure. Differences between means were determined using Tukey-Kramer mean separation test and significance was declared at $P < 0.05$. A trend towards significance was considered at $P < 0.10$.

7. Research accomplishments

Objective 1. To determine the effect of long-term exposure to mycotoxin-contaminated diets on growth performance of grower-finisher pigs

Two growth performance studies were conducted to examine the impact of long-term feeding of graded levels of DON in finisher (75 – 120 kg) and grower-finisher (35 – 120 kg) pigs. In finisher pigs we found that there was a rapid negative response to > 1 ppm DON intake, resulting in a decrease in average daily gain and feed intake as well as reduced body weight within the first week. The reduction in body weight was maintained throughout the study, however, after a period of approximately 4 weeks, the feed intake and average daily gain of all pigs had recovered. In grower-finisher pigs, the response to DON intake was less pronounced and not as rapid, resulting in variability in the response over time and across treatments. Overall there was reduction in average daily gain, feed intake, and body weight in pigs fed > 1 ppm DON, however, this negative effect was less than observed in finisher pigs. Overall, this study provides further evidence for an upper limit of 1 ppm DON in finished feed to avoid reduced performance. While there was an initial reduction in performance, pigs seem to be able to adapt to DON intake of > 1 ppm, and < 5 ppm).

Objective 2. To determine the effect of long-term exposure to mycotoxin-contaminated diets on nutrient utilization in grower-finisher pigs

Nitrogen-balance was determined in grower and finisher pigs during both studies to determine effects of DON intake on nutrient utilization. In finisher pigs, there was a reduction in nitrogen retention in pigs fed 3-5 ppm DON (Study 1), however, this reduction was less in grower pigs (Study 2) and was no longer evident in finisher pigs that had been fed DON-containing diets for 77d. Interestingly, this was not evident in measures of plasma urea nitrogen content (an indicator of nitrogen utilization) and on feed efficiency (gain:feed). Moreover, there was no impact of DON on carcass measures, indicating that exposure of pigs to DON at an earlier production stage may allow for recovery.

Objective 3. To determine the effect of long-term exposure to mycotoxin-contaminated diets on health status in grower-finisher pigs

We evaluated the effect of DON intake on health status via evaluation of indicators of kidney and liver function and health (Study 1 and 2) and a humoral response to vaccination (Study 2). There was no indication of negative effects of DON intake on any measure of pig health status determined in the current studies.

Objective 4. To determine estimates of mycotoxin exposure and absorption via biological samples

As mycotoxin analysis in feed and feedstuffs can be difficult and highly variable, we evaluated the potential for determining DON exposure via biological samples. We found that DON intake is highly correlated with DON content in blood samples and in urine samples taken from pigs exposed to DON containing diets. This provides another tool which producers can use to determine actual exposure of pigs to DON contamination in feed.

Objective 5. To determine the effect of long-term exposure to mycotoxin-contaminated diets on carcass characteristics

We evaluated the effect of long-term DON exposure in grower-finisher pigs (35 – 120 kg) on carcass quality. There was no impact of DON exposure on any carcass measures when fed for the entire grower-finisher period.

Objective 6. To determine the economic viability of feeding mycotoxin-contaminated diets in the grower-finisher period

In the current series of studies, we determined that the negative effects of DON intake in grower-finisher pigs appears to be due largely to reduced feed intake, and, therefore, growth performance, and not due to effects on nutrient utilization or health status. Moreover, while there is an initial reduction in growth performance upon DON intake, pigs are able to adapt to intake of DON contaminated feed over time. As there was also no impact on carcass quality, it would appear that if producers can make proper adjustments to account for the reduced growth performance (i.e., increased days to market) there is potential for use of DON-containing diets in

swine. Based on economic analysis, there is a significant decrease in margin over feed costs in pigs fed diets containing > 1 ppm DON. Adjustments would need to be made (e.g., reduced grain cost, increased days to market) in order to avoid financial losses.

8. Discussion

Study 1 (Objectives 1-4 and 6) – Effect of long-term feeding of graded levels of deoxynivalenol on finisher pig performance, nutrient utilization, and health status

Growth Performance

Growth performance data is presented in **Table 6**. Initial body weight (d 0) was not different among the dietary treatments ($P > 0.05$). Body weight was reduced in DON3 and DON5 fed pigs by d 7 of the study, 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 d 0-7, DON3 fed pigs had reduced growth compared to both CONT and DON1 fed pigs ($P < 0.05$) but was not different from CONT fed pigs from d 8-42 ($P > 0.05$). Pigs fed DON5 had reduced ADG from d 0-21 compared to all other dietary treatments ($P < 0.05$). From d 22-28, ADG of DON5 fed pigs was not different than DON1 and DON3 fed pigs ($P > 0.05$). From d 29-42 there were no differences in ADG among dietary treatments ($P > 0.05$). Overall (d 0-42), ADG was reduced in DON3 and DON5 fed pigs compared to both CONT and DON1, with the greatest reduction observed with DON5 ($P < 0.05$). There was no impact of DON1 on ADFI compared to CONT ($P > 0.05$). From d 0-7, DON1 fed pigs had reduced ADFI compared to both CONT and DON1 fed pigs ($P < 0.05$), after which no difference was observed ($P > 0.05$). In DON5 fed pigs, ADFI was reduced from d 0-28 compared to all other dietary treatments ($P < 0.05$), after which no difference was observed ($P > 0.05$). Overall (d 0-42), ADFI was only reduced in DON5 fed pigs ($P < 0.05$). Feed efficiency, measured as GF, was reduced in DON5 fed pigs from d 0-7 compared to all other dietary treatments ($P < 0.05$), which were not different from each other ($P > 0.05$). There was no effect of dietary treatment on GF from 8-42 or overall (d 0-42) ($P > 0.05$).

Relationship Between Dietary DON Intake and Performance

A linear regression model was applied to study the relationship between DON intake and performance (% BW gain, Fig. 1; ADG, **Fig. 2**). As indicated, there was a negative relationship between DON intake and BW gain, whereas DON intake increased, % BW gain was reduced. This negative relationship was also observed for ADG, as DON intake increased, there was a linear reduction in ADG. This relationship was consistent from d 0-35, however, the strength of the impact of DON intake on ADG reduced consistently and from d 28-35, the impact was lowest, with a slope not different than zero (Fig.2E). From d 35-42, the slope shows a positive relationship (Fig. 2F).

Nitrogen-Balance

The results for the N-balance study are presented in **Table 7**. Dry matter intake was not different across dietary treatments. ($P > 0.05$). Average daily N intake for both DON3- and DON5 was not different but both were lower ($P < 0.05$) than DON1 and CONT diet. The urinary N output from CONT, DON3, and DON5 diets were not different ($P > 0.05$) but the pigs fed DON1 show a significantly ($P < 0.05$) lower urinary N output compared to the other dietary treatments. The fecal N output was not different between DON1, DON3, and DON5, but was significant ($P < 0.05$) higher in the CONT diet. The apparent total tract digestibility (ATTD) of N was significantly ($P < 0.05$) lower in the CONT diet compared to all the other dietary treatments. The protein deposition (PD) for DON1 pigs was significantly higher ($P < 0.05$) than pigs fed CONT, DON3, and DON5 diets.

Plasma Metabolites

Key indicators of kidney and liver health and function are presented in **Table 8**. There was no effect ($P > 0.05$) of dietary DON content on the selected liver and kidney blood parameter. For most of the analyzed metabolites there was a significant effect of day. These differences are considered to not be related to dietary treatment as there was no significant diet × day interaction.

Concentration of DON in Biological Samples

A linear regression model was used to analyze the relationship between DON intake and DON and DON metabolite concentration in blood serum and recovery in urine as shown in Fig. 3, 4 and 5. As dietary DON intake increased, the amount of DON and DON metabolites in the blood increased. For urinary DON analysis, as dietary DON levels increased, there was an increase ($P < 0.05$) in DON metabolites in the urine. DON recovery in urine and serum was based on actual intake.

Study 2 (Objectives 1-6) – Effect of long-term feeding of graded levels of deoxynivalenol on grower-finisher pig performance, nutrient utilization, health status, and carcass quality

Growth Performance

The growth performance results are presented in Table 9. The initial BW was not significantly different ($P > 0.05$) between the dietary treatments. Pigs fed DON5 had reduced BW by d 21 and DON3 fed pigs by d 35 compared to CONT fed pigs ($P < 0.05$) and in general this observation remained for the duration of the study. There was no difference in BW between CONT and DON1 at any point in the study ($P > 0.05$).

From d 0-7, the ADG for pigs fed the DON3 and DON5 diets were lower ($P < 0.05$) than DON1 and CONT fed pigs. No effect ($P > 0.05$) of increasing dietary DON levels on ADG was observed from d 7-42. From d 42-49, ADG for pigs fed DON5 diet was reduced ($P > 0.05$) compared to the rest of the dietary treatments. Subsequently, no further treatment effects were observed ($P > 0.05$) on ADG from d 49-77. Overall only pigs fed the DON5 diet had reduced ADG compared to CONT with no difference in ADG among other treatments over the entire grower phase (d 0-42) and no treatment differences observed over the entire finisher phase (d 42-77). Over the entire growth performance study (d 0-77), both DON3 and DON5 resulted in reduced ADG ($P < 0.05$) compared to both CONT and DON1, which were not different ($P > 0.05$).

From d 0-7, ADFI of pigs fed DON5 diet was lower ($P < 0.05$) compared to pigs fed CONT, DON1, and DON3 diets. From d 7-21, there was no impact of dietary treatment ($P > 0.05$) on ADFI. However, from d 21-28, pigs fed DON-contaminated diets (DON1, DON3, and DON5) had a significantly higher ADFI ($P < 0.05$) compared to the CONT fed pigs. From d 28-42, no significant differences in ADFI were observed ($P > 0.05$). Over the entire grower period (d 0-42) there was no impact of diet on ADFI ($P < 0.05$) whereas in the finisher period (d 42-77), feeding of DON contaminated diets reduced ADFI compared to CONT ($P < 0.05$). Over the entire growth performance study, ADFI was reduced ($P < 0.05$) in DON3 and DON5 fed pigs compared to CON and DON1, which were not different ($P > 0.05$). There was no impact of dietary treatment on GF at any point in the study ($P > 0.05$). Carcass characteristics data is presented in Table 10. There was no significant effect ($P > 0.05$) of dietary treatments on parameters of carcass characteristics as measured.

Relationship Between Dietary DON Intake and Performance

The relationship between dietary DON intake and body weight gain was evaluated using a linear regression model as shown in **Fig. 7**. In general, there was a negative relationship between DON intake and ADG in the grower phase and over the entire growth performance study, however, there was only a weak relationship in the finisher phase.

The relationship between DON intake and body weight gain of the treatment groups (DON1, DON3, and DON5) relative to CONT are presented in **Fig 8**. The results indicated that as DON intake increased, the rate of BW gain relative to the CONT pigs reduced and increased the difference in BW relative to the control in a linear trend, hence a positive relationship. The observation above was present throughout the grower phase (d 0-42) and by the end of the finisher phase (d 42-77), there was the apparent difference between the experimental groups.

Nitrogen-Balance

Nitrogen-balance results are presented in **Table 11**. In the grower phase, the dry matter intake was not different ($P > 0.05$) between dietary treatments, however, N-intake of pigs fed DON3 and DON5 diets was lower ($P < 0.05$) compared to pigs fed CONT and DON1 diets. Digestibility of N was higher ($P < 0.05$) for CONT and DON3 pigs compared to DON1-fed pigs

but not different from DON5 fed pigs and fecal N output was higher ($P < 0.05$) in DON1 fed pigs compared to all other treatments. Urinary N output was not affected ($P > 0.05$) by dietary treatments. Nitrogen retention and PD were reduced ($P < 0.05$) in pigs fed DON3 and DON5 compared to CONT and DON1 fed pigs, which were not different. In the finisher phase, dry matter intake was reduced ($P < 0.05$) DON3 compared to CONT fed pigs, with no difference between all other treatments. Nitrogen intake was higher ($P <0.05$) in DON1 fed pigs compared to CONT and DON3, but not different from DON5. Nitrogen digestibility was lower ($P < 0.05$) in the all DON treatments (DON1, DON3, DON5) compared to CONT fed pigs resulting in increased fecal N output for all DON treatments. Urinary N output was not affected ($P < 0.05$) by dietary treatment. Overall, nitrogen retention and PD were not affected by dietary treatment in the finisher period ($P > 0.05$).

Plasma Metabolites

Key indicators of kidney and liver health and function are presented **Tables 12 and 13**. There was no effect ($P > 0.05$) of dietary DON content on the selected liver and kidney blood parameter. For most of the analyzed metabolites there was a significant effect of day. These differences are considered to not be related to dietary treatment as there was no significant diet × day interaction.

Concentration of DON in Biological Samples

A linear regression model shows a significant positive relationship ($P < 0.05$) between dietary DON intake and DON concentration in serum and recovery in urine (**Fig 9 & 10**).

General Discussion

Animal Performance: The reduction in average daily gain and feed intake was expected and in agreement with previous studies examining the effect of DON intake in pigs. In the current studies, there was limited or no effect on feed efficiency in pigs fed DON-contaminated diets. This suggests that reduced growth performance is largely due to the reduction in feed intake and not due to reduced nutrient utilization. This is in agreement with Pastorelli et al. (2012) who determined that in general, approximately 85% of the reduction in gain due to mycotoxicosis due to the observed reduction in feed intake, with the remainder due to feed efficiency. That we did not observe alterations in nutrient utilization in the current study may be due to the fact that we only studied one mycotoxin (i.e., DON) whereas Pastorelli et al. (2012) included studies examining different mycotoxins alone or in combination. The meta-analysis largely included studies examining mycotoxicosis in post-weaned pigs, indicating there may be a difference in response due to age and physiological stage.

Nitrogen-balance can be used as an indicator of the efficiency of nutrient utilization, specifically of dietary protein for protein deposition (i.e., lean gain). It has been suggested that DON intake can interfere with protein deposition (Swamy et al., 2003). Unfortunately, in the current study, we were not aware of DON contamination in our CONT diet used for N-balance. We, therefore,

lack a true control diet to compare the results of the other diets to. If we assume that the DON1 response is similar to a DON-free diet (based on similar growth performance results), then it would appear that DON intake does reduce protein deposition. It is interesting to see this response in the CONT-fed finisher pigs, as this would have been their first exposure to DON and this may indicate the initial response to a low level DON contamination. This response to DON intake was also observed in the grower pigs, although to a lesser extent, but there was no difference in finisher pigs that had been exposed to DON-contaminated diets for 77 d. In a study in starter pigs, Danicke et al. (2004) found no effect of DON on N-balance. Overall, it is possible that nitrogen utilization may be impaired with DON exposure, however, this is not observed through measurement of feed efficiency or carcass quality.

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). Likewise, in their meta-analysis, Dersjant-Li et al., (2003) demonstrated a strong linear relationship between DON intake in 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 recovery. 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 week study. This is similar to the observed recovery period in the current studies, in which ADG had started to recover by 4 weeks post-DON exposure. In a more recent study in grower-finisher pigs, 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 body weight gain observed in the grower and grower-finisher study is similar to that observed in a meta-analysis, Dersjant-Li et al. (2003) who determined approximately 20% reduction in body weight gain with 5 ppm DON. This degree of reduction in body weight gain was not observed in the grower-finisher study in which feeding of DON-contaminated diets was initiated at an earlier stage (i.e., 35 kg) and fed for a longer period of time (i.e., 77 d).

In the current study, there was less of an impact on final body weight when pigs were exposed to DON earlier, with exposure in the finisher period resulting up to ~6 reduction in final body weight compared to ~3% reduction with exposure in the grower period. The length of time available to recover post-exposure likely plays a role. For example, Serviento et al. (2018) observed a reduction in ADG and body weight when finisher pigs were exposed to DON at ~70 kg or ~95 kg body weight, however, body weight had largely recovered in the lighter pigs but not in the heavier pigs. Another possibility for this difference in response could be due a higher tolerance for DON intake in younger pigs compared to older pigs. In the current study, upon initial exposure to DON-contaminated diets there was a 27 and 53% reduction in ADG in the finisher pigs, however, only a 21 and 17% numerical (i.e., not significant) reduction in the grower pigs fed DON3 and DON5, respectively. Serviento et al. (2018) also observed a nearly 50% reduction in ADG when exposing finisher pigs to diets 3 ppm DON. In the current study,

we also observed a delayed and more variable response to DON intake in grower vs. finisher pigs, similar to a study in younger pigs by Chaytor et al. (2011), who also observed a more variable response in ADG and ADFI, with very little response in feed efficiency in young pigs fed diets containing low levels of DON and aflatoxin. Overall, this provides evidence that finisher pigs are much less tolerant to the effects of DON than younger animals.

Animal Health: 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 reduction in immune function and organ damage may not be evident in growth performance, we evaluated pig health via a number of additional assays. However, in the current study we saw no evidence for reduced immune function (as determined by humoral immune response) and kidney and liver damage (as evaluated using a standard veterinary liver/kidney health panel). Similarly, Chaytor et al. (2011) feeding diets containing DON and aflatoxin and Accensi et al. (2006), Goyarts et al. (2005), and Danicke et al. 2004 with DON-contaminated diets observed little effect of on hematological, biochemical, or immune response. In addition, 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. Reddy et al. (2018) found little effect of feeding DON on measures of muscle, liver, and kidney inflammation although there was some evidence for reduced IgG production and negative effects on kidney histology. In this study, however, diets contained 8 ppm DON, and this higher level may be required in order to result in the negative effects on pig health and organ function.

Biological Assessment: 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 feeding of a DON-contaminated diet, reaching a peak between 1.5 (Goyarts and Danicke, 2006) and 4 h (Danicke et al., 2004a) post-intake. In the current study, we confirmed that DON ingestion can be determined through analysis of DON in blood serum samples taken between 3-4 h post-ingestion. The level of DON in serum was also highly correlated to DON intake and could, therefore, be used to determine degree of DON exposure. While not shown here, the higher than expected DON content in the diets used for the finisher N-balance measures was confirmed by serum DON concentration, and even pigs fed the CONT diets, formulated to contain no DON, had detectable levels of DON in serum. The main route of excretion of ingested and absorbed DON is through urine. In the current study, DON concentration in urine was highly correlated to actual DON ingestion, with between 30-60% of ingested DON recovered in urine. Danicke et al. (2004b) determined that > 50% of DON was recovered in urine, confirming that urine is the major route of excretion. They also found that DON concentration in urine increased in DON-fed vs. control pigs. While they did not determine the linear relationship of DON excretion to DON intake overall several DON levels, with the results of the current study this shows that urinary DON can also be used to determine actual DON exposure in pigs.

Economic Assessment: The Prairie Swine Centre Enterprise Model was used to assess the economic impact of feeding DON-contaminated grain to pigs. It is important to note that the following assessment was based on the results of the current studies as well as a number of other

assumptions (e.g., grid, market weight, current market prices). Therefore, these results are meant only as an indicator of the potential economic impact and the specific economics will be dependent on individual production parameters. Producers need to weigh several factors when considering feeding DON contaminated grains in their operations, the most important being - What are the costs associated with it? Results from this project have shown, that pigs consuming high levels of DON, in complete diets, will be 5-8 kg lighter by the time they reach market weight. However, these pigs also consumed less total feed. Does this drop in feed consumption, and total feed cost, outweigh the drop in market revenue from the sale of hogs? The simple answer is no, however it depends when pigs are introduced to DON in their diets.

Figure 13 shows the margin over feed cost when pigs (at average market conditions) are fed varying levels of DON in complete diets and when DON is introduced at different stages in the production cycle. Results indicate little to no change in returns when pigs are fed diets containing 1 ppm of DON - regardless of when it was introduced. In both studies, no significance was found in final market weight between control and diets containing 1 ppm of DON. Results also indicate an inverse relationship between margin over feed cost and the level of DON in the diet for both studies, in other words increasing DON reduces producer returns. However, the negative impact on margin over feed cost is far greater when pigs are first introduced to DON in the finishing period. This indicates the negative impacts of DON are less when introduced earlier to pigs in the production cycle. Based on the results of this study we would estimate between a $2 -$7 per hog drop in revenue under average market conditions. Therefore, it would be in the producer's best interest to avoid contaminated grains when possible.

In order to balance the drop in returns (margin over feed cost), producers will need to buy DON contaminated grains at a discount, compared to clean grain, in order or make feeding DON contaminated grain a viable option. **Figure 14** shows the estimated drop in finished feed cost (per mt) for various levels of DON contaminated diets required to have no impact to margin over feed cost (returns) to the producer. The finished diet will need to drop in price between $11 - $63 per tonne, depending on level of contamination and exposure to DON, in order to have no change in margin over feed cost.

Figure 15 displays the drop in (DON contaminated) ingredient price required when that ingredient would make up 40% of the total finished diet. If we assume clean grain can be purchased at $225/mt - producers will need to purchase the DON contaminated ingredient at a significant discount, up to $155 mt, in order to justify feeding 3 or 5 ppm of DON in a diet. It is important to remember an ingredient containing 2.5 ppm making up 40% of the diet translates to 1 ppm in the final diet, and it would take 12.5 ppm of DON in an ingredient to achieve 5 ppm in a diet.

There are additional considerations that producers must take in account when feeding DON in their diets. In theory if we could simply purchase DON contaminated grains cheaper we could maintain margin over feed cost. However, as the level of DON increases pigs grow slower, if you need to achieve a specific weight range for your grading grid, pigs will need to be kept in the barn longer, reducing throughput. Adding 5 days to market adds approximately 4.5% to fixed costs, as fewer pigs can be marketed from the barn in a year. In farrow-to-finish operations, many facilities simply cannot afford to keep pigs 5 days longer. Logistics are another important consideration. If farms do not have the ability to separate the DON contaminated ingredient (wheat, barley) from clean grain, the entire herd would receive the DON ingredient – perhaps creating additional challenges in other parts of the production system. Finally, this analysis looks at the impact of feeding DON on one specific grading grid. Packers have different requirements – as such the change in margin over feed cost would be packer specific and shipping at lighter weights (associated with higher levels of DON) may be more detrimental in some cases.

There are a number of considerations that producer need to take in account when considering feeding high levels of DON in their diets, however, results indicate we can feed levels higher than previously thought if adjustments are made, such as additional time before market, earlier introduction of DON-contaminated diets, and reduced grain/diet costs. While currently there are no products available in Canada for the specific purpose of reducing the effects of mycotoxins in feed, producers may wish to consider these products if they become available as an additional method to allow for the use of DON-contaminated feedstuffs in swine diet.

Conclusions and Recommendations

1. In finisher pigs, feeding of diets with > 1 ppm DON results in an initial reduction in feed intake and average daily gain. This results in a reduction in body weight which is sustained over time. Growth performance recovers after a period of time, indicating that pigs may be able to adapt to DON intake. The response to DON appears to be reduced and more variable in grower pigs than in finisher pigs.

2. The negative effects of DON intake appear to be due largely to reduced feed intake. This is supported by lack of negative effects of DON intake on nutrient utilization, health status, and carcass quality.

3. Exposure to DON (DON intake) can be determined through analysis of DON in biological samples, such as blood and urine, which is highly correlated to actual DON intake.

4. Feeding diets containing > 1 ppm DON will result in reduced margin over feed cost. This reduction is greater when DON is first introduced in the finisher period compared to the grower period.

4. Producers may be able to feed DON-contaminated diets, up to 5 ppm, while adjusting for the negative impact of DON intake on growth performance.

Overall, these are the first studies to look at the effects of long-term, sustained feeding DON-contaminated diets in both grower-finisher and finisher periods. Previous studies have examined the effect of DON intake in younger pigs (e.g., post-weaning) and/or over short duration, with little evaluation of long-term effects and the potential for pigs to adapt to DON intake over time. This study also provides evidence that the negative effects of DON may be largely due to feed intake. This information can be used to mitigate the effects of DON by development of strategies that address this effect (i.e., increase feed intake).

Success stories/practical implications for producers or industry

In Canada, losses range between $50 to $300 million each year (direct and secondary losses) due to DON contamination (Alberta Agriculture and Forestry 2012). In the most recent World Mycotoxin Survey conducted by BIOMIN (2018), DON was reported to be the most prevalent mycotoxin in Canada and the United States in many ingredients of importance in swine, occurring in 86% of cereals (i.e., wheat, barley, oats, rice, sorghum), with an average contamination of 1,853 ppb DON. In a recent survey in Western Canada, it was found that 42 and 56% of wheat and barley samples, respectively, contained at least one mycotoxin with a total of 33 of wheat and 44% of barley samples containing DON (Shi et al., 2019). First quarter results from BIOMIN (2019) indicates that the incidence of DON, and other mycotoxins, is increasing. A sustainable livestock industry depends on using low-quality or downgraded grains. A large percentage of downgraded grain as a result of mycotoxin contamination is destined for the livestock feed market. Because of the widespread occurrence of contaminated grains being used as feeds producers require mitigation strategies and/or feeding strategies that are safe and inexpensive enough to be used on a routine basis.

The majority of studies examining the effects of mycotoxins in swine have been performed in young (e.g., weaned) animals with the assumption that the physiological effects of consuming mycotoxin contaminated feed is highest in the young animal. Previous studies have examined the impact of mycotoxins over a relatively short period of time (Dersjant-Li et al., 2003). We have shown that the response to DON is less when introduced in the grower period compared to the finisher period. We have also demonstrated that while there is an initial drop in performance upon exposure to DON-contaminated diets, pig performance recovers after a period of time. It may be possible to reduce the negative effects of DON through earlier introduction of DON-contaminated feed.

Consistent determination of actual DON contamination level in feed stuffs and feed is difficult. We have shown that DON in plasma and urine is highly correlated to actual DON intake. Producers may be able to use these biological samples as a better indicator of actual DON exposure in their pigs.

Mycotoxin contamination results in significant financial losses to the agriculture industry. The reduced performance observed with feeding diets containing > 1 ppm DON results in reduced

margin over feed costs. Producers should, therefore, expect loss in revenue when feeding DON-contaminated diets unless efforts are made to either mitigate the effects of DON in diets or to reduce costs, such as reduced feedstuff or overall diet costs.

Patents/IP generated/commercialized products – None

Technology transfer activities

Scientific publications (e.g., scientific journals); attach copies of any publications as an appendix to this final report

Wellington MO, Bosompem MA, Petracek R, Nagl V, and Columbus DA (2020) 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. J. Anim. Sci. Submitted 09-30-2020, Manuscript ID: JAS-2020-4989.

Bosompem MA, Wellington MO, Rodrigues LA, Sands JM, Petracek R, and Columbus DA (2021) Effect of long-term feeding of graded levels of deoxynivalenol on growth performance of grower-finisher pigs. J. Anim. Sci. In preparation.

Scientific presentations (e.g., posters, talks, seminars, workshops, etc.)

Wellington MO, Bosompem MA, Nagl V, and Columbus DA (2021) Analysis of deoxynivalenol (DON) and DON metabolites in swine biological samples using high performance liquid chromatogrpy-mass spectrometry technique. ASAS Midwest Section Meeting, March 8-10, Omaha, NE. Submitted.

Bosompem MA, Wellington MO, and Columbus DA (2021) Effect of long-term feeding of deoxynivalenol (DON) contaminated diets on performance of grower-finisher pigs. ASAS Midwest Section Meeting, March 8-10, Omaha, NE. Submitted.

Industry-oriented publications (e.g., agribusiness trade press, popular press, etc.)

Engele K, Bosompem MA, and Columbus DA (2020) Pig performance and economics of long-term feeding of DON-contaminated diets. Centred on Swine, Prairie Swine Centre, Saskatoon, SK. In press.

Columbus D (2019) What is the long-term impact of feeding DON to finisher pigs? Pages 1-3 In: Centred on Swine (Volume 26, Number 1), Prairie Swine Centre, Saskatoon, SK.

Columbus D, and Bosompem M (Summer 2019) What is the long-term production and economic impact of feeding deoxynivalenol-contaminated feed to finisher pigs? Page 6-9 In: Canadian Hog Journal, Edmonton, AB.

Engele K, Bosompem MA, and Columbus DA (2020) Pig performance and economics of long-term feeding of DON-contaminated diets. Centred on Swine (Volume 27, Number 3), Prairie Swine Centre, Saskatoon, SK. In press.

Bosompem MA, and Columbus DA (2021) The use of biological analysis to determine DON-intake in grower-finisher pigs. Centred on Swine, Prairie Swine Centre, Saskatoon, SK. In press.

Industry-oriented presentations (e.g., posters, talks, seminars, workshops, etc.)

Columbus DA (2019) Mycotoxins in Western Canadian diets. Red Deer Swine Technology Workshop. Red Deer, AB. Invited.

Bosompem MA, Wellington MO, Rodrigues LA, Sands JM, Petracek R, and Columbus DA (2020) Effect of long-term feeding of graded levels of deoxynivalenol on growth performance of grower-finisher pigs. Advances in Pork Production. 31:237. (Banff Pork Seminar)

Bosompem MA, Wellington MO, Rodrigues LA, Sands JM, Petracek R, and Columbus DA (2019) Long-term feeding of graded levels of deoxynivalenol in grower-finisher pigs. Saskatchewan Pork Industry Symposium, November 14-15, Saskatoon, SK.

Petracek, R, ter Borgh M, Bandaralage K, Krone JEC, and Columbus DA (2019) Long-term feeding of DON-contaminated diets to finisher pigs. Saskatchewan Pork Industry Symposium, November 14-15, Saskatoon, SK.

Petracek R, ter Borgh M, Bandaralage K, Krone JEC, and Columbus DA (2019) Long-term feeding of DON-contaminated diets to finisher pigs. Advances in Pork Production. 30:223. (Banff Pork Seminar)

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Is there a need to conduct follow up research?

Based on the current findings, further research is warranted. This includes:

1. In the current studies, it appears that the negative response to DON intake is largely due to a reduction in feed intake, with little negative effect on immune status or organ function. Further research should attempt to confirm these results, as a number of previous studies have shown an impact of DON intake on organ function, overall health, and nutrient utilization.
2. As the negative effects of DON intake appear largely due to feed intake, future research should determine if strategies that counteract the reduced feed intake can (e.g., adjustment of dietary nutrient content) mitigate this effect.

3. Further research is required in order to further estimate the variability in response to DON intake in pigs and identification of factors (e.g., physiological markers) that could be used to predict pigs' response.
4. Researchers should continue to evaluate potential diet additives that act to mitigate the effects of DON in diets.

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Appendices – Include any additional materials supporting the previous sections, e.g. detailed data tables, maps, graphs, specifications, literature cited.

Table 1 Composition of the diets used in Study 1 (as-fed basis)[1]

Ingredient, %	CONT[2]	DON1[3]	DON3[4]	DON5[5]
Wheat	40.0	33.3	20.0	6.7
DON wheat [6]	0.0	4.9	14.8	24.7
Wheat screenings[7]	0.0	1.7	5.2	8.6
Barley	44.0	44.0	44.0	44.0
Canola oil	3.5	3.5	3.5	3.5
Soybean meal	10.0	10.0	10.0	10.0
L-lysine-HCl	0.297	0.297	0.297	0.297
DL-methionine	0.070	0.070	0.070	0.070
L-threonine	0.100	0.100	0.100	0.100
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[8]	0.2	0.2	0.2	0.2
Calculated Nutrient Content				
ME, kcal/kg	3282	3282	3282	3282
NE, kcal/kg	2502	2502	2502	2502
Dry Matter, %	86.49	86.51	86.54	86.58
Crude Protein, %	15.9	15.9	15.9	16.0
Lysine, % SID[9]	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 diets				
Dry Matter, %	88.9	88.2	88.3	88.8
Crude Protein, %	14.6	14.2	13.5	14.8
DON[10], ppm[11]	0.11	1.34	3.58	5.72
Nitrogen-balance diets				
Dry Matter, %	88.5	88.1	88.9	88.7
Crude Protein, %	14.5	14.6	14.1	14.7
DON, ppm	1.56	1.32	3.09	4.94

[1]Nutrient content of diets based on the nutrient content of feed ingredients according to NRC (2012).
[2]CONT, 0 ppm DON Control diet
[3]DON1, 1 ppm DON diet
[4]DON3, 3 ppm DON diet
[5]DON5, 5 ppm DON diet
[6]DON wheat contained 6.9 ppm DON (Central Testing Laboratory, Winnipeg Manitoba).
[7]Wheat screenings contained 32.8 ppm DON (Central Testing Laboratory, Winnipeg Manitoba).
[8]Supplied per kg of complete diet; vitamin A, 8000 IU; vitamin D, 1500 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, 1mg.
[9]SID, standardized ileal digestible
[10]DON, deoxynivalenol content analyzed by BIOMIN
[11]ppm, parts per million

Table 2 Analyzed mycotoxin content (ppm) of diets used in Study 1 (as-fed basis)[1]

	Finisher Diet							
	Growth Performance Diets				Nitrogen-Balance Diets			
Mycotoxin, ppm	CONT[2]	DON1[3]	DON3[4]	DON5[5]	CONT	DON1	DON3	DON5
Deoxynivalenol	0.11	1.34	3.59	5.72	1.56	1.32	3.09	4.94
3-acetyldeoxynivalenol	ND[6]	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]Mycotoxin contents analyzed in diet samples by BIOMIN.
[2]CONT, 0 ppm DON Control diet
[3]DON1, 1 ppm DON diet
[4]DON3, 3 ppm DON diet
[5]DON5, 5 ppm DON diet
[6] ND, Not detected or below the limit of detection.

Table 3 Composition of the diets used in Study 2 (as fed basis)[1]

	Grower Diets				Finisher Diets			
Ingredient, %	CONT[2]	DON1[3]	DON3[4]	DON5[5]	CONT	DON1	DON3	DON5
Wheat	40.0	33.3	20.0	6.7	40.0	33.3	20.0	6.7
DON wheat[6]	0.0	4.9	14.8	24.7	0.0	4.9	14.8	24.7
Wheat screenings[7]	0.0	1.7	5.2	8.6	0.0	1.7	5.2	8.6
Barley	39.2	39.2	39.2	39.2	44.0	44.0	44.0	44.0
Canola oil	3.7	3.7	3.7	3.7	3.5	3.5	3.5	3.5
Soybean meal	14.0	14.0	14.0	14.0	10.0	10.0	10.0	10.0
L-Lysine- HCl	0.463	0.463	0.463	0.463	0.297	0.297	0.297	0.297
DL-Methionine	0.075	0.075	0.075	0.075	0.070	0.070	0.070	0.070
L-Threonine	0.144	0.144	0.144	0.144	0.100	0.100	0.100	0.100
Limestone	1.0	1.0	1.0	1.0	0.8	0.8	0.8	0.8
Dicalcium phosphate	0.8	0.8	0.8	0.8	0.5	0.5	0.5	0.5
Salt	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin/Mineral Premix[8]	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Calculated nutrient content								
ME, kcal/kg	3291	3291	3291	3291	3282	3282	3282	3282
NE, kcal/kg	2495	2495	2495	2495	2502	2502	2502	2502
Dry Matter, %	86.4	86.4	86.4	86.5	86.5	86.5	86.5	86.6
Crude Protein, %	17.5	17.5	17.5	17.5	15.9	15.9	15.9	16.0
Lysine, % SID[9]	0.98	0.98	0.98	0.98	0.76	0.76	0.76	0.76
Calcium, %	0.64	0.64	0.64	0.64	0.50	0.50	0.50	0.50
Phosphorus, %	0.53	0.53	0.53	0.53	0.48	0.48	0.48	0.48
Analyzed nutrient content								
Growth performance diets								
Dry Matter, %	89.2	88.4	82.9	89.4	90.5	90.2	86.7	90.2
Crude Protein, %	15.4	17.4	16.4	16.4	16.7	16.3	17.2	16.6
DON[10], ppm[11]	0.28	0.73	3.40	4.36	0.20	1.02	3.28	4.14
Nitrogen-balance diets								
Dry Matter, %	88.9	89.4	88.8	88.6	90.1	90.1	90.5	90.3
Crude Protein, %	17.8	18.8	18.2	17.3	16.0	15.7	16.5	16.5
DON, ppm	0.04	0.57	2.72	4.10	1.04	1.35	3.28	5.43

[1]Nutrient content of diets based on the nutrient content of feed ingredients according to NRC (2012).
[2]CONT, 0 ppm DON Control diet
[3]DON1, 1 ppm DON diet
[4]DON3, 3 ppm DON diet
[5]DON5, 5 ppm DON diet
[6]DON wheat containing 6.9 ppm DON (Central Testing Laboratory, Winnipeg Manitoba)
[7]Wheat screenings containing 32.8 ppm DON (Central Testing Laboratory, Winnipeg Manitoba)
[8]Supplied per kg of complete diet; vitamin A, 8000 IU; vitamin D, 1500 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, 1mg
[9]SID, standardized ileal digestible
[10]DON, deoxynivalenol content analyzed by BIOMIN
[11]ppm, parts per million

Table 4 Analyzed mycotoxin content of grower diets used in Study 2 (as-fed basis)[1]

	Growth Performance Diets				Nitrogen-Balance Diets			
Mycotoxin, ppm	CONT[2]	DON1[3]	DON3[4]	DON5[5]	CONT	DON1	DON3	DON5
Deoxynivalenol	0.28	0.73	3.4	4.36	0.043	0.57	2.72	4.10
3-acetyl-deoxynivalenol	ND[6]	ND	ND	ND	ND	ND	ND	ND
15-acetyl-deoxynivalenol	ND	ND	ND	ND	ND	ND	ND	ND
HT-2 toxin	ND	ND	ND	ND	ND	ND	ND	ND
Nivalenol	0.39	0.30	0.17	0.33	0.06	0.20	0.08	0.09
Ochratoxin A	0.01	0.014	0.03	0.02	ND	0.01	0.06	0.10
Zearalenone	ND	ND	0.011	0.007	ND	ND	0.005	0.010
Total Ergot alkaloids	0.53	0.73	1.13	0.69	1.01	0.63	0.98	1.78

[1]Mycotoxin content analyzed in diet samples by BIOMIN
[2]CONT, 0 ppm DON Control diet
[3]DON1, 1 ppm DON diet
[4]DON3, 3 ppm DON diet
[5]DON5, 5 ppm DON diet
[6] ND, Not detected or below the limit of detection

Table 5 Analyzed mycotoxin content of finisher diets used in Study 2 (as-fed basis)[1]

	Growth Performance Diets				Nitrogen-Balance Diets			
Mycotoxin, ppm	CONT[2]	DON1[3]	DON3[4]	DON5[5]	CONT	DON1	DON3	DON5
Deoxynivalenol	0.20	1.02	3.28	4.13	1.04	1.35	3.22	5.43
3-acetyl-deoxynivalenol	ND[2]	ND	0.03	0.04	ND	ND	ND	0.02
15-acetyl-deoxynivalenol	ND	ND	ND	ND	ND	ND	ND	ND
HT-2 toxin	ND	ND	ND	ND	ND	ND	ND	0.04
Nivalenol	0.53	0.63	0.55	0.16	0.10	0.09	0.12	0.09
Ochratoxin A	ND	0.02	0.02	0.07	0.01	0.03	0.09	0.12
Zearalenone	0.001	0.004	0.004	0.007	0.006	0.004	0.008	0.014
Total Ergot alkaloids	0.32	0.63	0.61	0.36	0.18	0.20	0.28	0.77

[1]Mycotoxin content analyzed in diet samples by BIOMIN
[2]CONT, 0 ppm DON Control diet
[3]DON1, 1 ppm DON diet
[4]DON3, 3 ppm DON diet
[5]DON5, 5 ppm DON diet
[6] ND, Not detected or below the limit of detection

Table 6 Growth performance of finisher pigs fed graded levels of deoxynivalenol[1]

	Dietary Treatment					
Item	CONT[2]	DON1[3]	DON3[4]	DON5[5]	SEM[6]	*P-value*
Body weight, kg						
Initial	76.9	77.0	76.3	76.0	1.18	NS[7]
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
Final	126.7[a]	126.9[a]	123.6[b]	118.5[c]	0.80	<0.001
Average daily gain, kg/d						
Day 0-7	1.27[a]	1.18[a]	0.93[b]	0.60[c]	0.05	<0.001
Day 8-14	1.40[ab]	1.49[a]	1.33[b]	1.13[c]	0.04	<0.001
Day 15-21	1.17[ab]	1.21[a]	1.06[b]	1.01[c]	0.04	0.004
Day 22-28	1.24[a]	1.17[ab]	1.15[ab]	1.04[b]	0.04	0.033
Day 29-35	1.08	1.12	1.01	1.06	0.04	NS
Day 35-42	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
Average daily feed intake, kg/d						
Day 0-7	2.59[a]	2.59[a]	2.22[b]	1.70[c]	0.06	<0.001
Day 8-14	2.98[a]	3.07[a]	2.89[a]	2.55[b]	0.07	<0.001
Day 15-21	3.03[a]	3.03[a]	2.88[a]	2.56[b]	0.05	<0.001
Day 22-28	3.25[a]	3.19[a]	3.13[a]	2.85[b]	0.05	<0.001
Day 29-35	3.22	3.20	3.19	3.04	0.06	NS
Day 35-42	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
Gain: Feed, kg/kg						
Day 0-7	0.49[a]	0.46[a]	0.41[a]	0.34[b]	0.02	<0.001
Day 8-14	0.47	0.49	0.47	0.44	0.01	NS
Day 15-21	0.38	0.40	0.37	0.40	0.01	NS
Day 22-28	0.38	0.36	0.37	0.36	0.02	NS
Day 29-35	0.33	0.35	0.32	0.35	0.01	NS
Day 35-42	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 with n=10 pens/treatment.
[2]CONT, 0 ppm DON Control diet
[3]DON1, 1 ppm DON diet
[4]DON3, 3 ppm DON diet
[5]DON5, 5 ppm DON diet
[6] SEM, Standard error of the means
[7]NS, Not significant
[a, b, c] Means without a common superscript are significantly different ($P < 0.05$)

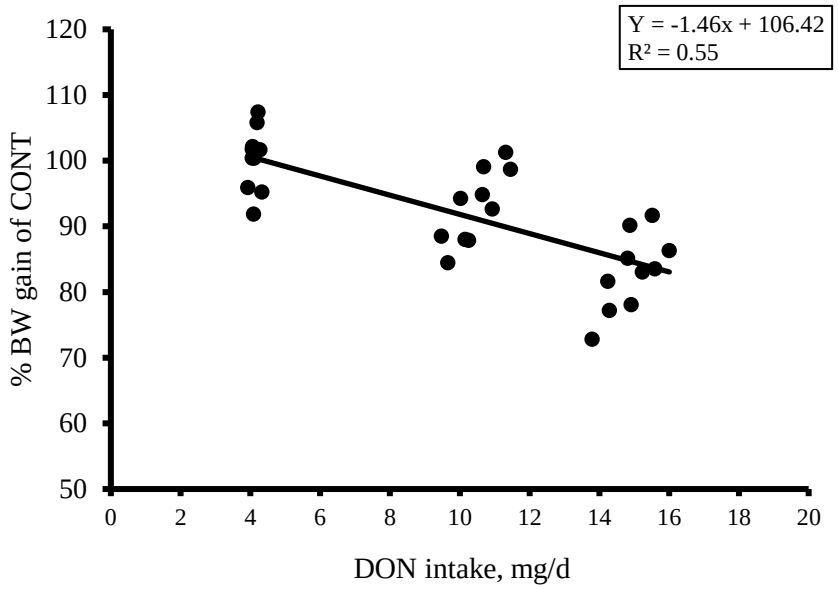

Figure 1 Effect of deoxynivalenol (DON) intake on body weight change (%) relative to control fed pigs

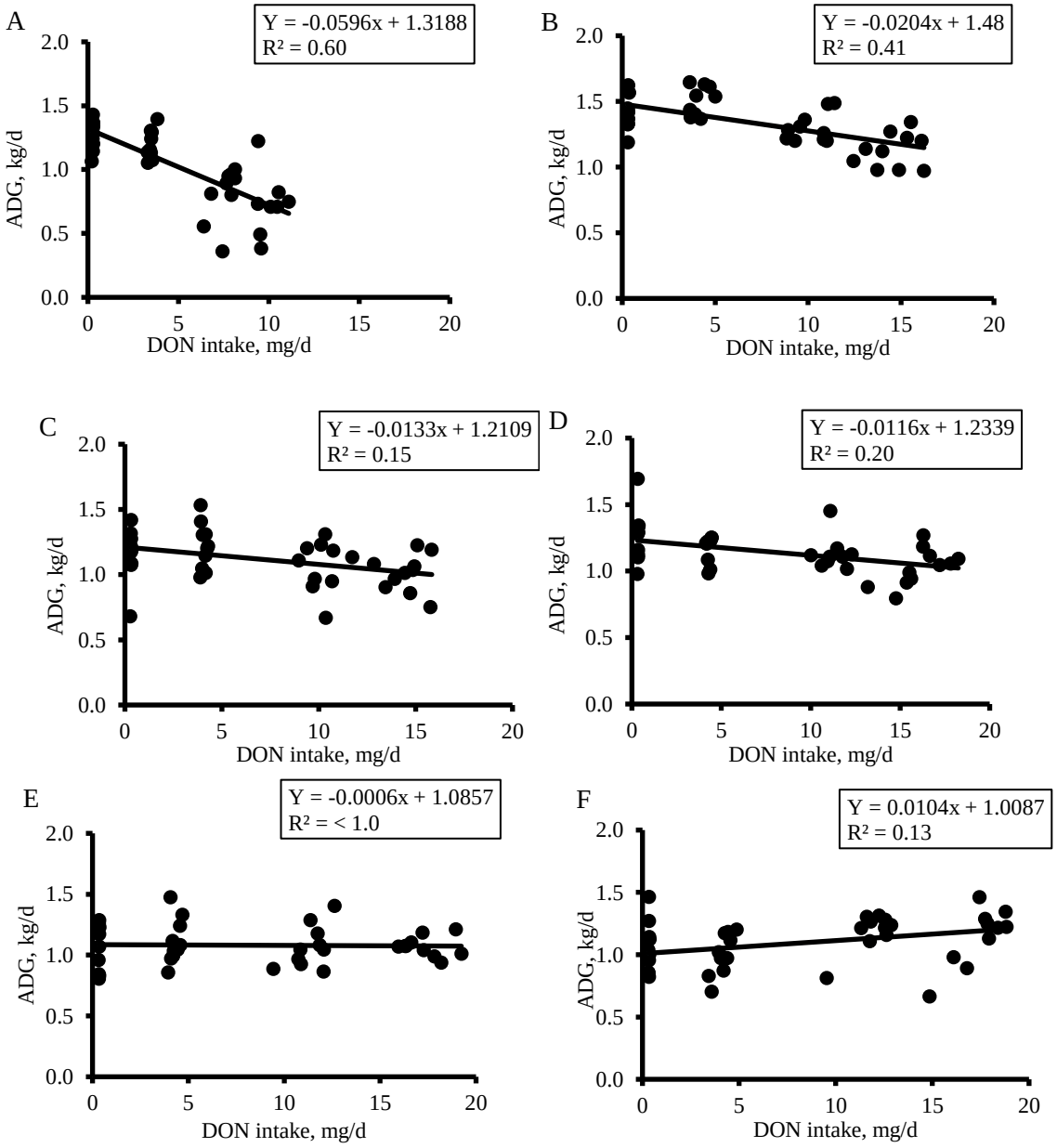

Figure 2. Relationship between average daily gain (ADG) and deoxynivalenol (DON) intake in finisher pigs from d 0-7 (A), 8-14 (B), 15-21 (C), 22-28 (D), 29-35 (E), and 36-42 (F).

Table 7 Nitrogen-balance in finisher pigs fed diets containing graded levels of deoxynivalenol (DON) for 42 d[1]

	Dietary Treatment					
Items	CONT[2]	DON1[3]	DON3[4]	DON5[5]	SEM[6]	*P-value*
Dry matter intake, g/d	2581	2574	2506	2393	48.5	NS[7]
N[8] intake, g/d	67.84[a]	68.45[a]	63.48[b]	63.32[b]	1.27	0.024
ATTD[9] of N, %	79.27[a]	87.12[b]	85.41[b]	85.44[b]	1.56	0.009
Fecal N output, g/d	14.10[a]	8.82[b]	9.28[b]	9.26[b]	1.05	0.005
Urinary N output, g/d	25.53[a]	15.19[b]	28.38[a]	22.69[ab]	3.59	0.015
N retained, g/d	28.30[b]	44.40[a]	25.85[b]	31.34[b]	3.17	<0.001
Protein deposition[10], g/d	176.89[b]	277.78[a]	161.56[b]	195.99[b]	19.81	<0.001

[1]Values are least squares means with n=10 pens/treatment
[2]CONT, 0 ppm DON Control diet
[3]DON1, 1 ppm DON diet
[4]DON3, 3 ppm DON diet
[5]DON5, 5 ppm DON diet
[6] SEM, Standard error of the mean
[7]NS, Not significant
[8]N, nitrogen
[9]ATTD, Apparent total tract digestibility
[10]Protein deposition calculated as N retained × 6.25
[a, b]Means without a common superscript are significantly different ($P < 0.05$)

Table 8 Plasma indicators of liver and kidney health and function in finisher pigs fed diets containing graded levels of deoxynivalenol[1]

		Dietary Treatment					*P*-value[7]
Item	Day	CONT[2]	DON1[3]	DON3[4]	DON5[5]	SEM[6]	Day
Sodium, mM	-1	144.43	144.08	144.50	144.48	0.700	<.001
	13	146.23	145.88	146.10	145.78	0.700	
	43	145.03	146.28	145.99	145.18	0.700	
Potassium, mM	-1	5.45	5.42	5.35	5.60	0.18	0.074
	13	5.32	5.05	5.01	5.44	0.18	
	43	5.21	5.32	5.28	5.32	0.18	
Chloride, mM	-1	97.36	97.89	97.30	97.38	0.70	<.0001
	13	98.95	99.69	99.10	99.58	0.70	
	43	98.75	99.89	99.56	98.88	0.70	
Bicarbonate, mM	-1	26.79	26.92	28.40	27.30	0.71	<.0001
	13	31.10	29.32	29.30	28.80	0.71	
	43	33.40	32.32	33.14	32.20	0.71	
Anion Gap, mM	-1	25.87	24.37	24.20	25.35	1.03	<.0001
	13	21.57	21.87	22.70	22.85	1.03	
	43	18.07	19.37	18.44	19.45	1.03	
Calcium, mM	-1	2.84	2.78	2.82	2.86	0.05	0.012
	13	2.82	2.74	2.74	2.80	0.05	
	43	2.75	2.95	2.92	2.88	0.05	
Phosphorus, mM	-1	2.99	2.97	3.03	3.00	0.90	<.0001
	13	3.05	3.06	3.05	3.14	0.90	
	43	2.52	2.52	2.54	2.58	0.90	
Magnesium, mM	-1	0.94	0.90	0.93	0.94	0.020	<.0001
	13	0.90	0.90	0.89	0.90	0.020	
	43	0.84	0.84	0.84	0.85	0.020	
Urea, mM	-1	4.03	3.96	3.64	3.70	0.300	<.0001
	13	4.74	4.40	4.09	4.12	0.300	
	43	4.91	4.81	5.00	4.67	0.300	
Creatinine, mM	-1	112.39	113.62	111.90	114.52	4.600	<.0001
	13	118.49	118.92	116.80	122.32	4.600	
	43	136.59	139.42	134.86	140.62	4.600	
Glucose, mM	-1	5.53	5.68	5.83	5.86	0.180	0.048
	13	5.54	5.30	5.45	5.44	0.180	
	43	5.34	5.69	5.65	5.21	0.180	
Total Bilirubin, mM	-1	1.10	1.10	1.09	1.17	0.095	<.0001
	13	0.72	0.84	0.86	0.73	0.095	
	43	0.39	0.50	0.43	0.44	0.095	
Direct Bilirubin, mM	-1	0.44	0.38	0.42	0.37	0.042	<.0001

	13	0.27	0.27	0.25	0.25	0.042	
	43	0.25	0.33	0.25	0.28	0.042	
Indirect Bilirubin, mM	-1	0.65	0.73	0.67	0.79	0.096	<.0001
	13	0.44	0.58	0.61	0.47	0.096	
	43	0.13	0.18	0.18	0.15	0.096	
GGT[8], mM	-1	39.68	36.83	41.60	38.94	3.4	0.173
	13	36.88	35.53	37.20	42.34	3.4	
	43	34.48	34.53	37.88	38.94	3.4	
GLDH[9], mM	-1	1.7	1.6	1.4	1.3	0.38	0.004
	13	1.9	1.7	2.0	2.8	0.38	
	43	1.2	1.2	1.2	1.2	0.38	
AST[10], mM	-1	30.27	28.17	23.00	25.9	3.1	<.0001
	13	25.57	29.27	25.50	33.5	3.1	
	43	19.97	18.87	17.42	19.5	3.1	
CK[11], mM	-1	3133.6	1558.5	1441.2	2035.13	698.4	0.073
	13	2545.0	3315.3	2207.6	3615.43	698.4	
	43	2578.7	1885.0	1116.1	2390.33	698.4	
Total Protein, mM	-1	68.01	63.85	63.30	64.13	1.7	0.003
	13	65.41	63.65	60.70	63.63	1.7	
	43	63.41	61.85	61.71	63.33	1.7	
Albumin, mM	-1	43.14	41.98	42.40	43.70	0.9	0.005
	13	41.44	41.48	41.00	42.30	0.9	
	43	42.04	42.98	43.31	43.50	0.9	
Globulin, mM	-1	24.88	21.91	20.90	20.43	1.9	0.001
	13	23.98	22.21	19.70	21.33	1.9	
	43	21.38	18.91	18.33	19.83	1.9	
Albumin:Globulin, mM	-1	1.93	2.04	2.14	2.18	0.18	0.002
	13	1.89	1.97	2.16	2.04	0.18	
	43	2.1	2.37	2.47	2.28	0.18	

[1]Values are least squares means with n=10 pens/treatment
[2]CONT, 0 ppm Control diet
[3]DON1, 1 ppm DON diet
[4]DON3, 3 ppm DON diet
[5]DON5, 5 ppm DON diet
[6] SEM, Standard error of the means
[7]There were no significant effects of diet or diet × day interaction
[8]GGT, Gamma-glutamyl transferase
[9]GLDH, Glutamate dehydrogenase
[10]AST, Aspartate aminotransferase
[11]CK, Creatine kinase

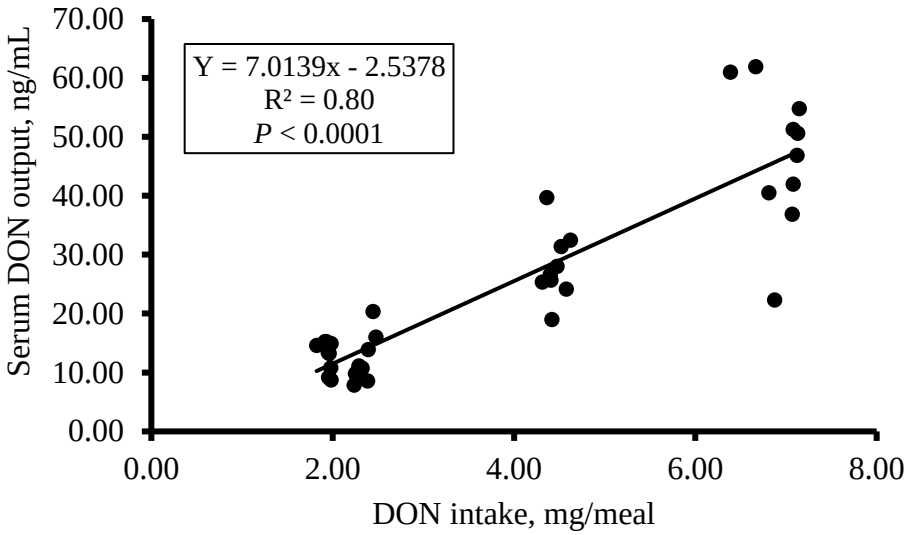

Figure 3 Regression analysis of the relationship between deoxynivalenol (DON) intake and serum DON concentration (ng/mL). The blood samples were taken during the nitrogen balance period (3-4 hours after a single meal) of the experiment and analyzed for DON concentration (n=10 pigs/treatment). Data is expressed as the DON intake after a single meal and the serum DON concentration (ng/mL) after that meal. The coefficient of determnation (R^2) of the regression curve is 0.80 at $P < 0.0001$.

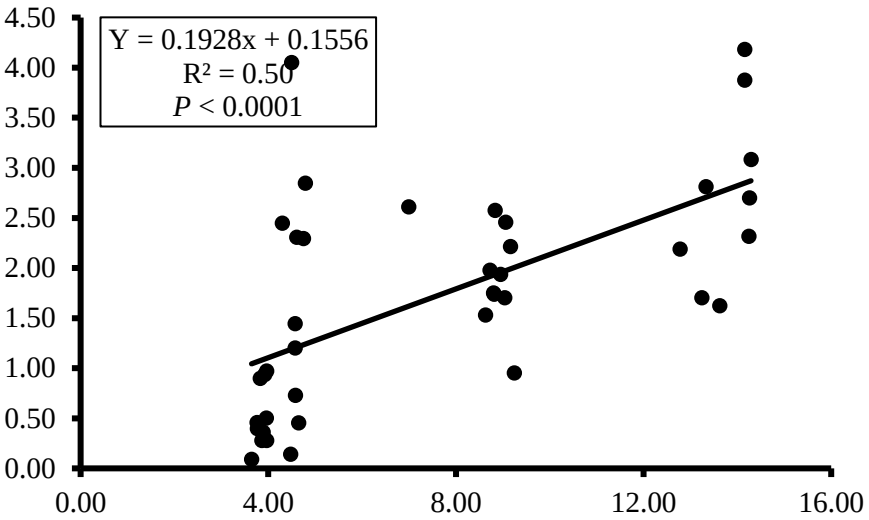

Figure 4 Regression analysis of the relationship between deoxynivalenol (DON) intake and DON output in urine (n=10 pigs/treatment). The urine samples were collected during the nitrogen balance period of the experiment over 24-h and analysed for DON content. Data is expressed as the DON intake per day (mg/d) and the urine DON output (mg/d) and the coefficient of determination (R^2) of the regression curve is 0.50 at $P < 0.0001$.

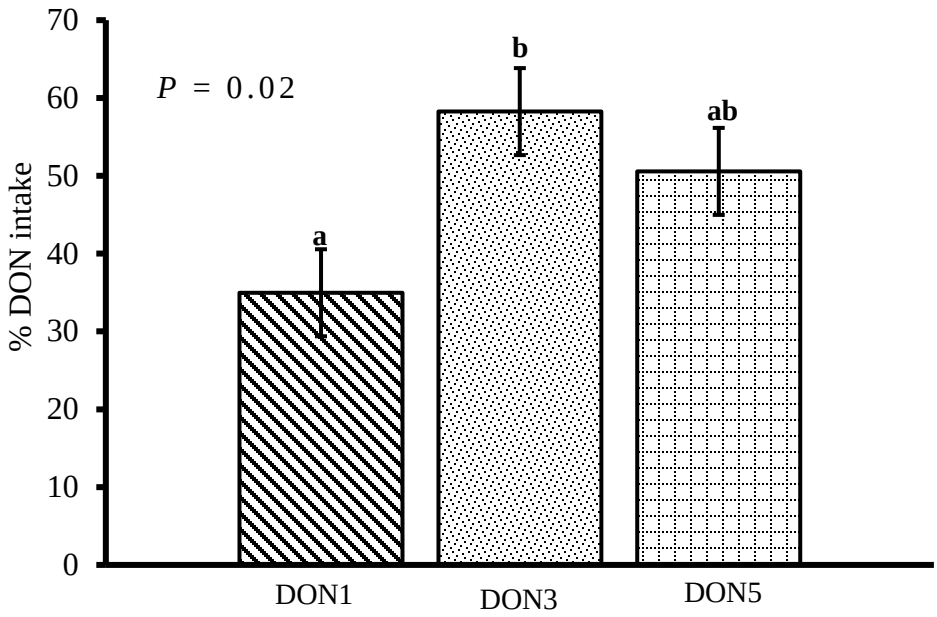

Figure 5 Deoxynivalenol (DON) recovery in urine of finisher pigs fed graded levels of deoxynivalenol

Table 9 Growth performance of grower-finisher pigs fed diets with graded levels of deoxynivalenol (DON)[1]

	CONT[2]	DON1[3]	DON3[4]	DON5[5]	SEM[6]	*P*-value
Body weight, kg						
Day 0	36.0	35.6	35.7	36.4	0.34	NS[7]
Day 7	42.5	41.6	40.7	41.7	0.44	NS
Day 14	50.1^{a}	49.8^{a}	47.8^{b}	49.2^{ab}	0.49	0.01
Day 21	58.0^{a}	57.7^{a}	55.7^{ab}	56.7^{b}	0.60	0.04
Day 28	68.1	67.6	65.4	65.7	0.84	NS
Day 35	75.9^{a}	74.5^{ab}	72.7^{b}	72.7^{b}	0.86	0.03
Day 42	85.2^{a}	83.7^{ab}	81.9^{b}	81.6^{b}	0.91	0.03
Day 49	94.7^{a}	93.1^{ab}	90.9^{bc}	89.8^{c}	0.96	0.005
Day 56	102.7^{a}	100.9^{ab}	98.3^{bc}	97.7^{c}	1.00	0.004
Day 63	110.6^{a}	108.6^{ab}	106.3^{bc}	105.0^{c}	0.91	<0.001
Day 70	118.4^{a}	116.2^{ab}	114.6^{bc}	112.9^{c}	0.91	0.001
Day 77	124.9^{a}	123.0^{ab}	121.0^{bc}	120.0^{c}	0.91	0.002
Average daily gain, kg/d						
Day 0-7	0.92^{a}	0.86^{a}	0.72^{b}	0.76^{b}	0.04	0.001
Day 7-14	1.09	1.17	1.02	1.08	0.04	NS
Day 14-21	1.14	1.13	1.12	1.06	0.03	NS
Day 21-28	1.44	1.42	1.38	1.30	0.06	NS
Day 28-35	1.15	1.12	1.14	1.11	0.04	NS
Day 35-42	1.32	1.32	1.32	1.27	0.04	NS
Day 42-49	1.37^{a}	1.34^{a}	1.28^{a}	1.17^{b}	0.04	<0.01
Day 49-56	1.13	1.11	1.05	1.13	0.06	NS
Day 56-63	1.13	1.11	1.15	1.04	0.05	NS
Day 63-70	1.13	1.08	1.18	1.13	0.04	NS
Day 70-77	0.93	1.03	0.91	1.00	0.06	NS
Day 0-42	1.17^{a}	1.15^{ab}	1.10^{bc}	1.08^{c}	0.02	<0.01
Day 42-77	1.14	1.13	1.11	1.10	0.01	NS
Overall (d 0-77)	1.15^{a}	1.14^{a}	1.11^{b}	1.09^{b}	0.01	<0.001
Average daily feed intake, kg/d						
Day 0-7	1.59^{a}	1.55^{a}	1.40^{b}	1.42^{b}	0.04	0.002
Day 7-14	1.90	1.98	1.78	1.81	0.07	NS
Day 14-21	2.03	1.95	1.93	1.95	0.06	NS
Day 21-28	2.37^{b}	2.58^{a}	2.49^{a}	2.49^{a}	0.03	0.002
Day 28-35	2.79	2.77	2.67	2.60	0.05	NS
Day 35-42	3.17	3.07	3.09	2.95	0.08	NS
Day 42-49	3.17^{a}	2.95^{a}	2.96^{a}	2.71^{b}	0.08	0.004
Day 49-56	3.19^{a}	3.06^{ab}	2.99^{b}	2.94^{b}	0.06	0.01
Day 56-63	3.02	2.80	2.89	2.88	0.09	NS
Day 63-70	3.19	3.05	3.06	2.97	0.05	NS
Day 70-77	3.05	2.99	2.94	2.91	0.07	NS
Day 0-42	2.29	2.27	2.20	2.18	0.03	NS
Day 42-77	3.12^{a}	2.97^{b}	2.96^{b}	2.88^{b}	0.05	<0.001

Overall (d 0-77)	2.62[a]	2.55[ab]	2.47[b]	2.47[b]	0.03	0.003
Gain:Feed, kg/kg						
Day 0-7	0.58	0.56	0.52	0.53	0.02	NS
Day 7-14	0.58	0.60	0.58	0.60	0.02	NS
Day 14-21	0.57	0.58	0.59	0.55	0.02	NS
Day 21-28	0.60	0.55	0.56	0.52	0.02	NS
Day 28-35	0.41	0.41	0.43	0.43	0.01	NS
Day 35-42	0.42	0.43	0.43	0.43	0.01	NS
Day 42-49	0.43	0.46	0.43	0.44	0.02	NS
Day 49-56	0.35	0.36	0.35	0.38	0.02	NS
Day 56-63	0.37	0.41	0.40	0.36	0.02	NS
Day 63-70	0.35	0.36	0.39	0.38	0.01	NS
Day 70-77	0.31	0.35	0.31	0.35	0.02	NS
Day 0-42	0.51	0.50	0.50	0.49	0.01	NS
Day 42-77	0.35	0.36	0.39	0.38	0.01	NS
Overall (d 0-77)	0.44	0.45	0.45	0.44	0.004	NS

[1]Values are least squares means with n=10 pens/treatment
[2]CONT, 0 ppm DON Control diet
[3]DON1, 1 ppm DON diet
[4]DON3, 3 ppm DON diet
[5]DON5, 5 ppm DON diet
[6] SEM, Standard error of the mean
[7]NS, Not significant
[a, b, c] Means without a common superscript are significantly different ($P < 0.05$)

Table 10 Carcass characteristics for pigs fed diets containing graded levels of DON for 77 d[1]

Item	CONT[2]	DON1[3]	DON3[4]	DON5[5]	SEM[6]	*P-value*
pigs/treatment	31	37	37	39		
Live weight, kg	122.65	121.48	119.42	116.68	1.62	NS[7]
Slaughter weight, kg	99.49	97.08	96.22	94.85	1.32	NS
Backfat thickness, mm	16.54	15.68	15.93	16.20	0.62	NS
Loin depth, mm	67.95	67.39	65.15	65.33	0.99	NS
Yield, %	61.91	62.36	62.07	61.97	0.30	NS
Dressing, %	81.40	80.00	80.56	81.29	1.07	NS

[1]Values are least squares means with n=10 pens/treatment
[2]CONT, 0 ppm DON Control diet
[3]DON1, 1 ppm DON diet
[4]DON3, 3 ppm DON diet
[5]DON5, 5 ppm DON diet
[6] SEM, Standard error of the mean

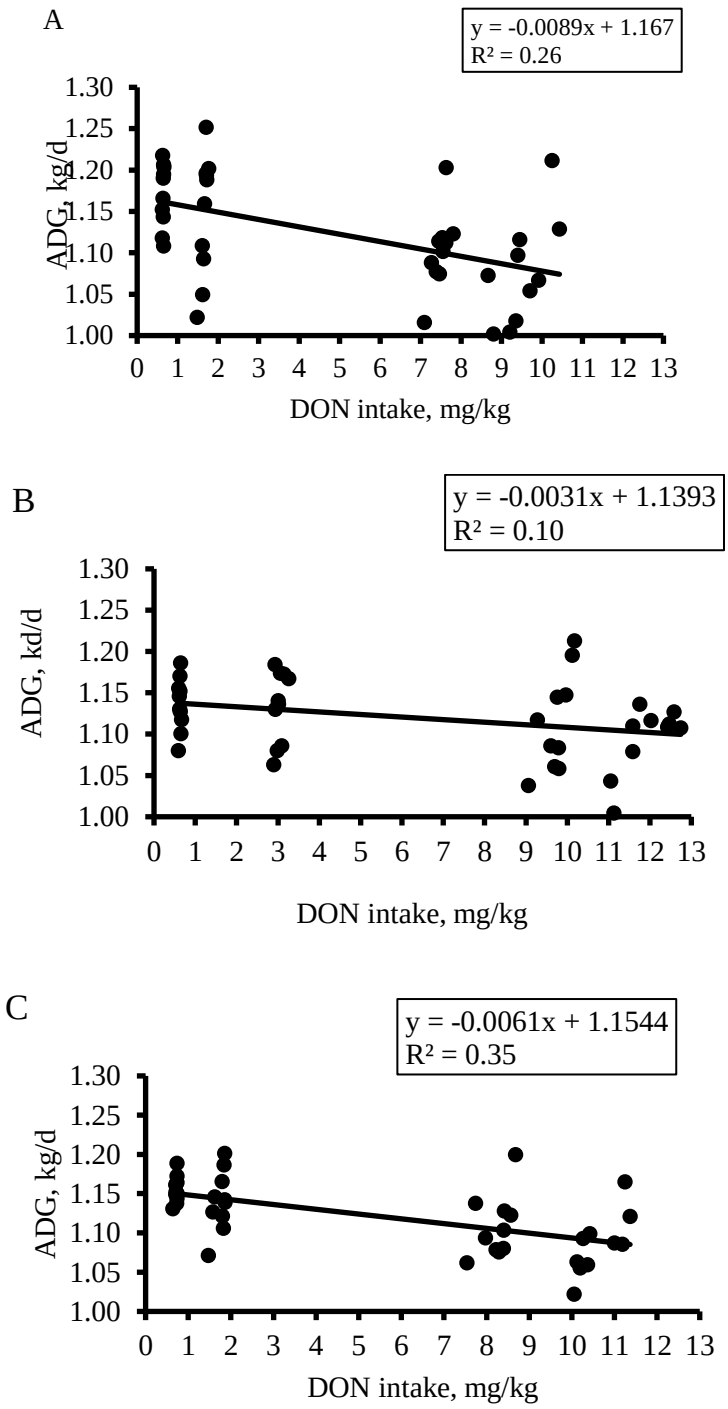

Figure 7. Relationship between deoxynivalenol (DON) intake and average daily gain (ADG) in pigs fed graded levels of DON from d 0-42 (A), d 42-77 (B), and d0-77 (C)

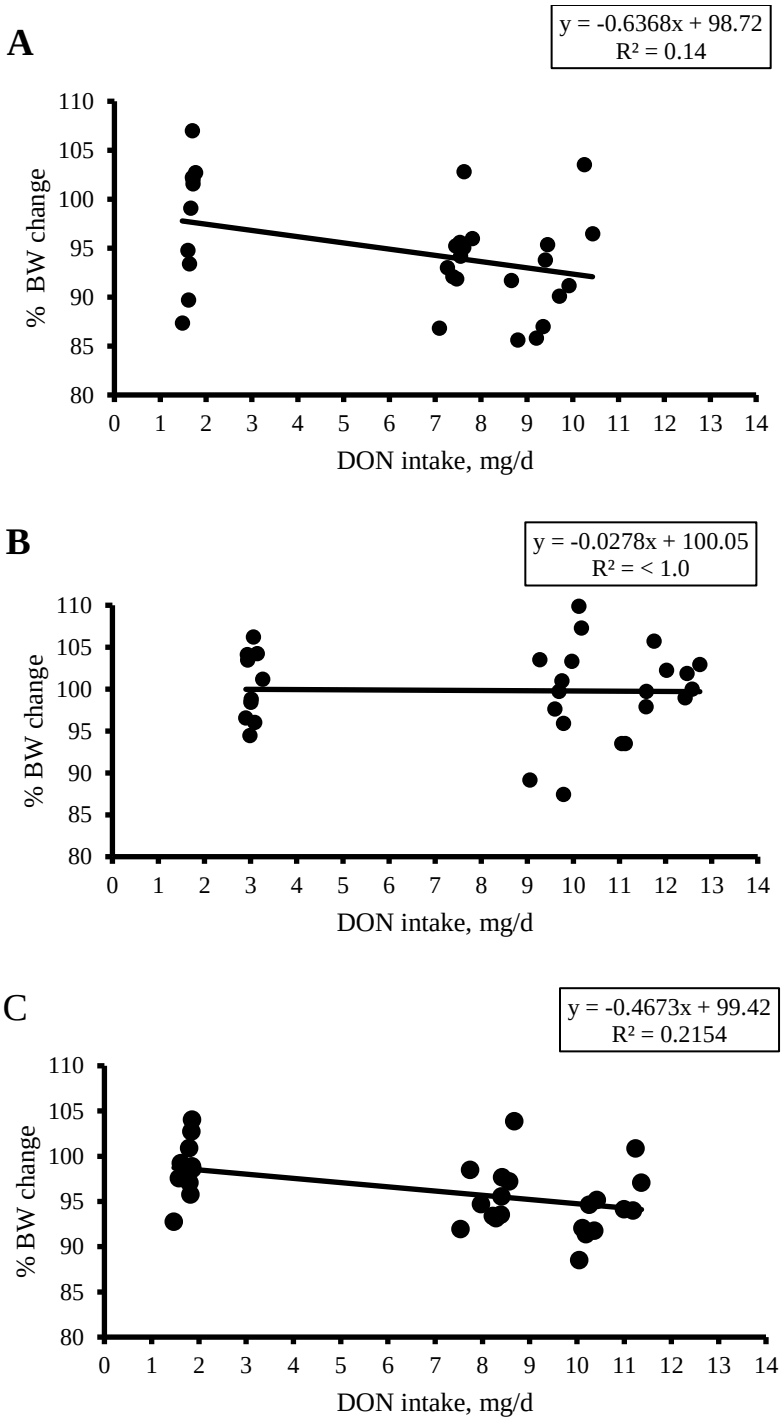

Figure 8 Relationship between deoxynivalenol (DON) intake and % body weight (BW) gain relative to pigs fed a control diet of pigs fed graded levels of DON from d 0-42 (A), d 42-77 (B), and d0-77 (C).

Table 11 Nitrogen-balance of grower-finisher pigs fed graded levels of deoxynivalenol (DON)[1]

	Dietary Treatment					
Items	CONT[2]	DON1[3]	DON3[4]	DON5[5]	SEM[6]	*P-value*
Grower pigs (d 0 - 42)						
Dry matter intake, g/d	2020	2060	2009	2034	50.82	NS[7]
N Intake, g/d	68.21[a]	66.95[a]	62.61[b]	58.58[b]	1.57	<0.0001
ATTD[8] of N[9], %	84.94[a]	79.95[b]	83.70[a]	82.68[ab]	0.92	<0.001
Urine N output, g/d	21.59	21.62	21.48	20.65	1.50	NS
Fecal N output, g/d	10.27[b]	13.36[a]	10.21[b]	10.12[b]	0.47	<0.0001
N retention, g/d	36.35[a]	31.99[ab]	30.91[b]	27.80[b]	1.75	<0.001
Protein deposition[10], g/d	227.2[a]	199.90[ab]	193.16[b]	173.73[b]	10.94	<0.001
Finisher pigs (d 42-77)						
Dry matter intake g/d	2804.[a]	2729.[ab]	2578.[b]	2751.[ab]	48.59	0.02
N Intake, g/d	77.95[b]	83.66[a]	75.16[b]	80.36[ab]	1.41	0.001
ATTD of N, %	91.18[a]	88.14[b]	87.56[b]	87.75[b]	0.52	<0.0001
Urine N output, g/d	35.65	32.46	31.52	34.49	1.86	NS
Fecal N output, g/d	6.88[b]	9.91[a]	9.40[a]	9.84[a]	0.46	<0.0001
N retention, g/d	35.42	41.29	34.24	36.04	2.17	NS
Protein deposition, g/d	221.38	258.07	213.97	225.25	13.50	NS

[1]Values are least squares means with n=10 pens/treatment
[2]CONT, 0 ppm DON Control diet
[3]DON1, 1 ppm DON diet
[4]DON3, 3 ppm DON diet
[5]DON5, 5 ppm DON diet
[6] SEM, Standard error of the mean
[7]NS, Not significant
[8]ATTD, Apparent total tract digestibility
[9]N, nitrogen
[10]Protein deposition calculated as N retention × 6.25
[a, b] Means without a common superscript are significantly different ($P < 0.05$)

Table 12 Plasma indicators of liver and kidney health and function in grower pigs (Study 2) fed diets containing graded levels of deoxynivalenol[1]

		Dietary Treatment					*P*-value[7]
Item	Day	CONT[2]	DON1[3]	DON3[4]	DON5[5]	SEM[6]	Day
Sodium, mM	0	144.58	144.53	142.90	142.81	0.77	<0.001
	14	145.88	145.93	145.40	142.91	0.77	
	42	145.46	146.05	145.20	147.47	0.77	
Potassium, mM	0	6.52	6.33	6.29	6.29	0.44	0.008
	14	5.96	6.21	5.46	5.46	0.44	
	42	5.60	5.24	5.60	5.60	0.44	
Chloride, mM	0	96.63	96.41	96.00	95.29	0.63	NS
	14	95.83	97.21	96.60	96.19	0.63	
	42	96.33	96.51	96.30	97.33	0.63	
Bicarbonate, mM	0	19.41	21.02	20.60	20.78	0.87	<0.001
	14	18.61	21.12	21.40	20.78	0.87	
	42	26.33	25.65	25.70	27.74	0.87	
Anion Gap, mM	0	35.36	33.41	32.60	33.58	1.23	<0.001
	14	37.46	33.81	32.80	32.08	1.23	
	42	29.06	29.88	28.90	28.32	1.23	
Calcium, mM	0	2.88	2.85	2.78	2.82	0.05	0.049
	14	2.95	3.04	2.88	2.78	0.05	
	42	2.87	2.83	2.83	2.88	0.05	
Phosphorus, mM	0	3.32	3.25	3.19	3.26	0.08	0.001
	14	3.38	3.19	3.10	3.13	0.08	
	42	3.12	2.98	3.11	3.18	0.08	
Magnesium, mM	0	0.84	0.83	0.77	0.78	0.03	0.04
	14	0.87	0.85	0.81	0.80	0.03	
	42	0.86	0.86	0.86	0.87	0.03	
Urea, mM	0	4.48	4.61	3.84	3.79	0.26	<0.001
	14	3.72	3.84	3.69	3.72	0.26	
	42	4.99	5.43	4.72	4.80	0.26	
Creatinine, mM	0	69.03	73.62	68.60	71.94	3.55	<0.001
	14	82.53	85.72	82.60	86.54	3.55	
	42	99.58	108.25	95.30	100.71	3.55	
Glucose, mM	0	6.57	6.23	6.41	6.36	0.00	NS
	14	6.92	6.14	5.97	5.97	0.00	
	42	5.82	6.10	5.85	6.20	0.00	
Total Bilirubin, mM	0	0.32	0.37	0.45	0.37	0.62	NS
	14	0.29	0.38	0.22	0.43	0.62	
	42	0.34	0.35	0.22	0.36	0.62	
Direct Bilirubin, mM	0	0.27	0.25	0.28	0.21	0.03	<0.001

	14	0.15	0.23	0.15	0.12	0.03	
	42	0.20	0.16	0.11	0.18	0.03	
Indirect Bilirubin, mM	0	0.05	0.12	0.17	0.16	0.05	NS
	14	0.14	0.15	0.07	0.31	0.05	
	42	0.14	0.22	0.11	0.18	0.05	
GGT[8], mM	0	32.39	30.11	32.60	35.92	2.78	NS
	14	34.19	32.61	34.00	34.82	2.78	
	42	34.15	33.74	33.90	38.41	2.78	
GLDH[9], mM	0	1.50	1.49	1.70	1.29	0.18	0.007
	14	1.60	1.49	1.50	1.29	0.18	
	42	1.33	1.10	0.90	1.11	0.18	
AST[10], mM	0	26.02	22.08	22.40	23.86	4.33	NS
	14	22.22	18.38	19.20	23.06	4.33	
	42	33.88	15.77	16.70	15.68	4.33	
CK[11], mM	0	2192.26	1290.74	1159.10	1444.64	1229.03	<0.001
	14	4144.16	2723.04	2387.00	2359.94	1229.03	
	42	8051.96	3621.78	8384.10	4199.66	1229.03	
Total Protein, mM	0	53.93	54.33	53.60	55.06	1.15	<0.001
	14	58.73	59.53	56.60	55.56	1.15	
	42	62.37	61.75	58.70	62.76	1.15	
Albumin, mM	0	13.07	13.56	13.60	15.41	0.97	0.001
	14	15.97	15.66	15.70	16.71	0.97	
	42	15.31	14.15	12.40	16.00	0.97	
Globulin, mM	0	13.07	13.56	13.60	15.41	0.97	0.001
	14	15.97	15.66	15.70	16.71	0.97	
	42	15.31	14.15	12.40	16.00	0.97	
Albumin:Globulin, mM	0	3.23	3.17	3.07	3.75	0.22	<0.001
	14	2.82	2.89	2.66	2.45	0.22	
	42	3.23	3.45	3.75	3.02	0.22	

[1]Values are least squares means with n=10 pens/treatment
[2]CONT, 0 ppm Control diet
[3]DON1, 1 ppm DON diet
[4]DON3, 3 ppm DON diet
[5]DON5, 5 ppm DON diet
[6] SEM, Standard error of the means
[7]There were no significant effects of diet or diet × day interaction
[8]GGT, Gamma-glutamyl transferase
[9]GLDH, Glutamate dehydrogenase
[10]AST, Aspartate aminotransferase
[11]CK, Creatine kinase

Table 13 Plasma indicators of liver and kidney health and function in finisher pigs (Study 2) fed diets containing graded levels of deoxynivalenol[1]

		Dietary Treatment					*P*-value[7]
Item	Day	CONT[2]	DON1[3]	DON3[4]	DON5[5]	SEM[6]	Day
Sodium, mM	42	147.54	148.44	149.50	148.59	2.15	SS
	56	143.94	145.02	144.30	144.99	2.15	
	84	144.63	149.22	147.99	149.09	2.24	
Potassium, mM	42	5.80	5.46	5.66	5.16	0.19	0.06
	56	5.25	5.54	5.35	5.32	0.19	
	84	5.13	5.22	5.44	5.07	0.20	
Chloride, mM	42	98.11	97.76	99.20	98.42	1.47	<0.01
	56	95.01	95.93	94.90	95.42	1.47	
	84	96.54	99.43	99.34	100.44	1.55	
Bicarbonate, mM	42	23.08	24.62	24.00	24.79	0.83	<0.001
	56	22.48	22.91	22.50	23.19	0.83	
	84	26.40	27.61	28.47	28.69	0.87	
Anion Gap, mM	42	32.31	31.34	32.00	30.60	1.24	<0.001
	56	31.71	31.40	32.20	31.90	1.22	
	84	27.09	27.00	25.81	25.14	1.27	
Calcium, mM	42	2.88	2.85	2.86	2.80	0.08	NS
	56	2.73	2.78	2.75	2.65	0.08	
	84	2.60	2.81	2.81	2.98	0.08	
Phosphorus, mM	42	3.15	3.06	3.07	3.06	0.08	<0.001
	56	2.95	2.93	2.84	2.82	0.08	
	84	2.63	2.71	2.43	2.28	0.08	
Magnesium, mM	42	0.80	0.78	0.83	0.78	0.03	<0.001
	56	0.81	0.77	0.73	0.75	0.03	
	84	0.87	0.91	0.87	0.88	0.03	
Urea, mM	42	5.96	5.80	6.12	6.10	0.33	0.02
	56	5.56	5.65	5.37	5.49	0.33	
	84	5.81	6.05	6.03	5.64	0.34	
Creatinine, mM	42	91.39	96.22	95.10	96.41	5.09	<0.001
	56	106.88	106.71	108.10	108.01	5.09	
	84	126.49	135.91	133.26	133.30	5.27	
Glucose, mM	42	5.83	5.69	6.00	5.56	0.24	<0.01
	56	6.19	5.25	5.43	5.26	0.24	
	84	5.34	5.25	5.49	4.96	0.26	
Total Bilirubin, mM	42	0.23	0.07	0.18	0.22	0.12	NS
	56	0.23	0.17	0.40	0.10	0.12	
	84	0.37	0.41	0.23	0.35	0.12	
Direct Bilirubin, mM	42	0.15	0.10	0.10	0.19	0.04	<0.01

	56	0.12	0.13	0.07	0.06	0.04	
	84	0.18	0.26	0.16	0.19	0.04	
Indirect Bilirubin, mM	42	0.08	0.00	0.08	0.03	0.11	NS
	56	0.11	0.04	0.33	0.04	0.11	
	84	0.19	0.15	0.06	0.16	0.12	
GGT[8], mM	42	34.15	33.69	29.10	32.86	2.73	0.04
	56	32.05	33.10	29.90	32.76	2.73	
	84	35.49	36.60	31.57	36.51	2.83	
GLDH[9], mM	42	34.15	33.69	29.10	32.86	2.73	NS
	56	32.05	33.10	29.90	32.76	2.73	
	84	35.49	36.60	31.57	36.51	2.83	
AST[10], mM	42	24.87	17.42	19.40	19.83	3.97	0.04
	56	27.07	18.44	26.40	23.53	3.20	
	84	15.81	20.84	17.97	16.87	4.09	
CK[11], mM	42	3589.67	3593.82	3706.30	3619.14	913.93	NS
	56	4110.77	2803.16	4151.60	3144.64	913.93	
	84	2409.62	2224.76	2808.23	2280.97	957.18	
Total Protein, mM	42	63.29	62.71	63.40	64.18	1.76	NS
	56	61.59	60.83	61.50	62.38	1.76	
	84	61.46	63.93	62.11	65.94	1.86	
Albumin, mM	42	48.96	48.27	47.60	47.90	1.15	NS
	56	47.66	46.46	46.50	46.70	1.14	
	84	47.48	48.66	47.30	47.85	1.19	
Globulin, mM	42	14.37	14.40	15.80	16.25	0.89	NS
	56	13.97	14.33	15.00	15.65	0.89	
	84	13.97	15.23	14.82	17.90	0.93	
Albumin:Globulin, mM	42	14.37	14.40	15.80	16.25	0.89	NS
	56	13.97	14.33	15.00	15.65	0.87	
	84	13.97	15.23	14.82	17.90	0.91	

[1]Values are least squares means with n=10 pens/treatment
[2]CONT, 0 ppm Control diet
[3]DON1, 1 ppm DON diet
[4]DON3, 3 ppm DON diet
[5]DON5, 5 ppm DON diet
[6]SEM, Standard error of the means
[7]There were no significant effects of diet or diet × day interaction
[8]GGT, Gamma-glutamyl transferase
[9]GLDH, Glutamate dehydrogenase
[10]AST, Aspartate aminotransferase
[11]CK, Creatine kinase

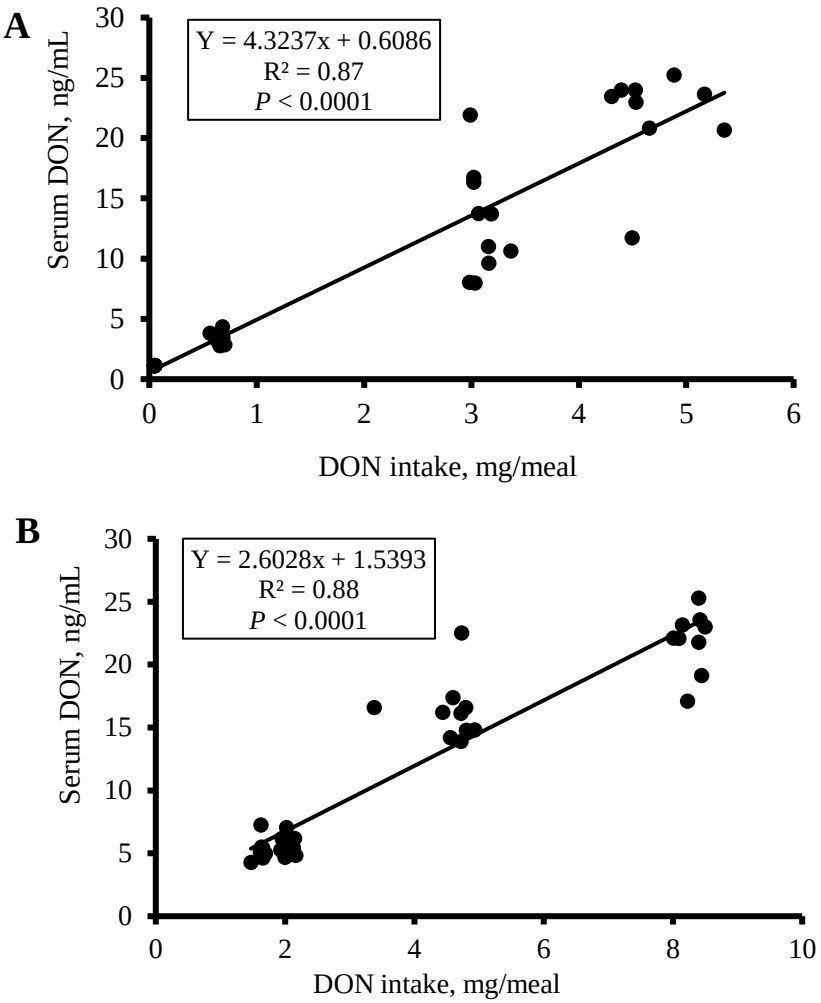

Figure 9 Regression analysis of the relationship between deoxynivalenol (DON) intake and DON output in serum (n=10 pigs/treatment). The serum samples were collected during the nitrogen balance period of the experiment 3-4h after a singel meal and analysed for DON content. The figure A represent the grower phase and figure B represents the finisher phase of the experimental animals. Data is expressed as the DON intake per meal (mg/d) and the serum DON output (ng/mL) and the coefficient of determination (R^2) of the regression curve is 0.87 and 0.88 for figure **A** (grower phase) and **B** (finisher phase) respectively at $P < 0.0001$.

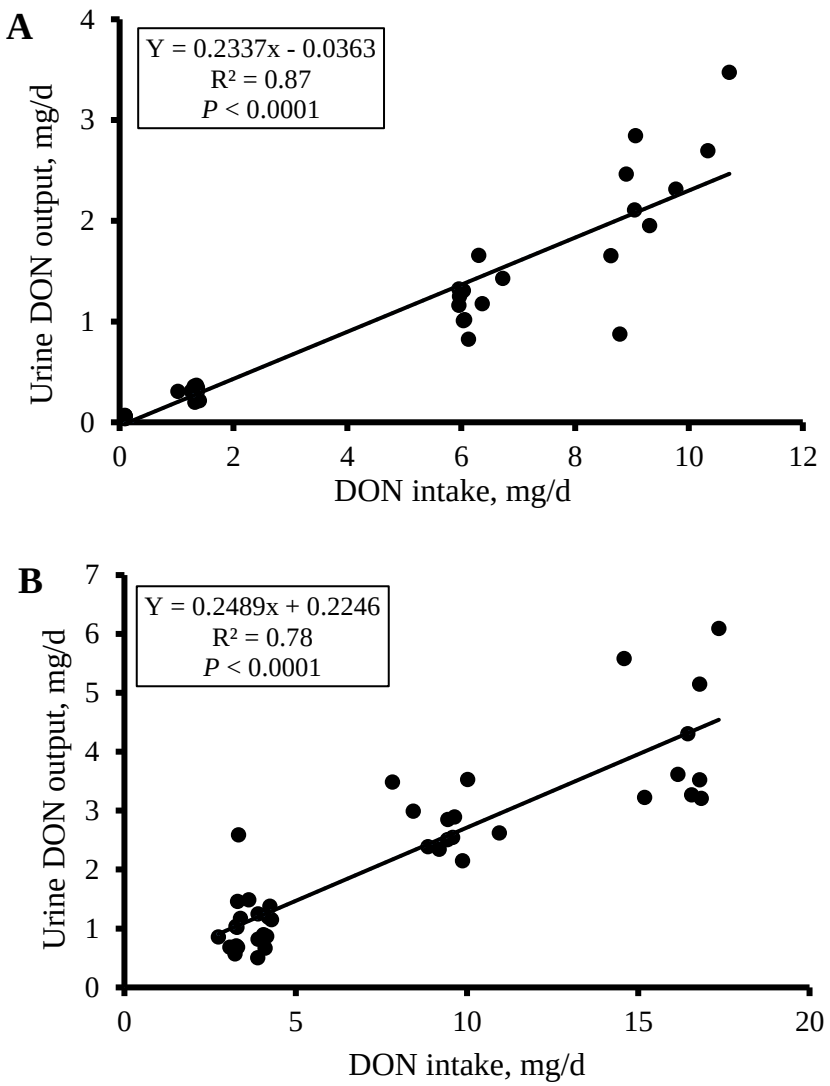

Figure 10 Regression analysis of the relationship between deoxynivalenol (DON) intake and DON output in urine (n=10 pigs/treatment). The urine samples were collected during the nitrogen balance period of the experiment over 24-h and analysed for DON content. The figure **A** represent the grower phase and figure **B** represents the finisher phase of the experimental animals. Data is 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 0.87 and 0.78 for figure A (grower phase) and figure B (finisher phase) respectively at $P < 0.0001$.

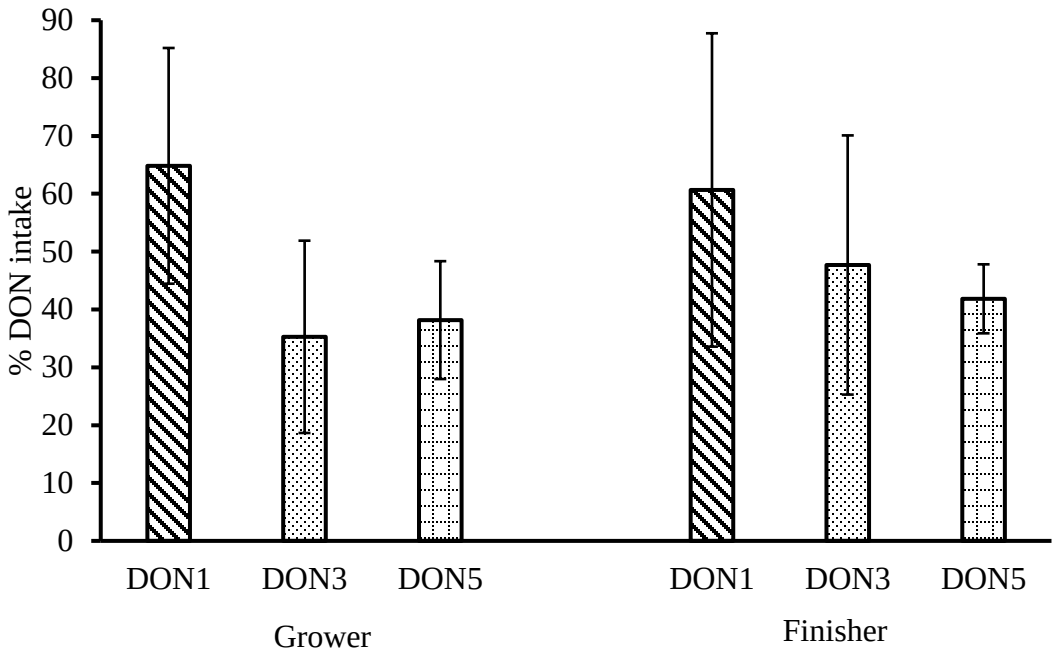

Figure 11 Deoxynivalenol (DON) recovery in urine of grower and finisher pigs fed graded levels of deoxynivalenol

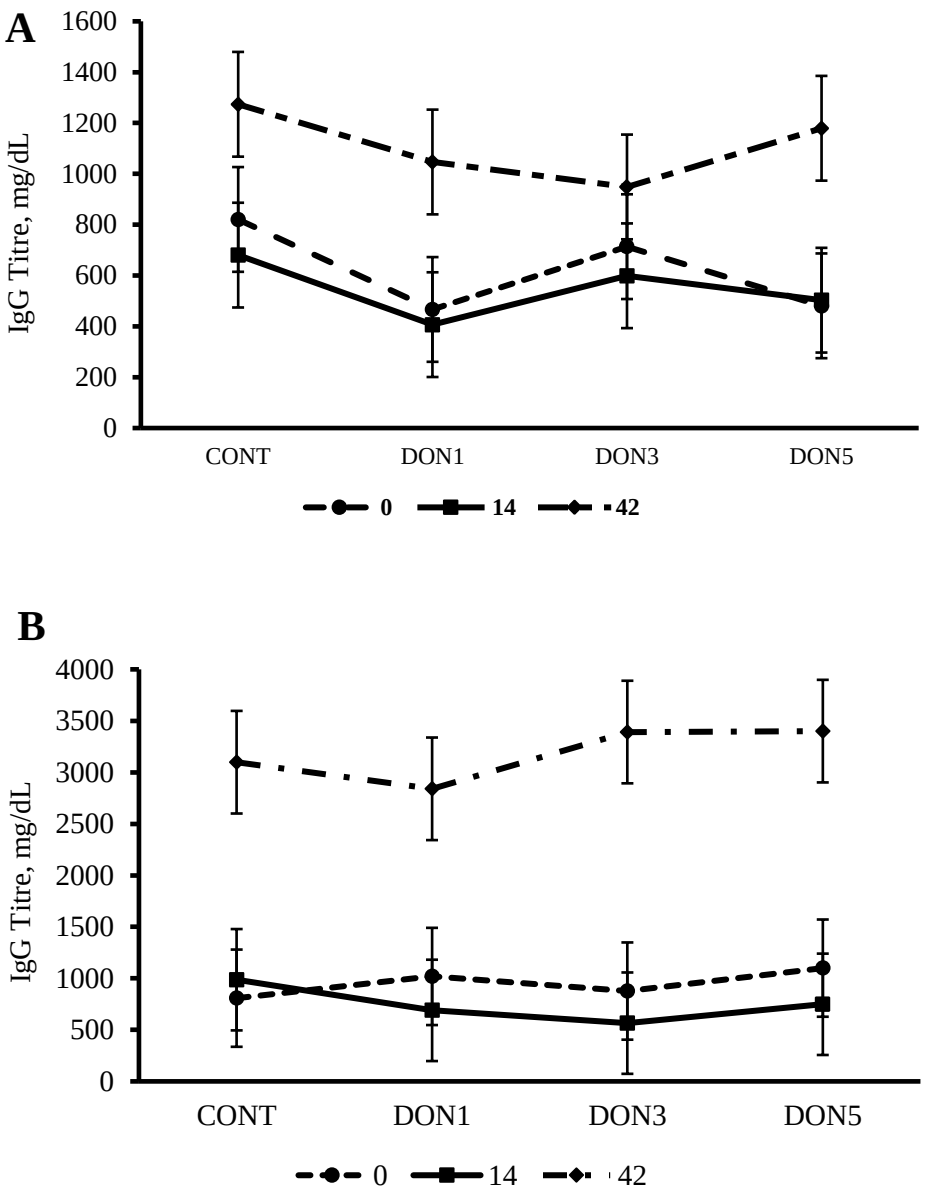

Figure 12 IgG titer in response to Leptospira spp. vaccine in grower (A) and finisher (B) pigs fed graded levels of deoxynivalenol.

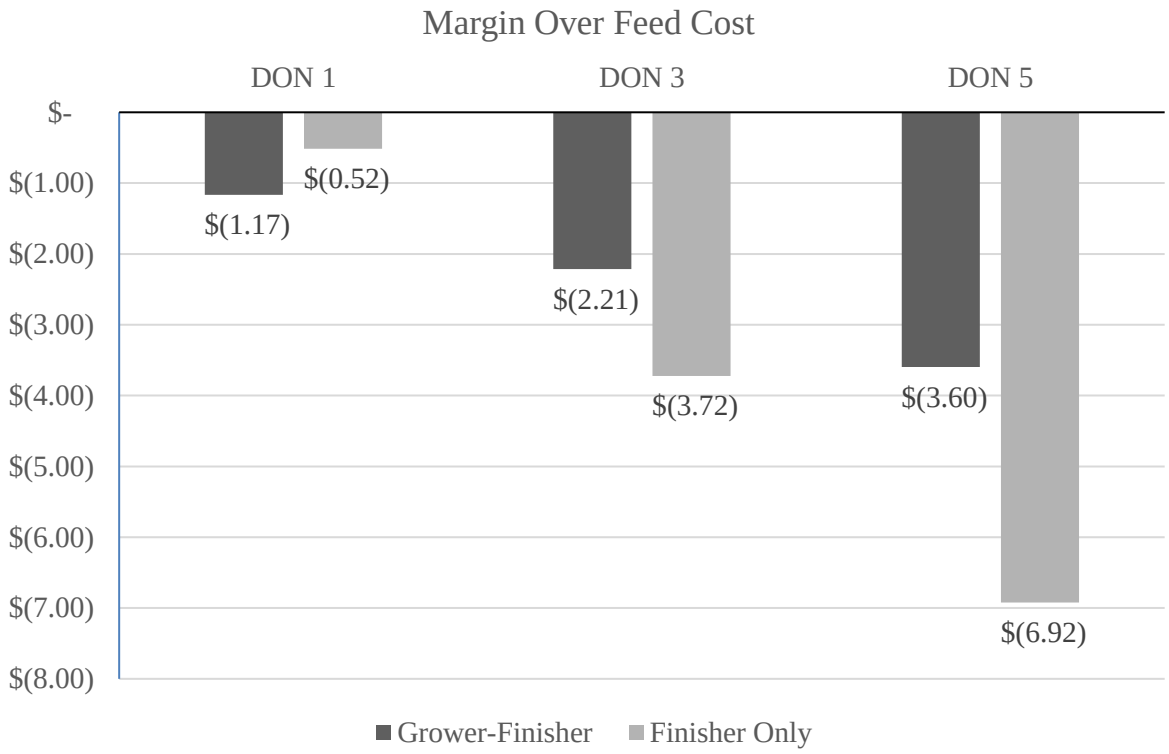

Figure 13 Margin over feed cost for diets containing various levels of DON

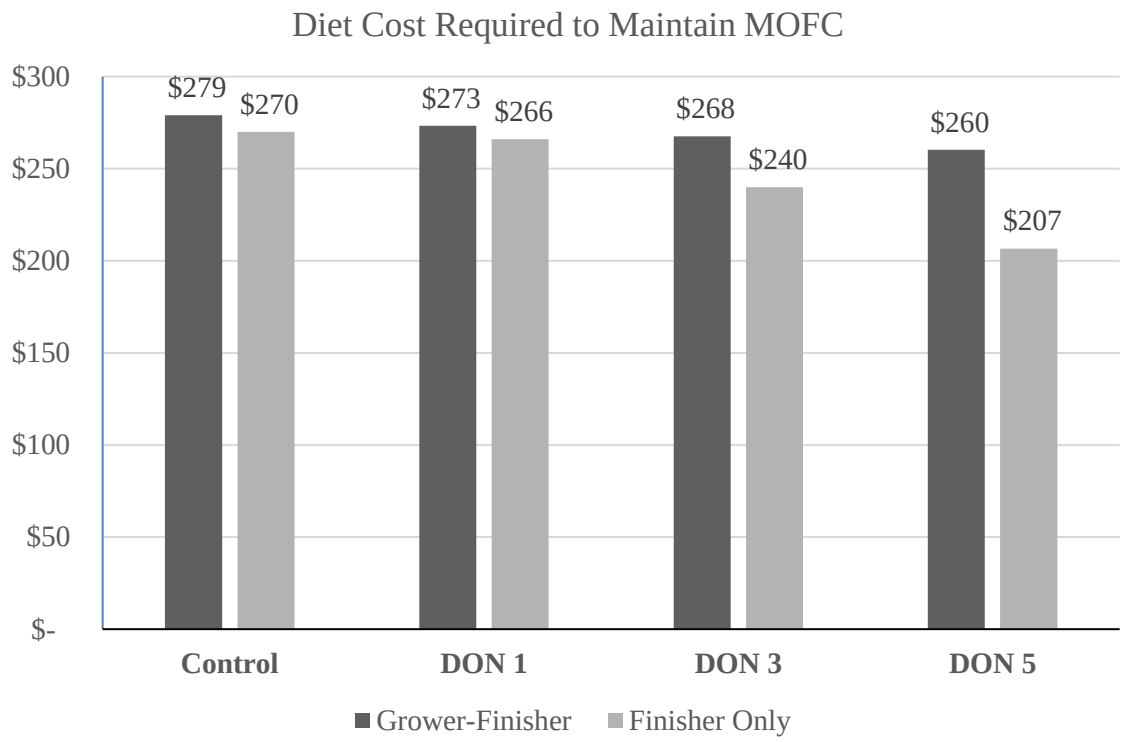

Figure 14 Diet cost required to maintain margin over feed cost to uncontaminated diets.

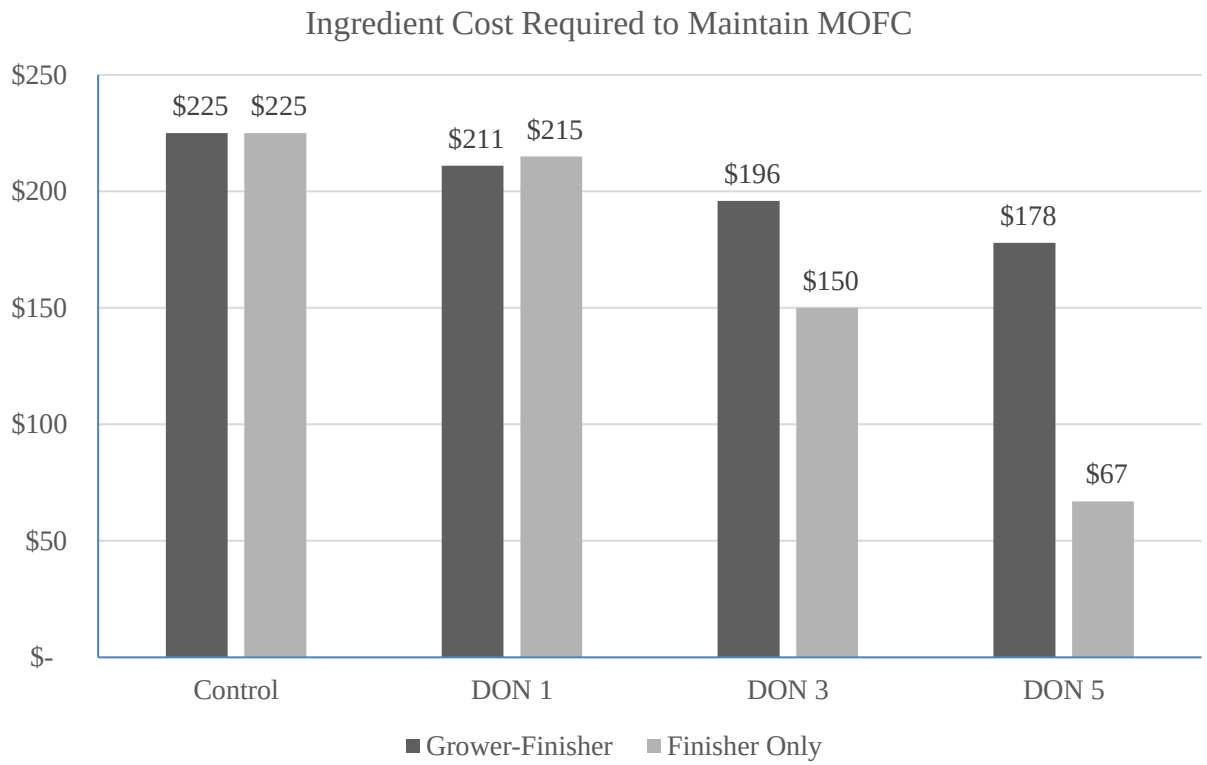

Figure 15 Ingredient price of contaminated grain, at 40% of the diet, required to maintain margin over feed cost at uncontaminated levels.

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