JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Deoxynivalenol Removal from Barley Intended as Swine Feed through the Use of an Abrasive Pearling Procedure

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Samples of naturally contaminated hulled barley, with varying deoxynivalenol concentrations, were subjected to an abrasive type dehulling procedure. The remaining grain fractions were analyzed for weight remaining (%), deoxynivalenol (ppm), crude protein (%CP), neutral detergent fiber (%NDF), ash (%ASH), gross energy (GE; kcal/kg), and calculated digestible energy values (DE; kcal/kg). Following the initial 15 s of pearling, 85% of the grain mass remained. Additional pearling resulted in a linear decline of grain mass. Following 15 s of pearling, the grain contained 34% of the initial deoxynivalenol content, irrespective of the initial level of contamination. Further pearling resulted in continued significant ($p < 0.05$) reductions in the percent of deoxynivalenol remaining to a level of 7.9% after 120 s but with significant losses in grain mass. Pearling can serve as an effective means of reducing the deoxynivalenol content of barley, with improvements in nutrient levels. However, the need to reduce the deoxynivalenol content of contaminated barley to less than 1 ppm for swine will necessitate the removal of a significant amount of the grain mass for heavily contaminated samples.

KEYWORDS: Mycotoxin; decontamination; *Fusarium*

INTRODUCTION

In cereal grains, the presence of deoxynivalenol, a trichothecene mycotoxin produced by *Fusarium* species (*1*), presents a challenge for livestock nutritionists and feed formulators. Swine, in particular, are known to be sensitive to the presence of deoxynivalenol due to the anorectic effects that this mycotoxin produces (*2*−*6*). In a recent study, we showed that feed intake was reduced by 7.6% for pigs that consumed barley-based diets with 2 ppm deoxynivalenol as compared to those pigs that consumed diets with no detectable deoxynivalenol (*7*). As a result of this anorectic effect of deoxynivalenol, swine performance may be compromised. Therefore, effective strategies for dealing with deoxynivalenol-contaminated grains are required in areas where infections by toxigenic *Fusairum* spp. are frequent, such as the Eastern Prairie region of Canada (*8*).

Several strategies have been employed for the detoxification of deoxynivalenol-contaminated grains. Biological detoxification strategies include the use of exogenous enzymes or bacteria to hydrolyze the 12−13 epoxide ring (**Figure 1**), which is responsible for the toxicological properties of deoxynivalenol (*9*). For example, the incubation of deoxynivalenol-contaminated corn with the cecal microflora from chickens reduced the anorectic potential of the corn and improved the performance of swine (*10*). Additional approaches to reduce the anorectic potential of deoxynivalenol-contaminated grains include the use of dietary additives, including compounds with purported mycotoxin binding properties. However, to date, these compounds have proved ineffective in improving feed intake or performance in swine consuming deoxynivalenol-contaminated grains (*11*).

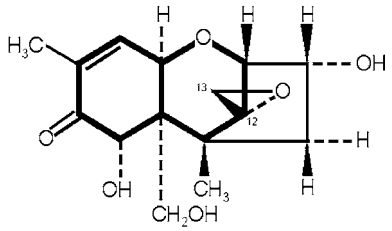

Figure 1. Chemical structure of deoxynivalenol.

Alternative approaches for the removal of deoxynivalenol from grains include the use of washing or soaking procedures (*12*) and physical methods, such as dehulling. With respect to the latter, sequential removal of the outer portions of barley kernels, through the use of a laboratory scale, abrasive pearling procedure, reduced the deoxynivalenol content of the resultant grain by 80−100% (*13*−*15*). While the latter studies provide evidence that pearling may be an effective means of removing deoxynivalenol from barley, the efficiency of the process for removing deoxynivalenol from grains with varying levels of contamination needs to be characterized before considerations can be given to scaling up the procedure to commercial levels. To this end, an experiment was designed with the objectives of characterizing the efficiency of deoxynivalenol removal from

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10.1021/jf034244p CCC: $25.00
Published on Web 07/17/2003

Table 1. Deoxynivalenol Content, Proximate Analyses, and Energy Content of Barley Samples Prior to Pearling[a]

		barley GRS99	barley GRS01	barley SS01
deoxynivalenol	ppm	4.8 (0.5)	9.8 (1.3)	21.1 (2.1)
crude protein	%	10.2 (0.2)	10.7 (0.1)	13.2 (0.3)
NDF	%	25.5 (0.6)	16.7 (0.4)	15.4 (0.4)
ash	%	2.6 (0.1)	2.4 (0.1)	2.5 (0.1)
GE	kcal/kg	3728 (28)	4031 (16)	3950 (30)
DE	kcal/kg	2734 (41)	3515 (52)	3328 (50)

[a] Values are means (SD) of three replicates.

barley samples, varying levels of contamination, through the use of an abrasive pearling procedure, and assessing the impact that pearling has on the nutritional profile of the resultant grain.

MATERIALS AND METHODS

Sample Acquisition. Three 10 kg samples of deoxynivalenol-contaminated barley were secured from crops grown in the Red River Valley region of Manitoba, Canada. Two samples were secured from barley grown at the Glenlea Research Station, University of Manitoba, during the 1999 (sample key = GRS99, six row Robust) and 2001 (sample key = GRS01, six row Robust) growing seasons, and one sample was secured from barley grown during the 2001 growing season by a local producer (sample key = SS01, six row, variety unknown). Samples were selected on the basis of initial deoxynivalenol measurements performed at harvest and chosen to reflect a range of deoxynivalenol contents. The deoxynivalenol contents at the time of trial were confirmed using the procedures outlined below.

Pearling Procedure. For each barley source, three replicate 100 g samples were subjected to an abrasive type, pearling procedure, using a Scott-Strong, laboratory scale barley pearling machine, complete with a 30 grit carborundum stone and a no. 7 mesh screen (Seedburo Equipment Co., Chicago, IL), for 0, 15, 30, 45, 60, 75, 90, 105, or 120 s. All other variables were held constant; only contact time varied. The polishings were discarded, and the pearled samples were collected, weighed, and retained for further analyses.

Analytical Techniques. Samples of hulled and pearled barley were analyzed for deoxynivalenol content by enzyme immunoassay (Ridascreen R-5902, R-Biopharm, Darmstadt, Germany). Samples (5 g) were extracted with 100 mL of distilled water for 3 min in a Waring blender. Following extraction, the samples were centrifuged at 15 000*g* for 5 min, and the supernatants were used for deoxynivalenol analysis. The limit of detection for the assay was 0.2 ppm. Initial barley samples were assayed for deoxynivalenol, 3-acetyl-deoxynivalenol, and 15-acetyl-deoxynivalenol by electron-capture gas chromatography of the trimethylsilyl ether derivative (*16*, *17*). The results indicated no 3-acetyl-deoxynivalenol or 15-acetyl-deoxynivalenol levels above the limits of detection of the assay (0.10 and 0.12 ppm, respectively). Samples were analyzed for crude protein (AOAC 990.03), using the LECO CNS-2000 (LECO Instruments, Mississauga, ON), and for dry matter (AOAC 934.01) and ash (AOAC 942.05) content according to standard procedures (*18*). Gross energy (GE) content was determined by bomb calorimetry. Neutral detergent fiber (NDF) content was determined as previously described (*19*). Digestible energy (DE) values for the barley samples were calculated using an equation derived for swine (*20*): DE = 949 + (0.789 × GE) − (43 × %ASH) − (41 × %NDF). The initial values for deoxynivalenol, proximate analysis measures, and energy are presented in **Table 1**.

Statistical Analyses. For each barley source, the data for deoxynivalenol, proximate analysis measures, and energy, obtained following pearling, were expressed as a percentage of the initial values presented in **Table 1**. Data were subject to analysis of variance (ANOVA), with main effects partitioned between the pearling time, the barley source, and the interaction term. Individual treatment means were compared to control (0 pearling time) values set at 100%, using Bonferroni's procedure with adjustment for the number of comparisons (*21*). Data for changes in the measured values for dry matter disappearance, deoxynivalenol, crude protein, ash, and NDF, relative to time, were fitted to an exponential decay curve ($y = ae^{-kx}$), and the decay constant was compared to 0, using the proc GLM function of SAS (*21*). The data for the percentage of deoxynivalenol remaining after pearling were plotted against the percentage of grain mass remaining for each barley sample, and the data were fitted to a three parameter, exponential growth equation ($y = y_o + ae^{bx}$), using SigmaPlot 2000 (*22*).

RESULTS AND DISCUSSION

The pearling procedure, where grain is placed in contact with a rotating abrasive disk for controlled time periods, proved to be an effective means of removing deoxynivalenol from hulled barley. The initial 15 s pearling period proved to be the most efficient time period for reducing deoxynivalenol levels, as removal of 15% of the grain mass led to a reduction in the deoxynivalenol content of 66% (**Table 2**). Fitting the data to exponential decay curves yielded negative decay constants that were significantly different ($P < 0.05$) from 0 (**Table 2**), providing strong evidence that increasing pearling times led to greater reductions in both grain mass and deoxynivalenol content. Because of the differences between barley sources in the absolute values for deoxynivalenol and the other nutrients listed in **Table 1**, representing changes in chemical composition as a percentage of control values permitted a meaningful comparison of values by way of ANOVA. In the ANOVA, the effect of barley source was significant ($P < 0.05$) for all of the measures observed, reflecting, primarily, the low analytical variability for replicate analyses. The regression of the independent observations for percentage of grain mass remaining against the percentage of deoxynivalenol remaining yielded the relationship presented in **Figure 2**. The data fit well ($r^2 = 0.99$) to a three parameter exponential growth equation, best explained by the following equation:

$$\%\ \text{deoxynivalenol remaining} = (7.36 \pm 0.6) + (0.0114 \pm 0.003)\, e^{0.090 \pm 0.003} \times \%\ \text{grain mass remaining}$$

The data in **Figure 2** highlight the uniformity, irrespective of initial deoxynivalenol content, in the relative reduction in deoxynivalenol levels with pearling. As such, the equation derived permits the calculation of the amount of grain needed to be removed, via pearling, from barley samples with varying levels of deoxynivalenol contamination, to reduce deoxynivalenol levels to within "acceptable" levels. For example, if a hulled barley sample presents with a deoxynivalenol content of 10 ppm and the desired level of deoxynivalenol is 1 ppm or less, a maximum of 10% of deoxynivalenol should remain, and this would necessitate the pearling of the barley to remove 40% of the grain weight. The value of 1 ppm represents the recommendation of Agriculture and Agri-Food Canada (*23*) for the maximal level of deoxynivalenol in the final feed for all classes of swine, on the basis of published literature (i.e., *2*−*6*). In a recent study, we documented reductions in feed intake of 7.6% when pigs were fed diets containing 2 ppm deoxynivalenol (*7*). However, in that study, growth performance was not impacted, and the only negative outcome in the trial was a slightly, but significantly, longer time required to reach 110 kg for gilts but not for barrows. The results from that study provide evidence that the limitations on deoxynivalenol levels in grower−finisher feeds could be relaxed. Increasing the tolerance level from 1 to 2 ppm in the final feed would require the removal of less grain mass from the contaminated barley, thus reducing the pearling time required.

The current study extends research conducted by others (*13*−*15*) on the potential for pearling to remove deoxynivalenol from

Table 2. Effect of Pearling Time on the Deoxynivalenol and Nutrient Profile of Barley

pearling time (s)	% of original values[b]						
	grain mass	DON[a]	crude protein	NDF[a]	ash	GE[a]	DE[a]
15	85.0*	34.0*	103.6*	60.3*	70.8*	101.2	113.3*
30	79.1*	21.9*	99.1	55.8*	63.6*	101	114.0*
45	74.2*	17.6*	95.3*	47.7*	58.1*	100.4	115.5*
60	69.4*	13.3*	92.5*	45.1*	51.8*	100.2	117.1*
75	64.7*	11.8*	89.3*	44.2*	49.1*	99.3	115.6*
90	60.1*	9.8*	86.6*	43.4*	46.5*	99.9	117.9*
105	56.5*	9.4*	83.2*	38.8*	42.4*	98.6*	116.3*
120	52.3*	7.9*	81.3*	40.7*	38.8*	97.8*	115.4*
SEM[c]	0.2	0.7	0.4	1.2	1.1	0.5	0.7
				P values			
time	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0
barley	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
barley × time	<0.0001	0.28	0.24	0.011	0.002	0.22	0.89
				exponential decay constants[d]			
a (intercept)	91.2 (1.0)	33.1 (1.0)	106.3 (1.0)	58.6 (1.1)	74.7 (1.0)	101.9 (1.0)	ND[e]
k (decay constant)	−0.0047 (0.0002)	−0.0131 (0.0007)	−0.0023 (0.0001)	−0.0042 (0.0009)	−0.0055 (0.0003)	−0.0003 (0.0001)	ND
P value; $k \neq 0$	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	ND

[a] Abbreviations: DON, deoxynivalenol. [b] Data are expressed as the least squares means calculated as a percentage of control values from respective barley sources. * signifies that the values within a column are significantly different from 100%, as determined by Bonferroni's procedure ($\alpha = 0.05$) adjusted for the number of comparisons. [c] SEM, standard error of the least squares means term. [d] Data were fitted to an exponential decay function ($y = ae^{kt}$) and describes time ≥15 s. Values are presented as the derived parameter estimate, with the standard error in parentheses. [e] ND, not determined; data did not follow exponential decay pattern.

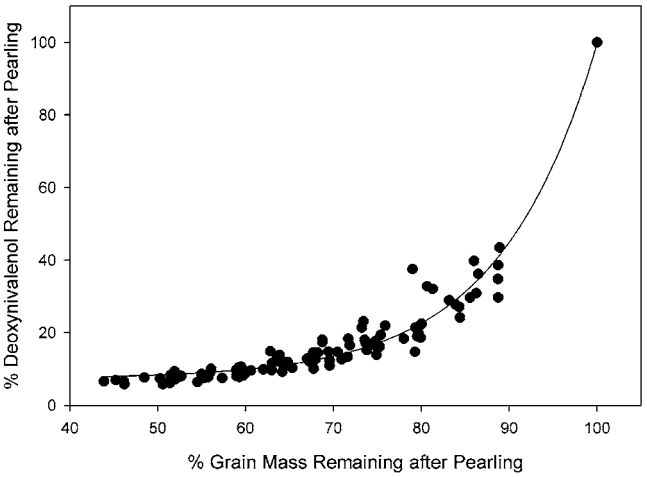

Figure 2. Relationship between the percentage of barley mass remaining following pearling and the percentage of deoxynivalenol left in the sample. Data were fitted to a three parameter, exponential growth equation, $y = y_0 + ae^{bx}$, yielding the following parameter estimates (mean ± SEM): $y_0 = 7.36 \pm 0.60$; $a = 0.0114 \pm 0.003$; $b = 0.090 \pm 0.003$; and $r^2 = 0.99$.

cereal grains such as barley. The novelty of the current research relates to the characterization of the efficiency of the pearling process. Furthermore, because of the fact that the end product is intended to be fed to swine, a preliminary assessment of the nutritive value of the grain was conducted. As given in **Table 2**, both crude protein and DE content increased significantly ($P < 0.05$), relative to 100% control values, by approximately 4 and 13%, respectively, following the initial 15 s of pearling. The initial 15 s of pearling was also the period during which the greatest percentage drop in grain mass, deoxynivalenol, NDF, and ash was observed. Beyond the initial 15 s of pearling, crude protein, NDF, ash, and GE declined and like deoxynivalenol, their reductions were best described using an equation for exponential decay. Unlike GE, DE remained significantly ($P < 0.05$) elevated over 100% control values, with the increase in DE reflecting, primarily, the sustained reductions in the NDF content of the pearled barley samples (*20*).

On the basis of chemical composition alone, pearling can have a significant impact on the nutritive value of hulled barley for swine, especially through the increase in calculated DE content. An increase in DE would be important as cereal grains represent the primary energy source in swine rations. However, in vivo determinations of the impact that pearling has on the content of DE and the digestibility of other nutrients, including protein and amino acids, are required before pearled barley can be incorporated effectively into feed formulations. Hulled barley contains β-glucans at levels ranging between 4 and 9% (*24*, *25*), and these carbohydrates may reduce nutrient digestibility in swine. Zheng et al. (*25*) examined the distribution of β-glucans in fractions collected from hull-less barley undergoing a sequential pearling process. They observed that the β-glucan content of the residual kernels, following removal of 70% of the kernel weight, was similar to that of the intact kernel. Miller et al. (*26*) documented a marginal reduction in measured DE values in swine for hulled barley diets formulated to contain higher levels of β-glucans. However, DE values for barley were more sensitive to changes in fiber levels, a fact supported by others (*24*). Future studies will examine the in vivo DE content of hulled barley undergoing the pearling process and will determine the impact that various carbohydrate classes have on nutrient digestibility.

As discussed, the pearling process uniformly removes the outer portions of the hulled barley kernel in a time-dependent fashion. On the basis of the pattern of responses observed, deoxynivalenol appears to be concentrated in the hull fraction, as was reported in hull-less barley (*15*), thus making pearling a viable decontamination option. The fact that a similar removal pattern, as a percentage of control values, was observed for barley sources with initial deoxynivalenol contents ranging from 4.8 to 21.1 ppm simplifies calculations for the amount of pearling required to reach a given level of deoxynivalenol in the final product. However, it also indicates that barley samples with higher levels of deoxynivalenol will require substantially more pearling to reduce deoxynivalenol to a given level. If a similar relationship was to exist with equipment designed for higher capacity pearling, as would be required in a commercial

setting, an increase in pearling time would increase the energy required for deoxynivalenol removal, with implications, ultimately, for the economic feasibility of the process. However, such an analysis remains to be performed through the systematic evaluation of the efficiency of large scale pearling units for removing deoxynivalenol from contaminated grains, such as barley.

The development of an efficient and cost effective strategy for the removal of deoxynivalenol from barley and other cereal grains will have significant implications for the feed industry in regions where this mycotoxin is endemic. For example, the presence of high levels of deoxynivalenol in barley in the eastern prairie region of Canada (*8*), due primarily to the proliferation of *Fusarium graminearum*, limits the utilization of locally produced feedstuffs in swine rations. The use of local feed resources is desirable in that transportation and handling costs are reduced, there is increased opportunities for the addition of value to cereal grains through the production of pork, and there is a reduction in the reliance on imported nutrients into a region, which potentially reduces the net accumulation of nutrients within an agroecosystem. The present study highlights the efficiency of a laboratory scale pearling procedure for the removal of deoxynivalenol from barley. Future studies with commercial pearling units will ascertain the cost effectiveness of this procedure for removing deoxynivalenol from feed grains.

ACKNOWLEDGMENT

We thank Diane Smith, Cereal Research Centre, Agriculture and Agri-Food Canada, for her assistance in conducting the deoxynivalenol analyses. Additionally, we thank Ricky Araneda, Katherine Hossack, Jason Neufeld, and Danielle Ludke, Department of Animal Science, for their technical help, and John Maltman, Manitoba Agriculture and Food, for his assistance in locating barley samples.

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Received for review March 13, 2003. Revised manuscript received June 2, 2003. Accepted June 6, 2003. We thank the Agri-Food Research and Development Initiative of the Province of Manitoba, the Manitoba Pork Council, and the University of Manitoba for their financial support.

JF034244P