



Evaluation of the Nutritive Value of Pearled Deoxynivalenol (DON)-contaminated Barley in Swine Nutrition

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ABSTRACT

Effect of commercial pearling on digestible energy (DE) value, crude protein (N) and amino acid (AA) digestibility of DON-contaminated barley fed to pigs was evaluated. Six barrows with an initial body weight (BW) of 52.5 ± 2.7 kg and fitted with a simple T-cannula at the distal ileum were assigned to three dietary treatments according to a replicated 3 x 3 Latin square design. The experimental diets contained pearled DON-contaminated barley: 1.2, 4.4 and 7.6ppm DON as the only source of energy and N in the diet. Chromic oxide (0.4%) was added as the digestibility marker. Daily feed allowance for the pigs were fixed at 2.6 x maintenance energy requirements based on the BW of the pigs at the beginning of each experimental period. Feed was offered at 8:00 and 16:00 h. Experimental periods lasted 8-day with 4-day of adaptation to experimental diets followed by 2-day each of fecal and digesta collections for energy, N and AA digestibility. DE values for 1.2 and 7.6ppm DON barley diets were significantly ($P < 0.05$) higher than their predicted values. DE of the 4.4ppm DON diet was similar ($P > 0.05$) to the predicted value. Apparent ileal N and AA digestibility (AID) values were mostly similar ($P > 0.05$) amongst diets except for arginine and methionine for the indispensable AAs and glutamic acid, glycine and proline for the dispensable AAs. It was concluded that commercial pearling enhances the nutritive value of DON-contaminated barley for swine.

INTRODUCTION

Barley grain is a common feedstuff used primarily as an energy source in swine diets (NRC, 2012). However, it also provides N and AAs to the animal requirements. Nevertheless, the presence of DON in barley makes its use difficult in swine diets as pigs are very sensitive to DON leading to feed refusal and reduced growth rate

(House *et al.*, 2002; Dersjant-Li *et al.*, 2003). House *et al.* (2003) using a small scale laboratory de-huller demonstrated that pearling was effective in removing DON from DON-contaminated barley. Additionally, they also showed that pearling was effective in removing fibres found in barley where DON concentrates during barley infection by DON. Therefore, apart from DON removal from DON-contaminated barley pearling efficacy

in removing fibres in the pearled barley resulted in improved predicted DE of the pearled barley versus the intact DON-contaminated barley in that study.

Since the study of House *et al.* (2003) was done at the small scale level, there is a need to evaluate the findings of House *et al.* (2003) at the commercial level before the pearling technology can be recommended for adoption by farmers. To this standpoint therefore, there is the need to evaluate the effect of commercial pearling strategy on DE values, ileal N and AAs digestibilities in the pig in order to characterize the process relative to the use of DON-contaminated barley in swine nutrition. To date no studies have determined the DE content and ileal N and AAs digestibility values of pearled DON-contaminated barley. Therefore, the objectives of this study are to determine the DE value of commercially-pearled DON-contaminated barley fed to growing pigs and to also determine the effect of commercial pearling on the ileal digestibility values of N and AAs of DON-contaminated barley fed to growing pigs.

MATERIALS AND METHODS

Barley Samples and Diets

Low DON barley (1.2 ppm) and high DON barley (7.6 ppm) were used to make a synthetic 4.4 ppm DON barley by mixing thoroughly the low and high DON barleys at a ratio of 1: 1 using the Marion mixing machine for 10-minutes. Each barley treatment: 1.2, 4.4 and 7.6 ppm were pearled by passing them through the commercial-scale Satake™ cereal abradar 3-times (passes). Prior to commencement of study, each barley sample was ground through a 1-mm screen and used to determine their DE, N and AAs contents prior to formulating the diet used in the current study.

The diets were formulated to contain 96.3% barley, 3.34% minerals and vitamins. 0.4% chromic oxide was added to the diets to serve as the indigestible marker for determining energy, N and AAs digestibilities (Table 1). The pearled barley type in each diet was the only source of energy, N and AAs in the diet. Vitamins and minerals were supplied at levels to meet or exceed the requirements for 50 – 70 kg BW pigs as defined by NRC, (1998).

Table 1: Composition of Experimental Diets (as-fed basis)

Ingredient	Experimental Diet (%)
Pearled Barley	96.26
Limestone	1.03
Dicalcium Phosphate	0.81
Mineral Premix ^a	0.50
Vitamin Premix ^b	0.50
Salt	0.50
Chromic Oxide	0.40
Nutrients	Calculated Levels
DE (kcal/kg)	3,285
CP (%)	10.26
Calcium (%)	0.61
Total Phosphorus (%)	0.50
Sodium (%)	0.19
Chloride (%)	0.36

^aProvided the following per kilogram of diet: Zn, 100mg; Fe, 80mg; Mn, 25mg; Cu, 50mg; I, 0.5mg; Se, 0.1mg.

^bProvided the following per kilogram of diet: vitamin A, 8250IU; vitamin D₃825IU; vitamin E, 40IU, menadione, 4mg, thiamine, 1mg; riboflavin, 5mg; d-pantothenic acid, 15mg, niacin, 35mg; vitamin B₁₂,0.025mg; d-biotin, 0.2mg; folic acid, 2mg. Calculated DE (kcal/kg) was based on the predicted value for pearled barley.

Animals and Housing

Six Cotswold barrows with an average initial BW of 52.5 ± 2.7kg were obtained from Glenlea Research Farm and housed in individual metabolism crates (118 cm x 146 cm) with smooth transparent plastic sides and tender foot floors in a temperature controlled room (20°C). After a 7-day adjustment period to their environment the pigs were surgically fitted with simple T-cannulae at the distal ileum according to the procedures of Sauer *et al.* (1983).

Feed was removed in the afternoon of the day preceding the surgery. After each surgery the pig was immediately returned to its crate. They were allowed 11-days to recover and regain their pre-surgery appetites. On the 1st day of surgery the pigs were fed 50g of their commercial grower diet in the evening after regaining their consciousness and thereafter feed allowance was gradually increased by 50g by the next feeding daily until they attained their pre-surgery appetites fully. Pigs had unlimited access to water. A day prior to and three days

after surgery each pig received Excenel (Upjohn company, Orangeville, Ontario, Canada) intramuscularly at a dose of 1 ml/17 kg live weight. Pigs were cleaned twice daily at 8:00 h and 16:00 h except the two days of digesta collections with Hibitane skin cleaner (Ayerst Laboratories, Division of Wyeth-Ayerst Canada Inc. Montreal, Canada) and then skin smeared with zincoderm (Rhone Merieux Canada Inc.) to minimize skin irritation due to the emissions of digesta around the cannula. The University of Manitoba Animal Care Committee approved the use of the pigs and experimental procedure of study and pigs were cared for according to the guidelines of the Canadian Council on Animal Care (CCAC, 1993).

Experimental Design and General Conduct of Study

The experiment was designed and carried out as a replicated 3 x 3 Latin Square Design (LSD). However, the experiment was initially planned to be 6 x 6 LSD with the six simple T-fitted ileal cannulae pigs involving the three intact DON-contaminated barley diets and their pearled counterpart diets. However, the experiment was re-designed to a replicated 3 x 3 LSD because the pigs refused to ingest the intact-DON barley diets even the low 1.2ppm DON diet; confirming the anorectic effects of DON in swine nutrition.

Each experimental period lasted 8-days. Pigs received their daily feed allowance in two equal portions at 08:00 and 16.00 h, respectively. Feed intake was closely monitored especially for orsts to be able to measure actual feed intake. Daily feed allowance was maintained at 2.6 x daily maintenance energy requirements based on the BW of the pigs at the beginning of each experimental period (Agricultural Research Council, 1981). Pigs had unlimited access to water at all times throughout the study period. During study, at the end of each period animals were usually removed from their individual metabolism crates to individual exercise pens for three days during which they were fed standard commercial grower diet. This was necessary to neutralize any possible stress the animal might have experienced due to confinement in the crates. Pigs were usually weighed before the beginning of each of the periods consisting of 8-days of 4-days adaptation period to diets, 2-days of fecal collections and 2-days of digesta collections, respectively. This means during any one period, pigs were fed their respective diets from day 1 to 4 (adaptation period to diet), followed by 2-days (d 5 and 6) fecal collections from 08:00 to 16:00 and another 2-days (d 7 and 8) of digesta collections starting from 08:00 to 20:00 h every 2 h intervals, respectively. Digesta were collected into plastic bags containing 10 ml of 10% formic acid solution to minimize microbial activity. Bags were usually attached to the cannulas with hose clamps and were changed every 2 h throughout the 2-days of digesta collections. Both fecal and digesta collected were immediately snap frozen at -23°C for later analyses.

Sample Processing and Chemical Analyses

Both fecal and digesta samples were thawed and pooled for each pig within a collection period. Digesta samples were thoroughly mixed for 15 s using a heavy duty blender (Model 38BL56, SERIAL No. 536024, Torrington, Connecticut, USA). After mixing 400 ml of each mixed digesta sample was collected in sample bags and they as well as the entire pooled fecal samples were freeze-dried. For the pooled and mixed digesta samples the remnants were collected in large sample bags and immediately frozen again at -23°C in case there would be a need for further analyses. Diet samples, freeze-dried fecal and digesta samples were ground in a Wiley mill through a 1-mm screen and thoroughly mixed before being used for the analyses.

Diet, fecal and digesta samples were analyzed for dry matter (DM) and chromic oxide. Fecal samples were further analyzed for gross energy (GE) by Adiabatic Bomb Calorimeter (Parr Instrument Company Inc. Moline, Illinois, USA). DM contents for diet, fecal and digesta were determined by weighing out 2 g of sample into a pre-weighed silica dish and dried to a constant weight at 105°C for 24 h (AOAC, 2000). Samples were then removed and allowed to cool down in a desiccator and re-weighed. DM determined as: (%DM = final weight / initial weight x 100). Crude protein (N) contents were determined as: (N x 6.25) for diets and ileal-digesta using Leco NS 2000 Nitrogen Analyzer (Leco Corporation, St. Joseph, MI, USA, and Model No. 602-000-500, Serial No. 3611). Chromium concentration in diets, digesta and faeces was determined according to the procedure of Williams *et al.* (1962). Amino acids (AA) concentration in diet and digesta was determined using the standard procedure of AOAC (2000). Tryptophan was not measured because it is destroyed by acid hydrolysis.

Digestibility Calculations

DE value for each barley type diet was calculated as described by Adeola (2001) using the index method. Accordingly, the digestibility coefficient (DC) of each diet was calculated as:

$$DC (\%) = 100 - [100 (M_{\text{feed}}/M_{\text{feces}}) \times (N_{\text{feces}}/N_{\text{feed}})]$$

where:

M_{feed} = marker (chromic oxide) concentration in the feed; M_{feces} = marker concentration in the feces; N_{feces} = concentration of energy component in feces (%) and N_{feed} = concentration of energy in feed (%). The component of interest in feces and feeds was gross energy (GE). The % DC obtained for the diets using the pig fecal samples from the diets within periods for individual pigs were converted to DE values for the three DON-contaminated pearled barley-based diets as: DE = %DC x gross energy (GE) of the diet.

Similarly, the apparent N digestibility and ileal AA digestibility of each AA acid for individual pigs were

calculated for each diet using marker content in feed and digesta as: N digestibility coefficient = $100 - [100 (M_{\text{feed}}/M_{\text{digesta}}) \times (N_{\text{digesta}}/N_{\text{feed}})]$ where M_{feed} is the concentration of marker in feed; M_{digesta} is the concentration of marker in digesta; N_{digesta} is the concentration of nutrient in digesta and N_{feed} is the concentration of nutrient in feed. Apparent ileal AA digestibility (AID) was derived as: $AID = 100 - [100 (AA_d/AA_f) \times (Cr_f/Cr_d)]$ where AA_d is the AA content in ileal digesta; AA_f is the AA content in feed; Cr_f is chromium content in feed and Cr_d is the chromium content in the ileal digesta. All analyses were performed on dry matter basis.

Statistical Analysis

Analysis of variance was carried out using the GLM of SAS (SAS Institute Inc., 1988). Bonferoni's test was used to compare means at α - level for significance of $P \leq 0.05$. The model used was $Y_{ijk} = \mu + D_i + P_j + A_k + E_{ij(k)}$; where Y_{ijk} = the digestibility of the k^{th} pig fed the i^{th} diet in the j^{th} period; μ = the population mean; D_i = the effect of the i^{th} diet; P_j = the effect of the j^{th} period; A_k = the effect of the k^{th} pig and $E_{ij(k)}$ = the residual error. The premise for comparisons of the actual DE values (sample means) obtained in the in vivo studies from the pigs for the three barley-based diets belong to the population mean (predicted or calculated DE values) obtained in vitro. A t -test was therefore conducted to compare the sample means with the population means using t_8 degree of freedom from the ANOVA table obtained from the proc GLM procedure (Table 2) and the standard error of the means (11.35). The null hypothesis was: there is no difference between sample and population means for each of the three barley diet types ($H_0: X - \mu = 0$); while

the alternative hypothesis is that sample and population means are not the same ($H_a: X - \mu \neq 0$); $\alpha = 0.05$. Since the test is 2-tailed, the value of $\alpha/2 = 0.025$ was used as the two-side critical region. Critical region of $t_{0.025(8)} = 2.306$. Accordingly, the decision rule was reject the H_0 if $t_{\text{calculated}} \geq 2.306$; formula = $X - \mu/\text{SEM}$. For the 1.2 ppm DON diet: $3803.5 - 3756.9/11.35 = 4.1$; 4.4 ppm DON diet = $3741.1-3752.8/11.35 = -1.033$ and 7.6 ppm DON diet = $3771.07-3662.69/11.35 = 9.549$.

RESULTS

The animals appeared healthy throughout the experimental duration and readily consumed their daily feed allowance without any feed rejection and thus were seen to be growing during the study period. This also confirmed that commercial pearling was effective in removing DON from the pearled barleys resulting in the elimination of anorectic effect of DON from the diets. This is further supported by the fact that the pigs grew as feed intakes were not perturbed during the experimental period leading to increased final average body weight of the pigs at the end of the study to 69.4 ± 3.5 kg. These observations are further cemented by the refusal of the pigs to consume the intact-barley diets but not their pearled counterpart diets. The DE contents of the three pearled barley-based diets are presented in Table 2. The DE contents of 1.2 ppm DON pearled barley diet was significantly ($P < 0.05$) greater than that of 4.4 ppm but not the 7.6 ppm DON pearled diet. Nevertheless, the DE of 4.4 ppm DON pearled diet was not different ($P > 0.05$) from that of 7.6 ppm DON diet.

Table 2: DE contents of pearled DON-contaminated barley diets fed to growing pigs

Item	1.2 DON	4.4 DON	7.6 DON	SEM ^c	P-value
Predicted					
DE (kcal/kg)	3756.9	3752.8	3662.7		
Determined					
DE (kcal/kg)	3803.5 ^a	3741.1 ^b	3771.1 ^{ab}	11.35	0.0144

^cStandard error of the mean; ^{a,b}DE values with different subscripts indicate significant ($P < 0.05$) differences between diets.

The corresponding predicted or calculated DE values of the three barley diets were based on barley swine prediction equation of Fairbairn *et al.* (1999). The summary of the hypotheses as they relate to the

comparisons between the actual (determined) and predicted DE values for the barley-based diets are presented in Table 3.

Table 3: Results of hypotheses and inferences for the three barley-diets

Barley Type	Sample mean (kcal/kg) n = 6	Population mean (kcal/kg) N = 2	Difference (kcal/kg)	T _{cal}	Inference made
1.2 ppm	3803.5	3756.9	46.5	4.1	Fail to accept H_0
4.4 ppm	3741.1	3752.8	- 11.7	- 1.033	H_0 accepted
7.6 ppm	3771.1	3662.7	108.4	9.549	Fail to accept H_0

The dry matter (DM), N and AA contents (%) of the pearled barley diets used in this study are shown in Table 4.

Table 4: Analyzed DM, N and AAs content (%) of pearled DON-contaminated barley-based diets (DM basis)

Item	DIETS		
	1.2 ppm	4.4 ppm	7.6 ppm
Dry matter	91.73	91.13	89.76
N	11.69	12.49	13.39
Indispensable amino acids			
Arginine	0.51	0.60	0.64
Histidine	0.32	0.33	0.33
Isoleucine	0.85	0.95	0.97
Leucine	0.40	0.48	0.55
Lysine	0.39	0.45	0.46
Methionine	0.14	0.18	0.14
Phenylalanine	0.63	0.68	0.76
Threonine	0.41	0.52	0.49
Valine	0.59	0.69	0.74
Dispensable amino acids			
Alanine	0.46	0.49	0.49
Aspartic acid	0.73	0.79	0.79
Cystine	0.25	0.27	0.28
Glutamic acid	3.13	3.58	3.63
Glycine	0.48	0.54	0.53
Proline	1.37	1.56	1.67
Serine	0.57	0.63	0.65
Tyrosine	0.34	0.35	0.40

The apparent ileal digestibilities of N and AA of the three pearled barley diets are presented in Table 5.

Table 5: Apparent ileal digestibilities of N and AAs in pearled DON-contaminated diets fed to growing pigs

Item	1.2 DON diet	4.4 DON diet	7.6DON diet	SEM ^d	P-value
N	72	74	77.3	1.6	0.1248
Indispensable AAs					
Arginine	72.8 ^b	77.1 ^{ab}	81.1 ^a	2.0	0.0479
Histidine	76.2	78.6	78.7	1.6	0.4956
Isoleucine	78.4	81.6	82.5	1.6	0.2119
Leucine	72.0	76.7	80.1	2.2	0.0843
Lysine	66.8	73.7	70.3	2.3	0.1670
Methionine	78.2 ^{ab}	85.3 ^a	75 ^b	1.8	0.0102
Phenylalanine	80.2	83.1	85.1	1.5	0.1310
Threonine	68.8	76.4	74.3	2.2	0.0877
Valine	72.9	78	78.3	1.7	0.0998
Dispensable AAs					
Alanine	66.7	70.5	67.7	2.1	0.4635
Aspartic acid	69.5	72.3	71	2.1	0.6438
Cystine	75.1	79.5	77.6	1.9	0.3185
Glutamic acid	85.1 ^b	88.1 ^{ab}	89.2 ^a	1.0	0.0447
Glycine	46.9 ^b	61.5 ^a	63.9 ^a	3.2	0.0122
Proline	56.2 ^b	68.2 ^{ab}	80.1 ^a	4.2	0.0115
Serine	74.4	78.9	78.8	1.6	0.1374
Tyrosine	75.4	80	83.6	2.3	0.0963

^dSEM = pooled standard error of the mean. ^{a,b}Means with same superscripts within the same row are not significantly ($P > 0.05$) different.

Apparent ileal N digestibilities were not different ($P > 0.05$) amongst diets. There were also no differences ($P > 0.05$) in the ileal digestibilities of indispensable AAs amongst diets except for arginine and methionine where

significant ($P < 0.05$) differences were observed. Arginine lowest value was with the 1.2ppm diet while the 7.6 ppm diet had the highest value. However, for methionine the lowest value was found with the 7.6 ppm

diet while the 4.4 ppm diet demonstrated the highest value. For the dispensable AAs, there were no differences ($P > 0.05$) amongst diets except for glutamic acid, glycine and proline where significant ($P < 0.05$) differences were observed. From these results it could be seen that AID for N and AA were high. This might be related to the high N and AA contents of the pearled barley. This is true because it is known that the apparent digestibility of AA increases exponentially with the ingested quantity because endogenous excretion as a percentage of total excretion decreases proportionally.

DISCUSSION

The results obtained in this study might be connected with DON and fibre components of each of the barley-based diets. Charmley and Prelusky (1994) demonstrated that DON during infection of the barley grain is evenly distributed and is mostly found predominantly near the exterior surface of the kernel. This pattern of DON distribution therefore would have resulted in the removal of most of the DON in the 1.2ppm diet compared with those of 4.4ppm and 7.6 ppm diets; although there was no sign of feed refusal amongst the diets. Removal of DON from these diets made them palatable and metabolizable by the pigs as a result of the eradication of impairment of nutrient metabolism associated with DON in swine nutrition (Rotter *et al.* 1996). DON interferes with metabolism of glucose and other nutrients in animals that ingested DON-contaminated diets (Hunder *et al.* 1991; Rotter *et al.* 1996).

The determined and the predicted DE values were compared primarily to measure the similarity between the actual DE values of the diets with their predicted counterparts based on the prediction equation of Fairbairn *et al.* (1999) derived for swine with barley where $DE = 3,526 - 92.8 \times \%ADF$. This equation depends largely on the amount of ADF in the barley. The 4.4ppm barley diet sample mean (determined DE value) was not significantly ($P > 0.05$) different from its population mean (predicted DE value). However, significant differences were found between the sample and population means for the 1.2ppm and 7.6 ppm diets. A number of factors could have contributed to the observed differences, including the appropriateness of the prediction equation and variables associated with analyses and biological phenomenon. Sample means were obtained from six pigs (3 x 3) replicated LSD whereas population means were derived with just a duplicate sample of ADF analyses suggesting that accuracy of the chemical measurement of ADF is of essence (Noblet and Perez, 1993). This however, does not erode the fact that individual pig differences in the digestibility of the diets could also be an important factor contributing to the differences observed between the actual and predicted DE values for diets 1.2ppm and 7.6ppm, respectively.

The differences observed was not also surprising because in the study of Fairbairn *et al.* (1999) the mean DE value for the barley used diets ranged from 2,686 to 3,133 kcal/kg with an overall mean of 2,934kcal/kg. The lower DE values obtained in the study of Fairbairn *et al.* (1999) could be due to high fibre contents in the barley types used in their study; whereas in our study pearling significantly polished away the fibrous hulls from the barley much in the same fashion as reported by Trenholm *et al.* (1991) and House *et al.* (2003). The high NDF and ADF contents of grains in pig rations reduce energy and nutrient digestibilities. Therefore, their removal would have enhanced energy and nutrient digestibilities in the pig. This would have also resulted in the higher energy digestibility observed in this study leading to the improvements observed in the actual or in vivo DE values. It is also worthy of note that the prediction equation of Fairbairn *et al.* (1999) was developed based on intact hulled barley and not pearled barley and therefore would also be important in explaining some of the observed differences.

In the pig biology, BW and age affect energy and nutrient digestibilities. Energy digestibility is improved with increased BW as the pig grows. In Fairbairn *et al.* (1999) from which the prediction equation was developed the initial and final BW of the pigs used were 35.3kg and 38.7kg, respectively. Thus, the wide BW differences between their pigs and ours could have contributed to the improvement in the actual DE values obtained in our study. Although we could not determine the actual DE values of the intact DON-barleys whose pearled counterparts were used in this study due to feed refusal of the intact DON-barley diets, it is not a gainsaying that commercial pearling was effective in removing DON and fibres from the DON-contaminated barleys resulting in improved energy digestibility.

The results obtained with AID of N and AAs in this study are in agreement and in some cases better than values found in the literature for regular barley for growing pigs. For instance, Green *et al.* (1987), Stein *et al.* (1999) and in this study, AID of N was 71.3%, 61% and 74.4%, respectively. Similar trends in favour of pearled barley were also observed for AA digestibilities. Nevertheless, for methionine the highest levels of digestibility were observed with the 1.2 and 4.4 ppm barley diets compared with the 7.6 ppm diet. This finding might be related to the interference of DON with methionine metabolism (Hunder *et al.*, 1991). Hunder *et al.* (1991) showed in male mice that DON inhibited nutrient metabolism leading to impaired intestinal absorption of essential nutrients, such as D-glucose and 5-methyltetrahydrofolic (5-MTH) acid. Removal of DON via pearling from the diets of the pigs would therefore alleviate these effects of DON and better support nutrient metabolism. Effective uptake of 5-MTH is very important in the metabolism of methionine. It was possible that more of the DON were removed from the 1.2ppm and 4.4ppm diets compared with the 7.6ppm diet. Therefore, the enhanced digestibility of methionine in the low and medium DON barleys was not surprising.

CONCLUSIONS

Commercial pearling is effective in removing both DON and fibre from DON-contaminated barley. Therefore, pearling can make DON-contaminated barley even up to 7.6 ppm possible for effective utilization by the growing pig when first pearled without deleterious effects, such as feed refusal and reduced nutrient digestibility. DE value of pearled DON-contaminated barley was improved over intact barley from literature data and in this study. Therefore, apart from removing the anorectic properties of DON-barley, pearling has the additional advantage of increasing the DE value with improved nutrient digestibilities. Thus, pearling technology can serve as an effective strategy for managing DON in barley for swine in any DON endemic region.

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