ONTARIO PORK

Ontario Pork Research Final Report (OP 19-004) Executive Summary

Reporting Date: October 15th, 2020.

Introduction:
Swine producers use medicated feeds for treating pig diseases and optimizing their performance. This use neglects the feed-drug interactions that curtail the efficacy of dietary drugs. We hypothesized that the water holding capacities (**WHC**) of feedstuffs predict their hindrance to the release of dietary chlortetracycline (**CTC**) and lincomycin (**LIN**).

Objectives:
1. Determine the WHC of feedstuffs commonly used in the composition of porcine diets.
2. Develop in vitro dissolution testing (**IVDT**) methods to determine the release of LIN and CTC from medicated feeds, using different combinations of drugs and feeds or feedstuffs.

Materials and Methods:
We measured the WHC of ground corn (**gC**), wheat (**gW**), rye (**gR**), soybean meal (**SBM**), corn dried distillers grains with solubles (**DDGS**) and meat and bone meal (**MBM**) after 24 hours of soaking in distilled water, and tested how their WHC was affected by feedstuff particle size, soaking time and acidity of the solvent.

We performed IVDT of LIN and CTC premixes alone or admixed to the above feedstuffs in simulated porcine gastric fluid (**SPGF**) for 2 hours, after which we raised the pH of the fluid to continue the IVDT in simulated intestinal fluid. We measured the concentrations of dissolving LIN and CTC with HPLC, respectively with mass spectrometry or UV detection.

Results and Discussion:
Objective 1. The WHC of our feedstuffs significantly depended on their potassium, tryptophan, lysine, aspartic acid and digestible energy contents, with significant effects of soaking fluid and feed particle size, but not soaking time.

Objective 2. The extent of in vitro dissolution of LIN and CTC decreased significantly as a function of the WHC of the tested feedstuffs. Noteworthy, SBM and gC exerted the greatest hindrances on the dissolution of both antibiotics.

Conclusions:
The WHC appears to be a promising indicator of the feedstuff-drug premix interactions for dietary LIN and CTC. Our findings initiate a novel precision-feeding approach for optimizing the use of oral drugs in swine production.

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Introduction: For the last 70 years, feed has been used as a convenient drug administration vehicle for group medication of pigs. Once they satisfy the requirements for regulatory approval, the dietary drug premixes may be admixed to any kind of feed, irrespective of its composition. Even though the demonstration of safety and therapeutic efficacy is mandatory for the regulatory approval of veterinary drugs, the outcome of oral medication in pigs is inconsistent [1]. A possible cause that has been poorly explored so far is the presence of feed-drug interactions that modify the oral absorption of dietary drugs [2]. Contrariwise to the manufacturers of oral drugs for humans or pets, the dietary drug premix manufacturers must document only their incompatibilities with other feed additives, not their interactions with the major feedstuffs used in formulating the medicated feeds. Because dissolution in the gastrointestinal fluids is the prerequisite to the absorption and therapeutic effect of oral drugs, we hypothesize that the water-holding capacity of feedstuffs applies to the gastrointestinal fluids too, and hinders the drug release from the medicated feeds used in pigs.

Objectives:

1. Determine the water-holding capacity (**WHC**) of feedstuffs commonly used in porcine feeds.
2. Develop in vitro dissolution testing (**IVDT**) methods to determine the drug release from porcine medicated feeds.

Materials and Methods:

1. FEEDSTUFFS AND DRUG PREMIXES

A local feed mill[1] provided 1-kg samples of freshly ground corn (**gC**), rye (**gR**), wheat (**gW**), dried distiller's corn grains with solubles (**DDGS**), soybean meal (**SBM**), and meat and bone meal (**MBM**), the results of their nutrient contents analysis, and 250-g samples of commercial feed-grade chlortetracycline[2] (**CTC**) and lincomycin[3] (**LIN**) premixes

[1] F. Ménard Inc. St-Pie-de-Baggot, Qc, Canada.
[2] Deracin 22% Granular Premix. Pharmgate LLC, Wilmington, NC, U.S.A.
[3] Lincomycin 110 G Premix. Bio Agri Mix LP, Mitchell, On, Canada.

2. WATER-HOLDING CAPACITY OF FEEDSTUFFS

All experiments were performed with three or more replicates. Precision-weighed (1 g) aliquots of each feedstuff were soaked for 24h in 100 mL of distilled water at room temperature, filtered with Whatman No. 1 paper disks [3], and precision weighed before (i.e. wet) and after (i.e dry) 24 h of lyophilisation, using both a tray[4] or a jar[5] apparatus. The effect of fluid type on WHC was determined identically, but comparing SPGF (pH=1.60±0.05, containing 0.2M HCl and 0.2M KCl) to distilled water as soaking media. The effect of water-soaking duration on WHC was determined identically, but lyophilization was performed at 2, 6, 12 and 24 h of soaking. The effect of particle size on WHC was determined using feedstuff fractions collected after gentle sifting in stacked sieve drums (1.00, 0.70, 0.50, 0.30, 0.20, 0.10, and 0.07 mm mesh size from top to bottom). We calculated the WHC as follows, where *W* is the weight of the feedstuff aliquot:

$$WHC = \left(W_{wet} - W_{dry}\right)/W_{dry}$$

3. IN VITRO DISSOLUTION TESTING OF DIETARY DRUGS

The dissolution experiments were performed in a type-2 (i.e. paddle) dissolution apparatus, where lid-covered flasks containing 500 mL of the simulated porcine gastric fluid (SPGF) were stirred at 70 RPM in a water bath set at 40°C, the postprandial body temperature of pigs. Premix particle hardness was tested prior to performing IVDT. Aliquots (40 mg) of the tested premixes were poured alone or admixed to 9.960 g of gC, gR, gW, DDGS or SBM, yielding 440 ppm of LIN or 880 ppm of CTC dietary concentrations. Filtered SPGF samples (1 mL) were taken serially in duplicates from the flask's mid-depth, a pair immediately before the addition of the medicated articles and the others on 15 occasions during the initial 120 min of dissolution testing. At 120 and 135 min and dissolution time, we added K_2HPO_4 to the SPGF to increase its pH to 5.8 and to 6.2, corresponding to the pH of the porcine small intestine. Filtered pairs of fluid samples were taken at 5-min intervals between 120 and 150 min.

4. ANALYSIS OF DRUGS IN SIMULATED GASTROINTESTINAL FLUIDS

We developed a high-performance liquid chromatography (HPLC) method for testing LIN using a mass spectrometry detector set at 407.2 mass/charge ratio. The stationary phase was a Luna C18 reverse-phase column (5 µm particle size; 4.5 mm width × 50 mm length), and the mobile phase a water-acetonitrile-formic acid 950:49:1 v/v mixture that was replaced progressively with an acetonitrile-formic acid 0.1% mixture. The limits of quantification (LOQ) and detection

[4] FTS Systems. Stone Ridge, NY, U.S.A.
[5] Labconco Corporation. Kansas City, MO, U.S.A.

(LOD) respectively were 3.4 and 1.1 µg/mL LIN, as determined with the signal to noise ratio. A 6-point calibration curve (0, 5, 10, 20, 40, and 50 µg/mL LIN) was used on each run. The accuracy and precision tested at 5, 20 and 40 µg/mL LIN respectively were 112.6% and 7.9%.

We developed a HPLC testing method for measuring the CTC concentrations using an UV detector set at 375 nm wavelength. The stationary phase was a Luna C18 Hypersil GOLD column (4.6 mm width × 150 mm length; 3 µm particle size), and the mobile phase a 0.01M oxalic acid solution in water mixed with acetonitrile at a 50:50 volume ratio and thermostated at 35°C. The limits of quantification (LOQ) and detection (LOD) respectively were 2.4 and 0.7 µg/mL, as determined with the signal to noise ratio. A 6-point calibration curve (0, 5, 10, 15, 20, and 30 µg/mL CTC) was tested on each analytical run. The accuracy and precision tested at 5, 20 and 40 µg/mL CTC respectively were 109.2% and 5.8%.

5. DATA ANALYSIS

The nutritional determinants of the WHC of feedstuffs were identified with stepwise linear regression at a p=0.15 threshold. The effects of lyophilisation method, soaking fluid, soaking time, and feedstuff particle size on WHC were analyzed with generalized linear mixed models for Gaussian distributed outcomes using the Laplace approximation of the likelihood function. The effects of time, WHC, solvent pH and feedstuff nutrient contents on the radially-smoothed random time-course of dietary LIN and CTC in vitro dissolutions were examined with generalized linear mixed model for Gaussian outcomes. All tests were carried at α=0.05 significance threshold, using the stepdown simulation procedure for containing the familywise type-1 and type-2 errors in planned multiple comparisons.

Results and Discussion:

1. DETERMINATION OF THE WHC OF FEEDSTUFFS

The feedstuff contents of lysine (p=0.0006) and potassium (p=0.03) decreased the WHC values, while those of tryptophan (p<0.0001), aspartic acid (p=0.001), and digestible energy (p=0.03) increased the WHC values, with an R^2=0.973 value for the final regression model. These results suggest that WHC is related to the contents of dietary proteins and/or carbohydrates, confirming previous findings using other types of feedstuffs [3-5].

When testing the effects of soaking medium on WHC (Fig. 1), we only recorded significant differences among feedstuffs and medium×feedstuff combinations (both p<0.0001). Noteworthy, WHC was highest for SBM, confirming previous reports [5]. In addition, the WHC of MBM was lower in SPGF than water, likely resulting from protein oxidation [6].

Similarly to previous reports [3], the effects of soaking time on WHC were negligible for all time×feedstuff combinations (both $p>0.50$), but WHC significantly differed among feedstuffs ($p<0.0001$), as shown in Fig. 2.

As expected from the relationship between particle volume and surface area, WHC decreased with increasing particle size for all feedstuffs, but their relationships significantly differed. Both their intercepts and linear slopes were significant ($p<0.0001$), and a significant quadratic slope was recorded for 3 of the 5 feedstuffs ($p<0.0001$). Only SBM and gW showed linear WHC decreases over the range of particle sizes (Fig. 3). This result suggests that the nutrient composition of their particle fractions is more homogeneous than those of gC, gW and DDGS, analogous to other study reports [7].

Fig. 1

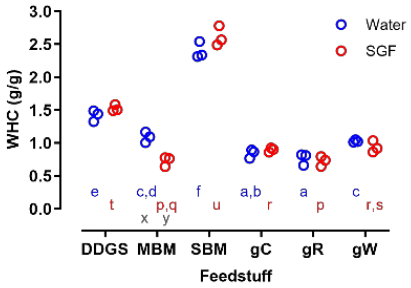

Fig. 1. Water-holding capacities of the tested feedstuffs in disilled water and in simulated porcine gastric fluid. Note: matched-color rows of subscripts with no common letter differ significantly.

Fig. 2

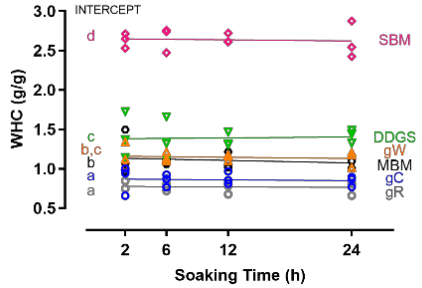

Fig. 2. Water-holding capacities of the tested feedstuffs in distilled water, in function of soaking time. Note: intercept subscripts with no common letter differ significantly.

Fig. 3

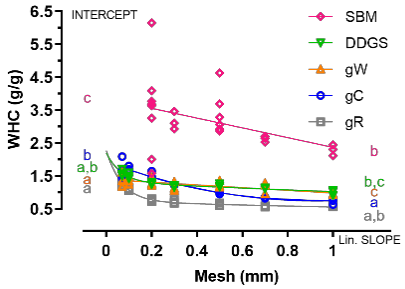

Fig. 3. Water-holding capacities of the tested feedstuffs, in function of feedstuff particle size. Note: intercept subscripts (left) and linear slope superscripts (right) with no common letter differ significantly.

2. IN VITRO DRUG DISSOLUTION OF DIETARY CHLORTETRACYCLINE (CTC) AND LINCOMYCIN (LIN)

Premix particle hardness was 7.5 N for LIN and 19.35 N for CTC. The time course of their respective drug dissolutions from the dietary premixes tested alone was completed within the 120 min of exposure to SPGF (Figs. 4 and 5). In contrast to LIN and regardless the presence of feedstuff, a small amount of CTC (<5%) dissolved almost immediately at the beginning of the experiment, after which its concentration stabilized for several minutes before rising again. This two-phase process suggests that CTC premix particles are dustier than those of LIN and highlight their difference in particle hardness: the latter slows the permeation of SPGF to its core, hence delaying the dissolution and release of drug molecules [8]. Neutralizing the pH slightly decreased the proportion of dissolved drug molecules in SPGF, an expected finding because CTC becomes anionic at pH>5 and forms insoluble complexes with Ca^{++} and other multivalent cations [9], while LIN becomes unionized and its calculated water solubility decreases by 60%. All feedstuffs decreased the rate and extent of drug release of both dietary drugs, but hindrances were larger for gW, gC and SBM than for DDGS and gR (Figs. 4 and 5). Noteworthy, the dissolution extents of CTC and LIN were similar when admixed to a given feedstuff.

Fig. 4

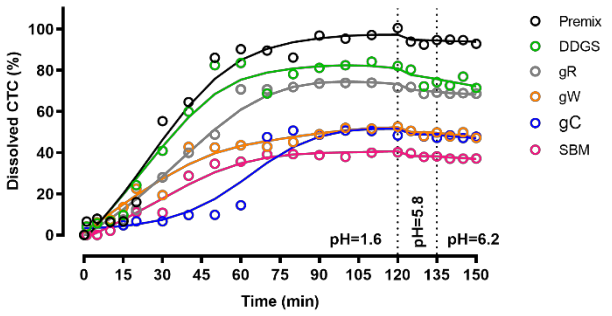

Fig. 4. Observed and model-predicted time-course of chlortetracycline released in simulated gastrointestinal fluids from the drug premix alone or from premix-fortified feedstuffs commonly used in the composition of porcine diets. Note: the solvent pH and the timing of buffer additions are depicted with two dotted lines.

Fig. 5

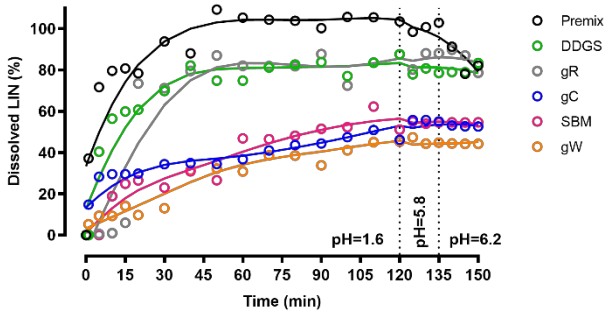

Fig. 5. Observed and model-predicted time-course of lincomycin released in simulated gastrointestinal fluids from the drug premix alone or from premix-fortified feedstuffs commonly used in the composition of porcine diets. Note: the solvent pH and the timing of buffer additions are depicted with two dotted lines.

The statistical testing of the fixed effects of WHC, pH and the WHC×pH interaction on the radially-smoothed random time-courses of CTC and LIN dissolution in SPGF was significant for WHC ($p=0.008$), but not for pH or WHC×pH ($p>0.69$). We refined the goodness of fit of this statistical model by adding the fixed effects of time, time×drug interaction, and ash content of the feedstuffs, which results are summarized in Table 1.

Table 1. Estimated coefficients of the linear predictor of the time-course of dissolved chlortetracycline (CTC) and lincomycin (LIN) in simulated porcine gastrointestinal fluids, boundaries of their 95% confidence intervals, and results of type III statistical testing. Note: the generalized linear mixed model for Gaussian-distributed outcomes additionally contains a 17-knot radial smoother of the random time-course of drug×feedstuff data.

Effect	Drug	Solutions for Fixed Effects				Type III Tests of Fixed Effects		
		Estimate	SE	Lo 95%C.I.	Hi 95% C.I.	Den DF	F Value	Pr > F
Intercept		93.3164	13.1683	66.9040	119.73			
Drug	**CTC**	-26.0491	17.0612	-60.2696	8.1714	53	2.33	0.1328
	LIN	0	.	.	.			
Time		0.1201	0.1531	-0.1818	0.4220	202.2	7.17	0.0080
Time×Drug	**CTC**	0.3383	0.2160	-0.08757	0.7642	202.2	2.45	0.1188
	LIN	0	.	.	.			
WHC		-119.35	17.0225	-153.50	-85.2106	53	49.16	<.0001
pH		-0.5490	0.5683	-1.6888	0.5907	53	0.93	0.3383
Ash		37.7662	6.0450	25.6414	49.8910	53	39.03	<.0001

According to these results, WHC was the single, most important factor that hindered the dissolution of both tested drugs ($p<0.0001$), while time and the ash content of feedstuffs significantly favored their release ($p\leq0.008$). The effect of time is unsurprising, as it denotes the solvent permeation inward the premix particles, but the effect of feedstuff ash content was unexpected: this indicator of the mineral content of feedstuffs may have operated a “salting-in” effect, whereby a slight increase of the solvent’s ionic strength favors the dissolution of poorly soluble drugs [10].

With regards to the other fixed effects in the model, Drug and Time×Drug respectively were an additional intercept and slope term for differentiating the tested drugs: their effects were marginally non-significant for CTC from LIN ($p \geq 0.12$), but they might be valuable for studying other drugs with greater physicochemical differences. Solvent pH was another additional slope term whose effect was not significant ($p=0.34$): it is possible that this result is associated with the short duration of fluid sampling at higher pH values, or with feedstuff's buffering effects. Indeed, the pH-associated changes in the extent of final drug dissolution were small for most medicated feedstuffs.

Conclusions:

We have verified our research hypothesis: the WHC of feedstuffs commonly used in manufacturing of medicated feeds predicts their hindrance to the in vitro dissolution of dietary LIN and CTC. This finding warrants further research, because it founds a new precision-feeding approach to the optimization of oral drug use in the Canadian swine sector, which will benefit the swine producers and specialists, the health of pigs, the value of pork products, and the environment.

References:

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Knowledge Transfer:

The results of this research project have not yet been disclosed to a large audience outside U. Montreal Veterinary College. So far, the M.Sc. dissertation of our graduate student (A. Jafarzadeh) is under review. We plan to submit the manuscripts of research reports and scientific vulgarization papers during the winter of 2021. We recently have contacted the London Swine Conference organizers to request a spot for a presentation in March 2021.