



The influence of pelleting and supplementing sodium metabisulfite ($\text{Na}_2\text{S}_2\text{O}_5$) on nursery pigs fed diets contaminated with deoxynivalenol[☆]



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ARTICLE INFO

Article history:

Received 2 April 2015

Received in revised form 16 August 2015

Accepted 22 September 2015

Keywords:

Deoxynivalenol

Growth

Pelleting

Sodium metabisulfite

Swine

Vomitoxin

ABSTRACT

Four experiments were conducted to ascertain the effects of hydrothermal treatment and sodium metabisulfite (SMB) on deoxynivalenol (DON)-contaminated corn dried distillers grains with solubles (DDGS). Experiment 1 evaluated SMB and heat (autoclaving) on high-DON DDGS (20.6 mg/kg). Six levels of SMB were tested: 0.0% (control), 0.5%, 1%, 2.5%, 5%, and 5% with 100 mL/kg distilled water. Autoclaving after 1 h at 121 °C alone elicited a 9.8% reduction in DON, whereas an 82% reduction was achieved when 5% SMB was added before autoclaving. Experiment 2 tested pelleting high-DON DDGS with SMB. Four batches of DDGS (20.5 mg/kg DON) were tested: 0 (control), 1.0, 2.5, and 5.0% SMB. Pelleted samples were collected at conditioning temperatures of 66 and 82 °C and retention times of 30 and 60 s within temperature. Pelleting conditions had no effect on DON levels, but as SMB inclusion increased in pelleted DDGS, DON levels were reduced (quadratic; $P < 0.001$). Experiments 3 and 4 evaluated pelleting and SMB on nursery pig growth. Both trials were arranged in a $2 \times 3 + 1$ factorial with 5 replicate pens per treatment. In Exp. 3, 987 pigs (13.0 ± 0.2 kg) were used with main effects of (1) diet form: meal or pellet and (2) SMB level: Negative Control (NC), NC + 0.25% SMB, or NC + 0.50% SMB. Negative Control diets were formulated to contain 3 mg/kg DON. Treatment 7 was a Positive Control (PC; < 0.5 mg/kg DON) fed in meal form. Pigs fed high-DON diets had reduced ($P < 0.001$) ADG and ADFI, but pelleting improved ($P < 0.001$) ADG and G:F. Adding SMB increased (linear; $P < 0.03$) ADG and tended to increase ($P < 0.10$) ADFI. In Exp. 4, 1180 pigs (11.1 ± 0.32 kg) were used with main effects of (1) diet form: meal or pellet and (2) DDGS source: PC (< 0.5 mg/kg DON), NC (5 mg/kg DON), or NC + DDGS pelleted and crumbled before mixing into the final diet. In meal form, treatment 7 included 2.5% SMB prior to pelleting DDGS (final diet contained 0.77% SMB). Overall, a 2-way interaction ($P < 0.04$) was observed within NC diets where pelleting the final diet improved G:F by a greater margin in high-DON diets than when the DDGS was pelleted, crumbled, and re-pelleted. DON reduced ($P < 0.002$) ADG and ADFI, and pelleting the diet improved ($P < 0.01$) ADG and G:F. Including SMB prior to pelleting DON-contaminated DDGS increased ($P < 0.01$) ADG and ADFI. Using SMB combined with thermal processing can mitigate DON effects in diets for nursery pigs.

Published by Elsevier B.V.

[☆] Contribution no. 13-056-J from the Kansas Agric. Exp. Sta., Manhattan, KS 66506-0210, United States.

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1. Introduction

Deoxynivalenol (DON), also known as vomitoxin, is produced by fungi of the *Fusarium* genus and is one of the key contaminants of cereal grains because it often occurs at levels high enough to cause adverse effects in farm animals. Among livestock species, pigs are the most sensitive, primarily because DON is rapidly absorbed and poorly metabolized (Etienne and Waché, 2008). The most obvious effect in pigs is reduced feed intake, which may be attributed to irritation of stomach mucosa (Rotter et al., 1994; Trenholm et al., 1994) and changes in brain transmitters (Prelusky, 1993; Swamy et al., 2002).

Levels of DON that elicit negative effects on growth (>1 mg/kg; Dänicke et al., 2001) are relatively common. Ethanol by-products are especially concerning because DON levels are approximately 3 times more concentrated in corn dried distillers grains with solubles (DDGS) than in the corn source. Because DON cannot be consistently removed, many types of detoxification have been evaluated. The majority of these treatments are either ineffective (Friend et al., 1984; Dänicke et al., 2004; Döll et al., 2005) or impractical in large-scale production (He et al., 1993; Li et al., 2011).

Other studies have shown more promising results. Young et al. (1987), for instance, showed that DON is converted to a 10-sulfonate adduct (DON-S) in the presence of sodium bisulfite and heat (autoclave); the resulting DON-S is non-toxic when fed to pigs. Research by Dänicke et al. (2005) reported similar DON-transformation using sodium metabisulfite (SMB) and hydrothermal treatment with a laboratory conditioner. We hypothesized that pelleting, particularly conditioning, could detoxify DON-contaminated feedstuffs. Using DON-contaminated DDGS, the aims of this study were to evaluate: (1) the ability of SMB to transform DON using an autoclave, (2) pelleting under varying conditions with SMB for reducing DON, and (3) the effects of pelleting either DDGS or final diets with SMB on nursery pig performance.

2. Material and methods

2.1. General

All experimental procedures and animal care were approved by the Kansas State Institutional Animal Care and Use Committee. Corn DDGS were provided by Hubbard Feeds (Mankato, MN), and the uncontaminated (POET Bio-refining, Bingham Lake, MN) and naturally DON-contaminated (POET Bio-refining, North Manchester, IN) DDGS originated from the same plants across all four experiments.

2.2. Experiment 1

The objective of this pilot study was to verify that DON levels in naturally DON-contaminated DDGS can be reduced using SMB (Samirian Chemical, Campbell, CA) in an autoclave. All samples used in this study were prepared at the Kansas State University Swine Nutrition Laboratory, with the samples autoclaved at the K-State Food Science Laboratory. Samples were prepared from a previously identified, uniform source of DDGS with a known DON concentration of 20.6 mg/kg. The DDGS were homogenized thoroughly prior to sample preparation to eliminate variation in DON content across samples.

This experiment used 6 treatments with DDGS containing either: (1) No SMB (control), (2) 0.5% SMB, (3) 1.0% SMB, (4) 2.5% SMB, (5) 5.0% SMB, or (6) 5.0% SMB with 100 mL/kg distilled water added to evaluate the role of water in the potential change in DON. Each treatment had a final weight of 500 g per sample except treatment 6 (550 g with water). Samples were split into two replicates and placed in covered aluminum trays but were not sealed airtight to allow steam interaction and gas release during the autoclave process. Samples were autoclaved at 121 °C for 60 min. After autoclaving, samples were dried in a 55 °C drying oven to convert to a DM basis before replicates were sent for a full 17-component mycotoxin analysis at the North Dakota State University Veterinary Diagnostic Laboratory (NDSU; Fargo, ND). Analyzed mycotoxin levels were adjusted by the proportion of DDGS in the original sample, then converted to an as-fed basis. Because replications were combined for mycotoxin analysis in Exp. 1, statistical analysis could not be conducted for this pilot study.

2.3. Experiment 2

The objective of this experiment was to evaluate the extent of DON reduction due to SMB when DDGS were pelleted under varying conditions. This experiment was conducted at the Kansas State University Grain Sciences and Industry Feed Mill. All personnel involved were required to wear respirators and safety goggles during the pelleting process, because sodium metabisulfite releases sulfur dioxide gas in the presence of heat and moisture and can irritate the eyes and respiratory tract.

Treatments comprised of 205-kg batches of DDGS after the addition of SMB. The DDGS were sourced from naturally DON-contaminated DDGS (averaging 20.6 ± 0.5 mg/kg). Four DDGS treatments contained either: (1) 0.0% (control), (2) 1.0% SMB, (3) 2.5% SMB, or (4) 5.0% SMB. Prior to the addition of SMB, each batch was mixed for 4 min in a paddle mixer (Forberg 500 L double-shaft) to homogenize the DDGS and eliminate any variation in initial DON concentration. After adding SMB, each batch was mixed for an additional 3 min before pelleting. The pellet mill (CPM Master Model 1000HD; Crawfordsville, IN) was set to a production rate of 454 kg/h to control conditioning temperature and retention time for each batch of DDGS. Within each treatment, the pellet conditioner was adjusted to conditioning temperatures of 66 and 82 °C and retention times of 30 and 60 s for each temperature, and 2-kg samples were collected at each temperature \times retention time combination.

Table 1
Mycotoxin and mineral analysis of diets, Exp. 3 (as-fed basis).

| Item | DDGS source ^a | | Experimental diets ^{a,b} | | | | | | | |
|-------------------------|--------------------------|-----------------------|-----------------------------------|------|------------------|--------|-----------------------------|--------|-----------------------------|--------|
| | Control | High-DON ^c | Positive Control | | Negative Control | | NC + 0.25% SMB ^d | | NC + 0.50% SMB ^d | |
| | | | Meal | | Meal | Pellet | Meal | Pellet | Meal | Pellet |
| Mycotoxin, mg/kg | | | | | | | | | | |
| DON | <0.5 | 11.7 | <0.5 | 3.2 | 3.3 | 3.1 | 1.7 | 2.4 | 0.8 | |
| 15-ADON ^e | <0.5 | 2.0 | <0.5 | 0.6 | 0.7 | 0.7 | 0.6 | 0.6 | 0.6 | |
| Total DON ^f | <0.5 | 14.0 | <0.5 | 3.8 | 4.0 | 3.8 | 2.3 | 3.0 | 1.4 | |
| Zearalenone | <0.5 | 2.0 | <0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | |
| Mineral, % ^g | | | | | | | | | | |
| Na | – | – | 0.24 | 0.28 | 0.37 | 0.32 | 0.30 | 0.36 | 0.37 | |
| S | – | – | 0.41 | 0.38 | 0.56 | 0.45 | 0.44 | 0.56 | 0.56 | |

^a Dried distillers grains with solubles and diet samples were sent to the North Dakota State University (NDSU) Veterinary Diagnostic Laboratory, Fargo, ND for a full 17-component toxin screen. Samples were analyzed using a variety of mass spectrometry, ELISA, and HPLC methods with a practical quantitation limit of 0.5 mg/kg.

^b Positive Control diet formulated to contain <0.5 mg/kg DON and all remaining diets formulated to contain 3 mg/kg DON.

^c High-deoxynivalenol (DON) DDGS were analyzed with both an ELISA test kit (8.9 mg/kg) and at NDSU (14.4 mg/kg). Levels were averaged due to variability.

^d Sodium metabisulfite (Samirian Chemical, Campbell, CA).

^e 15-Acetyldeoxynivalenol (15-ADON).

^f Total DON reported as a combination of DON and 15-ADON, because both DON metabolites have similar toxicity (Pestka, 1987).

^g Mineral analyses were conducted at MVTL Labs (New Ulm, MN).

Pellets were cooled prior to sampling, and the 4 corresponding samples from each batch were ground and individually sent for mycotoxin analysis at NDSU.

In Exp. 2, data were analyzed using sample within batch as the experimental unit. Analysis evaluated the linear and quadratic effects of SMB and interactions with conditioning temperature and retention time using Genstat (Release 11.1, VSN International Ltd., Hemel Hempstead, UK). Data collected from Exp. 3 and 4 were analyzed using the MIXED procedure of SAS, version 9.1 (SAS Inst. Inc., Cary, NC). Treatment effects were assessed within each experimental period using pen as the experimental unit. For statistical tests, significance and tendencies were set at $P < 0.05$ and $P < 0.10$, respectively.

2.4. Experiment 3

A total of 987 mixed-sex pigs (Fast/PIC × TR4; Fast Genetics, Saskatoon, SK, Canada; PIC, Hendersonville, TN), initially 13.0 ± 0.2 kg BW, were used in a 27-day growth experiment to evaluate the effects of supplementing SMB and pelleting on the performance of nursery pigs fed naturally DON-contaminated diets. There were 5 replicate pens per treatment, and average initial pig BW was used as the blocking factor. Pens were allotted to treatments based on initial pen weight with 28 pigs per pen (14 barrows and 14 gilts).

Based on results of mycotoxin analysis (Table 1) of the high-DON DDGS (11.7 mg/kg) at NDSU, DDGS were incorporated into experimental diets at 25.0% to achieve desired DON concentrations (Table 2). The study was arranged in a randomized complete block design with a $2 \times 3 + 1$ factorial. The main effects were diet form (meal or pellet) and SMB (0, 0.25, and 0.50%); therefore, the 7 experimental diets consisted of 4 diets: (1) Positive Control (PC; <0.5 mg/kg DON) in meal form only, (2) Negative Control (NC; 3.0 mg/kg DON) in pellet and meal form, (3) NC + 0.25% SMB (3.0 mg/kg DON) in pellet and meal form, and (4) NC + 0.50% SMB (3.0 mg/kg DON) in pellet and meal form. All diets were also medicated with chlortetracycline 400 at a rate of 441 mg/kg. Diets were formulated to meet or exceed all nutrient requirement estimates (NRC, 1998).

Feed manufacturing took place at Hubbard Feeds in Mankato, MN. Diets were pelleted using a CPM 7800 (California Pellet Mill, Crawfordsville, IN) through a stainless steel 635-mm-thick, 32-mm pellet die at a conditioning temperature of 61.4 ± 3.8 °C. Corn was ground using a roller mill, and the particle size for diets fed in meal form averaged 616 μ . Following diet manufacturing, a sample of each diet was collected, homogenized, and sent to NDSU for mycotoxin analysis. Diets were also analyzed for sodium and sulfur content at MVTL Laboratory (New Ulm, MN) due to concerns that incorporating SMB at high levels may negatively affect performance because of high dietary sodium or sulfur.

This experiment was conducted at the New Fashion Pork Research Nursery in Buffalo Center, IA. Each pen (1.75×4.05 m²) contained a 5-hole, dry self-feeder and provided ad libitum access to feed and water. Pig weights and feed disappearance were measured on days 0, 7, 14, 21, and 27 to determine ADG, ADFI, and G:F.

In Exp. 3, the experimental unit was pen. The statistical model included pelleting and SMB inclusion as fixed factors and block as the random factor. The preplanned contrasts in Exp. 3 were: (1) DON vs. non-contaminated; (2) diet form (pellet vs. meal); (3) linear and quadratic effects of increased levels of SMB; and (4) pelleting-SMB interaction. Statistical significance was set at $P < 0.05$ and trends at $P < 0.10$.

Table 2
Composition of experimental diets, Exp. 3 (as-fed basis).

| Item | Positive Control | Negative Control | NC + 0.25% SMB ^a | NC + 0.50% SMB ^a |
|--|------------------|------------------|-----------------------------|-----------------------------|
| Ingredient, % | | | | |
| Corn | 45.33 | 45.33 | 44.84 | 44.33 |
| Dried distillers grains with solubles (DDGS), 26.3% CP | 25.00 | – | – | – |
| Contaminated DDGS, 26.0% CP | – | 25.00 | 25.00 | 25.00 |
| Soybean meal, 46.5% CP | 24.90 | 24.90 | 24.95 | 25.00 |
| Choice white grease | 1.30 | 1.30 | 1.50 | 1.70 |
| Limestone | 1.06 | 1.06 | 1.05 | 1.05 |
| Salt | 0.44 | 0.45 | 0.45 | 0.45 |
| Monocalcium phosphate, 21% P | 0.55 | 0.55 | 0.55 | 0.55 |
| Trace mineral premix ^b | 0.08 | 0.08 | 0.08 | 0.08 |
| Copper sulfate | 0.07 | 0.07 | 0.07 | 0.07 |
| Selenium | 0.05 | 0.05 | 0.05 | 0.05 |
| Vitamin premix ^c | 0.03 | 0.03 | 0.03 | 0.03 |
| L-Lys HCl | 0.46 | 0.45 | 0.45 | 0.45 |
| Methionine hydroxy analog | 0.12 | 0.12 | 0.12 | 0.13 |
| L-Thr | 0.07 | 0.07 | 0.08 | 0.08 |
| Medication ^d | 0.40 | 0.40 | 0.40 | 0.40 |
| Mold inhibitor ^e | 0.10 | 0.10 | 0.10 | 0.10 |
| Phytase ^f | 0.04 | 0.04 | 0.04 | 0.04 |
| Sodium metabisulfite | – | – | 0.25 | 0.50 |
| Total | 100 | 100 | 100 | 100 |
| Calculated analysis | | | | |
| SID ^g amino acids, % | | | | |
| Lys | 1.20 | 1.20 | 1.20 | 1.20 |
| Ile:Lys | 61 | 61 | 61 | 61 |
| Leu:Lys | 145 | 144 | 143 | 143 |
| Met:Lys | 34 | 34 | 34 | 34 |
| Met and Cys:Lys | 58 | 58 | 58 | 58 |
| Thr:Lys | 60 | 60 | 60 | 60 |
| Trp:Lys | 17.5 | 17.5 | 17.5 | 17.5 |
| Val:Lys | 72 | 72 | 72 | 72 |
| Total Lys, % | 1.39 | 1.39 | 1.39 | 1.39 |
| ME, MJ/kg | 13.85 | 13.85 | 13.85 | 13.85 |
| SID Lys:ME, g/Mcal | 3.63 | 3.63 | 3.63 | 3.63 |
| CP, % | 21.80 | 21.80 | 21.79 | 21.78 |
| Ca, % | 0.66 | 0.66 | 0.66 | 0.66 |
| P, % | 0.58 | 0.58 | 0.58 | 0.58 |
| Available P, % | 0.30 | 0.31 | 0.31 | 0.31 |
| Na, % | 0.25 | 0.25 | 0.31 | 0.37 |
| Cl, % | 0.43 | 0.43 | 0.43 | 0.43 |
| Added S, % ^h | – | – | 0.08 | 0.16 |

^a Sodium metabisulfite (Samirian Chemicals, Campbell, CA).

^b Trace mineral premix provided per kilogram of premix: 13.3 mg of Cu, 1.40 mg of I, 134 mg of Fe, 53.3 mg of Mn, and 160 mg of Zn.

^c Vitamin premix provided per kilogram of premix: 22,046,000 IU of vitamin A, 5,291,000 IU of vitamin D₃, 97,002 IU of vitamin E, 10,288 mg of vitamin K, 88.2 mg of vitamin B₁₂, 79,366 mg of niacin, 61.7 mg of pantothenic acid, and 13,228 mg of riboflavin.

^d To provide chlortetracycline at 441 g/t.

^e Ammo Curb (Kemin Industries, Des Moines, IA).

^f Phyzyme 2500 (Danisco Animal Nutrition, St. Louis, MO).

^g Standardized ileal digestible.

^h Originating from sodium metabisulfite (Na₂S₂O₅), which is 33% sulfur.

2.5. Experiment 4

A total of 1180 mixed-sex pigs (Fast/PIC × TR4; Fast Genetics, Saskatoon, SK, Canada; PIC; Hendersonville, TN, initially 11.2 ± 0.3 kg BW) were used to evaluate the effects of pelleting, pelleting DON-contaminated DDGS, and supplementing SMB on nursery pig performance. A combined total of 9 or 10 replications per treatment were placed in a completely randomized design in a 2 × 3 + 1 arrangement. The experiment was conducted concurrently at two sites to evaluate these effects in both university and commercial conditions. The research sites were: (1) Kansas State University (KSU) Swine Teaching and Research Center in Manhattan, KS; and (2) New Fashion Pork (NFP) Research Nursery in Buffalo Center, IA. All diets were manufactured simultaneously at Hubbard Feeds in Mankato, MN, and diets for both sites were bagged and transported to the research locations.

At KSU, a total of 238 mixed sex pigs (PIC 337 × 1050; Hendersonville, TN, initially 11.5 ± 0.2 kg BW) were used in a 21-day growth trial with 5 replicates per treatment (pens) and 7 pigs (4 barrows, 3 gilts) per pen. Based on limited pen availability, 1 treatment (Positive Control, meal) only had 4 replicate pens. Pigs were allotted to pens by initial BW at weaning, and when pigs reached approximately 11.5 kg, they were re-weighed and average pig BW within pen was balanced across the 7 treatments. Each pen contained a 4-hole, dry self-feeder and a nipple waterer to provide ad libitum access to feed and water.

Table 3
Mycotoxin and mineral analysis of diets, Exp. 4 (as-fed basis).

| Item | DDGS source ^a | | Experimental diets ^{a,b} | | | | | | |
|------------------------|--------------------------|-----------------------|-----------------------------------|--------|------------------|--------|---------------------------------|--------|---------------------------------------|
| | Control | High-DON ^c | Positive Control | | Negative Control | | NC + crumbled DDGS ^d | | NC + crumbled DDGS w/SMB ^e |
| | | | Meal | Pellet | Meal | Pellet | Meal | Pellet | Meal |
| Mycotoxin, mg/kg | | | | | | | | | |
| DON | <0.5 | 16.0 | <0.5 | <0.5 | 3.5 | 3.3 | 3.4 | 3.5 | 1.6 |
| 15-ADON ^f | <0.5 | 2.2 | <0.5 | <0.5 | 0.6 | 0.6 | 0.6 | 0.6 | 0.6 |
| Total DON ^g | <0.5 | 18.2 | <0.5 | <0.5 | 4.1 | 3.9 | 4.0 | 4.1 | 2.2 |
| Zearalenone | <0.5 | 1.20 | <0.5 | <0.5 | <0.5 | <0.5 | <0.5 | <0.5 | <0.5 |
| Mineral, % | | | | | | | | | |
| Na | – | – | 0.28 | 0.34 | 0.29 | 0.29 | 0.28 | 0.31 | 0.23 |
| S | – | – | 0.39 | 0.46 | 0.43 | 0.43 | 0.43 | 0.46 | 0.57 |

^a Dried distillers grains with solubles and diet samples were sent to the North Dakota State University Veterinary Diagnostic Laboratory in Fargo, ND, for a full 17-component toxin screen. Samples were analyzed using a variety of mass spectrometry, ELISA, and HPLC methods with a practical quantitation limit of 0.5 mg/kg.

^b Positive Control diet formulated to contain <0.5 mg/kg DON and all remaining diets formulated to contain 5 mg/kg DON.

^c Deoxynivalenol (DON).

^d DDGS were pelleted then crumbled before being added back to final diet to prevent segregation.

^e Sodium metabisulfite (Samirian Chemicals, Campbell, CA) was added to DDGS at 2.5% prior to pelleting and crumbling into final diet. Final diet contained 0.77% SMB.

^f 15-Acetyldeoxynivalenol (15-ADON).

^g Total DON reported as a combination of DON and 15-ADON, because both DON metabolites have similar toxicity (Pestka, 1987).

At the NFP site, a total of 942 pigs (Fast/PIC × TR4, initially 10.9 ± 0.3 kg BW) were used in a 21-day growth trial with 5 replications per treatment and 28 pigs (14 barrows, 14 gilts) per pen. Pens of pigs were allotted to 1 of 7 treatments based on initial pen weight. Each pen (1.75 × 4.05 m²) contained a 5-hole, dry self-feeder and provided ad libitum access to feed and water.

The 7 experimental treatments consisted of 3 diets fed in meal or pellet form. Diets were: (1) Positive Control (PC; <0.5 mg/kg DON), (2) Negative Control (NC; 4.8 mg/kg DON), and (3) NC + crumbled DDGS (4.8 mg/kg DON). In the seventh treatment, high-DON DDGS were mixed with 2.5% SMB prior to pelleting. After pelleting, DDGS were crumbled and mixed into the diet (4.8 mg/kg DON), with a final dietary concentration of 0.77% SMB. Treatment 7 was fed in meal form and supplemental salt was removed due to concerns regarding the high sodium content in SMB.

Naturally contaminated DDGS with a known DON concentration (Table 3, 16.0 mg/kg) were incorporated at 30% to produce diets with the desired DON concentration. Ten subsamples of corn and the clean and contaminated DDGS source were collected and homogenized into a composite sample for a 17-component mycotoxin analysis at NDSU prior to diet formulation and manufacturing. Apart from DON and SMB content, diets were formulated to be identical in nutrient composition (Table 4). Because of the SMB addition, the inclusion rate of DDGS for the seventh treatment was increased to 31% so the final DDGS content was equivalent to the level in other diets. Diets were formulated to meet or exceed all nutrient requirement estimates (NRC, 1998). Diets were analyzed for sodium and sulfur content at MVTL Laboratory due to concerns that incorporating SMB at high levels may negatively affect performance because of high dietary sodium or sulfur (Til et al., 1972). To prevent segregation of final diet ingredients, diets with pelleted DDGS were crumbled before incorporation in the final diet.

Diets were pelleted at the same pellet mill as in Exp. 3 with conditioning temperatures averaging 63.2 ± 1.8 °C. Dietary corn was processed using a roller mill, and the particle size for diets fed in meal form averaged 650 μ. For treatment 7, DDGS were pelleted using the same pellet mill, then crumbled before adding prior to final diet preparation. Samples of each diet were collected, blended, and subsampled before sending them to NDSU. Experimental diets were fed for 21 days with ADG, ADFI, and G:F determined by weighing pigs and measuring feed disappearance on days 7, 14, and 21.

For statistical analysis, data from the two research sites were pooled and analyzed for location × treatment interactions. Due to a lack of significant interactions, the data were combined and analyzed with research location included in the model as a random effect. Pen was the experimental unit and the fixed factors in the model were pelleting and DDGS source (non-contaminated; contaminated; or contaminated, pelleted and crumbled). The planned contrasts in Exp. 4 included: (1) DON vs. non-contaminated, (2) diet form (pellet vs. meal), (3) pelleting vs. un-pelleted DDGS in NC diets, (4) interactions between pelleting final diets and DDGS source, and (5) interaction between pelleting and pelleting DDGS within NC diets. Finally, a pair-wise comparison contrast evaluated the effects of SMB when DON-contaminated DDGS were pelleted, crumbled, and incorporated into the final diet fed in meal form. For all statistical tests, significance and tendencies were set at $P < 0.05$ and $P < 0.10$, respectively.

2.6. Mycotoxin analysis

In Exp. 1, 2, and 4, feed samples were sent to the NDSU Veterinary Diagnostic Laboratory for a 17-component mycotoxin analysis. The analysis for tricothecene mycotoxins (DON, 15-acetyldeoxynivalenol [15-ADON], 3-Acetyl DON, nivalenol, and

Table 4
Composition of experimental diets, Exp. 4 (as-fed basis).

| Item | Positive Control | Negative Control | NC crumbled ^a | NC crumbled w/SMB ^b |
|-----------------------------------|------------------|------------------|--------------------------|--------------------------------|
| Ingredient, % | | | | |
| Corn | 41.36 | 41.36 | 41.36 | 40.44 |
| DDGS, 26.3% CP | 30.00 | – | – | – |
| Contaminated DDGS, 26.0% CP | – | 30.00 | 30.00 | 31.00 |
| Soybean meal, 46.5% CP | 24.15 | 23.95 | 23.95 | 24.00 |
| Choice white grease | 1.30 | 1.30 | 1.30 | 1.60 |
| Limestone | 1.10 | 1.10 | 1.10 | 1.10 |
| Salt | 0.43 | 0.43 | 0.43 | – |
| Monocalcium phosphate, 21% P | 0.45 | 0.45 | 0.45 | 0.45 |
| Trace mineral premix ^c | 0.08 | 0.08 | 0.08 | 0.08 |
| Copper sulfate | 0.07 | 0.07 | 0.07 | 0.07 |
| Selenium | 0.05 | 0.05 | 0.05 | 0.05 |
| Vitamin premix ^d | 0.03 | 0.03 | 0.03 | 0.03 |
| L-Lys HCl | 0.46 | 0.46 | 0.46 | 0.46 |
| Methionine hydroxy analog | 0.12 | 0.11 | 0.11 | 0.11 |
| L-Thr | 0.07 | 0.07 | 0.07 | 0.07 |
| Medication ^e | 0.40 | 0.40 | 0.40 | 0.40 |
| Mold inhibitor ^f | 0.10 | 0.10 | 0.10 | 0.10 |
| Phytase ^g | 0.04 | 0.04 | 0.04 | 0.04 |
| Total | 100 | 100 | 100 | 100 |
| Calculated analysis | | | | |
| SID ^h amino acids, % | | | | |
| Lys | 1.20 | 1.20 | 1.20 | 1.20 |
| Ile:Lys | 61 | 61 | 61 | 61 |
| Leu:Lys | 147 | 148 | 148 | 148 |
| Met:Lys | 34 | 34 | 34 | 34 |
| Met and Cys:Lys | 58 | 58 | 58 | 58 |
| Thr:Lys | 60 | 60 | 60 | 60 |
| Trp:Lys | 17.5 | 17.5 | 17.5 | 17.5 |
| Val:Lys | 73 | 73 | 73 | 73 |
| Total Lys, % | 1.40 | 1.40 | 1.40 | 1.40 |
| ME, MJ/kg | 13.85 | 13.85 | 13.85 | 13.85 |
| SID Lys:ME, g/Mcal | 3.63 | 3.63 | 3.63 | 3.63 |
| CP, % | 22.3 | 22.4 | 22.4 | 22.4 |
| Ca, % | 0.66 | 0.66 | 0.66 | 0.66 |
| P, % | 0.57 | 0.58 | 0.58 | 0.58 |
| Available P, % | 0.30 | 0.31 | 0.31 | 0.31 |
| Na, % | 0.25 | 0.25 | 0.25 | 0.30 |
| Cl, % | 0.43 | 0.43 | 0.43 | 0.17 |
| Added S, % ⁱ | – | – | – | 0.25 |

^a DDGS (dried distillers grains with solubles) were pelleted then crumbled and added back to final diet to prevent segregation.

^b Sodium metabisulfite (Samirian Chemicals, Campbell, CA); added to contaminated DDGS at 2.5% prior to pelleting and crumbling. SMB level of 0.77% in final diet.

^c Trace mineral premix provided per kilogram of premix: 13.3 mg of Cu, 1.40 mg of I, 134 mg of Fe, 53.3 mg of Mn, and 160 mg of Zn.

^d Vitamin premix provided per kilogram of premix: 22,046,000 IU of vitamin A, 5,291,000 IU of vitamin D₃, 97,002 IU of vitamin E, 10,288 mg of vitamin K, 88.2 mg of vitamin B₁₂, 79,366 mg of niacin, 61.7 mg of pantothenic acid, and 13,228 mg of riboflavin.

^e To provide chlortetracycline at 441 g/t.

^f Ammo Curb (Kemin Industries, Des Moines, IA).

^g Phyzyme 2500 (Danisco Animal Nutrition, St. Louis, MO).

^h Standardized ileal digestible.

ⁱ Originating from sodium metabisulfite (Na₂S₂O₅), which is 33% sulfur.

T-2 toxin) along with zearalenone and zearalenol was conducted according to a modified version of Groves et al. (1999) using gas chromatography coupled with mass spectrometry. Aflatoxins and fumonisins were analyzed by HPLC. Samples were tested on an as-fed basis; the practical quantitation limit for all mycotoxins was 0.5 mg/kg. In Exp. 3, feed samples were sent to MVTL Labs and tested for DON levels using an ELISA test kit (Neogen, 2007) with a range of quantitation between 0.5 and 5.0 mg/kg.

3. Results

3.1. Experiment 1

The DON-contaminated DDGS used in the autoclave pilot study had mycotoxin levels above the practical detection limit for DON (20.6 mg/kg), 15-ADON (3.3 mg/kg), and zearalenone (1.1 mg/kg), but the levels of all other mycotoxins screened were not detected above 0.5 mg/kg. The effects of autoclaving with SMB on levels of DON are shown in Table 5. Autoclaving without SMB addition reduced DON by 18.9% and 15-ADON by 33.3%. The addition of SMB further reduced DON, with 5.0%

Table 5

Effects of sodium metabisulfite (SMB) on mycotoxin concentration in corn dried distillers grains with solubles (DDGS) within an autoclave, Exp. 1 (as-fed basis)^{a,b}

| Sample | SMB ^c , % | Mycotoxin, mg/kg ^d | | | % DON remaining ^f | % 15-ADON remaining ^f |
|-----------------|----------------------|-------------------------------|----------------------|-------------|------------------------------|----------------------------------|
| | | DON ^e | 15-ADON ^e | Zearalenone | | |
| No | – | 20.6 | 3.3 | 1.1 | – | – |
| Yes | 0 | 16.7 | 2.2 | 0.8 | 81.1 | 66.7 |
| Yes | 0.5 | 15.7 | 2.2 | 1.0 | 76.2 | 66.7 |
| Yes | 1.0 | 13.6 | 1.9 | 0.9 | 66.1 | 57.6 |
| Yes | 2.5 | 7.3 | 1.9 | 1.1 | 35.4 | 57.6 |
| Yes | 5.0 | 3.6 | 1.8 | 1.1 | 17.5 | 54.5 |
| Yes + 10% water | 5.0 | 1.2 | 1.5 | 1.1 | 5.8 | 45.4 |

^a DDGS samples were autoclaved for 60 min at 121 °C. After autoclaving, samples were dried in a 55 °C drying oven. A full 17-component mycotoxin analysis was conducted at the North Dakota State University Veterinary Diagnostic Laboratory (Fargo, ND) and used a combination of mass spectrometry, ELISA, and HPLC methods. All mycotoxins present above detectable levels (>0.5 mg/kg) are reported herein.

^b The treatments were not replicated and are provided as observational data only.

^c Sodium metabisulfite (Samirian Chemicals, Campbell, CA); 100% by weight.

^d Levels adjusted back to an as-fed basis (90.1% DM) after drying.

^e Deoxynivalenol (DON) and 15-acetyldeoxynivalenol (15-ADON), respectively.

^f Percentage of mycotoxin relative to amount prior to autoclaving.

SMB reducing DON by 82.5%. Adding 10% water to the 5.0% level of SMB appeared to elicit an additive effect, reducing DON by 94.2%, an 11.7% increase from the 5.0% level alone. The effects of added SMB on 15-ADON were not as clear, but at 5.0% SMB, 15-ADON levels were 12.2% lower than by autoclaving alone, and adding 10% water further reduced 15-ADON by 9.1%. Zearalenone concentrations remained relatively constant regardless of SMB inclusion or the addition of water.

3.2. Experiment 2

The DON-contaminated DDGS averaged 20.5 mg/kg DON and 3.0 mg/kg of 15-ADON prior to pelleting. The effects of pelleting with SMB and conditioning temperature on DON reduction are shown in Table 6. No significant two- or three-way interactions occurred between temperature, retention time, and SMB. Altering the retention time from 30 to 60 s had no effect on DON or 15-ADON. Increasing temperature from 66 to 82 °C had no effect on both DON and 15-ADON. When DDGS were pelleted, increasing SMB inclusion reduced (quadratic; $P < 0.001$) analyzed DON levels with up to 83% reduction at 5.0% SMB. Nevertheless, pelleting with SMB had no effect on 15-ADON concentrations.

3.3. Experiment 3

Mycotoxin analyses found that the PC diet did not contain any mycotoxins above the practical quantification limit (<0.5 mg/kg). Negative Control diets averaged 3.3 mg/kg DON, 0.7 mg/kg 15-ADON, and 0.5 mg/kg zearalenone. When 0.50% SMB was added to the diet, DON was reduced by 26 and 75% for the meal and pelleted forms, respectively, but adding SMB did not affect the concentrations of other mycotoxins in test diets. Mineral analyses showed considerable variation in sodium and sulfur content in experimental diets, but as expected, sodium and sulfur levels generally were higher in diets containing SMB.

During the trial period (day 0–27), no significant pellet \times SMB interactions were observed for growth performance or pig BW (Table 7). Dietary DON levels of 3.3 mg/kg negatively affected ($P < 0.001$) ADG and ADFI and did not influence G:F, but pelleting the diet improved ($P < 0.001$) ADG and G:F without influencing ADFI. When SMB was added to the diet, pigs

Table 6

Effect of pelleting temperature (Temp) and level of sodium metabisulfite (SMB) on deoxynivalenol (DON) and 15-acetyldeoxynivalenol (15-ADON) on corn distillers dried grains with solubles (DDGS) naturally contaminated with DON, Exp. 2 (as-fed basis).

| Item, mg/kg ^c | Temp, °C | SMB, % | | | | SED ^a | Probability, $P < ^{**}$ | | |
|--------------------------|----------|--------|------|-----|-----|------------------|--------------------------|---------------------|-------------------|
| | | 0 | 1.0 | 2.5 | 5.0 | | Temp | Linear ^b | Quad ^b |
| DON ^d | 66 | 20.5 | 10.2 | 5.6 | 3.3 | 1.29 | 0.15 | 0.001 | 0.001 |
| | 82 | 18.7 | 9.0 | 4.2 | 3.6 | | | | |
| 15-ADON ^d | 66 | 2.7 | 2.6 | 2.7 | 2.8 | 0.42 | 0.74 | 0.45 | 0.64 |
| | 82 | 2.8 | 2.5 | 2.8 | 3.0 | | | | |

^a Standard error of the difference for the Temp \times SMB interaction. To obtain SED for effect of Temp and SMB, multiply by 0.50 and 0.71, respectively.

^b Linear and quadratic effects of SMB.

^c Samples analyzed at North Dakota State University Veterinary Diagnostic Laboratory (Fargo, ND) using a variety of mass spectrometry, ELISA, and HPLC methods.

^d DDGS batches; prior to pelleting averaged 20.5 and 3.0 mg/kg for DON and 15-ADON, respectively.

^{*} No significant effect ($P > 0.40$) for retention time in pellet conditioner; thus, data are not shown.

^{**} No significant interactions ($P > 0.69$) between Temp \times SMB.

Table 7
Effects of pelleting and sodium metabisulfite (SMB) on growth performance of nursery pigs fed deoxynivalenol (DON)-contaminated diets, Exp. 3.^a

| Diet form | Negative Control diets (NC; 3 mg/kg DON) ^b | | | | | | | | Probability, P< | | | | | |
|------------|---|-------|--------|-----------------------------|--------|-----------------------------|--------|-------|---------------------------|-------|-----------------|------------|------|--|
| | Positive Control | NC | | NC + 0.25% SMB ^c | | NC + 0.50% SMB ^c | | SEM | Pellet × SMB ^d | DON | Pellet vs. meal | SMB effect | | |
| | Meal | Meal | Pellet | Meal | Pellet | Meal | Pellet | | | | | Linear | Quad | |
| Day 0–27 | | | | | | | | | | | | | | |
| ADG, g | 652 | 596 | 663 | 607 | 675 | 615 | 681 | 10.0 | 1.00 | 0.001 | 0.001 | 0.03 | 0.76 | |
| ADFI, g | 1078 | 988 | 1006 | 1013 | 1017 | 1020 | 1025 | 18.6 | 0.87 | 0.001 | 0.43 | 0.08 | 0.68 | |
| G:F | 0.605 | 0.604 | 0.659 | 0.600 | 0.664 | 0.603 | 0.665 | 0.007 | 0.74 | 0.89 | 0.001 | 0.67 | 0.86 | |
| Pig BW, kg | | | | | | | | | | | | | | |
| Day 0 | 13.07 | 13.04 | 13.08 | 13.03 | 12.97 | 13.02 | 13.04 | 0.212 | 0.59 | 0.68 | 0.97 | 0.61 | 0.33 | |
| Day 7 | 16.62 | 15.85 | 16.44 | 15.98 | 16.38 | 15.91 | 16.51 | 0.241 | 0.62 | 0.001 | 0.001 | 0.55 | 0.99 | |
| Day 14 | 21.28 | 20.06 | 20.98 | 20.32 | 21.04 | 20.42 | 21.09 | 0.292 | 0.72 | 0.001 | 0.001 | 0.15 | 0.76 | |
| Day 21 | 26.07 | 24.85 | 26.04 | 25.21 | 26.16 | 24.90 | 26.34 | 0.327 | 0.44 | 0.001 | 0.001 | 0.35 | 0.35 | |
| Day 27 | 30.67 | 29.26 | 31.17 | 29.53 | 31.19 | 29.63 | 31.55 | 0.386 | 0.79 | 0.001 | 0.001 | 0.10 | 0.83 | |

^a A total of 987 mixed-sex pigs (initially 13.0 ± 0.2 kg BW) were used in a 27-day experiment with 5 replicate pens per treatment and 28 pigs per pen.

^b Analyzed mycotoxin levels in Negative Control diets averaged: 3.3 mg/kg DON, 0.7 mg/kg 15-ADON, and 0.5 mg/kg zearalenone.

^c Sodium metabisulfite (Samirian Chemicals, Campbell, CA).

^d Each contrast compared the following treatments: (1) “Pellet × SMB” evaluated the two-way interaction between pelleting diets and adding SMB in the 6 NC treatments; (2) “DON” compared Positive Control to Negative Control (NC), both meal and pellet form; (3) “Pellet vs. Meal” compared final diet form in treatments 2–7; and (4) “SMB effect” compared the linear and quadratic effects of adding increasing levels of SMB in treatments 2–7.

had increased (linear; $P < 0.03$) ADG and tended to have increased (linear; $P < 0.08$) ADFI, but had no impact on G:F. For pig BW, responses were consistent from day 7 through day 27 for DON and pelleting effects, with pigs fed DON-contaminated diets weighing less ($P < 0.001$) and pigs fed pelleted diets weighing more ($P < 0.001$). The effects of SMB on pig BW were non-significant on days 7, 14, and 21, but pigs fed diets containing SMB tended (linear; $P < 0.10$) to be heavier at the end of the trial.

3.4. Experiment 4

Mycotoxin analysis of the PC diets found no mycotoxin concentrations above the practical quantification limit (< 0.5 mg/kg). Although NC diets were formulated at 4.8 mg/kg DON based on the analysis of the contaminated DDGS (16.0 mg/kg), analyzed DON levels in NC diets averaged 3.4 mg/kg. Nevertheless, based on previous research (Frobose et al., 2015) these analyzed DON levels should elicit an approximate 10% or greater reduction in growth performance. Interestingly, the treatment 7 diet, which contained 0.77% SMB, had a lower DON level at 1.6 mg/kg, which suggests that DON may have been converted to another structural form such as DON-S (Young, 1986a) when SMB was present during the DDGS pelleting process. As in Exp. 3, adding SMB did not alter 15-ADON levels. For the mineral analyses, sodium and sulfur levels were relatively constant, except for treatment 7. In treatment 7, where no salt was added, analyzed sodium levels were slightly lower (0.23 vs. 0.29%) and sulfur levels were higher (0.57 vs. 0.44%) than pigs fed NC diets using the same DDGS.

Overall (day 0–21), a two-way interaction was observed within NC diets in which pelleting only the final diet improved G:F ($P < 0.04$) by a greater margin in high-DON diets than in treatments in which the DDGS was pelleted, crumbled, then re-pelleted in the final diet (Table 8). No other two-way interactions were detected for ADG, ADFI, or BW. Pigs fed diets containing high DON (3.4 mg/kg average) had decreased ($P < 0.002$) ADG and ADFI and pig BW throughout the test period, no differences occurred in feed efficiency. Conversely, pigs fed pelleted diets had increased ($P < 0.01$) ADG, BW, and improved ($P < 0.001$) G:F. Throughout the trial period, pelleting the DDGS prior to final diet manufacturing (meal or pellet form) had no effect on growth performance or pig BW. Finally, including SMB prior to pelleting DDGS and feeding diets in meal form increased ($P < 0.01$) ADG and ADFI, but feed efficiency was not affected by adding SMB. Sodium metabisulfite inclusion did not affect pig BW at day 7, but incorporating SMB to Negative Control diets (meal form) prior to pelleting DDGS resulted in heavier ($P < 0.03$) pig BW at day 14 and day 21.

4. Discussion

Previous research has shown that when DON is combined with SMB or its aqueous form (sodium bisulfite) in a hydrothermal environment, it is readily converted to DON-S in corn (Young, 1986a) and wheat (Dänicke et al., 2005). Using a combination of hydrophilic interaction chromatography and tandem-mass spectrometry, a more recent study by Beyer et al. (2010) verified that the decrease in DON concentration was directly correlated to an increase in DON-S. This conversion is especially important from a toxicological point of view, because Young et al. (1987) saw no acute toxic effects when DON-S was fed to pigs at concentrations equivalent to DON levels that elicited emesis.

Young et al. (1987) showed that in an autoclave environment, DON levels in corn could be reduced using aqueous sodium bisulfite. Experiment 1 of the present study, which used naturally contaminated DDGS, supports this research by confirming that DON concentrations could be reduced by adding SMB in an autoclave. Compared with the results of Young et al. (1987), the level of reduction seen in the current study was comparable when no SMB was added (10 vs. 12%) and showed a similar decline with increasing SMB levels, with a greater overall reduction at 5% SMB (81 vs. 65%). Although SMB alone effectively detoxified a large proportion of DON, adding water further reduced DON levels with 5% SMB, which suggests that structural modification of the DON molecule occurs more rapidly in the presence of water.

Although autoclaving alone reduced DON concentrations in naturally contaminated DDGS, pelleting alone did not alter DON or 15-ADON levels in naturally contaminated DDGS, which suggests that the reductions seen in the autoclave may be related to the increased duration or the additional heat present in the autoclave. Earlier research on the effects of hydrothermal treatment alone on DON have been contradictory: Dänicke et al. (2005) saw no effect in a laboratory conditioner at 100 °C with naturally contaminated wheat, but when DON-inoculated corn flour was cooked in an extruder (150 °C, 15% moisture), DON was reduced by 98% (Cazzaniga et al., 2001). In Exp. 2, pelleting alone did not alter DON or 15-ADON levels in naturally contaminated DDGS, which suggests that reductions seen in the autoclave and previous extrusion research may relate to the duration of exposure or the additional heat and moisture present. Levels of DON in pelleted DDGS decreased by approximately 50% with 1.0% SMB and reduced further at higher levels, but the response seemed to plateau somewhere between 2.5 and 5.0% SMB. Although the two conditioning temperatures (66 and 82 °C) did not significantly affect DON reduction, results suggest that at 82 °C, DON levels were lower than at 66 °C when intermediate levels of SMB were added. Dänicke et al. (2005) effectively reduced 7.5 mg/kg DON wheat to below 1 mg/kg within 3 min (the earliest data point) using a laboratory conditioner at 98–102 °C with a 1.0% addition of SMB. While retention time did not influence DON reduction in the present study, results of Dänicke et al. (2005) and Cazzaniga et al. (2001) suggest that if higher conditioning temperatures could be achieved in a pellet mill, further DON detoxification may occur. Although DON was reduced to a greater extent during pelleting than in an autoclave, 15-ADON levels were not affected by pelleting with SMB, for reasons that remain unclear. A final consideration is the release of sulfur dioxide gas when SMB is treated hydrothermally. When levels of 2.5%

Table 8Effects of pelleting, dried distillers grains with solubles (DDGS) source, and sodium metabisulfite (SMB) on growth performance of nursery pigs fed deoxynivalenol (DON)-containing diets, Exp. 4.^a

| Diet form | Positive Control | | Negative Control ^b | | NC + crumbled DDGS ^c | | NC + crumbled DDGS w/SMB ^d | SEM | Probability, <i>P</i> < ^e | | | | | |
|------------|------------------|--------|-------------------------------|--------|---------------------------------|--------|---------------------------------------|-------|--------------------------------------|---------------|-------|-----------------|----------------|-----|
| | Meal | Pellet | Meal | Pellet | Meal | Pellet | | | Meal | Pellet × DDGS | DON | Pellet vs. meal | Pelleting DDGS | SMB |
| Day 0–21 | | | | | | | | | | | | | | |
| ADG, g | 584 | 628 | 520 | 582 | 543 | 581 | 577 | 10.5 | 0.15 | 0.001 | 0.001 | 0.21 | 0.01 | |
| ADFI, g | 881 | 875 | 791 | 801 | 799 | 807 | 848 | 18.0 | 0.94 | 0.001 | 0.68 | 0.55 | 0.01 | |
| G:F | 0.664 | 0.718 | 0.657 | 0.729 | 0.680 | 0.720 | 0.681 | 0.020 | 0.04 | 0.45 | 0.001 | 0.33 | 0.95 | |
| Pig BW, kg | | | | | | | | | | | | | | |
| Day 0 | 11.21 | 11.22 | 11.14 | 11.19 | 11.12 | 11.15 | 11.24 | 0.317 | 0.97 | 0.65 | 0.84 | 0.86 | 0.60 | |
| Day 7 | 14.50 | 14.68 | 13.74 | 14.37 | 13.73 | 14.20 | 14.15 | 0.267 | 0.70 | 0.002 | 0.01 | 0.67 | 0.15 | |
| Day 14 | 17.98 | 18.45 | 16.84 | 17.92 | 16.92 | 17.62 | 17.66 | 0.358 | 0.41 | 0.001 | 0.001 | 0.63 | 0.03 | |
| Day 21 | 22.63 | 23.51 | 21.27 | 22.54 | 21.62 | 22.50 | 22.59 | 0.455 | 0.47 | 0.001 | 0.001 | 0.58 | 0.01 | |

^a A total of 1180 mixed-sex pigs (initially 11.2 ± 0.3 kg BW) were used in a 21-day study conducted concurrently at Kansas State University Swine Teaching and Research Center (Manhattan, KS) and New Fashion Pork Research Nursery (Buffalo Center, IA). At each location, there were 5 replicate pens per treatment with 7 and 28 pigs per pen, respectively.

^b Analyzed mycotoxin levels in Negative Control diets averaged 3.4 mg/kg DON and 0.6 mg/kg 15-ADON.

^c DDGS was pelleted, then crumbled and added back to final diet to prevent segregation.

^d Sodium metabisulfite (Samirian Chemicals, Campbell, CA) was added to DDGS at 2.5% prior to pelleting and crumbling into final diet. Final diet contained 0.77% SMB.

^e Each contrast compared the following treatments: (1) "Pellet × DDGS" evaluated the 2-way interaction between pelleting DDGS and pelleting final diets in the 4 Negative Control (NC) treatments; (2) "DON" compared Positive Control to NC without crumbling, both meal and pellet form; (3) "Pellet vs. Meal" compared final diet form in the first 6 treatments; (4) "Pelleting DDGS" compared the effect of pelleting DDGS and crumbling before final diet manufacturing in the 4 NC treatments; (5) "SMB" compared treatment 5 to treatment 7, where NC DDGS were pelleted, crumbled, and fed in meal form, isolating the effect of adding SMB.

and 5.0% SMB were used, air quality in the feed mill was such that all personnel wore safety goggles and respirators. Future research in which SMB is hydrothermally treated should take this gaseous release into account.

Feeding DON-contaminated feedstuffs treated with SMB has improved piglet growth in previous studies (Young et al., 1986b, 1987; Dänicke et al., 2005), but these studies used a limited number of animals. Yet, to the authors' knowledge, the present study is the first to incorporate SMB in final diet manufacturing and to evaluate the effects of SMB and pelleting together on pig growth in a commercial environment. The present data agree with Dänicke et al. (2005), who observed improvements in ADG and ADFI using a final dietary SMB concentration of 0.25%, but the improvement was more pronounced in the present study with 0.50% added SMB. Although SMB improved nursery pig growth in both meal and pelleted form compared with NC pigs in Exp. 3, growth was not restored to levels of the Positive Control. This result may be attributed to the remaining DON levels in Exp. 3, because adding only 0.25% and 0.50% SMB to final diets did not reduce DON greatly. When these diets were pelleted, analyzed DON levels decreased by 48% and 75%, but surprisingly, no two-way interactions occurred with pelleting and SMB on piglet growth. These results may be related to when hydrothermal SMB treatment is applied, because in this experiment it was applied only to final diets. In contrast, both Young et al. (1987) and Dänicke et al. (2005) reduced DON by over 90% by treating only the contaminated grain, which in both cases resulted in pig growth performance similar to those fed uncontaminated grain. We hypothesize that additional factors may have limited the effectiveness of SMB in Exp. 3, including any effects that SMB may have on diet palatability and the influence of higher levels of sulfur in the diet. However, in this experiment, pelleting alone improved ADG and G:F by 10 and 13%, which was higher than for pigs fed PC diets in meal form. These results suggest that pelleting DON-contaminated diets without SMB may provide another alternative to offset DON-associated effects on performance.

Experiment 4 was designed to further validate the effect of pelleting DON-contaminated diets as well as to evaluate pelleting the contaminated DDGS with and without SMB as a potential commercially applicable rapid method for detoxifying DON. Although diets were formulated to contain higher levels of DON (5 mg/kg), due to what we conclude was an initially inaccurate analysis of the contaminated DDGS, dietary levels of DON averaged 3.4 mg/kg in NC diets, which was still sufficient to reduce pig growth rate by 11% and 7.5% for diets fed in meal and pellet form, respectively. As in previous research (Etienne and Waché, 2008) and Exp. 3 of the present study, the biggest reduction in performance due to DON was during the initial 7-day period (16%), although pigs fed NC diets still grew 7% slower than pigs fed the PC from day 7–21. In accordance with the findings of Döll et al. (2007) and Trigo-Stockli et al. (2000), which used DON-contaminated wheat, as well as the results of Exp. 2 and 3 of the present study, the pelleting process did not alter the concentration of DON in the feed. This result was true whether the DON-contaminated DDGS were pelleted first and then re-ground and fed in either meal or pellet form or if the pelleting occurred only in the final diet. Pelleting DON-contaminated diets did improve growth performance to the level of pigs fed uncontaminated diets in meal form, but no two-way interactions occurred in which pelleting affected piglets' response to diets containing DON. A two-way interaction was observed for feed efficiency when high-DON DDGS were pelleted before adding to a meal diet, but not when added to a pelleted diet. Pigs fed DDGS that were crumbled back into a meal diet may have had enhanced feed efficiency from pelleting the DDGS, which could enhance nutrient digestibility. Additionally, Döll et al. (2007) suggested that because *Fusarium* infection in wheat causes other effects besides mycotoxin contamination, such as decreased starch content (Mätthaus et al., 2004), altering the grain composition by pelleting could alter the effect of DON. Although the effects of DON contamination on nutrient composition in DDGS went largely unconsidered in the present study, the lack of interaction for diet form and the inclusion of DON for both the DDGS alone and final diets makes it unlikely that any variation in the effects of DON were related to changes in the DDGS nutrient profile. In Exp. 4, SMB was added at a higher level (2.5%) than in Exp. 3 and was added prior to DDGS pelleting rather than during final diet manufacturing. Final diet DON levels were decreased (3.4 vs. 1.6 mg/kg) in this experiment by using 2.5% SMB when pelleting the DDGS, but only by 53%, which is less than the 75% detoxification seen with 0.5% SMB in Exp. 3 and the same result when 2.5% SMB was added in Exp. 2. The reason for the limited reduction is unknown, but this result may indicate that although pelleting with SMB can effectively reduce DON concentrations, variation in the extent of DON reduction that can occur in commercial pellet mills is considerable. Nevertheless, adding SMB prior to pelleting DDGS, re-grinding, and offering in meal form resulted in a 6% improvement in piglet growth rate, which was similar to pigs fed PC diets in meal form.

The introduction of additional sulfite and sodium into pig diets must also be taken into account when interpreting SMB utilization. Although sodium levels were not balanced in Exp. 3, analyzed sodium content of the diets showed only minimal increases in total sodium level when 0.25 or 0.50% SMB was added to the diet. Interestingly, when diets were balanced for sodium level during Exp. 4, analyzed sodium levels were slightly lower (0.23 vs. 0.30%) in pigs fed diets with SMB than in all other treatments. Nevertheless, this level is still above the 0.15% Na requirement of 10- to 20-kg pigs (NRC, 1998); therefore, influence of sodium on piglet performance appears to be unlikely in either study. Although not taken into account in the present growth experiments, the effect of additional sulfite in the diet when adding SMB is of greater concern. Til et al. (1972) conducted long-term studies on the toxicity of sulfite in pigs using SMB as the source and reported that growth performance was reduced at 0.83 and 1.72% SMB. Also noteworthy is the report of Til et al. (1972) that showed rapid losses of thiamine when SMB was prepared in wet diets prior to feeding. Sulfite destroys thiamine (Hermus, 1969; Joslyn and Leichter, 1968), and sulfur-induced thiamine deficiency has been implicated in reduced growth performance in pigs (Gibson et al., 1987). Although these previous studies lasted much longer, additional research is needed to evaluate the thiamine status of pigs fed diets using SMB for DON detoxification to develop recommended feeding levels and duration of inclusion in swine diets. In the present study, analyzed sulfur content did not exceed 0.57%, which is likely insufficient to cause thiamine deficiency;

however, higher levels may affect feed intake. Til et al. (1972) reported decreased feed intake due to palatability issues when 0.83% SMB was fed in a pair-feeding study. A similar decrease in ADFI was seen by Dänicke et al. (2005), but only in the pigs fed 2.5% SMB in uncontaminated wheat-based diets, whereas in a second experiment, pigs fed uncontaminated wheat with 1.0% SMB performed similarly to controls. Although SMB was not added to PC diets in the present study, SMB levels in diets did not exceed 0.77%, so it is unlikely that SMB-related palatability issues were a factor. Based on the results of Dänicke et al. (2005) and Til et al. (1972), feeding levels above 1.0% SMB for short periods or above 0.83% over long periods with supplemental thiamine may reduce growth performance.

In conclusion, DON from naturally contaminated DDGS can be greatly reduced when treated with SMB in a commercial pellet mill, although the influence of processing conditions on level of DON detoxification is negligible. Pelleting with SMB results in sulfur dioxide gaseous release, which is aversive to the eyes and respiratory tract. Future application of this method needs to account for adverse processing conditions. Adding 0.25–0.50% SMB to final diets improved growth performance in both meal and pelleted form. Pelleting 16 mg/kg DON-contaminated DDGS with SMB reduced analyzed DON and, when fed at 30% of the diet prepared in meal form (3.4 mg/kg), pigs performed similarly to those fed uncontaminated diets. Sodium metabisulfite did not affect other tricothecenes (15-ADON or zearalenone) present in naturally contaminated DDGS, however, additional research is needed to evaluate the effects of SMB on sulfur-induced thiamin deficiency and palatability of diets. Overall, when feeding high-DON diets, using pelleting to improve feed efficiency can help offset DON-related losses in growth performance; furthermore, including low levels of SMB and pelleting may serve as a management practice to utilize DON-contaminated feedstuffs without sacrificing growth performance.

Conflict of interest

The authors wish to state that there are no conflicts of interest with this manuscript.

Acknowledgements

Appreciation is expressed to Hubbard Feeds Inc. (Mankato, MN) for supplying the deoxynivalenol-contaminated DDGS; and New Fashion Pork (Jackson, MN) for providing animals and research facilities.

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