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Characterization of Feedstuff-Drug interactions in Swine

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Characterization of Feedstuff-Drug interactions in Swine

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Résumé

Les interactions aliment-médicaments nuisant aux processus de libération et/ou à la stabilité de leurs principes actifs doivent être caractérisées en raison de leurs conséquences adverses sur la biodisponibilité orale, l'efficacité thérapeutique et, dans le cas des agents anti infectieux, la sélection d'organismes résistants. Notre étude a évalué la relation entre l'efficacité de la libération in vitro de la chlortétracycline (CTC) et de la lincomycine (LIN) dans le liquide gastrique simulé de porc (LGSP) et la capacité de rétention d'eau (CRE) d'ingrédients couramment utilisés dans l'alimentation des porcs (tourteau de soja (TS), drèche de distillerie sèche avec solubles (DDS) et farine de viande et d'os (FVO), maïs, blé, et seigle), que l'on a fortifié avec 880 ppm de CTC ou 440 ppm de LIN. La CRE des ingrédients différait significativement ($p < 0,0001$) et était maximale avec le TS, aussi bien dans l'eau que le LGSP. Le FVO avait une CRE significativement plus basse dans le LGSP que dans l'eau ($p < .0001$). Les effets du temps de trempage sur la CRE étaient négligeables pour tous les ingrédients ($p > 0,50$). La CRE diminue avec l'augmentation de la taille des particules pour tous les aliments, mais leurs relations différaient significativement ($p<0.0001$). Tous les ingrédients alimentaires testés ont diminué la vitesse et l'étendue de la dissolution des prémélanges de CTC et de LIN. Le CRE était le principal facteur qui a empêché la dissolution des deux médicaments ($p < 0,0001$), tandis que le temps et la teneur en cendres des ingredients favorisaient significativement leur dissolution ($p \leq 0,008$). En comparaison des prémélanges dissouts seuls, le DDS et le seigle ont libéré 80% de ces antibiotiques, tandis que le TS, le blé et le maïs en ont libéré entre 40 et 50%. La neutralisation du LGSP au pH intestinal porcin a diminué les proportions dissoutes d'antibiotiques, mais pas significativement ($p>0.69$). La CRE des ingrédients utilisés dans la fabrication d'aliments médicamenteux serait donc un indicateur prometteur des interactions aliment-prémélange médicamenteux de LIN et de CTC. Afin d'augmenter leur libération et leur potentiel thérapeutique, la formulation des aliments médicamenteux pourrait être améliorée en utilisant des ingrédients alternatifs, dont la CRE est moindre.

Mot clés: Aliment médicamenteux; Capacité de rétention d'eau; Test de dissolution in vitro; Chlortétracycline; Lincomycine; libération de médicaments

Abstract

Food-drug interactions adversely affecting the release process and the stability of their active ingredients must be characterized because of their adverse consequences on oral bioavailability, therapeutic efficacy and, in the case of antibiotics, selection resistant organisms. Our study evaluated the relationship between the efficiency of the in vitro release of chlortetracycline (CTC) and lincomycin (LIN) in simulated pig gastric fluid (SPGF) and the water-holding capacity (WHC) of feedstuffs commonly used in pig feed (soybean meal (SBM), dry distillers' grain with solubles (DDGS) and meat and bone meal (MBM), corn (gC), wheat (gW), and rye (gR), which it was fortified with 880 ppm CTC or 440 ppm LIN. The WHC of ingredients differed significantly ($p < 0.0001$) among feedstuffs with the highest value for SBM both in water and SPGF and lowest value for MBM. The MBM had a significantly lower WHC in SPGF than in water ($p < .0001$). The effects of soaking time on WHC were negligible for all feedstuffs ($p > 0.50$). The WHC decreased with increasing particle size for all feedstuffs, but their relationships differed significantly ($p < 0.0001$). All the tested feedstuffs decreased the rate and extent of dissolution of the CTC and LIN premixes. WHC was the main factor that hindered the dissolution of both drugs ($p < 0.0001$), while the time and ash content of the ingredients significantly favored their dissolution ($p \leq 0.008$). Compared to the dissolved premixes alone, DDGS and gR released 80% of these antibiotics, while SBM, gW and gC released between 40% and 50%. Neutralization of SPGF at swine intestinal pH decreased the dissolved proportions of antibiotics, but not significantly ($p > 0.69$). The WHC of the used feedstuffs in the manufacture of medicated feed would therefore be a promising indicator of the feed-drug interactions of LIN and CTC. To increase their release and therapeutic potential, the formulation of medicated feeds could be improved by using alternative ingredients, with less WHC.

Keywords: Medicated feed; Water holding capacity; In vitro dissolution test; Chlortetracycline; Lincomycin; rate and extent of drug release

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List of Symbols & Abbreviations

ABC	Acid binding capacity
AOX	Aldehyde oxidase
Ca	Calcium
CMIB	Compendium of Medicating Ingredient Brochures
CRE	Capacité de rétention d'eau
CTC	Chlortetracycline
CYP	Cytochrome P450
DDGS	Distillers' dried corn grains with solubles
DDS	Drèche de distillerie sèche avec solubles
EC	Enteric coated
ER	Extended release
FDA	Food and Drug Administration
FVO	Farine de viande et d'os
gC	ground corn
GE	Gastric emptying
gR	Ground rye
gW	Ground wheat
IR	Immediate release
IVDT	in vitro dissolution drug testing method
IVIVC	in vitro-in vivo correlation
LC/MS	Liquid Chromatography/ Mass Spectrometry
LGSP	Liquide gastrique simulé de porc
LIN	Lincomycin
LOD	Limit of detection
LOQ	Limits of quantification
MBM	Meat and bone meal
MIB	Medicating Ingredient Brochures

MMC	Migrating myoelectric complex
MR	Modified release
PK	Pharmacokinetic
SBM	Soybean meal
SGF	Simulated gastric fluid
GIT	Gastrointestinal tract
F	Bioavailability
TS	Tourteau du soja
USP	United states pharmacopeia
VMP	Veterinary medicinal product
WHC	Water holding capacity
$D_{LIN}(t)$	% of active ingredient dissolved at the sampling time t
H	Sigmoidicity factor
D_0	% of active ingredient dissolved at time 0
D_{max}	Maximum dissolved %
Flag	Threshold factor
P_{max}	The asymptotic maximum precipitation of CTC that
k	First-order rate of CTC-metal complexation
t_{50}	The time required for the half of D_{max}

"To my wife"

"To my parents"

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Introduction 1

In pork production farming, the animals are reared intensively and housed in large groups. The primary health concerns in pig farms are bacterial infections, especially in the alimentary and respiratory tract (Glass-Kaastra, Pearl et al. 2013). They are the major clinical challenges and may be observed in almost all commercial pig farms resulting in a significant economic impact for the swine industry. As the animal density of conventional swine operations is high (Rosengren, Waldner et al. 2008), infectious disease may spread quickly within farm animals (van der Meulen, van der Werf et al. 2007). Outbreaks of bacterial diseases can be treated and controlled by antibiotics. Individual animal treatment is often impracticable in food-producing animals that are kept in groups of several hundred of pigs or more (Schwarz, Kehrenberg et al. 2001) and have a very low swineherd-to-animal ratio; in such cases, the basic therapeutic unit is often the group rather than the individual. The most common way of administering antibiotics for prophylactic or therapeutic purposes to pigs is by mixing the drug into the feed or by dissolving it in the drinking water (Nielsen and Gyrd-Hansen 1997). In most cases in feed medication is preferred because it is commercially available from the feed mill, not all drugs are sufficiently water-soluble to such a degree that it is possible to obtain a therapeutically effective daily dosage, and the feed may effectively hide the unpleasant taste of the drug (Nielsen and Gyrd-Hansen 1997). In addition, the administration of drugs

in water requires equipment which, for various reasons, is not always available in all pig farms.

In addition, pigs are intractable animals (Wilson, Harvey et al. 1972) thereby they are not cooperative to forced oral drug dosing with tablets or syrups, and they have highly acute olfactory (Brunjes, Feldman et al. 2016) and gustative (Danilova, Roberts et al. 1999) senses that prevent the animals from ingesting drugs and poisons (Garcia and Hankins 1975, Glendinning 1994).

When medicated feed is used, several potential problems can arise. An example is the reduced uptake of the therapeutic dose by animals due to reduced palatability of medicated feed (Van Boeckel, Brower et al. 2015).

In addition, in systemically infected pigs, endogenous and bacterial anorexigenic substances may decrease their appetite by increasing PGE2 concentration in the cerebrospinal fluid (Del Castillo, Laroute et al. 2006), therefore pigs tend to stop eating and drink less and will not take the required quantity of therapeutic dose (Lugarini, Hrupka et al. 2002).

By mixing the drug into feed, feed-drug interactions could influence the drug bioavailability. In general, the presence of food in the alimentary tract impairs the absorption of drugs and directly influences the pharmacokinetic processes leading to reduced bioavailability of orally administered drugs (Nielsen and Gyrd-Hansen 1997), the degree of impairment to oral bioavailability may depend on the drug in question as well as the animal species (Yu, Elvin et al. 1990, Baggot 1992, Sutter, Riond et al. 1993). Previous studies with tetracycline (TC) and chlortetracycline (CTC) (Nielsen and Gyrd-

Hansen 1996) have shown that the bioavailability of these two compounds decreases after oral administration to fed pigs.

To date, the literature on feed–drug and feed additive-drug interactions in swine is limited (Christiansen, Mullertz et al. 2015). Previous food-drug interaction studies pertaining to swine mostly have focused the oral bioavailability of a limited number of drugs in fasted or fed conditions (Nielsen and Gyrd-Hansen 1996) how the feed's moisture content (Sutter and Wanner 1990) and feed additives such as calcium, citric acid (Wanner, Nietlispach et al. 1990) or mycotoxin binders (De Mil, Devreese et al. 2016) affect the oral bioavailability of the tetracyclines. From a practical point of view, this is of major relevance as most pharmacokinetic studies were performed in fasted animals while in intensive production farms animals are never fasted before drug administration. Moreover, some of the studied drugs either are not approved or are not allowed for use in pigs in Canada.

In vitro drug dissolution testing (IVDT) method is an important tool in the drug product development phase that predict in vivo drug absorption (Sunesen, Pedersen et al. 2005, Jantratid, Janssen et al. 2008) and can ensure what is the limiting step to drug absorption and therapeutic efficacy (Fleisher, Sweet et al. 2004). This method can also be used to monitor the effects of drug formulation changes (Dressman, Amidon et al. 1998). This is a major determinant of oral drug efficacy and safety which is mandatory tested for human drugs. Feed-grade veterinary drug premixes are also subject to IVDT (Hunter, Lees et al. 2012), but the effects of feed composition on the rate and extent of veterinary drug

release are overlooked. There are a few previous studies evaluating the drug release from swine medicated feed (Del Castillo and Wolff 2006).

Neglecting the effects of food-drug interactions leads to significant health and economic disadvantages for the pig producer: if the release of drugs from the feed is incomplete, they will not be able to reach their site of action in sufficient quantities, which delays their recovery and increases the risk of disease relapse. Increased fecal waste of unabsorbed drug, prolonged treatment duration, and increased inter-individual variability in therapeutic efficacy all worsen the cost-benefit ratio of the medication (Peeters, Croubels et al. 2018). Beside the inappropriate use of antibiotics in human medicine, in agriculture, and in other species of domestic animals, excessive and inappropriate use of antimicrobials in swine medicine has contributed to the emergence of antimicrobial-resistant bacteria (ARBs) which has significant public health implications (Laxminarayan, Duse et al. 2013). For example, these ARBs can be transmitted to humans through the environment (Graham, Evans et al. 2009), through direct contact between the agriculture workers and the population at large (Smith, Gebreyes et al. 2013), and through the food products that they consume (Price, Johnson et al. 2005).

The problem of selecting resistant bacteria can not be prevented, because even if the antibiotic is better absorbed, it will be eliminated either in the urine or the feces, which are all collected and stored in manure pits, which also receive faecal bacteria. As they will be exposed to antibiotics in the slurry for a long time, they will be under pressure to select their antibiotic resistance genes. However, by reducing food-drug interactions, the amount needed to achieve the desired therapeutic effect will decrease, which involves

reducing the level of exposure of bacteria to antibiotics, which can decrease the pressure to select for antibiotic resistance.

To the authors' knowledge, there are no studies that consider the impact of commonly used feedstuffs on drug release. Therefore, the goal of this study was to perform in vitro testing methods that identify feedstuffs or manufacturing practices that may hinder the in vivo release of drug.

Review of Literature 2

2.1. Medicated feed

A medicated feed is a medicinal product that is made from a mixture of one or more drug premixes (which the United States Food & Drug Administration names type-A medicated articles) and a nutritionally balanced feed. Premixes are veterinary drug formulations, designed to be added homogenously in animal feed and maintain their stability before and after mixing and feed pelleting (Hunter, Lees et al. 2012). Some carriers and other bulk excipients are mixed with drug substance(s), to provide a uniform mixing of feed and premix. For example, the Tylvalosin medicated premixes, are composed of 20 % w/w Tylvalosin tartrate as a drug substance and ~80 % w/w of other excipients[1], and chlortetracycline premixes are composed of 10-30% w/w of rice hulls and 10-30% w/w of calcium sulfate dihydrate as a bulk excipient[2]. The particle size of these excipients must have the similar size with drug substances to promote uniformly distribution of drug substances throughout the final product. In addition, paraffin oils are another component of the chlortetracycline premixes that is added to their composition at the rate of 1-5% w/w. The paraffin oil may be added to aid uniform distribution and decrease the formation of airborne drug substance dust during production of medicated feed. The final product of premix may be granulated due to their milling characteristics, increased

[1] Pharmgate 2019, Material safety data sheet (Tylvalosin Medicated Premix): 1-5.
[2] Pharmgate (2017), Safety Data Sheet, Canadian WHMIS Standards & GHS Rev04, 1-8.

stability and homogeneously dispersion in feed (Del Castillo and Wolff 2006). Once its efficacy, safety and product quality meet regulatory approval requirements, the premixes can be used to manufacture of other types of medicated feed. Therefore, in the case of medicated feed the premix is always standardized but the other feed ingredients may vary a lot over time due to some factors such as (Kim and Hansen 201): the availability of used feedstuffs, the nutrient requirements of treated animals, cost effective feeding constraints and the equipment used in the manufacture of medicated feed. Therefore, the medicated feed the is a bulk product that contains nonmedicinal ingredient. Most ingredients are vegetal (e.g., ground corn and soybean meal) some are of animal origin (e.g., milk by-products) and some agro-industry co-products from brewing and distilling (e.g., Dried Distillers Grain with Soluble) which can used as main ingredients or additives. Medicated feeds are generally prepared by the feed manufacturer that holds a licence to manufacture medicated feed (Vandael, Filippitzi et al. 2019) because the facilities of the pharmaceutical industry are obviously inadequate to manufacture and deliver great quantities of medicated feed. In addition, medicated feed manufacturers are in possession of a good-controlled technology that permits the homogeneous incorporations of feed ingredients. All medicated feeds should pose specific labeling information clearly listing ingredients, feeding instructions, cautions or warnings, withdrawal information, and other relevant information.

The Compendium of Medicating Ingredient Brochures (CMIB) lists those medicating ingredients permitted by Canadian regulation to be added to livestock feed (Canadian Food Inspection Agency, 2018). In addition, it lists the indications for which the premixes

can be used, their concentrations in the feed, withdrawal time and compatibilities with other food additives. Currently, 24 different standard drug premixes have been approved to be added to swine feed. The results of safety, efficacy, stability testing of these drugs have been reviewed and approved by health Canada.

2.1.1. Types of medicated feeds

There are several categories of drug products approved for use in animal feeds as follows that is per FDA nomenclature:

- Type-A medicated article, which is the premix sold by the pharmaceutical company, is designed to be added to animal feed to produce either type-C medicated articles that is the final medicated feed or type B medicated articles that is concentrated medicated feed. The premix cannot be administered directly to the target animals unless used as a top-dress over a drug-free feed (Pittman JS 2019). Type-A medicated articles consist of one or more animal drug(s) with other excipients (e.g., calcium sulfate dihydrate, rice hull). For example, the lincomycin-spectinomycin granular premix approved for use in Canadian livestock feeds are composed of 92% calcium sulfate dihydrate as an excipient, lincomycin and spectinomycin each in an amount of 2.2% and 3.5% mineral oil[3]. Calcium sulfate dihydrate and rice hull at the amount of 10-30% w/w are the components of chlortetracycline premixes used in swine medicine.

[3] BioAgriMix (2016). SAFETY DATA SHEET (Lincomycin Spectinomycin 4.4% G Premix), Bio Agri Mix: 1-7.

- Type-B medicated articles are further diluted from either Type-A medicated articles or Type-B medicated articles and are used for manufacturing of another Type-B or Type-C medicated article. In addition to the animal drug(s), Type B medicated feeds contain a substantial quantity of nutrients (not < 25% nutritional content, W/W).
- Type-C medicated articles are intended to be fed directly to animals that are manufactured from Type A, Type-B, or other Type-C medicated articles. This is the final product for incorporation into the feed or direct administration to the animals and contains a lower concentration of drug than a Type A and B premixes. For example, lincomycin 110 premixes contain 11% w/w lincomycin hydrochloride as an active substance with 89 % w/w excipients. The incorporation of 1.0 kg lincomycin 110 premixes with a metric tonne (1000 kg) of complete feed, give a final concentration of 0.011% active substance that is used for the treatment of Swine Dysentery.

2.2. Drug release and absorption following oral administration

There are a wide variety of oral dosage form that are available for use in swine medicine; including: oral solution and suspension. Each of them has a different absorption pattern; for example, oral solutions provide rapid absorption. There are many factors that may affect the rate and extent of oral drug absorption. Before absorption, solid drugs formulations must disintegrate and then dissolve; dissolution often is the rate-limiting factor in oral drug absorption (Florence and Siepmann 2016). Once the drug has dissolved in GIT, multiple factors influence its absorption; (i) Drug characteristics including

solubility, stability, intestinal permeability, lipophilicity, pKa, surface area, particle size etc. As environmental pH is markedly variable among the different regions or compartments, drug pKa is particularly important in the GIT. (ii) Physiological factors determining oral absorption include GI motility, surface area (being greatest in the small intestine, which is the major site of drug absorption), transport and metabolizing proteins, environmental pH, and epithelial permeability. The GI motility plays an important role in the absorption of oral drugs (Talattof, Price et al. 2016); it influences the mixing of luminal contents, which is necessary for the dissolved drug to come into contact with absorptive surfaces. Gastric motility determines gastric emptying, which in turn influences the rate of drug absorption. Both efflux transport proteins (eg, P-glycoprotein) and drug-metabolizing enzymes located in the GI epithelium can dramatically decrease drug absorption, contributing to a first-pass effect (Deng, Zhu et al. 2017).

2.3. Feed-drug interactions of oral drugs

Interactions between food and drugs can markedly alter oral absorption of drugs by either diluting it, or more importantly, binding to it, so that it is not absorbed (Pijpers, Schoevers et al. 1991, Nielsen and Gyrd-Hansen 1997). The oral route of delivery is by far the most popular, mainly because it is natural and convenient for the patient and because it is relatively easy to administer. The medicated feed is often used because intensive pig production farms have a very low pig keeper-to-animal ratio. Also, as discussed in previous section, pigs are intractable animals (Wilson, Harvey et al. 1972), thereby they are not cooperative to forced oral drug dosing with tablets or syrups, and they have highly acute olfactory (Brunjes, Feldman et al. 2016), and gustative senses (Danilova, Roberts et

al. 1999) that prevent the animals from ingesting drugs and poisons (Garcia and Hankins 1975, Glendinning 1994). The magnitude of food-drug interactions may depend on the size and the composition of a meal. It was shown that, binding of drugs in premixes to components of vegetable feed decreased both rate and extent of absorption of ivermectin in sheep from the in-feed formulation, by affecting the dissolution rate of drug in vivo in GIT (Ali and Hennessy 1996). Similar interactions were also demonstrated for sulphonamides (SCP) and trimethoprim (TMP), which were a major cause of the limited bioavailability in horses (Van Duijkeren, Kessels et al. 1996). The most important pharmacokinetic food-drug interactions are caused by changes in the absorption of a drug because of physiological response to food intake (gastrointestinal motility, bile secretion) or the chemical reactions between the drug and food (Deng, Zhu et al. 2017). Another important issue is the food categories. In food divalent metal ions, such as Ca2+, Mg2+ reduces the drug absorption. The bioavailability of the tetracycline derivatives (tetracycline, oxytetracycline and chlortetracycline) was considerably low in pigs (Nielsen and Gyrd-Hansen 1996). These compounds are strong chelators that can form complexes with some multivalent cations (Ca^{2+}, Mg^{2+}, Zn^{2+}, Cu^{2+}, Fe^{2+}, Fe^{3+}, Al^{3+}) and then become either insoluble precipitates or soluble complexes, which both are hardly absorbed (Deng, Zhu et al. 2017). In addition, high-fat food may provide a lipophilic environment that increases the solubilization of fat-soluble drugs. Such as gefitinib, which a log octanol:water partition coefficient (LogP) is around 4 (Swaisland, Smith et al. 2005). This type of food may also stimulate the intestinal lymphatic transport pathway (Gershkovich and Hoffman 2007) that facilitates the absorption of highly fat-soluble drugs such as

acitretin (McNamara, Jewell et al. 1988). High-carbohydrate food can also affect oral bioavailability of drug. For example, tacrolimus (Bekersky, Dressler et al. 2001) and praziquantel (Castro, Medina et al. 2000) had an increased absorption rate when taken with high-carbohydrate food as compared to a high-fat meal, although this phenomenon remains unexplained. High-fiber food may have a significant effect on drug absorption. This type of foods can adsorb postprandial secreted bile acid which solubilizes lipophilic drugs and decrease the concentration of the bile acids resulting in decreased absorption for lipophilic drugs (Kern, Birkner et al. 1978, Deng, Zhu et al. 2017). High-fiber food may also prolong gastric emptying, further reduces the fluid volume available for drug dissolution in the upper GIT and increases the viscosity of luminal contents, consequently, lead to a reduced drug absorption percentage (Deng, Zhu et al. 2017). In the GIT, high-fiber food also undergoes fermentation via gut, and thus, there is a reduction in drug-metabolizing activity by intestinal bacteria (Deng, Zhu et al. 2017). For pigs, potential feed resources derive primarily from the vegetable foods (e.g., cereals, legume seed by-products), and other agro-industry co-products. These dietary sources have physicochemical characteristic such as water holding capacity.

2.3.1. Water-holding capacity of feedstuffs

Water holding capacity (WHC) is the ability of feedstuffs to prevent water from being released (Robertson and Eastwood 1981). The WHC determination procedures will be explained in the materials and methods section of this thesis. The water volume is an indispensable factor for the food effect of drugs. Because the first step in the intestinal absorption process from an orally administered dosage form is drug dissolution in the

gastrointestinal (GI) tract. There are no studies that consider the impact of water holding capacity (WHC) of feedstuff on drug bioavailability of orally administered veterinary drugs. To better understand how medicated feeds may be released and absorbed in the swine gastrointestinal (GI) tract, we have provided an update on the knowledge of the digestive anatomy and physiology in pig in this capture with emphasis on *in vivo* and *in vitro* methods for testing and prediction of medicated feed bioavailability.

2.4. Physiology and anatomy of swine digestive system

2.4.1.Stomach

The pig is a monogastric animal: its stomach consists of a simple compartment that shows four functionally and structurally different regions. A protrusion named *diverticulum ventriculi* is located at the top of the cardiac stomach. This protrusion, whose physiological function is yet unclear, has implications for orally administered drugs in the experimental setting: if the drug given by gavage tube, formulations can be accidentally locate into this pocket and gastric emptying and dissolution might be delayed (Suenderhauf and Parrott 2013). Another unique feature of the pig stomach is the pylorus that consists of a semilunar sphincter and a fibromuscular protuberance of fat and muscle fibers, the *torus pyloricus*. These components are suspected to act together as reinforcement of closure and to regulate the passage into the duodenum (Bal and Ghoshal 1972). The functional consequences of this anatomical section are yet unclear, but it is shown that passage of large, and non-dissolving particles is markedly slower than in other species (Hossain, Abramowitz et al. 1990). The relative stomach weight of pigs represents approximately 0.95% (180–252 g) of body weight for Gottingen minipigs and

0.3% (415–500 g) for landrace pigs (Bollen, Madsen et al. 2005). The volume of stomach in the adult Yucatan pig (~50 kg) is about 1300 mL (Tang and Mayersohn 2018). Gastric acid secretion occurs in the fundus and is regulated by neuronal (n. vagus) hormonal (histamine, acetylcholine, gastrin) and physiological stimulation as in humans (Schubert 2009). In the pig, acid secretion results as a function of stimuli, such as food intake (Schubert 2009, von Rosenvinge and Raufman 2010). It is reported that, under fasted condition, the cardiac gland produce a slightly alkaline secretion with high buffering capacity, reaching maximum activity along the night (Holler 1970). This shows that the cardiac stomach and diverticulum retain a basic pH (McLauchlan, Fullarton et al. 1989). Mucous, parietal (HCl secretion), and chief (protease secretion; primarily pepsin) cells are the primary secretory cells (Tang and Mayersohn 2018).

High inter individual and inter study variability reported in the in vivo study using telemetric devices to elucidate gastric fluid pH ranging from 1.6 to 4.3 (Oberle and Das 1994, Kararli 1995). In the other study Gastric fluid pH ranges from 1.2 to 4 (Hossain, Abramowitz et al. 1990). The mean gastric fluid pH in 24 h fasted condition has been reported to be 3.6 (Oberle and Das 1994). Gastrointestinal pH is an important factor for the assessment of oral drug delivery vehicle that influences drug ionization which in their turn can markedly affect oral drug absorption and bioavailability as it may have significant influence on drug dissolution and solubility, drug release, drug stability, and intestinal permeability (Abuhelwa, Williams et al. 2017). The variability of GIT pH influences the behavior of modified-release (MR) dosage forms (Ibekwe, Fadda et al. 2008). The dissolution of weakly basic or weakly acidic drug can be pH dependent. The dissolution of weakly

basic drug has been reported be much higher in the acidic environment of the stomach as compared to basic environment of the small intestine. On the other hand, weakly acidic drugs dissolution is minimal in the stomach but as they move towards small intestine that is more basic, their solubility increases (Abuhelwa, Williams et al. 2017).

2.4.2.Small intestine

The small intestine is the major site of drug absorption. The small intestine is located primarily on the right side of the abdomen (Tang and Mayersohn 2018) and constituted 81% of the length of the gut (Merchant, McConnell et al. 2011). In mammals, including the pig, duodenum and ileum are much shorter than the jejunum (Suenderhauf and Parrott 2013). The duodenum, jejunum and ileum represent about 5%, 90% and 5%, of the small intestine length, respectively, with a diameter of about 20 mm in a 50 kg Yucatan pig and 30 mm in a 50 kg domestic swine (Tang and Mayersohn 2018). However, the exact discrimination of these compartments is technically challenging, and we will therefore consider only total small intestinal length in the pig (Adeola and King 2006). In 3-week-old Göttingen minipigs the in vivo total small intestine length of 832 – 900 cm and an average diameter of 1 cm was measured (Suenderhauf and Parrott 2013). In the other post-mortem study, the length of small intestine was found a mean value of 840 cm ± 6.0 CV% in adult (6 months old) Gottingen minipigs (Suenderhauf and Parrott 2013). Need to know the intestine considerably elongates after death and effective in vivo length might be shorter (Suenderhauf and Parrott 2013). The duodenum of minipig shows typical circular folds (*plicae circulares*) and finger like villi that reduce in height towards the ileum (Suenderhauf and Parrott 2013). The villi represent the same cell types as found in the

human small intestine (goblet, enterocytes, and crypt cells) as well as Peyer's patches (Suenderhauf and Parrott 2013). The small intestinal fluid pH ranges from 5.7 to 7.2 (Tang and Mayersohn 2018). The total estimated surface area of the small intestine in a 47 kg pig ranges from 168 to 210 m^2 (Tang and Mayersohn 2018).

Table 2.1. *Gastrointestinal System Dimension of Pig, Minipig, Dog and Human*

	Small intestine (cm)		Cecum (cm)	
	Length	**Diameter**	**Length**	**Diameter**
Pig [A]	1500-2000	2.5-3.5	21-30	8-10
Minipig [B]	832-900	2	13-20	3.13±15cv%
Dog [A]	150(62); 414	2-2.5	12-15	-
Human [A, C]	300-325	3-4	3-4	7

A: (Dressman and Yamada 1991), B:(Suenderhauf and Parrott 2013), C : (Kararli 1995)

2.4.3.large intestine

The large intestine is located in the left upper quadrant of the abdomen and is tightly coiled (spiral colon) in swine (Tang and Mayersohn 2018). The large intestine of the pig is about 20% the length of the small intestine (Tang and Mayersohn 2018) with a diameter indicated in the Table 2.1. The cecum in the pigs, is more developed as compared to other omnivorous animals. The length of cecum and colon in the adult Landrace pig (~240 kg) is about 21–23 and 450 cm, respectively (Tang and Mayersohn 2018). Total cecum length is reported to be 13 cm in adult Gottingen minipigs weighting about 80± 95 kg (McRorie, Greenwood-Van Meerveld et al. 1998). In the other study, in Gottingen minipigs (~ 29 kg, 15-30 month of age) total colon length was measured at 303 cm (Suenderhauf and Parrott 2013) and the colonic diameter was measured at 2.7 ±10 cm in adult minipigs (Suenderhauf and Parrott 2013). The small intestine and colon are designed to secrete biochemicals, process food, and provide efficient absorption of digested materials (Tang and Mayersohn 2018). In addition to water and electrolyte reabsorption, the porcine large intestine efficiently ferments

carbohydrates, similarly to stomach digestion in ruminants (Argenzio and Lebo 1982). The comparison of GI system dimension of laboratory animal species, as well as human is indicated in table 2.1. The pig's colon has a fluid pH of about 7.1 and the cecum has a fluid pH of about 6.8 (Tang and Mayersohn 2018).

Table 2.2. *Composition of biliary bile acid in pigs (Holm, Mullertz et al. 2013)*

Bile salt	Pig[A]	Pig[B]	Pig[C]	Pig[D]
THC	3.5 ± 0.4	1.2 ± 0.9	6.8 ± 0.3	4.8 ±1.3
THDC		3.2 ± 0.6	1.1 ± 0.1	2.1 ± 0.2
TC				
TDC				
TCDC	3.0 ± 0.3	4.2 ± 0.5	6.8 ± 0.3	4.9 ± 1.0
GHC	12.6± 1.5		44.4 ± 0.9	36.7 ± 4.7
GHDC	48.2 ± 3.6	13.2 ± 6.4	8.2 ± 0.7	14.2 ± 5.2
GC	1.3 ± 0.2			
GDC				
GCDC	31.3 ± 2.8	33.3 ± 3.2	31.2 ± 0.8	33.0 ±6.5
G-3α6keto- 5β-c			1.8 ± 0.2	3.5 ± 1.1

Abbreviations: GC: glycocholate; GCDC: glycochendeoxycholate; GDC: glycodeoxycholate; GHC: glycohyocholate; GHDC: glycohyodeoxycholate; G- 3α6keto-5β-C: Glyco 3α 6keto 5β cholanate; TC: taurocholate; TCDC: taurochendeoxycholate; TDC: taurodeoxycholate; THC: taurohyocholate; THDC: taurohyodeoxycholate.
A: (Alvaro, Cantafora et al. 1986) B: (Scanff, Monti et al. 1999) C: (Kuramoto, Miyamoto et al. 2000) D: (Scanff, Grison et al. 1997)

2.4.4. Pancreas

The porcine pancreas is composed of three lobes: splenic lobe, duodenal lobe, and connecting lobe. The splenic lobe is attached to the spleen and the stomach. The duodenal lobe is C-shaped, located adjacent to the duodenum. The connecting lobe is an extension of the pancreas which is attached to the anterior aspect of the portal vein (Ferrer, Scott et al. 2008). The pancreas fulfils multiple vital metabolic functions. The secretions of the exocrine pancreas (digestive enzymes) are essential for the utilisation of nutritional components. Peptide therapeutics is generally regarded after oral administration starting from stomach due to pepsin and continuing in the small intestine by proteolytic enzymes present in the pancreatic juice and associated with the brush

border membrane (Pereira de Sousa and Bernkop-Schnurch 2014). The most relevant proteolytic enzymes of the pancreatic juice secreted as inactive precursors into the duodenum are trypsin, carboxypeptidases A and B (exopeptidases), elastase (serine endopeptidases) and α-chymotrypsin (Kavutharapu, Nagalla et al. 2012). Enzymes associated with the brush border membrane are active at neutral pH and include mainly endopeptidases, carboxypeptidases, and aminopeptidases (Pereira de Sousa and Bernkop-Schnurch 2014).

Table 2.3. *The gastrointestinal tract of pig and minipig with respect to anatomy and physiological parameters of relevance for drug absorption studies*

Variable	Landrace pig	Minipig
pH fasted	Stomach: 1.2–4.0 (Hossain et al. 1990)	Stomach: 0.3–1.7 (Oberle and Das, 1994) SI: 7–8 (Oberle and Das, 1994) LI: 6.3-6.8(Tang and Mayersohn 2018)
pH fed	Stomach: 4.4 (Merchant et al. 2011) SI: Duo: 4.7–6.1 Jej: 6.0–6.5 Ile:6.3–7.2 (Merchant et al. 2011) LI: 6.1–6.6 (Merchant et al. 2011)	Stomach: 2.94 (McAnulty, Dayan et al. 2011) SI: 6.1-7.5 (Oberle and Das, 1994)
Transit time fasted	Stomach: 1–28 days ,SI: <1–3 days (Hossain et al. 1990) LI: <1–3 days (Hossain et al. 1990)	
Transit time fed	Stomach: Solution/pellets 1.4–2.2 tablet 1.5– 6.0 (Davis et al. 2001; Wilfart et al. 2007) SI: 3–4 h (Davis et al. 2001; Wilfart et al. 2007) LI: 24–48 h (Davis et al. 2001; Wilfart et al. 2007)	Stomach: solid: >24 (Suenderhauf and Parrott 2013)
Bile concentration	42–55 mM (Juste et al. 1983)	
Metabolic activities	Phase I: CYP1A1,1A2,2A6,3B6,2C9,2D6,2E1,3A4 Phase II: UGT, SULT, GST (Suenderhauf and Parrott 2013)	Phase I: CYP1A2,2A6, E1,3A4
Major drug Transporters	P-gp, BCRP, MRP2, OATP	
Water volumes	Stomach: Wetmass: _250 g (Merchant et al. 2011) SI: Wetmass: _500 g (Merchant et al. 2011) LI: wetmass: _750 g (Merchant et al. 2011)	

SI: Small intestine; LI: Large intestine

2.5. Physiological factors influencing oral drug absorption.

2.5.1. Gastric emptying and gastrointestinal transit

Gastric emptying (GE) is an important element of gastrointestinal physiology that may impact drug absorption from the pig GIT. During fasting, GE is controlled by the migrating myoelectric complex (MMC) which also known as interdigestive. The MMC in pigs recur at intervals of 75-80min (≈18MMC/day) during fasting. Numerous factors including type and volume of meal, viscosity, osmolarity, etc., will affect emptying rate in the fed condition (Tang and Mayersohn 2018). Small frequent meals do not appear to interfere with the MMC during the day (Ruckebusch and Bueno 1976), whereas with one or two large meal per day, the postprandial contractile activity in pigs lasted 6h (13/MMC/day) or 3h (16MMC/day). There are high inter-individual and inter study variability in the gastric emptying rates in various strain of pigs. some investigators reported a long gastric emptying rate in landrace pig (1-2 years old; ~ 29kg) in the range of 6-24 h (Oberle and Das 1994). In contrast to this, Davis, Illum et al. 2001 observed a gastric emptying time of 6 h in landrace pigs (90–100 kg BW) after a light meal.

Differences in feeding patterns may influence GE and drug absorption. Gastric emptying of food follows a bimodal pattern; About 30-40% of ingesta passes into small intestine within 15min of eating in adult pigs with subsequent emptying during the next hour. However, food can remain in the stomach for the entire day, and emptying may be incomplete (Tang and Mayersohn 2018).

The effect of dietary fibre on gastric emptying is controversial and is not very clear. It has been reported that, dietary fibre (both soluble and/or insoluble fibre) delays GE (Van Leeuwen, Van Gelder et al. 2006). Rainbird & Low, (1986) indicated that, dietary fiber has

no effect on GE. In other studies (Potkins & Lawrence, 1984; Guerin et al. 2001), the authors reported that, dietary fiber accelerates GE. It was reported that the rate of gastric emptying during the meal for both dry matter (DM) and liquids was positively and linearly correlated with body-weight (Gregory, McFadyen et al. 1990).

Davis et al. (2001) measured GE and intestinal transit of pharmaceutical dosage forms (liquid, pellet, and tablet formulations) in the Landrace pigs (90–100 kg; fed twice a day), using gamma scintigraphy under fasted conditions (18 hours prior to dosing and 6 hours following dosing). The mean time for 50% emptying (t50) of a test liquid and solid pellets were 1.4 and 2.2 hours, respectively. Whereas for the tablet (Non-disintegrating capsule-shaped with a size of 22.0×8.7×5.1 mm), in three pigs tested, the tablet emptied between 5 and 6 h in two pigs, whereas in the third pig, gastric emptying occurred between 1.5 and 2 h (Davis, Illum et al. 2001). It was demonstrated that the size and density of non-dissolving dosage forms had an impact on gastric transit times in pigs (6-8 months old; 45 kg). Prolonged gastric residence (>5 days) was found for enteric-coated nondisintegrating magnesium hydroxide caplets: density, 1.5 g/ml; size, 19.6×9.5 mm; weight, 1.2 g (Hossain, Abramowitz et al. 1990). The gastric transit time of non-disintegrating dosage form in the pig under fasted condition, is reported to be significantly retained (1-28 days) with high variability. The transit time in small and large intestine is reported to be shorter (<1-3 days) and less variable (Hossain, Abramowitz et al. 1990). The Heidelberg pH capsules that is a pH monitoring device had a gastric residence time of >6 days (144 h), a small intestine transit time of >2 days and a large intestine transit time of >1 day (Hossain, Abramowitz et al. 1990). Gastric emptying of non-disintegrating caplet is much slower in

pig compared to human; It seems that MMC is less efficient in emptying large indigestible solids from the stomach, or the mechanism in pigs is entirely different (Hossain, Abramowitz et al. 1990).

Corresponding data for total transit time from literature vary strongly among studies suggesting a relation between digesta passage and age (or BW) of the pig. Van Leeuwen et al. (2006) measured a total transit time of 75 h for passage of digesta (diet ingredient size:2mm) through the total GIT in pigs with a mean BW of 46 kg in the first experimental period increasing to 119 kg in the fourth period. Le Goff et al. (2002) reported mean residence times (MRT) of 81 h in sows (252 kg BW), of 37 h determined in finishing pigs (78 kg BW) and a MRT of 33 h in growing pigs (33 kg BW) feeding diets of the same composition (Le Goff, van Milgen et al. 2002). Some investigators reported an MRT of 25 to 38 h for passage through the total GIT in pigs with a mean BW of 24 kg (Potkins, Lawrence et al. 1991). The small and large intestine transit time of the pig is somewhat influenced by intestinal content, for example a high fiber meal transited in 26 h while other solid and liquid had a transit time of 25- 29 h (Van Leeuwen, Van Gelder et al. 2006). Transit times for the small intestine of the pig are much more homogeneous than for the stomach. Regardless of the dosage form, transit time of the various dosage forms (liquids and solids) through the small intestine of landrace pigs (90–100 kg) was approximately 3–4 h (Davis, Illum et al. 2001). In the large intestine, the transit time of 24.9 h was measured for fluids and high fiber meal, while solids and other fluids had transit times ranging between 35 and 49 h in pigs with a mean BW of 46 (Van Leeuwen, Van Gelder et al. 2006).

2.5.2.Metabolism

The elimination of xenobiotics and several endogenous compounds includes phase oxido-reductive and / or phase conjugative. The purpose of these phases is to facilitate the excretion of the parent compound. Oxido-reductive Phase involves the addition of polar functional group (s) via an oxidation (via cytochrome P450) reaction and convert a parent drug to more polar (water soluble) active metabolites (Burkina, Rasmussen et al. 2017). Therefore, the cytochrome P450 (P450) family of enzymes is a major player in the metabolism of xenobiotics and a wide range of endogenous compounds (Achour, Barber et al. 2011). The cytochrome P450 enzymes have been extensively studied in the pig (Soucek, Zuber et al. 2001, Achour, Barber et al. 2011). There have been limited studies examining phase conjugative processes. The UDP-glucuronosyltransferases (UGT) play an important role in metabolic elimination of endobiotics, and xenobiotics via glucuronidation (Monshouwer, Van't Klooster et al. 1998). The gastrointestinal tract is the most important extrahepatic site of drug metabolism (Helke and Swindle 2013). Quantitative information on metabolizing enzymes in the GIT of the pig is scarce, although CYP3A has been observed in the small intestine of Göttingen minipig along with the presence of P-gp (Tang and Mayersohn 2018). P-glycoprotein (P-gp) is an ATP-dependent drug efflux transporter, functioning as an efflux pump against xenobiotic substrates (Van Peer, 2014). The amount of P-gp, which varied about a 10-fold range, was found to increase from the proximal duodenum to the distal ileum (Tang, Pak et al. 2004). Shulman et al. (1988) have suggested that the minipig reflects the GIT membrane content of enzymes (sucrase, lactase, maltase, acid β-galactosidase, and glucoamylase). Sucrase activity was highest in the jejunum and lowest in the duodenum, whereas Lactase activity

was highest in the jejunum and lowest in the ileum. Maltase activity was highest in the jejunum. Acid P-galactosidase activity in the miniature pig, is higher in the upper small bowel, where the activity is greatest in the ileum. Glucoamylase activity was similar among the small intestinal segments (Shulman, Henning et al. 1988). Intestinal microbiota plays a key role in drug metabolism. Although, the liver is known as a main organ responsible for drug metabolism and biotransformation, the metabolism of drug may begin at the intestine earlier than that at liver (Sun, Chen et al. 2019). The metabolic response performed by liver is completely different from that by the gut microbiota. The liver primarily generates polar and high-molecular weight byproducts through oxidative and conjugative metabolism while the microbiota mainly produces non-polar low-molecular weight metabolites through hydrolytic and reductive metabolism (Joh and Kim 2010, Kim 2015). Therefore, the intestinal microbiota influences drug absorption, and changes therapeutic effects of drugs. Glucosamine, for instance, was shown to be metabolized by rat gut flora microbial flora. Therefore, the microbial flora is responsible for the low oral bioavailability of glucosamine (Ibrahim, Gilzad-kohan et al. 2012). Additionally, several other studies have been reported that the drug metabolized directly by intestinal microbes. It was reported for Omeprazole (Watanabe, Yamashita et al. 1995), Zonisamide (Kitamura, Sugihara et al. 1997) and Lactulose (Elkington, Floch et al. 1969).

Nutritional factors can dramatically alter drug metabolism rates by affecting phase I and phase II metabolism. The activity of drug metabolizing enzymes has been shown to depend on nutritional status (Parke 1978, Anderson, Conney et al. 1979, Yang, Welling et

al. 1995, Harris, Jang et al. 2003). For example, high protein diets may speed up the metabolism of some drugs by stimulating cytochrome P-450, and diets low in protein, calcium, magnesium and zinc decrease the activity of microsomal enzymes responsible for drug metabolism (Parke 1978). In the other study, the high-protein diet increases the metabolic clearance of antipyrine in human as compared to low-protein diet, whereas a carbohydrate rich diet decreases the rates of drug metabolism (Anderson, Conney et al. 1979). It was also reported that, dietary fat influences the composition of the endoplasmic reticulum of the liver and gastrointestinal mucosa, and enzymatic activity of several components of the drug metabolizing enzyme system (Parke 1978). Diets that disrupt the microbial flora can dramatically affect the metabolism of drugs.

2.6. In vitro testing methods of drug release and bioavailability

2.6.1. Dissolution

Over the past 50 years, dissolution testing has been employed as a quality control procedure in R&D to detect the influence of critical manufacturing variables and in comparative studies for in vitro-in vivo (IVIV) correlation (Zhang 2004). Broadly speaking, there are three main reasons for performing dissolution tests (Dressman, Amidon et al. 1998): (i) to make sure consistency of output throughout manufacture, (ii) to assess the factors may affect the bioavailability of the drug, (iii) to create predictions regarding the performance of the delivery system in vivo. Therefore, it is a very important test from a clinical perspective. Bioequivalence (BE) studies focus on the drug release from the formulation and subsequent absorption into the systemic blood circulation consist of both in vivo and in vitro studies (Pillay and Fassihi 1998). Considering that in vitro

dissolution may be applicable to the prediction of in vivo BE, and to save time and cost, in vitro dissolution testing method (IVDT) must perform to compare formulations (Polli 2008). Also, the impact of changing formulation and manufacturing process variables can be assessed with dissolution testing.

A biowaiver is a procedure for the regulatory approval of generic pharmaceutical forms allowing the drug manufacturers to replace the in vivo bioavailability and bioequivalence studies with an in vitro dissolution testing that compares their product to the reference drug formulation. This procedure significantly reduces development time and costs by avoiding lengthy and expensive in vivo trials. Biowaiver of in vivo bioavailability and bioequivalence for are based on the Biopharmaceutics Classification System (BCS) of the active ingredient (Yu, Amidon et al. 2002). The BCS is a scientific approach for classifying drug substances based on two factors, aqueous solubility, and intestinal permeability (Amidon, Lennernas et al. 1995).

- **Class I:** High Solubility – High Permeability: e.g., Metoprolol, Propranolol

These compounds exhibit a high dissolution and absorption rate and extent. For these drug compound formulated as immediate release products, dissolution rate exceeds gastric emptying time. Thus, BA is expected to approach 100%. In vivo bioequivalence data should not be necessary to ensure product comparability.

- **Class II:** Low Solubility – High Permeability: e.g., Danazol, Ketoconazole

These compounds have a high absorption rate and extent but are likely to be dissolution rate limited.

- **Class III**: High solubility – Low Permeability: e.g., Cimetidine, Captopril

For these compounds dissolution is likely to be rapid, but permeability is rate limiting for drug absorption. Waiver criteria similar to those for Class I compounds may be appropriate provided test and reference formulations do not contain agents that can modify drug permeability or GI transit time.

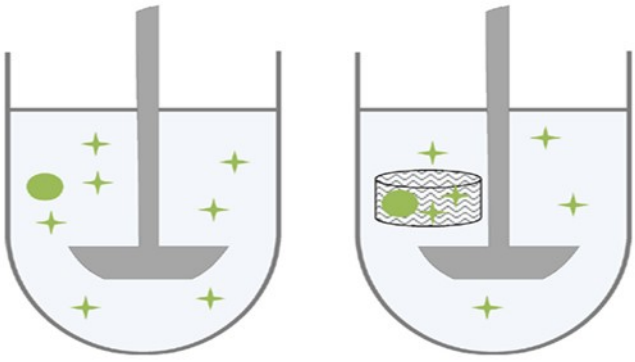
- **Class IV:** Low solubility – Low Permeability: Furosemide

These compounds have a poor BA, tend to be very difficult to formulate and can exhibit large inter and intra-subject variability in BA.

The connection between the in vitro dissolution test and in vivo performance of drugs is based on the fact that before an active pharmaceutical dosage form can be absorbed, it must first be dissolved in the GIT fluid. Based on the in vitro drug release profiles, in vitro – in vivo correlation (IVIVC) allows prediction of the in vivo performance of a drug. In vitro in vivo correlation in this sense refers to the relationship between the in vitro dissolution of the drug in the test apparatus and the release or absorption of the drug in vivo. In the pharmaceutical industry the establishment of an effective IVIVC has important implications in quality control and regulatory compliance and drug dissolution testing is commonly used to provide critical in vitro drug release information to assess batch-to-batch consistency of solid oral dosage forms such as tablets, and to predict in vivo drug release profiles. It is a process by which drug released from solid dosage from and immediately goes into molecular solution. The term "drug dissolution" is defined as mixing the two phases and forming another homogeneous phase (IUPAC, 1997). It should be noted that the terms "drug dissolution" and "drug release" are not synonyms (Fig 2.1), although these terms are often not appropriately distinguished in the literature

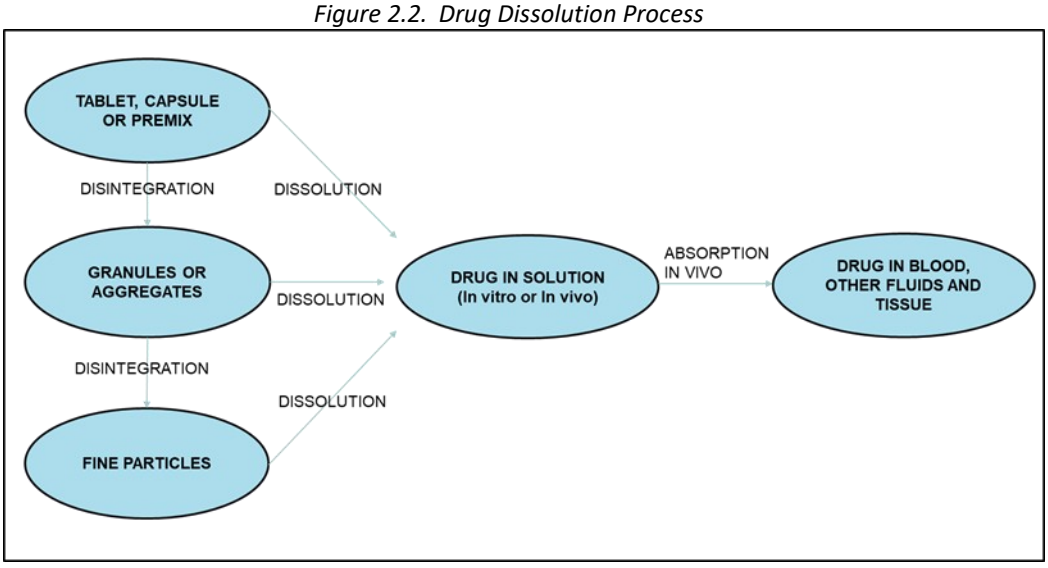
(Siepmann and Siepmann 2013). The term "dissolution" in clearly defined by IUPAC and generally encompasses the five major mass transport steps (Siepmann and Siepmann 2013): Wetting of the particles surface with water (i), breakdown of solid-state bonds in drug particle (ii), solvation of the individual species, such as ions or molecules (iii), diffusion through the liquid unstirred boundary layer (iv), convection within the well-stirred bulk fluid (v). In contrast, the term "drug release" most often refers to a much more complex phenomenon, one part of which is "drug dissolution". Upon contact with aqueous fluid of dissolution medium, water gets diffused into the core of the matrix of the delivery system and dissolves the drug. The dissolved drug species subsequently diffuse out of the matrix due to concentration gradient. In addition, the matrix of delivery system might undergo several changes including swelling as soon as critical water content is reached and eventually dissolves itself in the aqueous medium. Therefore, several phenomena can be involved in the process of drug release, only one of them being "drug dissolution" (Siepmann and Siepmann 2013).

Figure 2.1. Drug dissolution and drug release. The green circles represent drug particles (e.g., crystals), whereas the green stars represent dissolved (individualized) drug molecules/ions/atoms (Siepmann and Siepmann 2013)



Oral drug delivery is the most preferred and convenient drug delivery vehicle, especially for those kept in a very large group. The effectiveness of such dosage forms depends on the drug dissolving in the fluids of the gastrointestinal tract prior to absorption. The rate of dissolution of the drug (tablet, capsule, dietary drug premix) is therefore crucial. One of the problems facing the pharmaceutical industry is to optimize the dose fraction that reaches the general bloodstream following its extravascular administration, i.e., its bioavailability. insufficiency of bioavailability can mean that the treatment is ineffective (Kostewicz, Abrahamsson et al. 2014). Solid dosage form may disintegrate when they interact with gastrointestinal fluid following oral administration depending on their design. Disintegration plays a key role in the dissolution process since it determines to a large extent the area of contact between solid and liquid.

Figure 2.2. Drug Dissolution Process



2.6.1.1. paddle and basket apparatus

The paddle and basket apparatus (Fig 2.3) were the first dissolution standard testing equipment. These methods can be used for all dosage forms. With respect to immediate

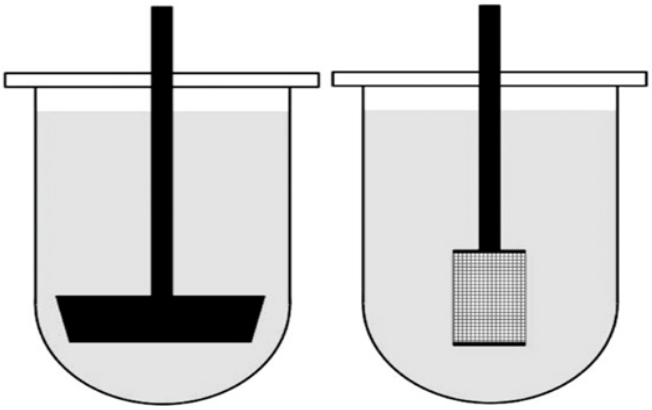
release (IR) products, tablet can usually be tested with both apparatuses without any additional hardware, while capsules require a sinker to hold the capsule in the medium when tested with the paddle and for this reason the basket apparatus be preferred. For the enteric coated (EC) product, the basket method is suitable. Also, these methods are appropriate for modified release (MR) dosage form if the formulation is robust to changes in the physiology as its proceeds through the gastrointestinal tract. These methods generally use media volumes in the range of 500 to 1000 mL that generate the sink condition for dissolution of the drug (Kostewicz, Abrahamsson et al. 2014). In pharmaceutics, sink condition is a term mostly related to the dissolution testing procedure. It means using a sheer volume of solvent, usually 5 to 10 times greater than the volume at the saturation point of the drug contained in the drug delivery system being tested (Phillips, Pygall et al. 2012).

The active substance of the drugs should be considered highly soluble with the highest drug product's strength soluble in 250 mL or less aqueous media over the pH range of 1–7.5 at 37°C that is derived from bioequivalence studies (Martinez and Papich 2012). Therefore, the dosage form should be ingested in the fasted state with the glass of water in humans. In animal this is impracticable as they do not drink voluntarily water after ingestion of an oral medication. Thus, dissolution of an oral medication in animal depends on residual gastrointestinal water. The gastric volume is unlikely to exceed 250 mL and therefore the volume used in the dissolution test is too high to accurately reflect condition in the stomach (Schiller, Frohlich et al. 2005). The hydrodynamic patterns for these methods generated by several computers simulation models (McCarthy, Kosiol et al.

2003). Since the in vivo condition have not been considered in the design of these method and variable hydrodynamic condition within the dissolution vessel, the hydrodynamic of these methods is problematic (Kostewicz, Abrahamsson et al. 2014). There are several reports to stablish relationship between stirring and in vivo hydrodynamics (Scholz, Kostewicz et al. 2003), and results are inconsistent. For example, the authors reported that, for predict behavior in Labrador dogs, the paddle speed for micronized felodipine should be 75 rpm (Scholz, Kostewicz et al. 2003). Other studies indicated that for paracetamol tablets, a paddle speed of 30 rpm would achieve the best IVIVC (Howgate, Rowland Yeo et al. 2006). It was reported that in the individual case, the in vitro dissolution at various stirring rates in these methods and in vivo bioavailability data is successful (Abrahamsson et al. 1996). Coning effect is another major issue for the commonly used paddle apparatus that can occur in the bottom of the vessels. This effect can also occur in the basket apparatus for particles small enough to pass through the basket mesh (Kostewicz, Abrahamsson et al. 2014). This problem is frequently happened during in vitro dissolution method development, and it has been proved that coning effect observed in vitro are unlikely indicative of a similar phenomenon in vivo (Kostewicz, Abrahamsson et al. 2014). For example, in a study in AstraZeneca two different modified release pellets formulations gave different in vitro profiles in a paddle method. Coning effect was observed for the slower releasing formulation; when the dissolution study was repeated using a ''peak'' vessel, no significant difference was observed (Kostewicz, Abrahamsson et al. 2014). It is a vessel whose bottom has a symmetrical protuberance at its center, which points towards the axis of the paddle. This protuberance is designed to

prevent cone formation during the test. To overcome this problem, increasing the stirring speed to 75 or 100 rpm in the paddle apparatus or replacing the round-bottomed dissolution vessels with peak vessels can be a solution (Kostewicz, Abrahamsson et al. 2014). To minimize variability of hydrodynamic effects, several factors need to be considered when using these (basket or paddle) methods (Gray, Kelly et al. 2009).

Figure 2.3. paddle (left) and basket (right) of a dissolution apparatus.



2.6.1.2. Dissolution testing of medicated feed

Since 1968, drug dissolution testing provides the pharmaceutical industry with critical in vitro drug release information for both drug development and quality control. Dissolution methodology initially developed for immediate release (IR) solid oral dosage forms such as tablets or capsules and then for alternate human dosage forms including patches, suspensions, and medicated devices (Mattocks and Thakker 2017). In the case of veterinary medicated feed, the application of dissolution is a relatively new application with consequently limited literature information. Medicated feeds have a wider range of attributes than human products which presents unusual challenge. As shown in Table 2.4,

there are significant differences between typical solid oral dose product and medicated feed.

Table 2.4. *Comparison of product attributes: medicated feed vs solid oral dose*

Medicated feed	Solid oral dose
Bulk product	Discrete unit product
Insoluble excipients	Soluble excipients
Dose may be variable	Well-defined dose
Aqueous dispersion limited	Easily disperses in aqueous media
Release in non-sink condition	Release in sink condition
Hard premix drug particles	Soft pure drug powder
Large sample mass typically measured in g	Small sample mass typically measured in mg

Unlike solid oral dosage forms with discrete unit doses, the sample size for medicated feed must be selected and consequently must be limited because of limited dissolution vessel volume for typical USP apparatus 1 and 2 which range from 500 to 4000 ml. During in vitro dissolution test determining of the actual dose to model depend on some factors including, species size, consumption of medicated feed by animal and the typical duration of dosing. Because of the complexity, Food and Drug Administration (FDA) has provided a draft guidance for estimating dosage-adjusted drug solubility based on the wide range of applicable gastric volumes (Food and Drug Administration, 2016). For medicated feed which pose high turbidity due to insoluble and soluble ingredients, the traditional analytical syringe filters may be impractical. The use of prefilter layer is a logical way to prohibit of rapid clogging of the filters due to high particle concentration. Considering that medicated feed is dense material that rapidly clump at bottom of the vessel, may experience significant coning in USP apparatus 2 systems and reduced both sufficient wetting of the whole sample and uniform dispersion throughout the vessel. The clumping of sample may limit active release even with high paddle speed. All these challenges together may require flexible approaches to method development. Dissolution of

medicated feed is complex and typical development strategies may not be appropriate. Although, USP apparatus 1 and 2 method development with some modification can be successfully executed.

2.6.1.3. Drug dissolution process analysis

2.6.1.3.1. Parametric approaches

The dissolution rate is generally defined as the change in the concentration of dissolved drug in the bulk fluid (dc), in the time interval (dt).

$$Dissolution\ rate = \frac{dc}{dt}$$

The rate of dissolution of a solid substance is directly proportional to its solubility in the dissolution medium. The most complex case is that of crystallized products which are more organized than amorphous products. We distinguish in the case of crystalline products a disorganization reaction at the solid-liquid interface; and secondly, diffusion of molecules or ions from the solid surface to the dissolution medium. The analysis of dissolution processes is demonstrated assuming some models such as the ones formulated by Noyes & Whitney, Nernst & Brunner, Hixson & Crowell equation or “cube-root law”. The original and most famous model was developed over a century ago by Noyes and Whitney in 1897 (Siepmann and Siepmann 2013). Based on observation of two quite different materials dissolving in distilled water, Noyes and Whitney deduced the general law:

$$\frac{dc}{dt} = K(Cs - Ct)$$

Where dc/dt is "drug dissolution rate"; dc is the change in the concentration of dissolved drug in the bulk fluid in the time interval dt; K is first-order dissolution constant, Cs is the

solubility concentration of the substance, and Ct is the concentration of dissolved drug at time t.

The basic hypothesis of this model is that the diffusional mass transport step through the liquid, unstirred boundary layer is the rate limiting process. To evaluate the validity of their hypothesis, these authors experimentally measured the solubility of benzoic acid and lead chloride in water. Based on the experimentally titrated concentration of dissolved lead chloride and benzoic acid in water, they calculated the K value for each time point.

Additional work by Nernst and Brunner elucidated the experimental factors that contributed to the proportionality constant k in Equation (Brunner 1903, Nernst 1904) leading to the form of the Noyes–Whitney equation that is still used today:

$$\frac{dm}{dt} = \frac{S.D}{\delta}(Cs - Ct)$$

where dm is amount of substance which dissolves in the time interval dt; S denotes the surface area available for diffusion/dissolution; D is the diffusion coefficient of the drug within the liquid unstirred boundary layer; δ is the thickness of this layer; Cs and Ct are the solubility of the drug in the bulk fluid and the concentration of dissolved drug in the bulk fluid at time t, respectively.

An alternative version of Equation is the Noyes–Whitney–Nernst–Brunner (NWNB) equation, which describes the change in concentration of dissolved solid with time:

$$\frac{Dc}{dt} = \frac{DA}{Vh}(St - C)$$

where V is the volume of bulk solvent. In either case, the model assumes that any drug molecule that dissolves at the surface of the solid must then diffuse through a stagnant

layer of saturated drug solution surrounding the solid before it moves into the bulk solvent. Another dissolution model was published by Hixson and Crowell (Hixson and Crowell 1931). These authors specifically addressed the fact that the surface of a dissolving substance often changes with time in practice, for example if spherical particles dissolve in well-agitated bulk fluids the radius of the spheres continuously decreases. Parallel to Noyes–Whitney and Nernst–Brunner, these authors started with the assumption that the rate at which the substance dissolves, dm/dt, is proportional to the available surface area, St, and the difference in concentration "cs–ct":

$$\frac{dm}{dt} = -K'.St.(Cs - Ct)$$

where dm is amount of substance which dissolves in the time interval dt; K' is the dissolution constant; Cs is the solubility of the drug in the bulk fluid and Ct is the concentration of the dissolved drug at time t. Several other notable models for dissolution have been published, but the NWNB model is still the model commonly used.

2.6.1.3.2. Comparison methods

There are some methods for comparing dissolution profiles (LeBlond, Altan et al. 2016):

- Statistical approaches,
- Model dependent method,
- Independent model method

Statistical approaches are based on analysis of variance, which assesses the assumption that the two profiles are statistically similar. The model-dependent method is mainly used for clarifying the mechanisms of dissolution or release under different experimental conditions. The dependent model method can be applied to dissolution profiles obtained

with dissolution programs with non-identical sampling, while the independent model method known as the “Fit Factor” method which requires identical sampling points for the calculation of two factors from individual raw data from two profiles. These two factors (difference factor ƒ1 and similarity factor ƒ2), have been adopted by regulatory agencies, and have been included in the guidelines for quality control of dissolution assays. The difference factor ƒ1 measures the relative error (in percentage) between two dissolution curves and at all points in time, the ƒ1 can be determined by below equation:

$$f_1 = \frac{\sum_{i=1}^{K} |R_i - T_i|}{\sum_{i=1}^{K} R_i} \times 100$$

where K is the number of points in time, R_i is the dissolved percentage of the reference at time i, and T_i is the dissolved percentage of the test form at time i.

The similarity factor ƒ2 measures the similarity of the dissolved percentage between the two curves. It can be determined by below Equation:

$$f_2 = 50\ log_{10}\left\{100\left(1 + \frac{1}{k}\sum_{i=1}^{k} W_i\,(R_i - T_i)^2\right)^{-1/2}\right\}$$

Where, W_i is an optional weight factor, R_i is the dissolved percentage of the reference shape at time i, T_i is the dissolved percentage of the test at time i.

The acceptable range of ƒ1 is <15 and ƒ2> 50. It should be noted that only dissolution data between 0 and 85% are included in the analysis with these methods and the method is not applicable when the final extent of dissolution is less. From a technical point of view, the following recommendations are given in the FDA guidelines for the calculation of ƒ1 and ƒ2:

- A minimum of three points in time (zero excluded).
- 12 individual values for each point in time for each formulation.

2.7. Absorption and bioavailability

Good absorption of drug compounds from the gastrointestinal tract depends on biopharmaceutical factors including suitable drug solubility in the gastrointestinal fluids, adequate intestinal permeability, and metabolic stability at a level suited for the drug within the intestine. From an oral drug absorption aspect, the most important features of the gastrointestinal tract are the stomach, small intestine and proximal large intestine. Moreover, secretions from the various organs (pancreas and gall bladder) that supply the small intestine play a considerable role. Drug absorption has been reported to decrease in the presence of micelle lipids due to reduction of thermodynamic activity. Therefore, drugs can be distributed differently between the colloidal phases of the intestinal tract after eating. In this case, rendering different solubility and dissolution patterns strongly affects the rate and amount of intestinal absorption and the bioavailability of Class II (Yu, Amidon et al. 2002).

The results of a swine in vivo study report that the dietary lipids increase the absorption of poorly soluble drugs by inhibiting the efflux mechanisms located at the apex of the enterocytes, such as P-glycoprotein (Persson, Nordgren et al. 2008). It has been reported that the bioavailability (BA) of midazolam in pigs is 5-14%, similar to that in rat and dog but lower than that of humans (34%), because of the greater systemic clearance of this drug in pigs, which approaches their liver blood flow (Lignet, Sherbetjian et al. 2016). Due to intestinal first-pass effect in the minipigs, it is reported that cimetidine had a higher

metabolic clearance explaining its lower oral bioavailability in pigs (~33%) as compared to humans, for whom this parameter is ~78% (Lignet, Sherbetjian et al. 2016). In pigs, finasteride has an oral BA of 40% compared to 80% in human, this has been explained by a greater liver/intestinal extraction in the pig (Lundahl, Hedeland et al. 2011). Antipyrine has a low systemic clearance in both pigs and humans and shows a sex-related difference in two species. The oral bioavailability of antipyrine reportedly is ~30% in male and female pigs, much lower than in humans (~100%). This difference can be explained by lower absorption in pigs or higher extraction in the gastrointestinal tract. Bioavailability of diclofenac in pigs was much greater than humans. This is explained by a greater hepatic first-pass effect in humans due to the higher CYP2C activity in humans (Willis, Kendall et al. 1979) that the porcine CYP2C were lower than in human, reaching only 16% of total CYP content (Achour, Barber et al. 2011).

The bioavailability of the tetracycline derivatives (tetracycline, oxytetracycline and chlortetracycline) was considerably low in both fed and fasted pigs than in human (Nielsen and Gyrd-Hansen 1996). Because tetracycline and oxytetracycline are eliminated primarily by renal excretion and have good solubility at the pH of the gastrointestinal fluids, first-pass effects cannot explain the difference. These compounds are strong chelators that may form insoluble complexes with heavy metals present in the gut that reduces absorption, which, there is an idea that if the cation composition of the pig gut are different from human, it can be explain the lower bioavailability (Nielsen and Gyrd-Hansen 1996). Food effect on oral absorption of pravastatin and atazanavir in the minipigs was studied for the evaluation of food effects on drug absorption in humans (Christiansen,

Mullertz et al. 2015). The result showed that, there is no considerable difference between the fed and fasted groups of minipig for either of the investigated compounds.

Drug formulation release and dissolution are the first step for achieving bioavailability for oral used drugs. A wide in vitro - in vivo extrapolation (IVIVE) study was performed in the minipigs with seven compounds (Antipyrine, atenolol, cimetidine, diazepam, hydrochlorothiazide, midazolam, and theophylline) These drugs were selected based upon their absorption, metabolism, and elimination routes in humans. In vitro data were generated on protein binding, blood to plasma partitioning, hepatocellularity and intrinsic clearance determinations. The estimated in vitro intrinsic clearance and in vivo intrinsic clearance illustrated an overall good correlation between minipigs and humans (Lignet, Sherbetjian et al. 2016).

Objectives & Hypothesis 3

3.1. Hypothesis

- Feedstuffs sponge the gastrointestinal fluids to different extents, which may become the limiting factor to dietary drug release.
- The water-holding capacity (WHC) of feedstuffs may predict the hindrance of drug release from medicated feeds.

3.2. Objectives

- Determine the water holding capacity of commonly used feed ingredients (soybean meal (SBM), ground corn (gC), wheat (gW) and rye (gR), dried distillers' corn grains with solubles (DDGS), and meat and bone meal (MBM).
- Perform an IVDT using the USP type-2 (i.e., paddle) apparatus to determine the drug release from medicated feeds, using different combinations of drugs and feedstuffs.

Methodology 4

4.1. Water holding capacity measurements

4.1.1. Introduction

Water holding capacity is the ability of the feedstuffs to incorporate water within its matrix. Potential feed resources used for animals in Canada and worldwide, derive primarily from the vegetable foods (e.g., cereals, legume seed by-products), and other agro-industry co-products (e.g., dairy by-products). Depending on their respective nutrient contents such as fiber (Ramanzin, Bailoni et al. 1994), proteins (Traynham, Myers et al. 2007), and sugars (Ngoc, Len et al. 2012), these feedstuffs all could absorb water and the amount of feed relative to the premix may limit the rate and extent of drug dissolution inside the gastrointestinal tract. There are several methods in the literature for measuring WHC (Kneifel, Paquin et al. 1991), but it is usually measured by centrifugation at high speed (McConnell et al., 1974; Robertson and Eastwood, 1981) and sometimes by filtration. The filtration method is robust and easy to perform and has been suggested to follow more closely the condition likely found in the gastro-intestinal tract and should resemble normal physiological conditions (Robertson and Eastwood 1981).

4.1.2. Tested feedstuff

We obtained from a local feed mill[4] samples of soybean meal (SBM), ground corn (gC), wheat (gW) and rye (gR), dried distillers' corn grains with solubles (DDGS), and meat and bone meal (MBM), which nutritional contents are listed in Table 4.1.

[4] F. Ménard Inc., St-Pie-de-Baggot, Qc, Canada.

Table 4.1. Nutrient contents (as percent of dry matter, except digestible energy in MJ/kg) of the tested feedstuffs

			Feedstuff					
Abb.	Name	Unit	gC	gW	gR	SBM	MBM	DDGS
DM	Dry matter	g/100 g	87.96	88.63	87.35	89.31	94.08	90.81
CP	Crude protein	% DM	7.45	12.62	8.49	48.33	55.56	28.11
EE	Ether extract	% DM	3.65	2.01	1.54	2.19	11.85	9.39
CF	Crude Fiber	% DM	2.21	2.45	1.94	4.25	-	6.57
NDF	Neutral detergent fiber	% DM	10.47	11.78	14.17	11.84	-	39.84
ADF	Acid detergent fiber	% DM	2.95	3.21	3.17	6.89	-	12.67
Starch	-	% DM	64.34	60.48	56.13	0.82	-	5.01
Sugar	-	% DM	1.34	2.16	4.97	9.11	-	1.39
K	Potassium	% DM	0.30	0.38	0.43	2.08	0.43	1.01
Na	Sodium	% DM	0.00	0.00	0.00	0.01	0.71	0.17
Mg	Magnesium	% DM	0.09	0.10	0.08	0.30	0.20	0.29
Ash	-	% DM	1.17	1.71	1.52	6.66	24.84	4.49
Ca	Calcium	% DM	0.01	0.04	0.04	0.30	7.81	0.03
P	Phosphor	% DM	0.22	0.27	0.24	0.59	3.60	0.79
DE	Digestible energy	MJ/kg	14.39	14.43	13.48	15.39	13.54	11.90
Lys	Lysine	% DM	0.23	0.35	0.32	2.92	2.41	0.83
Met	Methionine	% DM	0.16	0.2	0.14	0.64	0.66	0.56
Cys	Cystine	% DM	0.17	0.28	0.2	0.69	0.74	0.53
Thr	Threonine	% DM	0.27	0.35	0.28	1.86	1.81	1.04
Trp	Tryptophan	% DM	0.06	0.16	0.09	0.65	0.34	0.23
Arg	Arginine	% DM	0.36	0.6	0.45	3.53	3.72	1.28
Ile	Isoleucine	% DM	0.25	0.42	0.28	2.2	1.67	1.01
Leu	Leucine	% DM	0.88	0.83	0.53	3.67	3.36	3.17
Val	Valine	% DM	0.35	0.53	0.39	2.29	2.47	1.34
His	Histidine	% DM	0.22	0.28	0.19	1.25	0.85	0.73
Phe	Ph-alanine	% DM	0.35	0.57	0.37	2.47	1.87	1.35
Tyr	Tyrosine	% DM	0.27	0.38	-	1.8	-	-
Gly	Glycine	% DM	0.29	0.51	0.38	2.04	6.81	1.12
Ser	Serine	% DM	0.35	0.57	0.37	2.41	2.69	1.33
Pro	Proline	% DM	0.66	1.22	0.76	2.44	4.67	2.21
Ala	Alanine	% DM	0.55	0.44	0.37	2.08	3.66	1.99
Asp	Aspartic acid	% DM	0.49	0.64	0.61	5.5	3.86	1.83
Glu	Glutamic acid	% DM	1.33	3.51	1.84	8.67	6.29	4.74

4.1.3. General procedure

Feedstuffs were tested in triplicate by the filtration method (Robertson and Eastwood 1981): succinctly, A 1-g precision-weighed sample was soaked for 24 h at room temperature in 100 mL of distilled water without agitation, and then filtered through Whatman No. 1 filter paper. The wet samples were precision-weighed (wet weight), dehydrated by means of lyophilisation apparatus for 24 h and precision-reweighed (dry

weight). We performed some supplementary tests for SBM. In this case the samples of the SBM were dried overnight (24 h) in an oven at 105°C. The WHC was calculated using following formula:

$$WHC = \frac{Wet\ weight - Dry\ weight}{Dry\ weight}$$

We tested the effect of the type of lyophilisation apparatus by comparing the paired WHC results obtained with both an FTS Systems[5] or a Labconco Corporation[6].

4.1.4. Effect of Particle size on WHC

Samples (15-20 g) of each feedstuff were sifted for 10 min through seven stacked-up sieves of 1000, 700, 500, 300, 200, 100 and 70 µm mesh size (from top to bottom). The fraction remaining atop of each sieve was weighted and labeled as 70 µm, 100 µm, 200 µm, 300 µm, 500 µm, 700 µm and 1000 µm samples. Each fraction was used for WHC testing per the general procedure described in section 4.1.3. The fractions that were collected in the receptacle at the bottom of the sieves (particles <70 µm) were not tested.

4.1.5. Effect of soaking time on WHC

The samples were soaked without agitation in distilled water for 2, 6, 12 and 24 h at room temperature. Then, the WHC of each sample was measured as described in section 4.1.3.

4.1.6. Effect of porcine simulated gastric fluid on WHC

A suitable buffer solution simulating for the porcine gastric fluids was prepared (50 mL 0.2M KCl + 32.4 mL 0.2M HCl + 917.6 mL distilled H2O, for a total of 1000 mL), based on the pH of porcine gastric fluid (pH=1.6) in fasted condition (Hossain, Abramowitz et al.

[5] Stone Ridge, NY, USA
[6] Kansas City, MO, USA

1990). Then, WHC of feedstuff in SGF was measured as general procedure described in section 4.1.3.

4.2. Dissolution

4.2.1. Model drugs

The lincomycin[7] and chlortetracycline[8] feed-grade premixes within their respective expiration dates were donated by F. Menard feed mill company.

4.2.2. characteristics of premixes

4.2.2.1. Lincomycin

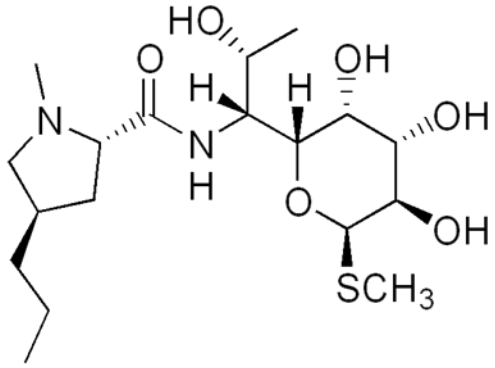
Lincomycin is a sulfur-containing pyranoside belonging to the lincosamide family of narrow-spectrum antimicrobials, which is synthesized by *Streptomycin lincolnensis*. Lincomycin has the empirical formula $C_{18}H_{34}N_2O_6S.HCL$ with a molecular weight of 443 g/mol. It is a basic compound possessing a single tertiary amine group whose conjugated acid has a reported pKa ranging from 7.5 to 7.8 (Qiang and Adams 2004). The free base is soluble in water and most organic solvents other than the hydrocarbons: its water solubility is 29.3 mg/mL. The octanol:water partition coefficient $K_{o/w}$ (which value commonly is log-transformed – symbol: log P) is a measure of the lipophilicity of a chemical substance. This parameter can be a valuable indicator of the biological properties of drugs, since liposolubility is a major determinant of their absorption and disposition by living organisms. Lincomycin has an experimental log-P value of 0.20. It is stable in the dry state and in aqueous solution for at least 24 months. It is also stable in

[7] Lincomycin 110 G Granular Premix; lot #. Bio Agri Mix LP (Mitchell, On, Canada)
[8] Deracin 22% Granular Premix; lot #. Pharmgate LLC (Wilmington, NC, U.S.A.)

the presence of air and light. The concentration of the drug in premix was 110 g/kg of lincomycin (as lincomycin hydrochloride) in the premix. Level of medicating ingredient in a complete feed depends on the indication approved in pigs

- 44 mg/kg feed for control of Swine Dysentery
- 110 mg/kg feed for treatment of Swine Dysentery
- 110 mg/kg feed for treatment of lleitis associated with *Lawsonia intracellularis.*

Figure 4.1. Structure of Lincomycin



4.2.2.2. Chlortetracycline

In Canada and in the United States, chlortetracycline (CTC) is one of the most used antibiotics in swine production (Barza 2002). Chlortetracycline is the first-discovered molecule of the tetracyclines family of broad-spectrum antimicrobials that is active against Gram-positive and Gram-negative bacteria, and atypical bacteria such as: chlamydiae, rickettsiae, and mycoplasmas, and against coccidia unicellular parasites (Chopra and Roberts 2001). The hydrochloride salts of these drugs are used as a standard to measure the level of activity of their formulations. Originally isolated from Streptomyces aureofaciens, chlortetracycline hydrochloride ($C_{22}H_{23}ClN_2O_8 \cdot HCl$) has a molecular weight of 515 g/mol. It has three ionizing groups: an amide attached to the

center of a β-ketoenol (acid), a phenol (acid), and a tertiary amine (basic). The acid dissociation constant (pKa) of the acid groups and of the conjugated acid of the basic group respectively are approximatively 3.2, 7.6 and 8.77 at 25°C depending on the nature of the substitutions present on the tetracycline backbone (Pulicharla, Hegde et al. 2017). Therefore, the tetracyclines are amphoteric drugs that are ionized at all pH values, with an isoelectric point at a pH-value around 5. Therefore, the zwitterionic form of the drug is predominant between the pH-values of 4 and 7 (Colaizzi and Klink 1969). Its water solubility at room temperature in water is reportedly about 8.6 mg/mL. The lipid solubility of CTC is highest at pH values of 5.5, which corresponds to the segment of pH values where the zwitterion is dominant. With respect to the log P values, chlortetracycline is less hydrophilic than oxytetracycline and tetracycline and more hydrophilic than doxycycline. The maximum value of the partition coefficients of CTC, tetracycline and doxycycline are respectively 0.41, 0.056 and 0.95 at a pH of 5.6. For oxytetracycline, the maximum log-p value is 0.087 at the pH of 6.6 (Colaizzi and Klink 1969).

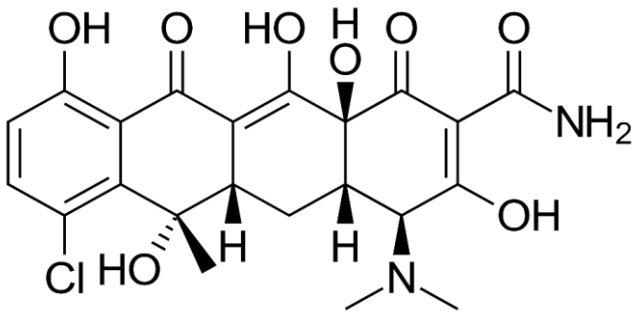
Their formulation as salts aims to stabilize them rather than solubilize them. In fact, the main salt used is the CTC-Ca^{++} complex which is insoluble in neutral or alkaline aqueous solvents. They are fluorescent and sensitive to light. Tetracyclines possess two chromophores and show strong UV absorption around 270 and 360 nm in neutral and acidic solutions. Tetracyclines are stable in dry form, but they are unstable in solution, especially under alkaline conditions but also under acidic conditions. Chlortetracycline is more stable than other tetracyclines in high temperature (Hsieh, Shyu et al. 2011) but, as compared to its congeners, more readily degrades in basic mediums at a rate that

increases with Ph (Li-ying 2010). In slightly acidic solvents (pH between 4 and 7) the neutrally charged zwitterion predominates, which helps the tetracyclines to precipitate in the dissolution media with pH between 5 and 7. On the brush border of enterocytes in the small intestine, where the pH is about 5.3 (Baggot and Brown, 1998), more than 90% of the tetracycline molecules in solution are believed to be in the form of zwitterion; Its null net charge favors its passage across cell membranes.

The nominal concentration of the CTC premix was equivalent to 220 g/kg of Chlortetracycline hydrochloride. Level of medicating ingredient in a complete feed depends on the indication approved in pigs:

- 55 mg/kg feed for the prevention of bacterial enteritis.
- 110 mg/kg feed for the treatment of bacterial enteritis.
- 22 mg / kg bw for the prevention of ileitis caused by *Lawsonia intracellularis*, depending on the daily intake and the weight of the animal, feed can be fortified between 220 ppm and 1375 ppm.

Figure 4.2. Structure of Chlortetracycline



4.2.2.3. Drug premix hardness

The granules hardness of drug premixes was measured using the manual tablet hardness testing instrument[9] to see the effect of granule hardness on dissolution behavior of drug premixes.

4.2.3. Analytical method development for drug dissolution

4.2.3.1. Lincomycin

There are many HPLC analytical methods reported for lincomycin in the literature, with ultraviolet (UV) at low wavelength range (208 nm) without derivatization step (Dousa, Sikac et al. 2006), and mass spectrophotometry (MS) detection for the determination of lincomycin (Bladek, Gajda et al. 2010). It was reported that, sulfur-containing antibiotics that do not contain fully oxidized sulfur can be detected electrochemically. Method for quantitation of lincomycin residues in tissues by ion-pair reversed-phase LC with electrochemical detection (Luo, Hansen et al. 1996) and in milk and tissues by reversed-phase LC on a C18 column and using UV detection is highly selective for lincomycin (Moats 1991). Liquid chromatography coupled with mass spectrometry is rapidly becoming the method of choice for the determination of lincomycin in feeds.

We used the HPLC-MS method for lincomycin detection in premixes and feeds. In comparison with described methods this is simple, rapid, and enough sensitive for lincomycin determination. We used Luna C18 reverse-phase column as stationary phase because the use of this column increases analyte retention and reduces matrix interference (Bladek, Gajda et al. 2010). In addition, we used the formic acid in our mobile

[9] PTB 111EP, Pharma Test, Hainburg, Germany

phase to increase overall sensitivity, provided good peak shape, and improve the reproducibility of the analyte retention time.

4.2.3.2. Chlortetracycline

Highly selective and sufficiently sensitive methods based on different analytical techniques are available for detection and quantification of Chlortetracycline (CTC) in animal feedstuffs including microbiological assays, thin-layer chromatography (Naidong, Hua et al. 2003), high-performance liquid chromatography (Wang, Yang et al. 2008) and liquid chromatography-mass spectrometry (Beaudry and del Castillo 2005). Tetracyclines are strong chelators and can form complexes with metal ions such as calcium. To avoid forming chelate, using mobile phases containing various acids including oxalic, phosphoric, citric, tartaric and EDTA have been reported. Based on the literature, a mobile phase containing oxalic acid is more efficient to improve the separation and the peak symmetry of doxycycline (Fiori, Grassigli et al. 2005). To obtain optimal separation conditions for tetracyclines, various combinations of methanol, acetonitrile, and aqueous oxalic acid solution were used in several study (Oka and Suzuki 1984, Ikai, Oka et al. 1987, Oka, Ikai et al. 1987).

Tetracyclines show strong UV absorption around 270 and 360 nm in neutral and acidic solutions, therefore, the most conventional detection method for tetracycline is the use of a UV detector (Oka, Ito et al. 2000). Highly sensitive detection of tetracyclines in HPLC has been carried out by detecting fluorescence after degradation of the tetracyclines under alkaline conditions and the formation of a metal chelate (Croubels, Vanoosthuyze et al. 1997). The mass spectrometric technique is also used as a highly sensitive detection

method of tetracyclines in food (Gavilán, Nebot et al. 2015). The LC-ESI/MS/MS method is used to detect of chlortetracycline or oxytetracycline in swine plasma where either drug can be used as internal standard for determining the other (Beaudry and del Castillo 2005).

We used HPLC-UV method for determination of in premix and in feed CTC. As they have strong UV absorbance, we choose this detection apparatus for CTC determination. As stationary phase we used Hypersil gold column because of its excellent resolution, efficiency, and sensitivity. As the CTC is strong chelators and can form complexes with metal ions such as calcium, oxalic acid was used as a mobile phase modifier for the HPLC separation that can acts as an acidifying agent.

4.2.4. Dissolution media

We prepared a water solution containing 0.2 M hydrochloric acid and 0.2 M potassium chloride (50 mL 0.2M KCl + 32.4 mL 0.2M HCl + 917.6 mL distilled H2O, for a total of 1000 mL), which acidity was adjusted to pH 1.6 ± 0.05 for simulating porcine gastric lumen conditions before adding the samples and adjusted constantly over the 120 min, then stepwise neutralized to pH 5.8 and 6.2 by adding K_2HPO_4 in the successive amounts of 5 and 3 mL, respectively, for simulating the early duodenum lumen conditions. These pH increases occurred immediately after taking the 120- and 135-min fluid samples as detailed below.

4.2.5. Dissolution Procedure

All dissolution tests were performed using a USP type-2 (i.e., paddle) apparatus[10]set at 70 rpm stirring speed, with 500 mL dissolution media that was poured in the vessels, then heated and constantly kept in a water bath set at 40°C, matching the normal body temperature of grower pigs during the postprandial period (Ingram and Legge 1970). The samples of the premixes (lincomycin and Chlortetracycline) were added into the vessels, either alone or admixed to each feedstuff (Table 1); 40 mg of either LIN or CTC with 9.960 g of each feedstuff to have 10 g of sample to have 440 ppm of LIN or 880 ppm of CTC dietary concentrations. The vessels were always covered with a plastic lid to minimize evaporation. Mid-depth samples of dissolution medium (1 mL) were aspirated 5cm below the surface of the liquid and 3 cm away from the motor axis of the pallet with single-use 3ml polypropylene syringes[11] fitted with a stainless steel cannula at 0, 1, 5, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 125, 130, 135, 140, 145, and 150 min dissolution time, and filtered immediately with PTFE syringe filter[12], 45 µm pore size.

4.3. Analysis the samples for dissolved drug

4.3.1. Chemicals and materials

Chemical reference Lincomycin hydrochloride (≥95.0%) and Chlortetracycline hydrochloride (≥91.0%) were purchased from sigma-Aldrich[13]. Solvents, acetonitrile, oxalic acid, and formic acid were of HPLC grade[14]. Other chemicals were of analytical grade. The Milli-Q system was used for water purification.

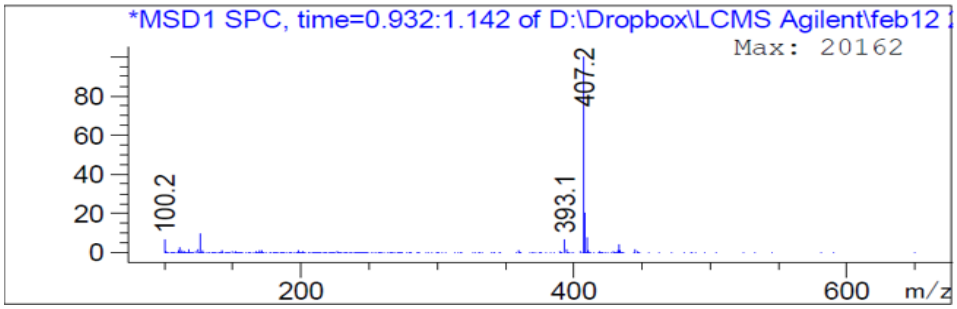
[10] Model 2500 Dissolution System, Distek, Inc., North Brunswick, NJ, USA
[11] Sigma-Aldrich, St. Louis, Missouri, USA
[12] Agilent, Santa Clara, CA
[13] Sigma-Aldrich, St. Louis, Missouri, USA
[14] Sigma-Aldrich, St. Louis, Missouri, USA

4.3.2. Instrumentation

4.3.2.1. Lincomycin

The serial lincomycin concentrations in the dissolution medium were measured with a high-performance liquid chromatography method using a mass spectrometry detector set at 407.2 mass/charge ratio (Fig.4.3). The HPLC separations were performed on a Luna C18 reverse-phase column[15](particle size of 5 µm; 4.6 mm width × 50 mm length).

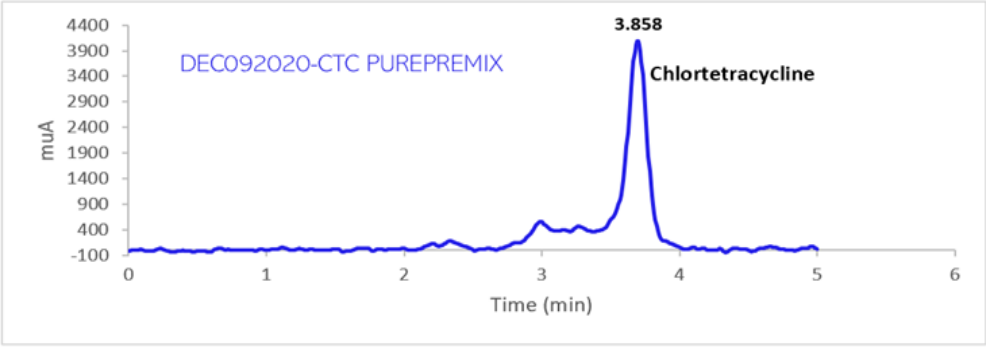
Figure 4.3. A mass spectrum of lincomycin (1 µg ml−1 in 0.1% formic acid in water and acetonitrile)



4.3.2.2. Chlortetracycline

The measurements of drug concentration were carried out in a Shimadzu high performance liquid chromatography (HPLC) system, equipped with an ultraviolet detector (UV). The HPLC separations were performed on a C18 selectivity Hypersil GOLD column[16] (particle size of 3 µm; 4.6 mm width × 150 mm length).

Figure 4.4. The HPLC spectrum for Chlortetracycline.



[15] Phenomenex, Torrance, CA, USA
[16] Thermo Fisher Scientific, Grand Island, NY, USA

4.3.3. Chromatographic conditions

4.3.3.1. Lincomycin

Mobile phase (MP) A water: acetonitrile: formic acid (950:49:1 v/v) and mobile phase B; Acetonitrile-formic acid 0.1% (99.9:1 v/v). Gradient transition from 0 to 100% MP B was achieved in two minutes. The flow rate was fixed at 1 mL/min. A 10 µL aliquot of the extracted sample was injected and the total run time was set to 6 min at room temperature.

4.3.3.2. Chlortetracycline

The mobile phase was a 0.01M oxalic acid solution in water mixed with acetonitrile at a 50:50 volume ratio. The detection wavelength was 375 nm (Wang, Wei et al. 2010), and the flow rate were fixed at 1 mL/min. A 50 µL aliquot of the sample was injected and the total run time was set to 6 min and the column was thermostated at 35 °C.

4.3.4. Calibration Curve

4.3.4.1. Lincomycin

An analytical grade lincomycin standard stock solution (1000 µg/mL) was diluted to get final concentration of 0, 5, 10, 20, 40, and 50 µg/mL. A calibration curve was tested on each analytical run. The limits of quantification (LOQ) and limit of detection (LOD) were 0.04 µg/mL and 0.025 µg/mL, respectively, as determined based on the standard deviation of the response and the slope of a weighted linear regression.

4.3.4.2. Chlortetracycline

Standard stock solution of CTC (1000 µg/mL) was prepared and then diluted 0, 5, 10, 15, 20, and 30 µg/mL. A calibration curve was tested on each analytical run. The limits of

quantification (LOQ) and limit of detection (LOD) were 0.02 µg/mL and 0.006 µg/mL, respectively, measured based on the standard deviation of the response and the slope of a weighted linear regression.

4.3.5. Precision and Accuracy

Model samples of CTC and lincomycin premixes were prepared to test the accuracy of the method. To evaluate the repeatability of the method, three samples of drugs (lincomycin & CTC) at the nominal concentration of 5, 20 and 40 µg/mL in three analytical runs were analysed. The accuracy of the method was calculated as a percentage recovery xi/µ×100%, where xi is the analysed amount of CTC and/or lincomycin in the sample, and µ is the known amount of the substance in the sample. The results are tabulated in Table 4.2.

Table 4.2. Precision and accuracy of the drug quantifications

Parameter	abbreviation	Lincomycin (µg/ml)			Chlortetracycline (µg/ml)		
		5	20	40	5	20	40
Number	n	6	6	6	6	6	6
Mean	Mean	5.28	22.53	43.17	5.45	19.51	42.48
Std. deviation	SD	0.20	1.8	2.23	0.31	0.35	1.75
Precision	CV%	3.7	7.9	5.2	5.8	1.75	4.2
Accuracy	NOM%	105.4	112.6	107.9	109.02	97.5	106.2

4.4. Rates and extents of dissolved LIN and CTC

To estimate the amount of drug release over time, non-linear regression was performed using ADAPT 5[17]. The regressions model used for lincomycin is as follows:

$$D_{LIN}(t) = D_0 + \frac{D_{max} \cdot t^H}{t_{50}^H + t^H} + \varepsilon$$

In this equation:

[17] ADAPT, Version 5.0.061; Biomedical Simulations Resource (BMSR), Los Angeles. CA. USA

- D_{LIN}(t) is the% of active ingredient dissolved at the sampling time t,
- D_0 is the % of active ingredient dissolved at time 0,
- D_{max} is the maximum dissolved % which comes from inside the premix granules,
- t_{50} is the time required for the half of D_{max} be dissolved, and
- H is a sigmoidicity factor that is <1 if the dissolution is slow and gradual (e.g., LIN in gW) and> 1 if the initial dissolution is negligible then increases rapidly to reach D_{max} (e.g., LIN in gR) and
- ε is an error function which is:
    - Constant for all measured concentrations: this is the case for Premix, gC, gW, DDGS and SBM.
    - A combination of constant errors and proportional to the concentration: this is the case with gR.

For the CTC, the regression model was modified as follows:

$$D_{CTC}(t) = D_0 + \frac{Dis_{max} \cdot t^H}{t_{50}^H + t^H} - Flag \cdot P_{max} \cdot \left(1 - e^{-k \cdot (t-120)}\right) + \varepsilon$$

the symbols D_{CTC}(t), D_0, D_{max}, t_{50}, H and ε have the same meanings as in the original equation. In addition, this modified regression equation additionally models the rate and extent of insoluble complex formation of CTC with multivalent cations following the pH increase by using with the following terms:

- *"Flag"* is a threshold factor that is equal to 0 during the first 120 min of dissolution, or equal to 1 at later times,

- P_{max} is the asymptotic maximum precipitation of CTC that follows the neutralization of simulated gastric fluid at 120 min and
- K is a first-order rate of CTC-metal complexation.

The goodness of the fit of both models were compared with their calculated Akaike Information Criterion (AIC).

4.5. Statistical analysis

First, Exploratory Data Analysis (EDA) was performed using GraphPad Prism[18] to assess the distribution of the data, identify obvious errors, outliers and other anormalities, and assess their bivariate relationships. All confirmatory statistical analyses were performed with the SAS software[19].

4.5.1. Nutritional determinants of WHC

The stepwise linear regression was used to identify the relationship between the WHC of tested feedstuffs and their nutrient contents presented in Table 4.1, using a p=0.15 threshold for the inclusion or exclusion of predictors in the regression model.

4.5.2. Effect of soaking fluid on WHC

The effect of soaking fluid on WHC was analyzed with generalized linear mixed models for Gaussian distributed outcomes using the Laplace approximation of the likelihood function where the feedstuffs, solvent, and feedstuff×solvent were the fixed effect variables and the replicate in the combination of feedstuff×solvent was the random effect. Then, we performed the following multiple comparisons with the familywise α error rate adjusted

[18] GraphPad prism version 8.4.3; (San Diego, CA, USA)
[19] SAS version 9.4 for Windows 10_x64. SAS Institute Inc. (Cary, NC, USA).

with the step-down simulation method (Westfall 1997): comparison of the WHC values of the tested feedstuffs saturated with water, comparison of their WHC values when saturated with simulated swine gastric fluid, and paired comparison of WHC values using water and SGF for each feedstuff.

4.5.3. Effect of soaking time on WHC

Generalized linear mixed models for Gaussian distributed outcomes using the Laplace approximation of the likelihood function were built to test the effect of soaking time on WHC, where the feedstuffs, time, and feedstuff×time were the fixed effect variables and the replicate in the combination of feedstuff×time was the random effect. Then the comparison of different feedstuffs was performed with the familywise α error rate adjusted with the step-down simulation method (Westfall 1997)

4.5.4. Effect of particle size on WHC

The effect of particle size on WHC was analyzed with generalized linear mixed models for Gaussian distributed outcomes using the Laplace approximation of the likelihood function. The fixed-effect variables of this model were the feedstuffs, mesh, feedstuff×mesh and poly2×feedstuff×mesh2, the latter coding for a quadratic effect of particle size on WHC that was identified for 3 feedstuffs (gC, DDGS, gR) during the exploratory data analysis. Finally, the replicate was used as a random effect in this model.

4.5.5. Effect of WHC on in vitro dissolution profiles

4.5.5.1. Initial model

The effects of WHC, pH of the solution and the WHC×pH interaction on radially-smoothed random time-courses of CTC and LIN dissolution were examined using a generalized linear

mixed-effects model for Gaussian outcomes, where the WHC of the feedstuffs and the pH of the solution are fixed-effect variables and time, the feedstuff and the antibiotic are random variables. The random effect of time on the dissolution of the premix-feedstuff combination is approximated with a radial smoothing spline that is a semi-parametric regression technique (Diggle, Liang et al. 1994). We assumed that the WHC of the antibiotic premixes is 0, that their dry matter content is 100% and they do not contain any nutrients.

This model containing WHC, pH, and WHC×pH interaction as fixed-effect variables and time, antibiotic and feedstuff as random-effect variables was refined by sequentially adding the feedstuff's content of specific nutrients, as disclosed below.

4.5.5.2. Refined model 1

We refined the initial model by testing the addition of the ash content as a new fixed-effect factor.

4.5.5.3. Refined model 2

We refined the initial model by replacing the feedstuffs' ash with a valine content into the list of fixed-effect factors. The other fixed-effects (i.e., antibiotic, WHC, pH, WHC×pH interaction) and random-effect (i.e., time, drug, and feedstuff) variables of the initial model remained unchanged.

4.5.5.4. Refined model 3

Finally, we refined the initial model by adding time, antibiotic, and antibiotic×time interaction and re-tested the effect of the nutrients. The antibiotic, time, antibiotic×time

interaction, WHC, pH and ash are the fixed-effect variables and time, antibiotic and feedstuff are random variables.

Results 5

5.1. Water Holding Capacity of different feedstuffs

5.1.1. Nutritional determinants of the WHC of tested feedstuffs

Table 5.1 shows the order in which the predictors included into or excluded from the regression model and how the R-squared improved as a consequence of modifying the model.

Table 5.1. *Stepwise Selection Summary*

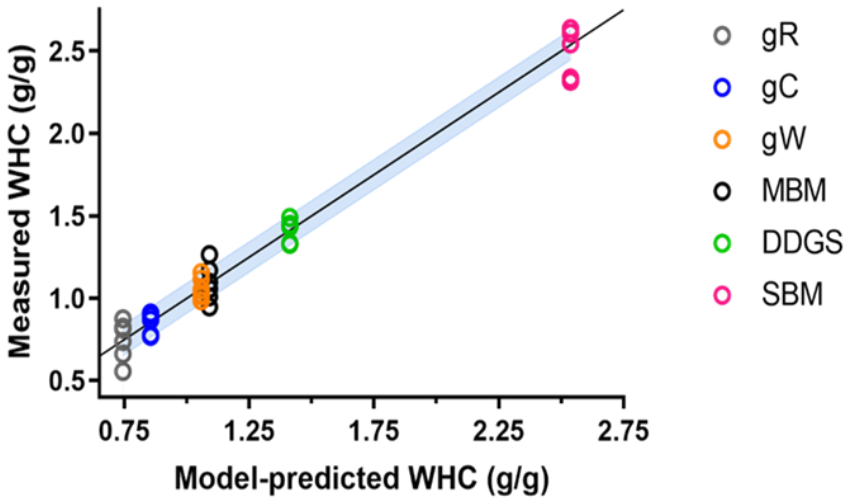
Step	Predictor	Added/With drawn	R square partial	R square model	C(p)	F value	Pr > F
1	**K**	Added	0.9303	0.9303	44.6374	453.65	<0.0001
2	**Trp**	Added	0.0212	0.9515	23.3568	14.40	0.0006
3	**Lys**	Added	0.0090	0.9605	15.4294	7.31	0.0109
4	**Asp**	Added	0.0075	0.9680	9.1855	7.26	0.0113
5	**DEgrPig**	Added	0.0047	0.9727	6.0000	5.19	0.0301

Among the nutrient contents that significantly predicted the WHC values of tested feedstuffs, lysine (p=0.0006) and potassium (p=0.03) decreased the WHC values while tryptophan (p<0.0001) aspartic acid (p=0.001) and digestible energy (p=0.03) increased the WHC values (table. 5.2). As shown in Figure 5.1, a well-fitting curve reveal our regression model resulted in predicted values close to the observed data values.

Table 5.2. *Estimated coefficients of the linear predictor of the effect of feedstuff contents on WHC.*

Variable	Estimate	SE	F value	Pr > F
Intercept	-0.10439	0.30441	0.12	0.7340
Lys	-3.40081	0.87988	14.94	0.0006
Trp	3.74930	0.83226	20.29	<0.0001
Asp	2.00402	0.55371	13.10	0.0011
K	-0.82944	0.36409	5.19	0.0300
DEgrPig	0.05442	0.02390	5.19	0.0301

Figure 5.1. *Predicted vs. observed Water Holding Capacity of feedstuff*



Legend: DDGS, dried distillers' (corn) grains with solubles; gC, ground corn; gR, ground rye; gW, ground wheat; MBM, meat and bone meal; SBM, soybean meal.

5.1.2. Effect of soaking fluid on WHC

The results of type-3 statistical testing are presented in table 5.3. We recorded significant differences among feedstuffs and medium×feedstuff combinations.

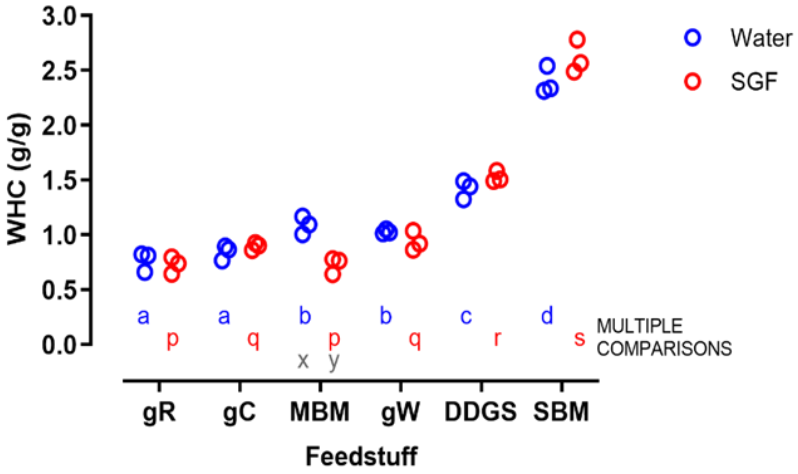
Table 5.3. *Type III Tests of Fixed Effects*

Effect	Den DF	F value	Pr > F
feedstuff	24	319.17	<0.0001
solvent	24	0.66	0.4258
feedstuff×solvent	24	12.98	<0.0001

The results of familywise error-corrected multiple comparison testing are tabulated in the table 1.A in the appendix 1. The WHC in water varies greatly between the tested feedstuffs with the highest value recorded for soybean meal followed by DDGS. The MBM and gW had a midrange WHC, and gR and gC had the lowest WHC (gR ≈ gC <MBM ≈ gW <DDGS <SBM). For the WHC in simulated swine gastric fluid, SBM had the highest value followed by DDGS. The midrange WHC was recorded for gW and gC, and MBM and gR had the lowest WHC (gR ≈ MBM < gC ≈ gW < DDGS < SBM). Except the WHC of MBM that was significantly ($p<0.0001$)

lower in SPGF than water (Table 1.A), no significant differences were observed between water and SPGF for the WHC the WHC of other feedstuff.

Figure 5.2. *Water-holding capacities of the tested feedstuffs in water and in simulated porcine gastric fluid. Note: matched-color rows of subscripts with no common letter differ significantly*



Legend: DDGS, dried distillers' (corn) grains with solubles; gC, ground corn; gR, ground rye; gW, ground wheat; MBM, meat and bone meal; SBM, soybean meal.

5.1.3. Effect of soaking time on WHC

As shown in Fig.5.3. the effects of soaking time on WHC were negligible for all time×feedstuff combinations ($p>0.50$), but their intercept values significantly differed among feedstuffs ($p<0.0001$).

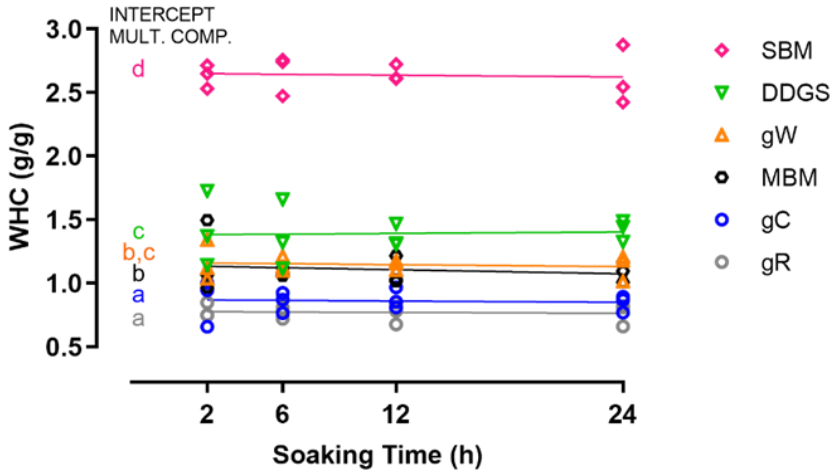
Table 5.4. *Type III Tests of Fixed Effects*

Effect	Den DF	F value	Pr > F
Feedstuff	60	147.96	<0.0001
Time	60	0.31	0.5769
Time×Feedstuff	60	0.08	0.9946

The multiple comparison of intercepts revealed significant differences between tested feedstuffs which are gR ≈ gC <MBM ≈ gW ≤ DDGS <SBM. Statistically non-significant differences were observed between the WHC of gC and gR. The intercept of gW did not

differ as DDGS and MBM, however the intercepts of DDGS and MBM are significantly different. The detailed results are tabulated in the table 2. A in the appendix 2.

Figure 5.3. *Water-holding capacities of the tested feedstuffs in distilled water, in function of soaking time. Note: intercept subscripts with no common letter differ significantly.*



Legend: DDGS, dried distillers' (corn) grains with solubles; gC, ground corn; gR, ground rye; gW, ground wheat; MBM, meat and bone meal; SBM, soybean meal.

Table 5.5. *Estimated coefficients of the effect of soaking time on WHC*

Effect	Feedstuff	Estimate	SE	Pr > |t|	Lo 95%C.I.	Hi 95%C.I.
Intercept		1.1620	0.05612	<0.0001	1.0497	1.2742
Feedstuff	**DDGS**	0.2185	0.07937	0.0078	0.05977	0.3773
Feedstuff	**MBM**	-0.02331	0.07937	0.7700	-0.1821	0.1355
Feedstuff	**SBM**	1.4897	0.07937	<0.0001	1.3309	1.6484
Feedstuff	**gC**	-0.2914	0.07937	0.0005	-0.4502	-0.1327
Feedstuff	**gR**	-0.3842	0.07937	<0.0001	-0.5430	-0.2255
Feedstuff	**gW**	0	.	.	.	.
Time		-0.00128	0.004072	0.7547	-0.00942	0.006867
Time×Feedstuff	**DDGS**	0.002241	0.005758	0.6986	-0.00928	0.01376
Time×Feedstuff	**MBM**	-0.00139	0.005758	0.8103	-0.01291	0.01013
Time×Feedstuff	**SBM**	0.000085	0.005758	0.9883	-0.01143	0.01160
Time×Feedstuff	**gC**	0.000435	0.005758	0.9401	-0.01108	0.01195
Time×Feedstuff	**gR**	0.000701	0.005758	0.9035	-0.01082	0.01222
Time×Feedstuff	**gW**	0	.	.	.	.
Scale		0.01373	0.002288	.	.	.

5.1.4. Effect of particle size on WHC

The 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 ($p<0.0001$).

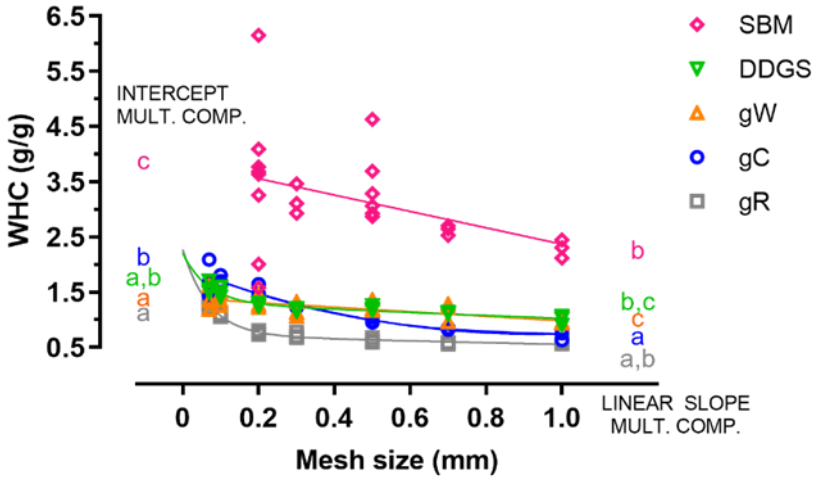
Table 5.6. *Type III Tests of Fixed Effects*

Effet	Den DF	Valeur F	Pr > F
Feedstuff	15	151.04	<0.0001
Mesh	79	206.76	<0.0001
Mesh×Feedstuff	79	25.39	<0.0001
poly2×mesh2×Feedstuf	79	20.76	<0.0001

Only SBM and gW showed linear WHC decreases over the range of particle sizes. As shown in the figure 5.4, the multiple comparison of intercepts revealed significant differences between tested feedstuffs which are gR ≈ gW ≤ DDGS ≤ gC < SBM.

There were no significant differences between the intercepts of gR, gW, and DDGS, while they were significantly different from the ones of gC and SBM. The intercept of gC was not significant from DDGS, but it was significantly different from the other feedstuffs. There were no significant differences between the linear slopes of SBM, DDGS, and gR, while they were significantly different from the slope of gW. The slope of gC was not significantly different from the gR. The detailed results are tabulated in the table 3. A in the appendix.

Figure 5.4. *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*



Legend: DDGS, dried distillers' (corn) grains with solubles; gC, ground corn; gR, ground rye; gW, ground wheat; MBM, meat and bone meal; SBM, soybean meal.

Table 5.7. *Estimated coefficients of the effect of particle size on WHC.*

Effect	Feedstuff	Estimate	SE	Pr > |t|	Lo 95%C.I.	Hi 95%C.I.
Intercept		1.5846	0.08441	<0.0001	1.4047	1.7645
Feedstuff	**SBM**	2.2606	0.1178	<0.0001	2.0094	2.5118
Feedstuff	**gC**	0.3512	0.1188	0.0098	0.09783	0.6045
Feedstuff	**gR**	-0.2856	0.1197	0.0306	-0.5407	-0.03058
Feedstuff	**gW**	-0.1890	0.1112	0.1099	-0.4260	0.04805
Feedstuff	**DDGS**	0	.	.	.	.
Mesh		-1.1888	0.2907	0.0001	-1.7675	-0.6101
Mesh×Feedstuff	**SBM**	-0.2312	0.3453	0.5051	-0.9185	0.4561
Mesh×Feedstuff	**gC**	-1.3829	0.4104	0.0012	-2.1999	-0.5660
Mesh×Feedstuff	**gR**	-1.0015	0.4107	0.0170	-1.8189	-0.1840
Mesh×Feedstuff	**gW**	0.7753	0.2994	0.0114	0.1793	1.3713
Mesh×Feedstuff	**DDGS**	0	.	.	.	.
poly2×mesh2×Feedstuf	**SBM**	0	.	.	.	.
poly2×mesh2×Feedstuf	**gC**	1.3874	0.2717	<.0001	0.8467	1.9282
poly2×mesh2×Feedstuf	**gR**	1.5085	0.2719	<.0001	0.9674	2.0496
poly2×mesh2×Feedstuf	**gW**	0	.	.	.	.
poly2×mesh2×Feedstuf	**DDGS**	0.6322	0.2725	0.0229	0.08980	1.1747

5.2. Hardness of the drug premixes

The hardness of premix granules was significantly ($p \leq 0.0001$) different from each other.

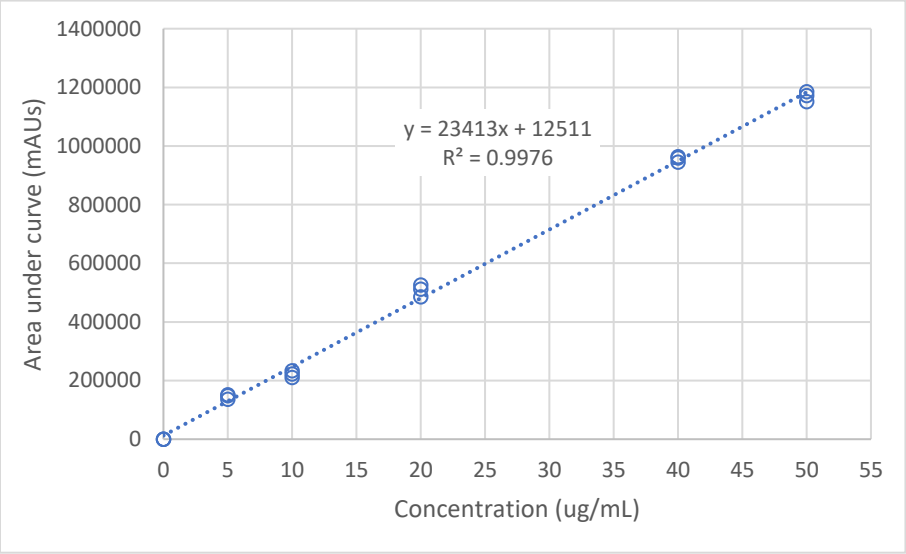
The hardness of 7.54±0.13 and 19.35±0.35 N was recorded for lincomycin and CTC, respectively.

5.3. Dissolution analysis of drugs

5.3.1. Lincomycin calibration curve analysis

A linear regression (values weighted with 1/concentration) was judged to fit adequately the concentration-signal relationship (Fig. 5.5). The regression model used was determined using the sum of the squares of the deviation. The calculated coefficients of correlations (r^2) were better than 0.997 for an analytical range set from 5 to 50 µg/mL.

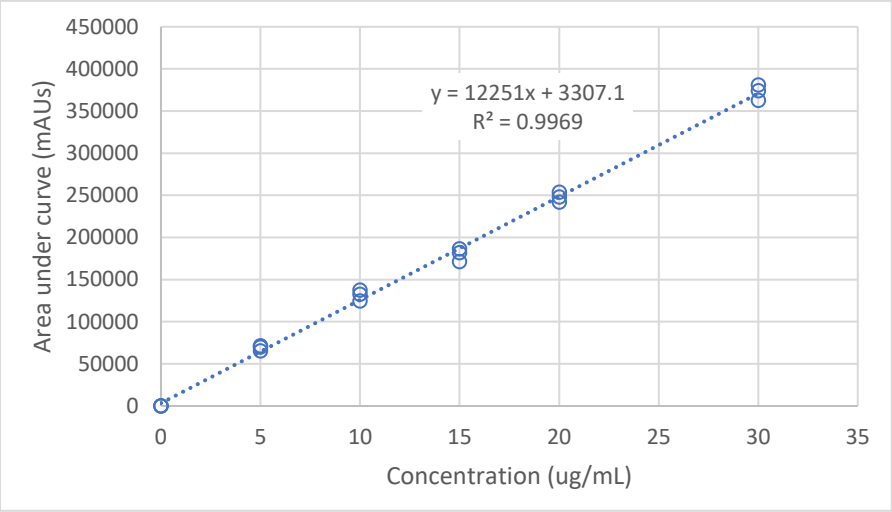
Figure 5.5. *Representative calibration curve for lincomycin*



5.3.2. Chlortetracycline calibration curve analysis

A linear regression (values weighted with 1/concentration) was judged to fit adequately the concentration-signal relationship (Fig. 5.6). The regression model used was determined using the sum of the squares of the deviation. The calculated coefficients of correlations (r^2) were better than 0.996 for an analytical range set from 5 to 30 µg/mL.

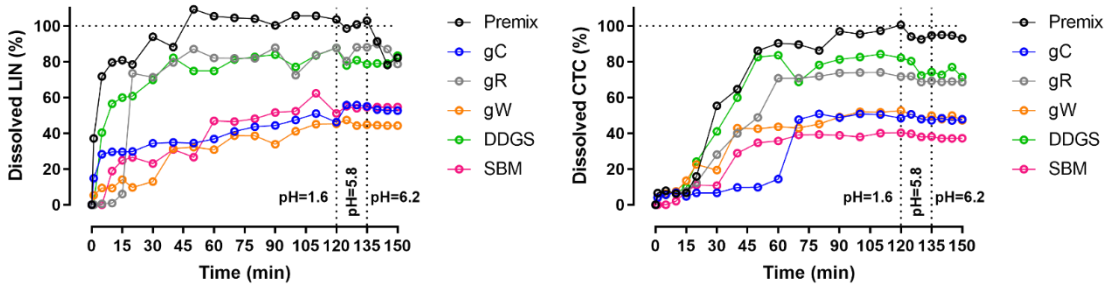
Figure 5.6. *Representative calibration curve for Chlortetracycline*



5.3.3. In vitro dissolution profile of two tested drugs

The time-course of in vitro drug dissolution with the USP type-2 apparatus revealed considerable interactions of the tested feedstuffs on the rate and extent of two tested drug release (Figure 5.7).

Figure 5.7. *In vitro dissolution profiles of lincomycin (left) and chlortetracycline (right) from their feed-grade premixes tested either alone or admixed to different feedstuffs.*

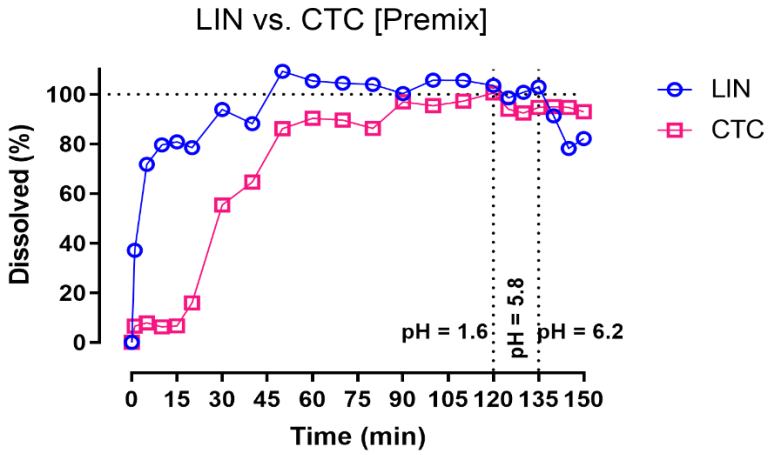


The specified amount (grams) of premix (alone or admixed to feedstuffs) were immersed in 500 mL of simulated gastric fluid at 40 ° C, then stirred at 70 rpm with constant pH measurement, then the pH was increased to 5.8 at 120 min by adding 5 mL of K2HPO4, then increased again to 6.2 at 135 min by addition of 3 mL of K2HPO4.

Legend: CTC, chlortetracycline; DDGS, dried distillers' (corn) grains with solubles; gC, ground corn; gR, ground rye; gW, ground wheat; LIN, lincomycin; SBM, soybean meal.

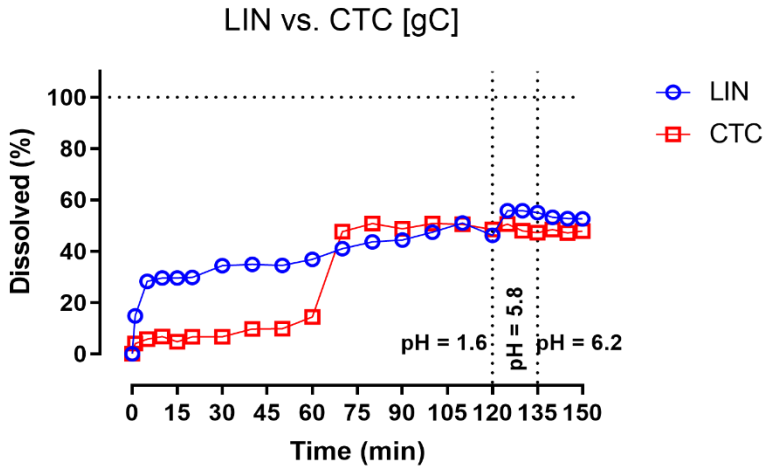
As indicated in Figure 5.8, lincomycin premixes dissolves faster than chlortetracycline in the simulated gastric fluid, and the maximum extent of lincomycin release was higher than chlortetracycline premix.

Figure 5.8. *In vitro dissolution profile of lincomycin (LIN) and chlortetracycline (CTC) from feed-grade premixes tested alone.*



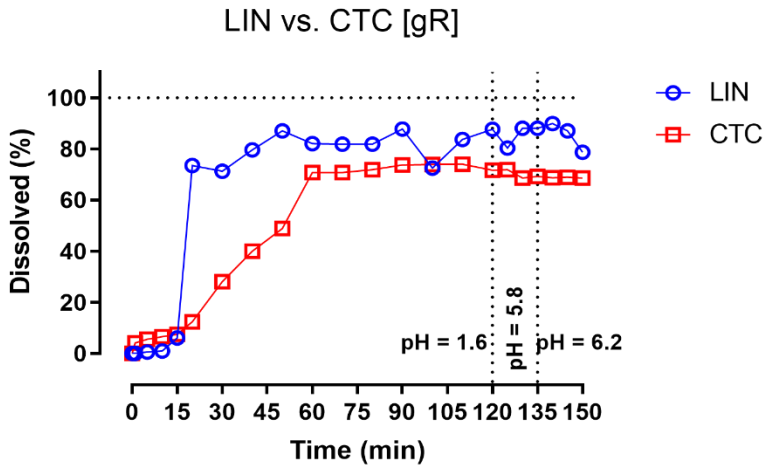
The maximum extent of drug release after 120 min was similar for two tested drugs, but the rate of LIN release was considerably faster than CTC. As shown in figure 5.9, an initial lag time was observed for CTC.

Figure 5.9. *In vitro dissolution profile of lincomycin (LIN) and chlortetracycline (CTC) from feed-grade premixes admixed to ground corn.*



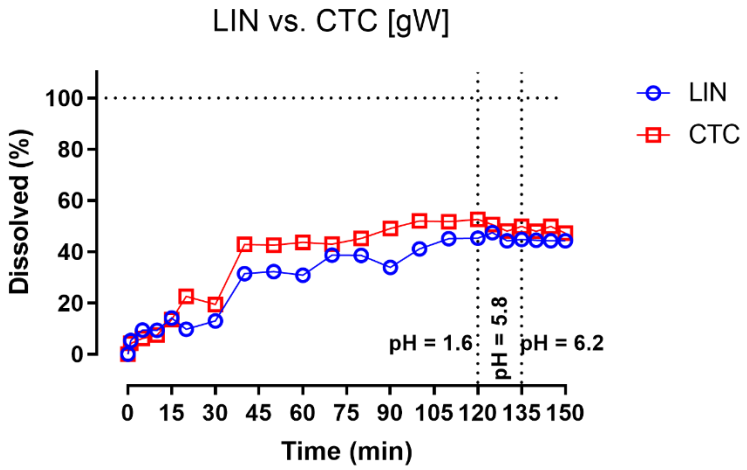
The extent of drug release at pH = 1.6 reached a maximum of approximately 80% and 70% when the LIN and CTC premixes were admixed to ground rye. The rate of drug release from CTC premix was slower than LIN (fig 5.10).

Figure 5.10. *In vitro dissolution profile of lincomycin (LIN) and chlortetracycline (CTC) from feed-grade premixes admixed to ground rye.*



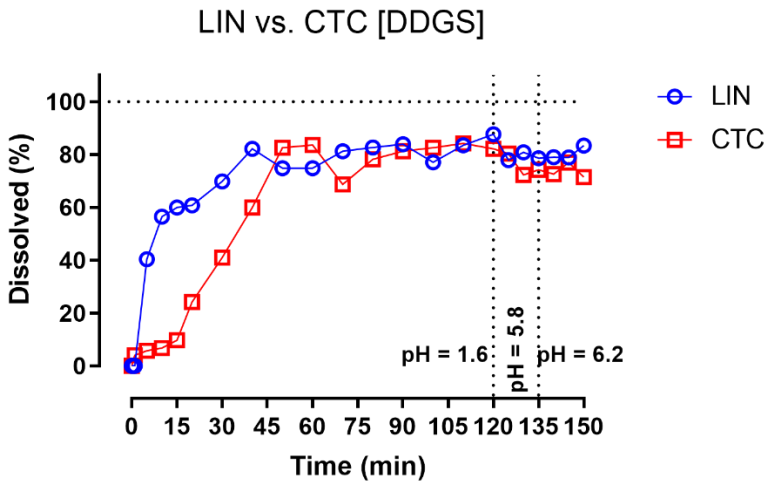
The extent of LIN release was slightly higher than CTC premix admixed to gW. The rate of LIN release was also slightly faster than CTC (Fig 5.11).

Figure 5.11. *In vitro dissolution profile of lincomycin (LIN) and chlortetracycline (CTC) from feed-grade premixes admixed to ground wheat.*



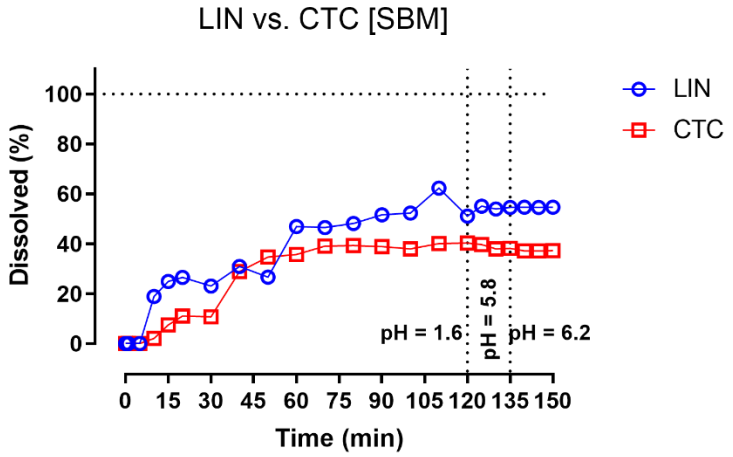
As shown in the figure 5.12, the extent of drug release from medicated DDGS was similar for two tested drugs, but the rate of drug release was faster for LIN as compared to CTC in simulated gastric fluid.

Figure 5.12. *In vitro dissolution profile of lincomycin (LIN) and chlortetracycline (CTC) from feed-grade premixes admixed to DDGS.*



The extent of drug release from LIN premix admixed to SBM was slightly higher than CTC premix. The rate of drug release was faster for LIN as compared to CTC. The initial lag period was observed for two tested drugs (fig 5.13).

Figure 5.13. *In vitro dissolution profile of lincomycin (LIN) and chlortetracycline (CTC) from feed-grade premixes admixed to soybean meal.*



5.3.4. The rate and extent of drug release over time

5.3.4.1. The rate and rate and extent of drug premix

As indicated in table 5.8 a maximum dissolution (D_{max}) of 101.9% was obtained for LIN premix. A t_{50} of 2.025 min was recorded for lincomycin pure premix.

Table 5.8. *Estimated dissolution parameters for the dietary lincomycin premix.*

Parameter (unit)	Estimated	Final value	SE (CV%)	95% CI	
D_{max} (%)	Yes	101.9	4.549	92.19	111.7
$t_{50\ (min)}$	Yes	2.025	29.89	0.7532	3.297
H (dimensionless)	Yes	0.8379	27.38	0.3558	1.320
D0 (%)	No	0	-	-	-

Legend: CI, confidence interval; CV%, percent coefficient of variation; D_0, percent dissolved at time 0; D_{max}, maximum percent dissolved; H, sigmoidicity factor; t_{50}, time required to dissolve half the maximum percent dissolved (i.e., D_{max}).

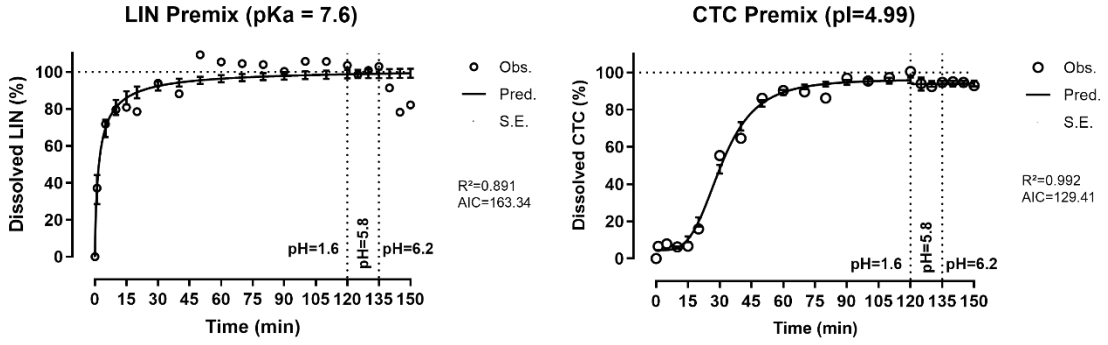
The maximum dissolution of 91.85% and t_{50} of 30.85 min was recorded for CTC. The maximum amount of precipitated CTC was 2.065%. Figure 5.14 shows dissolution profiles of the CTC premixes in SPGF.

Table 5.9. *Estimated dissolution parameters for the dietary chlortetracycline premix.*

Parameter (unit)	Estimated	Final value	SE (CV%)	95% CI	
D_{max} (%)	Yes	91.85	1.942	88.7	95.63
t_{50} (time)	Yes	30.85	3.132	28.81	32.90
H (dimensionless)	Yes	3.734	10.04	2.939	4.529
P_{max} (%)	Yes	2.065	110.1	-2.754	6.884
Kp	Yes	3.455	3.412E+8	-0.25E+08	0.25E+08

Legend: CI, confidence interval; CV%, percent coefficient of variation; D_0, percent dissolved at time 0; D_{max}, maximum percent dissolved; H, sigmoidicity factor; Kp, 1st-order precipitation rate constant; P_{max}, maximum percent precipitated; t_{50}, time required to dissolve half the maximum percent dissolved (i.e., D_{max}).

Figure 5.14. *Observed and model-predicted time-course of the dissolved drug fraction from the lincomycin (left) and chlortetracycline (right) dietary premixes*



5.3.4.2. The rate and rate and extent of drugs admixed to gC.

Figure 5.15 shows observed and model-predicted time-course of LIN and CTC dissolution profiles in SPGF. The D_{max} of 57.69% and Dt_{50} of 17.07 was recorded for LIN when the premix was admixed to gC (Table 5.10).

Table 5.10. *Estimated dissolution parameters for ground corn fortified with 440 mg/kg lincomycin*

Parameter (unit)	Estimated	Final value	SE (CV%)	95% CI	
D_{max} (%)	Yes	57.69	4.618	52.07	63.31
t_{50} (min)	Yes	17.07	13.96	12.10	22.04
H (dimensionless)	No	1	-	-	-
D0 (%)	No	0	-	-	-

Legend: CI, confidence interval; CV%, percent coefficient of variation; D_0, percent dissolved at time 0; D_{max}, maximum percent dissolved; H, sigmoidicity factor; t_{50}, time required to dissolve half the maximum percent dissolved (i.e., D_{max}).

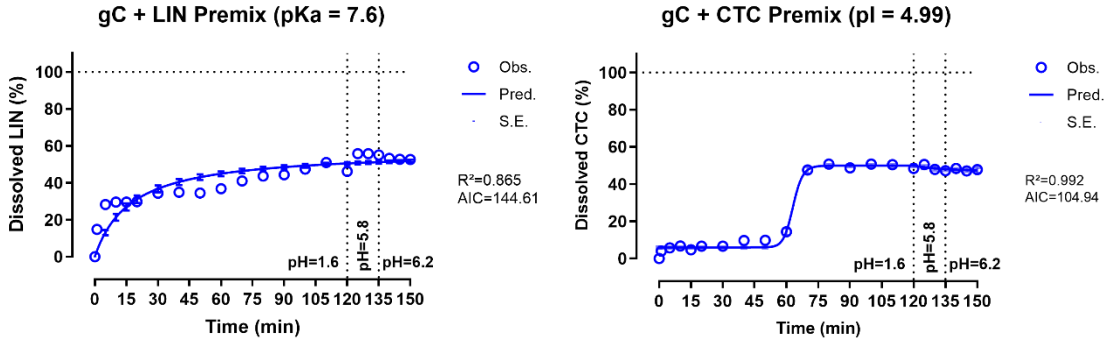
When the CTC premix admixed to gC, the maximum dissolution of 43.94% and t50 of 63.17 min were recorded for CTC (Table 5.11). The maximum precipitated CTC was 2.921% for medicated gC.

Table 5.11. *Estimated dissolution parameters for ground corn fortified with 880 mg/kg chlortetracycline*

Parameter (unit)	Estimated	Final value	SE (CV%)	95% CI	
D_{max} (%)	Yes	43.94	2.43	41.67	46.22
t_{50} (time)	Yes	63.17	1.33	61.38	64.96
H (dimensionless)	Yes	27.80	22.34	14.57	41.04
P_{max} (%)	Yes	2.921	135.2	-5.492	11.33
Kp	Yes	0.6738E-01	296.9	-0.359	0.49

Legend: CI, confidence interval; CV%, percent coefficient of variation; D_0, percent dissolved at time 0; D_{max}, maximum percent dissolved; H, sigmoidicity factor; Kp, 1st-order precipitation rate constant; P_{max}, maximum percent precipitated; t_{50}, time required to dissolve half the maximum percent dissolved (i.e., D_{max}).

Figure 5.15. *Observed and model-predicted time-course of drug released from medicated ground corn (gC).*



5.3.4.3. The rate and rate and extent of drug release admixed to gR.

A maximum dissolution of 82.98% and Dt_{50} of 17.63 (Table 5.12) was obtained for lincomycin premix when admixed to gR. Figure 5.16 shows dissolution profiles of the LIN admixed to gR in SPGF.

Table 5.12. *Estimated dissolution parameters for the ground rye fortified with 440 mg/kg lincomycin*

Parameter (unit)	Estimated	Final Estimate	SE (CV%)	95% CI	
D_{max} (%)	Yes	82.98	1.560	80.25	85.71
t_{50} (min)	Yes	17.63	1.989	16.89	18.37
H (dimensionless)	Yes	15.84	12.35	11.71	19.97
D0 (%)	No	0	-	-	-

Legend: CI, confidence interval; CV%, percent coefficient of variation; D_0, percent dissolved at time 0; D_{max}, maximum percent dissolved; H, sigmoidicity factor; t_{50}, time required to dissolve half the maximum percent dissolved (i.e., D_{max}).

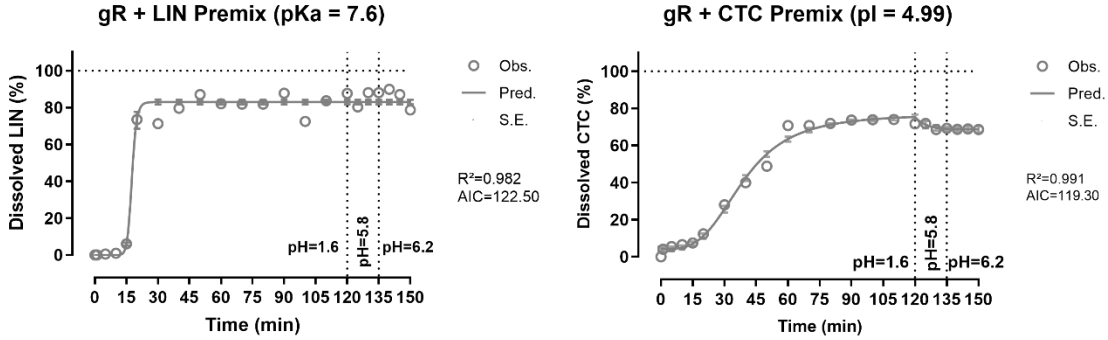
We recorded a D_{max} of 72.61% and t_{50} of 38.81 min for CTC when the premix was admixed to gR. The P_{max} of 7.530 was obtained for CTC admixed to gR. Figure 5.16 shows dissolution profiles of the LIN admixed to gR in SPGF.

Table 5.13. *Estimated dissolution parameters for the ground rye fortified with 880 mg/kg chlortetracycline*

Parameter (unite)	Estimated	Final value	SE (CV%)	95% CI	
D_{max} (%)	Yes	72.61	3.572	67.08	78.14
t_{50} (time)	Yes	38.81	3.482	35.93	41.69
H (dimensionless)	Yes	3.397	11.23	2.584	4.210
P_{max} (%)	Yes	7.530	30.98	2.559	12.50
Kp	Yes	0.1710	97.25	0.1833	0.5253

Legend: CI, confidence interval; CV%, percent coefficient of variation; D_0, percent dissolved at time 0; D_{max}, maximum percent dissolved; H, sigmoidicity factor; Kp, 1st-order precipitation rate constant; P_{max}, maximum percent precipitated; t_{50}, time required to dissolve half the maximum percent dissolved (i.e., D_{max}).

Figure 5.16. *Observed and model-predicted time-course of drug released from medicated gR.*



5.3.4.4. The rate and rate and extent of lincomycin admixed to gW.

As shown in table 5.14. the maximum dissolution of 63.32% and t_{50} of 57.19 min were recorded when the lincomycin premix was admixed to gW. Figure 5.17 shows dissolution profiles of the LIN admixed to gW in SPGF.

Table 5.14. *Estimated dissolution parameters for the ground wheat fortified with 440 mg/kg lincomycin*

Parameter (unit)	Estimated	Final value	SE (CV%)	95% CI	
D_{max} (%)	Yes	63.32	20.88	35.54	91.11
t_{50} (min)	Yes	57.19	45.65	2.334	112.0
H (dimensionless)	Yes	1.063	23.06	0.5482	1.578
D0 (%)	No	0	-	-	-

Legend: CI, confidence interval; CV%, percent coefficient of variation; D_0, percent dissolved at time 0; D_{max}, maximum percent dissolved; H, sigmoidicity factor; t_{50}, time required to dissolve half the maximum percent dissolved (i.e., D_{max}).

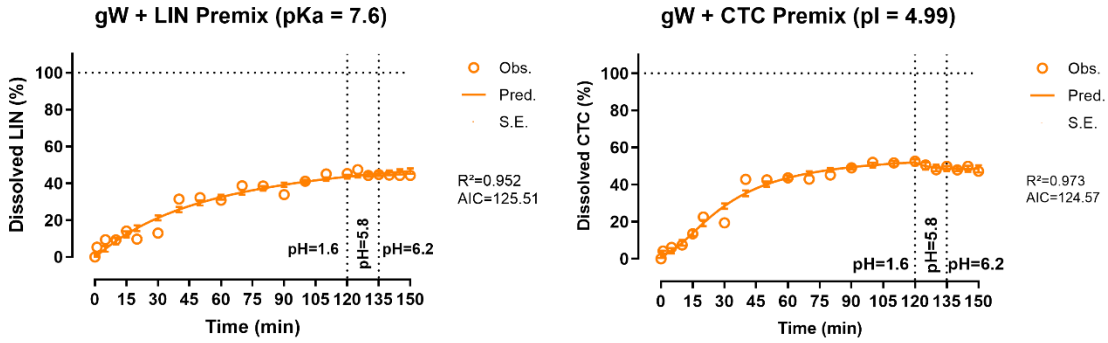
The extent of drug release reached a maximum of approximately 54.13% when the CTC premix was admixed to gW. The time required for dissolution of half of the maximum dissolution was 31.34 and the P_{max} of 4.978 was recorded for CTC premix admixed to gW.

Table 5.15. *Estimated dissolution parameters for the ground wheat fortified with 880 mg/kg chlortetracycline*

Parameter (unit)	Estimated	Final value	SE (CV%)	95% CI	
D_{max} (%)	Yes	54.13	9.554	43.11	65.15
t_{50} (time)	Yes	31.34	12.57	22.94	39.73
H (dimensionless)	Yes	1.785	19.25	1.053	2.517
P_{max} (%)	Yes	4.978	66.71	2.098	12.05
Kp	Yes	0.1213	178.8	0.3410	0.5837

Legend: CI, confidence interval; CV%, percent coefficient of variation; D_0, percent dissolved at time 0; D_{max}, maximum percent dissolved; H, sigmoidicity factor; Kp, 1st-order precipitation rate constant; P_{max}, maximum percent precipitated; t_{50}, time required to dissolve half the maximum percent dissolved (i.e., D_{max}).

Figure 5.17. *Observed and model-predicted time-course of drug released from medicated ground wheat (gW)*



5.3.4.5. The rate and rate and extent of lincomycin admixed to DDGS

The extent of drug release reached a maximum of approximately 82.80% when the lincomycin premix was admixed to DDGS. Figure 5.18 shows dissolution profiles of the LIN admixed to DDGS in SPGF.

Table 5.16. *Estimated dissolution parameters for the DDGS fortified with 440 mg/kg lincomycin*

Parameter (unit)	Estimated	Final value	SE (CV%)	95% CI	
D_{max} (%)	Yes	82.80	2.111	79.13	86.47
t_{50} (min)	Yes	6.262	10.01	4.945	7.580
H (dimensionless)	Yes	1.272	13.57	0.9089	1.634
D0 (%)	No	0	-	-	-

Legend: CI, confidence interval; CV%, percent coefficient of variation; D_0, percent dissolved at time 0; D_{max}, maximum percent dissolved; H, sigmoidicity factor; t_{50}, time required to dissolve half the maximum percent dissolved (i.e., D_{max}).

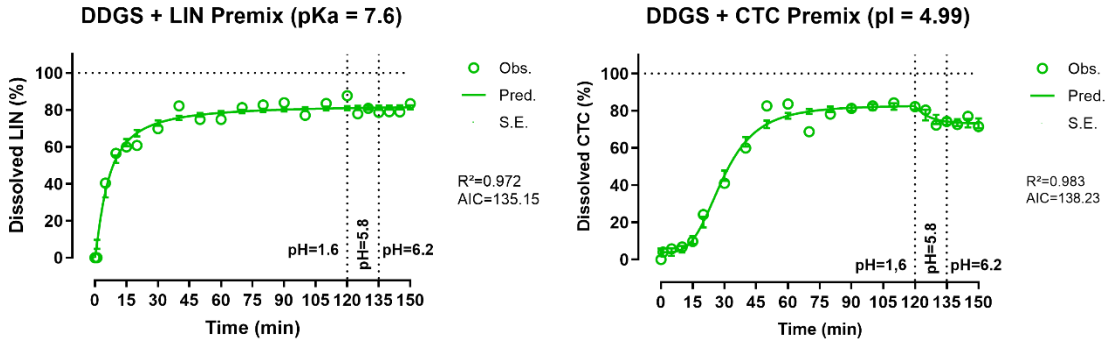
The extent of drug release reached a maximum of approximately 79.09% when the CTC premix was admixed to DDGS. The time required for dissolution of half of the maximum dissolution was 29.29 and the P_{max} of 9.680 was recorded for CTC premix admixed to gW. Figure 5.18 shows the observed and predicted rate and extent of the CTC admixed to gW in SPGF.

Table 5.17. *Estimated dissolution parameters for the ground wheat fortified with 880 mg/kg chlortetracycline*

Parameter (unit)	Estimated	Final value	SE (CV%)	95% CI	
D_{max} (%)	Yes	79.09	4.096	72.19	85.99
t_{50} (time)	Yes	29.29	4.860	26.26	32.33
H (dimensionless)	Yes	3.555	14.70	2.441	4.669
P_{max} (%)	Yes	9.680	37.88	1.866	17.49
Kp	Yes	0.1385	119.6	-0.214	0.4914

Legend: CI, confidence interval; CV%, percent coefficient of variation; D_0, percent dissolved at time 0; D_{max}, maximum percent dissolved; H, sigmoidicity factor; Kp, 1st-order precipitation rate constant; P_{max}, maximum percent precipitated; t_{50}, time required to dissolve half the maximum percent dissolved (i.e., D_{max}).

Figure 5.18. *Observed and model-predicted time-course of drug released from medicated DDGS.*



5.3.4.6. The rate and rate and extent of lincomycin admixed to SBM

Figure 5.19 shows dissolution profiles of the LIN admixed to SBM. The maximum extent of 63.24 and the t_{50} of 33.45 min was recorded for LIN when mixed to SBM.

Table 5.18. *Estimated dissolution parameters for the soybean meal fortified with 440 mg/kg lincomycin*

Parameter (unit)	Estimated	Final value	SE (CV%)	95% CI	
D_{max} (%)	Yes	63.24	5.005	56.61	69.86
t_{50} (min)	Yes	33.45	15.61	22.52	44.38
H (dimensionless)	Yes	1.340	11.26	1.024	1.656
D0 (%)	No	0	-	-	-

Legend: CI, confidence interval; CV%, percent coefficient of variation; D_0, percent dissolved at time 0; D_{max}, maximum percent dissolved; H, sigmoidicity factor; t_{50}, time required to dissolve half the maximum percent dissolved (i.e., D_{max}).

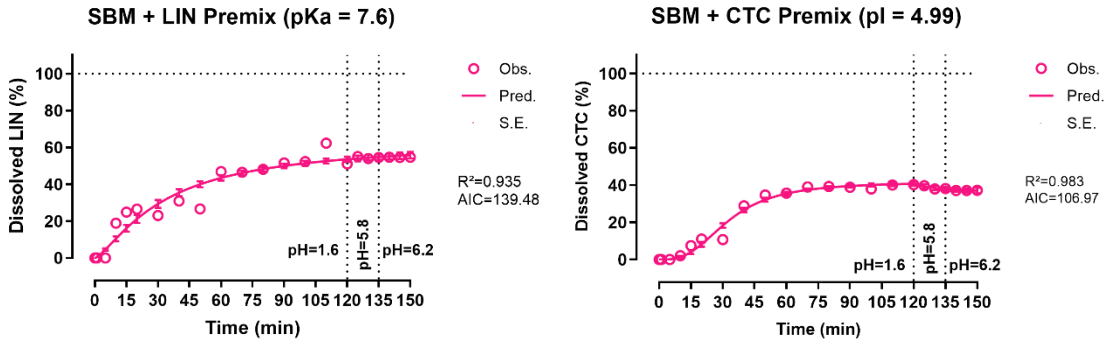
Figure 5.12 shows dissolution profiles of the CTC admixed to SBM. The maximum dissolution of 41.70% and t50 of 32.78 min was recorded for CTC premix admixed to SBM. The P_{max} of 4.582 was recorded for CTC premix admixed to SBM.

Table 5.19. *Estimated dissolution parameters for the soybean meal fortified with 880 mg/kg chlortetracycline*

Parameter (unit)	Estimated	Final value	SE (CV%)	95% CI	
D_{max} (%)	Yes	41.70	3.572	38.55	44.86
t_{50} (time)	Yes	32.78	5.397	29.03	36.53
H (dimensionless)	Yes	2.854	13.14	2.059	3.649
P_{max} (%)	Yes	4.582	75.72	2.773	11.94
Kp	Yes	0.7854E-01	180.6	0.2223	0.3793

Legend: CI, confidence interval; CV%, percent coefficient of variation; D_0, percent dissolved at time 0; D_{max}, maximum percent dissolved; H, sigmoidicity factor; Kp, 1st-order precipitation rate constant; P_{max}, maximum percent precipitated; t_{50}, time required to dissolve half the maximum percent dissolved.

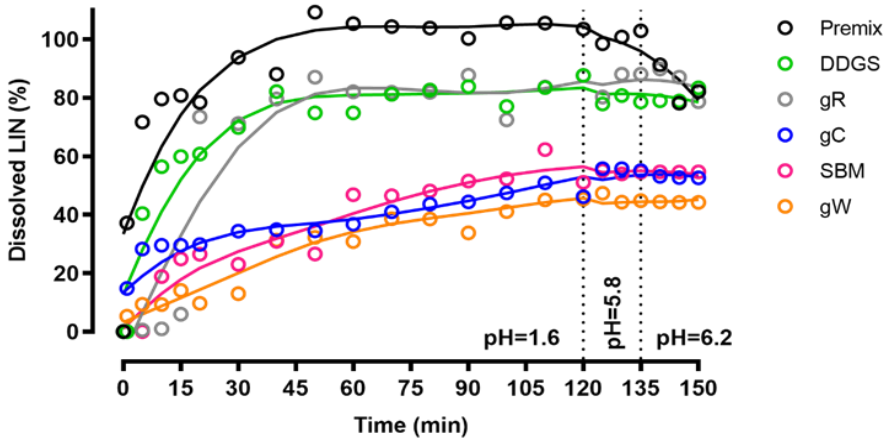
Figure 5.19. *Observed and model-predicted time-course of drug released from medicated soybean meal.*



5.3.5. Statistical determinants of the time-course of in vitro drug dissolution.

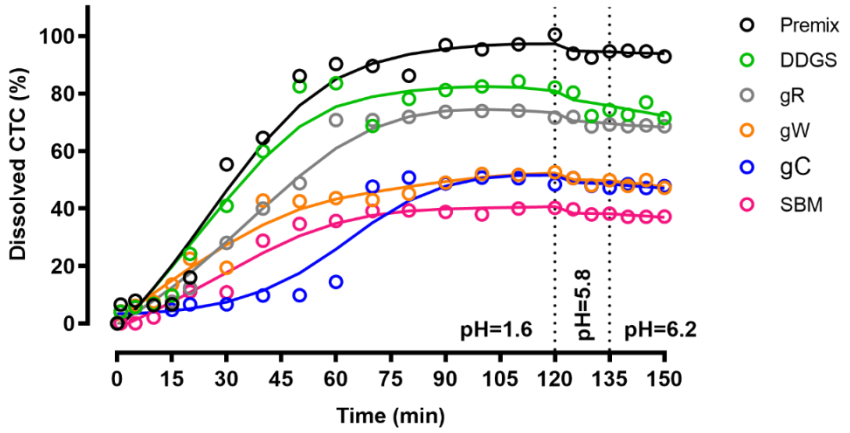
The results of statistical analysis are tabulated in the table 5.20. The statistical analysis of the results showed that the WHC, time and the ash content of the feedstuffs were the main determinants of this extent of dissolution kinetics. The WHC significantly decreased the dissolution ($p<0.0001$), while time and the ash content of feedstuffs significantly increased their dissolution ($p\leq0.008$). The type of drug and Time×Drug were an additional intercept and slope term for differentiating the tested drugs: their effects were marginally non-significant ($p\geq0.12$). Solvent pH was another additional slope term whose effect was not significant ($p=0.34$). The statistical testing of the fixed effect of WHC×pH interaction on the radially smoothed random time-courses of CTC and LIN dissolution in SPGF was not significant ($p>0.69$). Figure 5.20 and Figure 5.21 shows the observed and radially smoothed time-courses of in vitro release of LIN and CTC from the pure premix, and the medicated swine feedstuffs.

Figure 5.20. *Observed (circles) and radially smoothed predicted (lines) time-courses of in vitro dissolution of lincomycin from the pure premix, and the medicated feedstuffs.*



Legend: DDGS, gC, gR, gW, SBM, LIN. Succinct description of the IVDT.

Figure 5.21. *Observed (circles) and radially smoothed (lines) time-courses of in vitro dissolution of Chlortetracycline from the pure premix, and the medicated swine feedstuffs.*



Legend: DDGS, gC, gR, gW, SBM, LIN. Succinct description of the IVDT.

Table 5.20. *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.*

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		93.3	13.2	66.9	119.7			
Drug	**CTC**	-26.0	17.1	-60.27	8.2	53	2.33	0.1328
	LIN	0	.	.	.			
Time		0.12	0.15	-0.18	0.42	202.2	7.17	0.0080
Time×Drug	**CTC**	0.34	0.22	-0.09	0.76	202.2	2.45	0.1188
	LIN	0	.	.	.			
WHC		-119.4	17.0	-153.5	-85.2	53	49.16	<.0001
pH		-0.55	0.57	-1.69	0.59	53	0.93	0.3383
Ash		37.8	6.0	25.6	49.9	53	39.03	<.0001

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.

Discussion 6

6.1. Introduction

Oral group medication is the most common route of administration in pig farms that could achieved by mixing the drug into the feed or by dissolving it in the drinking water. However, it is a complex process affected by numerous factors such as feed-drug interactions which can pose a challenge for therapeutic efficacy. The therapeutic efficacy of medicated feed varies greatly between pig farms. The variability in therapeutic efficacy is partially due to the variability in the factors affecting the absorption along the gastrointestinal tract such as GI time and GI pH. Furthermore, it could be associated with changes in medicated feed formulation. One of the aspects that has not yet been investigated in food drug interaction studies is the influence of WHC of commonly used feedstuffs on the drug release behaviour of active substance present in medicated feed. This study aimed to identify feedstuffs or manufacturing practices that may interfere with in vitro drug release. Our hypothesis states that the WHC of the swine feedstuffs sponges the gastrointestinal fluids and limits the dissolution of drugs present in medicated feed. The results of this study showed that the WHC and ash content of the feedstuffs were the main determinants of the extent of dissolution. The findings of the study support our hypothesis that the WHC of the feedstuffs interferes with the dissolution of the active substances contained in the medicated feed. This physical measurement appears to be a promising indicator of food-drug interactions in pigs.

6.2. Water-holding capacity of feedstuffs

The WHC values were significantly ($p \leq 0.05$) highest for SBM, mid-range for DDGS, lower for gW and MBM, and lowest for gR and gC. The order of these feedstuffs according to the WHC is in agreement with the results of a study whose authors reported the highest WHC for SBM, medium for gW and low for gC with values of 3.22, 1.98, and 1.62 g/g, respectively (Giger-Reverdin 2000). Differences in the absolute value can be attributed to the alteration of the physical structure of feedstuffs during the milling and sieving that changes their WHC where the dietary fiber was ground with a hummer mil and the sieving was carried out through a series of sieves of mesh sizes decreasing from 1000 µm to 50 µm (Auffret, Ralet et al. 1994). Another study reached very similar results to our study with a WHC of 2.93 and 1.27 g/g for SBM and gC, respectively (Ramanzin, Bailoni et al. 1994), however the WHC of feedstuffs was estimated with different method.

Differences in WHC among feedstuffs can be associated with the feedstuff nutrient contents. However, these nutrient contents clearly have a different influence on WHC. In this experiment, among the feedstuff nutrient contents, lysine and potassium significantly decreased the WHC, while those of tryptophan, aspartic acid and digestible energy increased the WHC. In the other studies the WHC were largely affected by the fiber content of feedstuffs, mainly NDF. Ramanzin et al (1994) have shown that the amount of cellulose and hemicellulose increased the WHC of feedstuffs (Ramanzin, Bailoni et al. 1994). Ngoc et al (2012) further indicated that the feedstuffs with high amount in soluble non-starch polysaccharides, can retain more water due to the occurrence of more gaps within their cell matrix and increase the WHC (Ngoc, Len et al. 2012). Moreover, the high

correlation of WHC with non-starch polysaccharides was reported, as feedstuffs with a high pectin content had a higher WHC as compared to feedstuffs with low pectin levels (Giger-Reverdin 2000).

The results on water holding capacity of SBM in relation with particle size are in agreement with those of Ngoc et al (2012): these authors reported a water holding capacity value of 4.8± 0.23 g/g for SBM milled through a 0.5 mm screen, implying that their particle sizes were smaller than 0.5 mm (Ngoc, Len et al. 2012). In the present study, SBM had a water holding capacity of 2.4±0.13 g/g prior to sieving, and a WHC value of 3.2± 0.32 g/g or higher for particle sizes lower or equal than 0.5 mm. The differences in absolute values can be explained by some factors related to sieving: Sieving procedures enrich some nutrient contents of feedstuffs as the ingredient has a heterogeneous composition: the bran is rich in fiber, the germ is rich in protein, and the endosperm is rich in starch. As their respective harnesses differ, sieving concentrates the softer nutrients in the finer particles at the expense of the harder nutrients, which will affect the WHC of the particles (Challa, Srinivasan et al. 2010). Additionally, sieving produces small particles which increases the surface area exposed to fluids. Other investigators further shown that the physicochemical properties of feedstuff alter during grinding processes (Brachet, Arroyo et al. 2015) which could results in a change in WHC. In another study the authors have shown that experimental parameters such as stirring alter the physical structure of the feedstuff which resulted in a large change in WHC (Auffret, Ralet et al. 1994). In addition, the particle size distribution can also explain these differences, in particle size smaller than 0.5 mm there are different particle size (e.g., 0.1 mm), and we

only tested the particles that remained on top of the sieve, not those that fell into the bottom receptacle, which are smaller than 0.07mm in size.

Interestingly, MBM had significantly ($p<0.0001$) lower WHC in simulated gastric fluid than water, a result that may reflect the denaturation of animal proteins caused by the hydrochloric acid (Novák and Havlíček 2016). Addition of acid destroys the hydration layer of proteins, reduces the repulsive forces between the protein molecules, and remarkedly decreases the solubility of proteins, resulting in protein clustering and precipitation (Novák and Havlíček 2016). Moreover, protein oxidation exposed to acid can alter physical and chemical properties of proteins including solubility, and water holding capacity (Zhang, Xiao et al. 2013).

The feedstuff particle size was negatively associated with WHC, an expected finding because the grinding of feedstuffs increases their surface area exposed to water (Stephen and Cummings 1979, Auffret, Ralet et al. 1994). The authors reported that the hydration properties including WHC is depends on porosity of the feed particle; the more porous, the greater amount of water uptake.

Among feedstuffs, only SBM and gW showed linear decreases over the range of particle sizes, suggesting that the nutrient composition of their particles is more homogeneous than the ones of gC, gW and DDGS. We did not provide results for the water holding capacity of MBM in different particle sizes, since MBM was not available in different particle sizes. It is associated with the manufacturing process: meat grinder, which forces the material to pass through a grid with single diameter perforations. This by-product is rendered to produce a nutritional and economical feed ingredient. In a process known as

rendering, the bone meal, meat meal and blood meal are heated to remove moisture and release fat followed by crushing and grinding the material. Finally, the material is heated to reduce moisture content and eliminate any microorganisms.

For all feedstuffs, WHC yielded stable values after 6 h soaking time. This revealed that each feedstuff has a finite capacity to hold the water. This is in agreement with previous studies (Robertson, and Eastwood, 1981).

6.3. In vitro drug dissolution testing

The WHC of tested feedstuffs was the single, most important factor that hindered the release of both tested drugs, as the amount of available water which is necessary to dissolve their drug content was captured by feedstuffs and decreased the release of drugs over time. The time and feedstuff ash content significantly favored the release of both tested drugs. Time was an expected discovery as it drives the solvent to permeate through the premix particles to increase dissolution, 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 ionic strength of a solution increases the solubility of a solute and favors the dissolution of poorly soluble drugs (Long and McDevit 1952).

The pH neutralization visibly decreased the extent of lincomycin release from the pure premix suggesting that lincomycin with a pKa 7.6 (Qiang and Adams 2004) dissolve more readily in the acidic environment as it is presented in its ionized form but when the pH increases, their solubility is reduced which may result in drug precipitation, as reported by other authors (Abuhelwa, Williams et al. 2017). Several other studies in the literature

reported pH-dependent dissolution and bioavailability of some weakly basic drugs (itraconazole, fluconazole and dipyridamole) in both animals and humans (Blum, D'Andrea et al. 1991, Zhou, Moench et al. 2005, Pang, Dalziel et al. 2013). In contrast to the pure premix, the pH neutralization had a negligible effect on the extent of lincomycin release from medicated feed, suggesting that the feedstuffs may have a buffering effect. Among the tested feedstuffs, SBM and MBM have the highest acid binding capacity (ABC) with the values of 642 and 595 meq/Kg respectively (Lawlor, Lynch et al. 2005). Corn distillers has a low acid binding capacity, 96 meq/Kg as compared to wheat and corn that their ABC are 108 and 111 meq/Kg respectively (Lawlor, Lynch et al. 2005).

The extent of CTC release was also minimally affected by neutralization of the pH at time 120 and 135 min, but in any case, decreased their release extent. Our study suggests it may be due to affinity between divalent cations and CTC molecule that increases with pH. The calculated isoelectric point (pI) of the CTC is at a pH value = 4.99. At higher pH values, the keto-enol groups of CTCs will preferentially complex with multivalent cations. It was reported that the binding affinity of tetracyclines increases with pH (Pulicharla, Hegde et al. 2017). Therefore, the use of simulated intestinal fluid to dissolve CTC formulation, renders lower CTC concentration at dissolution media as compared to acidic pH. It is visible especially with the pure premix or mixed with DDGS, gR or SBM. For gC or gW, the difference is imperceptible: maybe these feedstuffs have a buffering effect that the others do not. The term of buffering capacity is used to describe the ability of a feedstuff to resist a change in pH after the addition of an acidic or a basic solution (Giger-Reverdin, Duvaux-Ponter et al. 2002, Lawlor, Lynch et al. 2005).

The buffering capacity of feedstuffs used in swine diets is another factor that could affect the dissolution behavior of medicated feed. Inside the dissolution medium, a feedstuff with a high acid binding capacity absorbs a large amount of acid and makes it difficult for the pH of the medium to be more acidic. It can also alter the local pH of the gastrointestinal tract in swine and change the dissolution rate followed by bioavailability. For cereal source of feedstuffs such as corn and wheat, low acid binding capacity was reported by several authors (Giger-Reverdin, Duvaux-Ponter et al. 2002, Lawlor, Lynch et al. 2005). As mentioned before, among the tested feedstuffs of current study, the highest acid binding capacity was reported for SBM and MBM (Lawlor, Lynch et al. 2005), as the acid binding capacity of feedstuffs are positively correlated with their protein and ash contents (Lawlor, Lynch et al. 2005).

The in vitro release of chlortetracycline and lincomycin from the dietary premixes tested alone was completed within the 120 min but was faster for lincomycin. A small fraction (about 5%) of chlortetracycline dissolved almost immediately after the beginning of the experiment, following which the concentrations plateaued for some minutes before a second phase of drug release. This two-phase release suggests that the CTC premix is dustier and harder than the lincomycin premix, and therefore the solvent diffuses slower through the premix particle to release its active substance. It was reported that, the increase in hardness of a solid dosage form (tablet) resulted in slower release rate that may be due to slower penetration rate of water into matrix of granules (Kitazawa, Johno et al. 1975, Saravanan, Nataraj et al. 2002). The granules with higher hardness contain compact mass of carrier (e.g., calcium carbonate) with relatively less pore, resulting in

slower release. In the other study, the authors have shown that the type of premix is the greatest determinant of rate of drug release, with significant differences between the premixes reported for the four investigated premixes (Del Castillo and Wolff 2006).

The maximum release of lincomycin was slightly higher than CTC from their premixes, that could be due to the hardness of CTC premix as compared to lincomycin premix. In addition, one of the main excipients in the composition of the CTC premix is calcium Sulfate dihydrate, which yields lots of Ca ions in the dissolution medium and can form a complex with dissolved CTC molecules (Weinberg 1957). High affinity of CTC for metallic ions also was reported by other authors. Albert (1953) reported a strong complexes of CTC with metallic ions, such as Al, Co and Zn (Albert 1953). Oxford (1953) reported that the CTC binds to metallic ions including Ca, Cu, Mg, and Cu, and form a complex, with the highest affinity recorded for Ca and Cu (Oxford 1953). In the other study, the CTC has been introduced as a potent and specific Ca ionophore (White and Pearce 1982).

The particle size of the premix is another factor that could affect dissolution rate of tested drugs. The particle size of drug premixes is inversely proportional to the area occupied by them, as the specific surface area increases with decreasing particle size (Chu, Lee et al. 2012). The rate of dissolution of a drug is directly proportional to the area of contact of the particles with the dissolution medium. Therefore, the geometric shape of the particle affects the contact surface and subsequently the dissolution rate. It was reported that the particle size of tested drug in dissolution media strongly affected the dissolution profiles, and the smaller particle size of drug dissolved rapidly in dissolution media due to the larger specific surface area (Chu, Lee et al. 2012).

In general, excipients are added to the oral drug formulations for control of disintegration rate of the solid dosage form (Sekiguchi and Obi 1961, Simonelli, Mehta et al. 1969). It was reported that the excipients present in the drug formulation has an important effect on the dissolution of the active substance by alteration of the disintegration time and dissolution rate (O'Connor and Corrigan 2002). They have also an ability to change the local pH that could influence drug dissolution rate. Drug-excipients interactions may be physical or chemical that have a beneficial or detrimental effect on drug release (Fathima, Mamatha et al. 2011). It was shown that, the mechanism of the active substances release depends on the excipients contained in solid dosage form (Kasperek, Bacz et al. 2014). Therefore, due to intimate contact of the active substance with the excipients, evaluation of possible interactions between the excipients and active substance has a vital role in drug development.

In addition, due to thermodynamic behavior of the drugs such as solubility, the effect of the excipient on dissolution depends on the active ingredient it contains, and the excipients used in drug formulation are selected based on their BCS classification. Surprisingly, in current study, despite the differences between the chemical structure of two tested drug, the effect of the feedstuff on dissolution was similar for active ingredients that they contain.

The Hill equation with some modification has been used to describe the in vitro dissolution kinetics of the medicated feed over time that was able to describe satisfactorily the rate and extent of drug dissolution over time. The Hill function is an equation to describe asymptotic behavior that has been widely used in pharmacology for

many PK–PD models describing the static and dynamic effects (Goutelle, Maurin et al. 2008). This equation was also used for predicting of drug dissolution behavior (Mendyk, Jachowicz et al. 2012). These authors revealed that this equation is able perfectly predict the rate and extent of drug release over time using nonlinear regression.

The US Food and Drug Administration's (FDA) guidelines indicate that in vitro release equipment should mimic an in vivo release situation as closely as possible, therefore, the good in vitro: in vivo comparisons can be obtained (FDA 1997). The development of a dissolution procedure requires fastidious selection of the dissolution equipment, dissolution medium, sample preparation, sampling schedule, hydrodynamics of agitation, and other aspects of dissolution methodology. In general, dissolution methods are considered the gold standard of in vitro dissolution testing, which is usually performed under perfect sink conditions, defined as, a sheer volume of solvent, usually about 5 to 10 times greater than the volume present in the saturated solution of the targeted chemical by the USP (USP 2015). In current study, all experiments carried out by USP apparatus 2 (paddle apparatus) dissolution test employing simple experimental conditions, e.g., sink conditions using a single well defined medium and volume at a constant pH; the amount of feed in dissolution media was minimal as compared to the volume of simulated gastric fluids and generated a perfect sink condition.

The in vivo dissolution behaviour of medicated feed is dependent on many factors and it cannot be fully obtained in vitro. In the fed gastrointestinal tract, the feed/gastric fluid ratio is much lower and drug dissolution likely occurs in non-sink conditions. However,

our dissolution procedure using Apparatus 2 was successfully executed and we confirmed our hypothesis that WHC of feedstuffs is a major hindrance of both tested drugs release. Nevertheless, designing non-sink conditions for medicated feed formulation is deemed appropriate as demonstrated in the US Food and Drug Administration's (FDA) guidelines on dissolution testing, including that "sink conditions are desirable but not mandatory" (FDA 1997). Therefore, various dissolution methods, with some modification, may be appropriate to evaluate drug release from medicated feed under non-sink conditions. In this case, the extent of non-sink conditions for medicated feed depends on their dose, sample size, dissolution volume, and dissolution condition.

The use of larger vessels designed between 2 and 4 L, making it possible to reflect more closely the in vivo environment for dissolution of the medicated feed. This modification can provide a realistic volume of porcine gastric medium. It also allows us to use the large sample size of medicated feed based on the swine average daily medicated feed intake. Although, in this case due to high concentrations of insoluble excipients in the dissolution media, they will present another challenge for filtration; because of the high turbidity caused by insoluble excipients, the use of typical analytical syringe filters may be impractical for samples and the filters become clogged. Introduction of a prefiltration step prior to filtration is an option to produce appropriate samples for HPLC analysis (Mattocks and Thakker 2017). To prevent the adsorption of the drug(s) onto the prefilter, the prefilter material should be evaluated prior to sampling.

Keep in mind that, the medicated feed is chewed by the pig before it reaches the stomach. The problem of USP apparatus type 2 is that there is no mechanical movement that would

help to drug release. In the apparatus designed for in vitro drug release testing of medicated chewing gums (Kvist, Andersson et al. 1999), there is a chewing module with the mechanical movements of lower and upper surface for chewing procedure. The chewing procedure consists of up and down strokes of the lower surface in combination with a twisting movement of the upper surface which provides a mastication of the chewing gum test medium (Kvist, Andersson et al. 1999). The combination of these two methods may provide a more realistic in vitro environment for prediction of in vivo drug release. Such test condition is not easy to perform, but if we can do it appropriately, they will give us the outstanding results to develop an in vitro/in vivo prediction.

In addition, for an in vitro release test of medicated feed, it is of fundamental importance that the test apparatus be able to stir the whole medicated feed samples uniformly to obtain reproducible results. Agitation accelerates dissolution by renewing the liquid at the interface. Agitation rate of 75 rpm is usually acceptable for dosage forms that exhibit coning. If it was inefficient 100 rpm may be practical to reduce coning.

Lincomycin and CTC are the most common used oral antimicrobial in food-producing animals including pigs (Toutain, Ferran et al. 2016). Chlortetracycline has very low oral bioavailability in pigs, with reported values typically between 20 and 28% (Kilroy, Hall et al. 1990). For drenched lincomycin, the oral bioavailability was determined to be 73% and 41% in fasted and fed pigs, respectively (Nielsen and Gyrd-Hansen 1998). Swine microbiota in the distal sections of the GIT (cecum and colon) is exposed to high concentration (85–95%) of unabsorbed fraction of the drug administered (Hansen, Aarestrup et al. 2002, Toutain, Ferran et al. 2016). Thereafter, the unabsorbed fraction is

excreted in feces into the environment. It was also reported for other antibiotics in pig, for example the oral bioavailability of ampicillin in pigs is very low (10%) leading to dramatic increase in antimicrobial resistance (Bibbal, Dupouy et al. 2007). The dissolution testing method using paddle apparatus was executed to determine the in vitro dissolution of CTC in simulated swine gastric fluid (pH=1.6) by other authors (Del Castillo and Wolff 2006). These authors have shown complete drug release when the premix was tested alone, and there was approximately 60% of CTC release when the premix admixed to swine feed. They also supplemented the in vitro dissolution data with in vivo pharmacokinetic data with administration of CTC medicated feeds to pigs and confirmed feed drug interactions.

Results from the present study reconfirmed feed drug interaction and shows that type of the feedstuffs is a major determinant of feed drug interaction and deepen this situation. Therefore, it is an important aspect to be considered in order attain complete drug absorption. These results suggest that, in any case, the use of feedstuffs with high WHC and high level of feed drug interactions, represent a crucial risk factor for antimicrobial resistance development and therapeutic failure. Of critical importance for swine producers, the hindrance to lincomycin and CTC release was greatest with gC and SBM, the feedstuffs most often used in the manufacturing of swine rations around the world. This is obviously an overlooked aspect in pig production farms and swine medicine applications which directly affects antibiotic dosage dissolution and absorption leading to therapeutic failure and increased antimicrobial resistance (Herrick, Haynes et al. 2014,

Toutain, Ferran et al. 2016) and drug dissemination in the environment (Cheng, Hao et al. 2014).

These results recognized potentially hindering feed ingredient, but our investigation in this area is still ongoing. We are in the process of testing the dissolution testing of lincomycin and CTC from a different composition of feedstuffs, for example from the mixture of gC and SBM, or a mixture where gC and/or SBM are replaced with DDGS and/or MBM, while keeping constant the crude protein and digestible energy contents.

A gC–SBM combination is traditional staple ingredients used in feeding most pigs that meets their nutrient requirements. Recently, soybean meal and corn are increasingly used in the manufacture of ethanol which is used as a biofuel (Lee, Featherstone et al. 2019). Soybean meal is the high-quality protein source in swine due to their amino acid profile, balance, and digestibility that is better than any other plant protein source, but as previously mentioned it has ability to capture high amount water resulting in a decrease in drug release amount. The MBM is the minerals and animal protein source that we can use in swine diet to minimize SBM inclusion. Using MBM instead of SBM would have a better effect on drug dissolution, because MBM had the lowest WHC and the highest ash content, which is concentrated by approximately 4 times in MBM compared to SBM.

Corn is the main energy sources in swine diets that contains greater energy level than the other feedstuffs. Replacement of gC with other energy sources of feedstuffs such as barley and sorghum as the alternative feedstuffs could also have a better effect on drug release due to their ash contents that are higher in barley and sorghum than that of gC, however the WHC of barley was reported to be slightly higher than that of gC (Giger-

Reverdin 2000). It has been reported that the replacement of corn by barley may not decrease growth performance under all circumstances (Woyengo, Beltranena et al. 2014). Oats is another energy source of swine diet that has the favorable amino acid balance, and their insoluble fiber content may improve gut health and decrease postweaning diarrhea in pigs. It was reported that oats have significantly higher amount of ash content as compared to other energy source feedstuffs (Giger-Reverdin 2000), therefore the use of oats as an alternative feedstuff for corn could have the better effect on drug release, however their high fiber content limits their application in swine diets (Stein, Lagos et al. 2016).

Another issue to consider is the effects of the particle size and size distribution of the premixes on the dissolution rate. As reported by several authors, the particle size of crystallized drugs has a dramatic effect on the dissolution behavior (Chu, Lee et al. 2012). The premixes used in the current study were provided in different particle sizes. Sifting through different sieves and separating them into different particle sizes will allow us to perform the in vitro dissolution test to see the effect of premix particle sizes on the dissolution behavior of the drug.

We will further set up an in vivo experiment involving administration of chlortetracycline, lincomycin and other medicated feeds to pigs, and finally we will develop in vitro in vivo correlation method to assess the efficiency of oral drug release from medicated feed.

Conclusion 7

There are marked differences in the water holding capacity between potential feed ingredients from vegetal, animal origin, and agro-industry co-products. We have verified our research hypothesis: the WHC of commonly used feedstuffs in manufacturing of medicated feeds predicts their hindrance to the in vitro dissolution of dietary LIN and CTC. There is a need for further research to identify the appropriate alternative feedstuffs in different ration and assessment of different factors that could affect drug release from medicated feed. It could create a novel application of precision-feeding for optimizing the use of oral drugs in swine medicine. Identifying the factors hindering drug release and using the suitable feedstuffs can result in efficient pharmacological effect by means of administrating a lower dose, that will have a significant cost and sanitary implication.

References

Abuhelwa, A. Y., D. B. Williams, R. N. Upton and D. J. Foster (2017). "Food, gastrointestinal pH, and models of oral drug absorption." Eur J Pharm Biopharm **112**: 234-248.

Achour, B., J. Barber and A. Rostami-Hodjegan (2011). "Cytochrome P450 Pig liver pie: determination of individual cytochrome P450 isoform contents in microsomes from two pig livers using liquid chromatography in conjunction with mass spectrometry [corrected]." Drug Metab Dispos **39**(11): 2130-2134.

Adeola, O. and D. E. King (2006). "Developmental changes in morphometry of the small intestine and jejunal sucrase activity during the first nine weeks of postnatal growth in pigs." J Anim Sci **84**(1): 112-118.

Albert, A. (1953). "Avidity of terramycin and aureomycin for metallic cations." Nature **172**(4370): 201-201.

Ali, D. N. and D. R. Hennessy (1996). "The effect of level of feed intake on the pharmacokinetic disposition and efficacy of ivermectin in sheep." J Vet Pharmacol Ther **19**(2): 89-94.

Alvaro, D., A. Cantafora, A. F. Attili, S. Ginanni Corradini, C. De Luca, G. Minervini, A. Di Biase and M. Angelico (1986). "Relationships between bile salts hydrophilicity and phospholipid composition in bile of various animal species." Comp Biochem Physiol B **83**(3): 551-554.

Amidon, G. L., H. Lennernas, V. P. Shah and J. R. Crison (1995). "A theoretical basis for a biopharmaceutic drug classification: the correlation of in vitro drug product dissolution and in vivo bioavailability." Pharm Res **12**(3): 413-420.

Anderson, K. E., A. H. Conney and A. Kappas (1979). "Nutrition and oxidative drug metabolism in man: relative influence of dietary lipids, carbohydrate, and protein." Clinical Pharmacology & Therapeutics **26**(4): 493-501.

Argenzio, R. A. and D. Lebo (1982). "Ion transport by the pig colon: effects of theophylline and dietary sodium restriction." Can J Physiol Pharmacol **60**(7): 929-935.

Auffret, A., M.-C. Ralet, F. Guillon, L. Barry and F. Thibault (1994). "Effect of Grinding and Experimental Conditions on the Measurement of Hydration Properties of Dietary Fibres." LWT **27**(2): 166-172.

Baggot, J. D. (1992). "Clinical pharmacokinetics in veterinary medicine." Clin Pharmacokinet **22**(4): 254-273.

Bal, H. S. and N. G. Ghoshal (1972). "Histomorphology of the torus pyloricus of the domestic pig (Sus scrofa domestica)." Zentralbl Veterinarmed C **1**(4): 289-298.

Barza, M. (2002). "Potential mechanisms of increased disease in humans from antimicrobial resistance in food animals." Clin Infect Dis **34 Suppl 3**: S123-125.

Beaudry, F. and J. R. del Castillo (2005). "Determination of chlortetracycline in swine plasma by LC-ESI/MS/MS." Biomed Chromatogr **19**(7): 523-528.

Bekersky, I., D. Dressler and Q. A. Mekki (2001). "Effect of low- and high-fat meals on tacrolimus absorption following 5 mg single oral doses to healthy human subjects." J Clin Pharmacol **41**(2): 176-182.

Bibbal, D., V. Dupouy, J. P. Ferre, P. L. Toutain, O. Fayet, M. F. Prere and A. Bousquet-Melou (2007). "Impact of three ampicillin dosage regimens on selection of ampicillin resistance in Enterobacteriaceae and excretion of blaTEM genes in swine feces." Appl Environ Microbiol **73**(15): 4785-4790.

Bladek, T., A. Gajda, M. Gbylik, A. Posyniak and J. Żmudzki (2010). "Analytical pocedure for the determination of lincomycin in honey by liquid chromatography-mass spectrometry." Bull Vet Inst Pulawy **54**: 205-209.
Blum, R. A., D. T. D'Andrea, B. M. Florentino, J. H. Wilton, D. M. Hilligoss, M. J. Gardner, E. B. Henry, H. Goldstein and J. J. Schentag (1991). "Increased gastric pH and the bioavailability of fluconazole and ketoconazole." Ann Intern Med **114**(9): 755-757.
Bollen, P. J., L. W. Madsen, O. Meyer and J. Ritskes-Hoitinga (2005). "Growth differences of male and female Gottingen minipigs during ad libitum feeding: a pilot study." Lab Anim **39**(1): 80-93.
Brachet, M., J. Arroyo, C. Bannelier, A. Cazals and L. Fortun-Lamothe (2015). "Hydration capacity: a new criterion for feed formulation." Anim. Feed Sci. Technol. **209**: 174-185.
Brunjes, P. C., S. Feldman and S. K. Osterberg (2016). "The Pig Olfactory Brain: A Primer." Chem Senses **41**(5): 415-425.
Brunner, E. (1903). Reaktionsgeschwindigkeit in heterogenen Systemen, Georg-Augusts-Universitat, Gottingen.
Burkina, V., M. K. Rasmussen, N. Pilipenko and G. Zamaratskaia (2017). "Comparison of xenobiotic-metabolising human, porcine, rodent, and piscine cytochrome P450." Toxicology **375**: 10-27.
Castro, N., R. Medina, J. Sotelo and H. Jung (2000). "Bioavailability of praziquantel increases with concomitant administration of food." Antimicrob Agents Chemother **44**(10): 2903-2904.
Challa, R., R. Srinivasan and F. To (2010). "Fractionation of soybean meal, cottonseed meal and wheat middlings using combination of sieving and air classification." Anim. Feed Sci. Technol **159**(1-2): 72-78.
Cheng, G., H. Hao, S. Xie, X. Wang, M. Dai, L. Huang and Z. Yuan (2014). "Antibiotic alternatives: the substitution of antibiotics in animal husbandry?" Front Microbiol **5**: 217.
Chopra, I. and M. Roberts (2001). "Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance." Microbiol Mol Biol Rev **65**(2): 232-260 ; second page, table of contents.
Christiansen, M. L., A. Mullertz, M. Garmer, J. Kristensen, J. Jacobsen, B. Abrahamsson and R. Holm (2015). "Evaluation of the use of Gottingen minipigs to predict food effects on the oral absorption of drugs in humans." J Pharm Sci **104**(1): 135-143.
Chu, K. R., E. Lee, S. H. Jeong and E.-S. Park (2012). "Effect of particle size on the dissolution behaviors of poorly water-soluble drugs." Arch Pharm Res **35**(7): 1187-1195.
Colaizzi, J. L. and P. R. Klink (1969). "pH-Partition behavior of tetracyclines." J Pharm Sci **58**(10): 1184-1189.
Croubels, S. M., K. E. Vanoosthuyze and C. H. van Peteghem (1997). "Use of metal chelate affinity chromatography and membrane-based ion-exchange as clean-up procedure for trace residue analysis of tetracyclines in animal tissues and egg." J Chromatogr B Biomed Sci Appl **690**(1-2): 173-179.
Danilova, V., T. Roberts and G. Hellekant (1999). "Responses of single taste fibers and whole chorda tympani and glossopharyngeal nerve in the domestic pig, Sus scrofa." Chem Senses **24**(3): 301-316.
Davis, S. S., L. Illum and M. Hinchcliffe (2001). "Gastrointestinal transit of dosage forms in the pig." J Pharm Pharmacol **53**(1): 33-39.
De Mil, T., M. Devreese, S. De Saeger, M. Eeckhout, P. De Backer and S. Croubels (2016). "Influence of Mycotoxin Binders on the Oral Bioavailability of Doxycycline in Pigs." J Agric Food Chem **64**(10): 2120-2126.
Del Castillo, J., V. Laroute, P. Pommier, C. Zémirline, A. Keïta, D. Concordet and P.-L. Toutain (2006). "Interindividual variability in plasma concentrations after systemic exposure of swine to

dietary doxycycline supplied with and without paracetamol: a population pharmacokinetic approach." J Anim Sci **84**(11): 3155-3166.
Del Castillo, J. and T. Wolff (2006). Therapeutic lung exposure to feedadministered chlortetracycline is premix brand dependent. Proceedings.
Deng, J., X. Zhu, Z. Chen, C. H. Fan, H. S. Kwan, C. H. Wong, K. Y. Shek, Z. Zuo and T. N. Lam (2017). "A Review of Food-Drug Interactions on Oral Drug Absorption." Drugs **77**(17): 1833-1855.
Diggle, P., K.-Y. Liang and S. L. Zeger (1994). "Longitudinal data analysis." OUP **5**: 13.
Dousa, M., Z. Sikac, M. Halama and K. Lemr (2006). "HPLC determination of lincomycin in premixes and feedstuffs with solid-phase extraction on HLB OASIS and LC-MS/MS confirmation." J Pharm Biomed Anal **40**(4): 981-986.
Dressman, J. B., G. L. Amidon, C. Reppas and V. P. Shah (1998). "Dissolution testing as a prognostic tool for oral drug absorption: immediate release dosage forms." Pharm Res **15**(1): 11-22.
Dressman, J. B. and K. Yamada (1991). "Animal models for oral drug absorption." In: Welling, P. G., Tse, F. L. S., Dighe, S. V (eds) Pharmaceutical Bioequivalence, Dekker, New York **48**: 727- 739.
Elkington, S. G., M. H. Floch and H. O. Conn (1969). "Lactulose in the treatment of chronic portal-systemic encephalopathy. A double-blind clinical trial." N Engl J Med **281**(8): 408-412.
Fathima, N., T. Mamatha, H. K. Qureshi, N. Anitha and J. V. Rao (2011). "Drug-excipient interaction and its importance in dosage form development." J. Appl. Pharm. Sci. **1**(06): 66-71.
FDA (1997). "Guidance for industry: dissolution testing of immediate release solid oral dosage forms." Center for Drug Evaluation and Research (CDER), US Department of Health and Human Services.
Ferrer, J., W. E. Scott, 3rd, B. P. Weegman, T. M. Suszynski, D. E. Sutherland, B. J. Hering and K. K. Papas (2008). "Pig pancreas anatomy: implications for pancreas procurement, preservation, and islet isolation." Transplantation **86**(11): 1503-1510.
Fiori, J., G. Grassigli, P. Filippi, R. Gotti and V. Cavrini (2005). "HPLC-DAD and LC-ESI-MS analysis of doxycycline and related impurities in doxipan mix, a medicated premix for incorporation in medicated feedstuff." J Pharm Biomed Anal **37**(5): 979-985.
Fleisher, D., B. Sweet, A. Parekh and J. Boullata (2004). "Drug Absorption with Food. Handbook of Drug-Nutrient Interactions." Totowa, NJ, USA, Humana Press: Ch. 8, 209-241.
Florence, A. T. and J. Siepmann (2016). Factors Influencing Oral Drug Absorption and Drug Availability. Modern Pharmaceutics, Two Volume Set, CRC Press**:** 135-172.
Garcia, J. and W. G. Hankins (1975). "The evolution of bitter and the acquisition of toxiphobia." Proceedings of the 5th International Symposium. Academic Press, New York: 39–45.
Gavilán, R. E., C. Nebot, J. M. Miranda, Y. Martín-Gómez, B. Vázquez-Belda, C. M. Franco and A. Cepeda (2015). "Analysis of Tetracyclines in Medicated Feed for Food Animal Production by HPLC-MS/MS." Antibiotics (Basel) **5**(1).
Gershkovich, P. and A. Hoffman (2007). "Effect of a high-fat meal on absorption and disposition of lipophilic compounds: the importance of degree of association with triglyceride-rich lipoproteins." Eur J Pharm Sci **32**(1): 24-32.
Giger-Reverdin, S. (2000). "Characterisation of feedstuffs for ruminants using some physical parameters." ANIM FEED SCI TECH **86**(1-2): 53-69.
Giger-Reverdin, S., C. Duvaux-Ponter, D. Sauvant, O. Martin, I. N. Do Prado and R. Müller (2002). "Intrinsic buffering capacity of feedstuffs." ANIM FEED SCI TECH **96**(1-2): 83-102.
Glass-Kaastra, S. K., D. L. Pearl, R. J. Reid-Smith, B. McEwen, S. A. McEwen, R. Amezcua and R. M. Friendship (2013). "Describing antimicrobial use and reported treatment efficacy in Ontario swine using the Ontario Swine Veterinary-based Surveillance program." BMC Vet Res **9**: 238.
Glendinning, J. I. (1994). "Is the bitter rejection response always adaptive?" Physiol Behav **56**(6): 1217-1227.

Goutelle, S., M. Maurin, F. Rougier, X. Barbaut, L. Bourguignon, M. Ducher and P. Maire (2008). "The Hill equation: a review of its capabilities in pharmacological modelling." Fund Clin Pharmacol **22**(6): 633-648.
Graham, J. P., S. L. Evans, L. B. Price and E. K. Silbergeld (2009). "Fate of antimicrobial-resistant enterococci and staphylococci and resistance determinants in stored poultry litter." Environ Res **109**(6): 682-689.
Gray, V., G. Kelly, M. Xia, C. Butler, S. Thomas and S. Mayock (2009). "The science of USP 1 and 2 dissolution: present challenges and future relevance." Pharm Res **26**(6): 1289-1302.
Gregory, P. C., M. McFadyen and D. V. Rayner (1990). "Pattern of gastric emptying in the pig: relation to feeding." Br J Nutr **64**(1): 45-58.
Hansen, L. H., F. Aarestrup and S. J. Sorensen (2002). "Quantification of bioavailable chlortetracycline in pig feces using a bacterial whole-cell biosensor." Vet Microbiol **87**(1): 51-57.
Harris, R. Z., G. R. Jang and S. Tsunoda (2003). "Dietary effects on drug metabolism and transport." Clinical pharmacokinetics **42**(13): 1071-1088.
Helke, K. L. and M. M. Swindle (2013). "Animal models of toxicology testing: the role of pigs." Expert Opin Drug Metab Toxicol **9**(2): 127-139.
Herrick, J. B., R. Haynes, S. Heringa, J. M. Brooks and L. T. Sobota (2014). "Coselection for resistance to multiple late-generation human therapeutic antibiotics encoded on tetracycline resistance plasmids captured from uncultivated stream and soil bacteria." J Appl Microbiol **117**(2): 380-389.
Hixson, A. and J. Crowell (1931). "Dependence of reaction velocity upon surface and agitation. II. Experimental procedure in study of surface." Ind. Eng. Chem **23**: 1002-1009.
Holler, H. (1970). "Untersuchungen über Sekret und Sekretion der Cardiadrüsenzone im Magen des Schweines: ." TRANSBOUND EMERG DIS **17**(10): 857-873.
Holm, R., A. Mullertz and H. Mu (2013). "Bile salts and their importance for drug absorption." Int J Pharm **453**(1): 44-55.
Hossain, M., W. Abramowitz, B. J. Watrous, G. J. Szpunar and J. W. Ayres (1990). "Gastrointestinal transit of nondisintegrating, nonerodible oral dosage forms in pigs." Pharm Res **7**(11): 1163-1166.
Howgate, E. M., K. Rowland Yeo, N. J. Proctor, G. T. Tucker and A. Rostami-Hodjegan (2006). "Prediction of in vivo drug clearance from in vitro data. I: impact of inter-individual variability." Xenobiotica **36**(6): 473-497.
Hsieh, M., C. Shyu, J. Liao, C. Franje, Y. Huang, S. Chang, P. Shih and C. Chou (2011). "Correlation analysis of heat stability of veterinary antibiotics by structural degradation, changes in antimicrobial activity and genotoxicity." Vet Med (Praha) **56**(6): 274-285.
Hunter, R. P., P. Lees, D. Concordet and P. L. Toutain (2012). "Establishing bioequivalence of veterinary premixes (Type A medicated articles)." J Vet Pharmacol Ther **35(Suppl. 1)**: 53-63.
Ibekwe, V. C., H. M. Fadda, E. L. McConnell, M. K. Khela, D. F. Evans and A. W. Basit (2008). "Interplay between intestinal pH, transit time and feed status on the in vivo performance of pH responsive ileo-colonic release systems." Pharm Res **25**(8): 1828-1835.
Ibrahim, A., M. H. Gilzad-kohan, A. Aghazadeh-Habashi and F. Jamali (2012). "Absorption and bioavailability of glucosamine in the rat." J Pharm Sci **101**(7): 2574-2583.
Ikai, Y., H. Oka, N. Kawamura, M. Yamada, K. Harada and M. Suzuki (1987). "Improvement of chemical analysis of antibiotics. XIII. Systematic simultaneous analysis of residual tetracyclines in animal tissues using thin-layer and high-performance liquid chromatography." J Chromatogr **411**: 313-323.
Ingram, D. and K. Legge (1970). "The thermoregulatory behavior of young pigs in a natural environment." PHYSIOL BEHAV **5**(9): 981-987.

Jantratid, E., N. Janssen, H. Chokshi, K. Tang and J. B. Dressman (2008). "Designing biorelevant dissolution tests for lipid formulations: case example--lipid suspension of RZ-50." Eur J Pharm Biopharm **69**(2): 776-785.
Joh, E. H. and D. H. Kim (2010). "A sensitive liquid chromatography-electrospray tandem mass spectrometric method for lancemaside A and its metabolites in plasma and a pharmacokinetic study in mice." J Chromatogr B Analyt Technol Biomed Life Sci **878**(21): 1875-1880.
Kararli, T. T. (1995). "Comparison of the gastrointestinal anatomy, physiology, and biochemistry of humans and commonly used laboratory animals." Biopharm Drug Dispos **16**(5): 351-380.
Kasperek, R., H. T. Bacz, Ł. Zimmer and E. Poleszak (2014). "The Effect of Excipients on the Release Kinetics of Diclofenac Sodium and Papaverine Hydrochloride From Composed Tablets." Acta Pol. Pharm. **71**(3): 439.
Kavutharapu, S., B. Nagalla, V. Abbagani, S. K. Porika, J. Akka, P. Nallari and V. Ananthapur (2012). "Role of proteases and antiprotease in the etiology of chronic pancreatitis." Saudi J Gastroenterol **18**(6): 364-368.
Kern, F., Jr., H. J. Birkner and V. S. Ostrower (1978). "Binding of bile acids by dietary fiber." Am J Clin Nutr **31**(10 Suppl): S175-s179.
Kilroy, C., W. Hall, D. Bane, R. Bevill and G. Koritz (1990). "Chlortetracycline in swine-bioavailability and pharmacokinetics in fasted and fed pigs." J. Vet. Pharmacol. Ther **13**(1): 49-58.
Kim, H. H. (2015). "Gut Microbiota-Mediated Drug-Antibiotic Interactions." Drug Metab Dispos **43**(10): 1581-1589.
Kitamura, S., K. Sugihara, M. Kuwasako and K. Tatsumi (1997). "The role of mammalian intestinal bacteria in the reductive metabolism of zonisamide." J Pharm Pharmacol **49**(3): 253-256.
Kitazawa, S., I. Johno, Y. Ito, S. Teramura and J. Okada (1975). "Effects of hardness on the disintegration time and the dissolution rate of uncoated caffeine tablets." J. Pharm. Pharmacol **27**(10): 765-770.
Kneifel, W., P. Paquin, T. Abert and J.-P. Richard (1991). "Water-holding capacity of proteins with special regard to milk proteins and methodological aspects—A review." Journal of Dairy Science **74**(7): 2027-2041.
Kostewicz, E. S., B. Abrahamsson, M. Brewster, J. Brouwers, J. Butler, S. Carlert, P. A. Dickinson, J. Dressman, R. Holm, S. Klein, J. Mann, M. McAllister, M. Minekus, U. Muenster, A. Mullertz, M. Verwei, M. Vertzoni, W. Weitschies and P. Augustijns (2014). "In vitro models for the prediction of in vivo performance of oral dosage forms." Eur J Pharm Sci **57**: 342-366.
Kuramoto, T., J. Miyamoto, M. Konishi, T. Hoshita, T. Masul and M. Une (2000). "Bile acids in porcine fetal bile." Biol Pharm Bull **23**(10): 1143-1146.
Kvist, C., S. B. Andersson, S. Fors, B. Wennergren and J. Berglund (1999). "Apparatus for studying in vitro drug release from medicated chewing gums." Int J Pharm **189**(1): 57-65.
Lawlor, P. G., P. B. Lynch, P. J. Caffrey, J. J. O'Reilly and M. K. O'Connell (2005). "Measurements of the acid-binding capacity of ingredients used in pig diets." Ir Vet J **58**(8): 447.
Laxminarayan, R., A. Duse, C. Wattal, A. K. Zaidi, H. F. Wertheim, N. Sumpradit, E. Vlieghe, G. L. Hara, I. M. Gould, H. Goossens, C. Greko, A. D. So, M. Bigdeli, G. Tomson, W. Woodhouse, E. Ombaka, A. Q. Peralta, F. N. Qamar, F. Mir, S. Kariuki, Z. A. Bhutta, A. Coates, R. Bergstrom, G. D. Wright, E. D. Brown and O. Cars (2013). "Antibiotic resistance-the need for global solutions." Lancet Infect Dis **13**(12): 1057-1098.
Le Goff, G., J. van Milgen and J. Noblet (2002). "Influence of dietary fibre on digestive utilization and rate of passage in growing pigs, finishing pigs and adult sows." Animal Science **74**(3): 503-515.
LeBlond, D., S. Altan, S. Novick, J. Peterson, Y. Shen and H. Yang (2016). "In vitro dissolution curve comparisons: a critique of current practice." Dissolut Technol **23**(1): 14-23.

Lee, J. Y., A. Featherstone, R. M. Nayga and D. B. Han (2019). "The long-run and short-run effects of ethanol production on US beef producers." Sustainability **11**(6): 1685.
Li-ying, Z. (2010). "Hydrolysis Kinetics of the Chlortetracycline." Guangzhou Chemical Industry(12): 69.
Lignet, F., E. Sherbetjian, N. Kratochwil, R. Jones, C. Suenderhauf, M. B. Otteneder, T. Singer and N. Parrott (2016). "Characterization of Pharmacokinetics in the Gottingen Minipig with Reference Human Drugs: An In Vitro and In Vivo Approach." Pharm Res **33**(10): 2565-2579.
Long, F. and W. McDevit (1952). "Activity coefficients of nonelectrolyte solutes in aqueous salt solutions." Chem. Rev **51**(1): 119-169.
Lugarini, F., B. J. Hrupka, G. J. Schwartz, C. R. Plata-Salaman and W. Langhans (2002). "A role for cyclooxygenase-2 in lipopolysaccharide-induced anorexia in rats." Am. J. Physiol. Regul **283**(4): R862-R868.
Lundahl, A., M. Hedeland, U. Bondesson and H. Lennernas (2011). "In vivo investigation in pigs of intestinal absorption, hepatobiliary disposition, and metabolism of the 5alpha-reductase inhibitor finasteride and the effects of coadministered ketoconazole." Drug Metab Dispos **39**(5): 847-857.
Luo, W., E. B. Hansen, Jr., C. Y. Ang and H. C. Thompson, Jr. (1996). "Determination of lincomycin residue in salmon tissues by ion-pair reversed-phase liquid chromatography with electrochemical detection." J AOAC Int **79**(4): 839-843.
Martinez, M. N. and M. G. Papich (2012). "Drug solubility classification in the dog." J Vet Pharmacol Ther **35 Suppl 1**: 87-91.
Mattocks, D. and K. Thakker (2017). "A practical approach to dissolution testing of Type A medicated articles." Dissolution Technol **24**(1): 16-19.
McAnulty, P. A., A. D. Dayan, N.-C. Ganderup and K. L. Hastings (2011). The minipig in biomedical research, CRC press.
McCarthy, L. G., C. Kosiol, A. M. Healy, G. Bradley, J. C. Sexton and O. I. Corrigan (2003). "Simulating the hydrodynamic conditions in the United States Pharmacopeia paddle dissolution apparatus." AAPS PharmSciTech **4**(2): E22.
McLauchlan, G., G. M. Fullarton, G. P. Crean and K. E. McColl (1989). "Comparison of gastric body and antral pH: a 24 hour ambulatory study in healthy volunteers." Gut **30**(5): 573-578.
McNamara, P. J., R. C. Jewell, B. K. Jensen and C. J. Brindley (1988). "Food increases the bioavailability of acitretin." J Clin Pharmacol **28**(11): 1051-1055.
McRorie, J., B. Greenwood-Van Meerveld and C. Rudolph (1998). "Characterization of propagating contractions in proximal colon of ambulatory mini pigs." Dig Dis Sci **43**(5): 957-963.
Mendyk, A., R. Jachowicz, K. Fijorek, P. Dorozynski, P. Kulinowski and S. Polak (2012). "KinetDS: an open source software for dissolution test data analysis." Dissolut Technol **19**(1): 6-11.
Merchant, H. A., E. L. McConnell, F. Liu, C. Ramaswamy, R. P. Kulkarni, A. W. Basit and S. Murdan (2011). "Assessment of gastrointestinal pH, fluid and lymphoid tissue in the guinea pig, rabbit and pig, and implications for their use in drug development." Eur J Pharm Sci **42**(1-2): 3-10.
Moats, W. A. (1991). "Determination of Lincomycin in Milk and Tissues by Reversed-Phase Liquid Chromatography." J. Agric. Food Chem **39**(10): 1812-1816.
Monshouwer, M., G. A. Van't Klooster, S. M. Nijmeijer, R. F. Witkamp and A. S. van Miert (1998). "Characterization of cytochrome P450 isoenzymes in primary cultures of pig hepatocytes." Toxicol In Vitro **12**(6): 715-723.
Naidong, W., S. Hua, E. Roets and J. Hoogmartens (2003). "Assay and purity control of tetracycline, chlortetracycline and oxytetracycline in animal feeds and premixes by TLC densitometry with fluorescence detection." J Pharm Biomed Anal **33**(1): 85-93.
Nernst, W. (1904). "Theorie der Reaktionsgeschwindigkeit in heterogenen Systemen." Z Phys Chem **47**(1): 52-55.

Ngoc, T. T., N. T. Len and J. E. Lindberg (2012). "Chemical Characterization and Water Holding Capacity of Fibre-rich Feedstuffs Used for Pigs in Vietnam." Asian-Australas J Anim Sci **25**(6): 861-868.
Nielsen, P. and N. Gyrd-Hansen (1996). "Bioavailability of oxytetracycline, tetracycline and chlortetracycline after oral administration to fed and fasted pigs." J Vet Pharmacol Ther **19**(4): 305-311.
Nielsen, P. and N. Gyrd-Hansen (1997). "Bioavailability of enrofloxacin after oral administration to fed and fasted pigs." Pharmacol Toxicol **80**(5): 246-250.
Nielsen, P. and N. Gyrd-Hansen (1998). "Bioavailability of spiramycin and lincomycin after oral administration to fed and fasted pigs." J Vet Pharmacol Ther **21**(4): 251-256.
Novák, P. and V. Havlíček (2016). Ch 4 - Protein Extraction and Precipitation. Editor(s): P. Ciborowski, J. Silberring, Proteomic Profiling and Analytical Chemistry (Second Edition),
, Elsevier**:** 51-62.
O'Connor, K. M. and O. I. Corrigan (2002). "Effect of a basic organic excipient on the dissolution of diclofenac salts." J. Pharm. Sci. **91**(10): 2271-2281.
Oberle, R. L. and H. Das (1994). "Variability in gastric pH and delayed gastric emptying in Yucatan miniature pigs." Pharm Res **11**(4): 592-594.
Oka, H., Y. Ikai, N. Kawamura, K. Uno, M. Yamada, K. Harada and M. Suzuki (1987). "Improvement of chemical analysis of antibiotics. XII. Simultaneous analysis of seven tetracyclines in honey." J Chromatogr **400**: 253-261.
Oka, H., Y. Ito and H. Matsumoto (2000). "Chromatographic analysis of tetracycline antibiotics in foods." J Chromatogr A **882**(1-2): 109-133.
Oka, H. and M. Suzuki (1984). "Improvement of chemical analysis of antibiotics. VII. Comparison of analytical methods for determination of impurities in tetracycline pharmaceutical preparations." J Chromatogr **314**: 303-311.
Oxford, A. (1953). "A colorimetric method, based on metallic complex formation, for the detection of aureomycin in presence of amino-acids and proteins." Nature **172**(4374): 395-396.
Pang, J., G. Dalziel, B. Dean, J. A. Ware and L. Salphati (2013). "Pharmacokinetics and absorption of the anticancer agents dasatinib and GDC-0941 under various gastric conditions in dogs--reversing the effect of elevated gastric pH with betaine HCl." Mol Pharm **10**(11): 4024-4031.
Parke, D. (1978). "The Effects of Diet and Nutrition On the Metabolism of Drugs." Royal Society of Health Journal **98**(6): 256-261.
Peeters, L. E. J., S. Croubels, G. Rasschaert, H. Imberechts, E. Daeseleire, J. Dewulf, M. Heyndrickx, P. Butaye, F. Haesebrouck and A. Smet (2018). "Effect of residual doxycycline concentrations on resistance selection and transfer in porcine commensal Escherichia coli." Int J Antimicrob Agents **51**(1): 123-127.
Pereira de Sousa, I. and A. Bernkop-Schnurch (2014). "Pre-systemic metabolism of orally administered drugs and strategies to overcome it." J Control Release **192**: 301-309.
Persson, E. M., A. Nordgren, P. Forsell, L. Knutson, C. Ohgren, S. Forssén, H. Lennernäs and B. Abrahamsson (2008). "Improved understanding of the effect of food on drug absorption and bioavailability for lipophilic compounds using an intestinal pig perfusion model." Eur J Pharm Sci **34**(1): 22-29.
Phillips, D. J., S. R. Pygall, V. B. Cooper and J. C. Mann (2012). "Overcoming sink limitations in dissolution testing: a review of traditional methods and the potential utility of biphasic systems." J Pharm Pharmacol **64**(11): 1549-1559.
Pijpers, A., E. J. Schoevers, H. van Gogh, L. A. van Leengoed, I. J. Visser, A. S. van Miert and J. H. Verheijden (1991). "The influence of disease on feed and water consumption and on pharmacokinetics of orally administered oxytetracycline in pigs." J Anim Sci **69**(7): 2947-2954.

Pillay, V. and R. Fassihi (1998). "Evaluation and comparison of dissolution data derived from different modified release dosage forms: an alternative method." J Control Release **55**(1): 45-55.
Pittman JS (2019). " Essentials of farm pharm – or the basics of antibiotic pharmacology in swine." Proceedings, American Association of Swine Veterinarians Annual Meeting: 50:53-18.
Polli, J. E. (2008). "In vitro studies are sometimes better than conventional human pharmacokinetic in vivo studies in assessing bioequivalence of immediate-release solid oral dosage forms." AAPS J **10**(2): 289-299.
Potkins, Z. V., T. L. Lawrence and J. R. Thomlinson (1991). "Effects of structural and non-structural polysaccharides in the diet of the growing pig on gastric emptying rate and rate of passage of digesta to the terminal ileum and through the total gastrointestinal tract." Br J Nutr **65**(3): 391-413.
Price, L. B., E. Johnson, R. Vailes and E. Silbergeld (2005). "Fluoroquinolone-resistant Campylobacter isolates from conventional and antibiotic-free chicken products." Environ Health Perspect **113**(5): 557-560.
Pulicharla, R., K. Hegde, S. K. Brar and R. Y. Surampalli (2017). "Tetracyclines metal complexation: Significance and fate of mutual existence in the environment." Environ Pollut **221**: 1-14.
Qiang, Z. and C. Adams (2004). "Potentiometric determination of acid dissociation constants (pKa) for human and veterinary antibiotics." Water Res **38**(12): 2874-2890.
Ramanzin, M., L. Bailoni and G. Bittante (1994). "Solubility, water-holding capacity, and specific gravity of different concentrates." Journal of dairy science **77**(3): 774-781.
Robertson, J. A. and M. A. Eastwood (1981). "An examination of factors which may affect the water holding capacity of dietary fibre." Br J Nutr **45**(1): 83-88.
Rosengren, L. B., C. L. Waldner, R. J. Reid-Smith, J. C. Harding, S. P. Gow and W. L. Wilkins (2008). "Antimicrobial use through feed, water, and injection in 20 swine farms in Alberta and Saskatchewan." Can J Vet Res **72**(2): 143-150.
Ruckebusch, Y. and L. Bueno (1976). "The effect of feeding on the motility of the stomach and small intestine in the pig." Br J Nutr **35**(3): 397-405.
Saravanan, M., K. S. Nataraj and K. S. Ganesh (2002). "The effect of tablet formulation and hardness on in vitro release of cephalexin from Eudragit L100 based extended release tablets." Biol Pharm Bull **25**(4): 541-545.
Scanff, P., S. Grison, P. Monti, C. Joubert, N. M. Griffiths and P. Gourmelon (1997). "Whole-body gamma irradiation modifies bile composition in the pig." Radiat Res **148**(2): 175-180.
Scanff, P., P. Monti, C. Joubert, S. Grison, P. Gourmelon and N. M. Griffiths (1999). "Modified bile acid profiles in mixed neutron and gamma-irradiated pigs." Int J Radiat Biol **75**(2): 209-216.
Schiller, C., C. P. Frohlich, T. Giessmann, W. Siegmund, H. Monnikes, N. Hosten and W. Weitschies (2005). "Intestinal fluid volumes and transit of dosage forms as assessed by magnetic resonance imaging." Aliment Pharmacol Ther **22**(10): 971-979.
Scholz, A., E. Kostewicz, B. Abrahamsson and J. B. Dressman (2003). "Can the USP paddle method be used to represent in-vivo hydrodynamics?" J Pharm Pharmacol **55**(4): 443-451.
Schubert, M. L. (2009). "Gastric exocrine and endocrine secretion." Curr Opin Gastroenterol **25**(6): 529-536.
Schwarz, S., C. Kehrenberg and T. R. Walsh (2001). "Use of antimicrobial agents in veterinary medicine and food animal production." Int J Antimicrob Agents **17**(6): 431-437.
Sekiguchi, K. and N. Obi (1961). "Studies on Absorption of Eutectic Mixture. I. A Comparison of the Behavior of Eutectic Mixture of Sulfathiazole and that of Ordinary Sulfathiazole in Man." Chem. Pharm. Bull. **9**(11): 866-872.
Shulman, R. J., S. J. Henning and B. L. Nichols (1988). "The miniature pig as an animal model for the study of intestinal enzyme development." Pediatr Res **23**(3): 311-315.

Siepmann, J. and F. Siepmann (2013). "Mathematical modeling of drug dissolution." Int J Pharm **453**(1): 12-24.
Simonelli, A., S. Mehta and W. Higuchi (1969). "Dissolution rates of high energy polyvinylpyrrolidone (PVP)-sulfathiazole coprecipitates." J. Pharm. Sci. **58**(5): 538-549.
Smith, T. C., W. A. Gebreyes, M. J. Abley, A. L. Harper, B. M. Forshey, M. J. Male, H. W. Martin, B. Z. Molla, S. Sreevatsan, S. Thakur, M. Thiruvengadam and P. R. Davies (2013). "Methicillin-resistant Staphylococcus aureus in pigs and farm workers on conventional and antibiotic-free swine farms in the USA." PLoS One **8**(5): e63704.
Soucek, P., R. Zuber, E. Anzenbacherova, P. Anzenbacher and F. P. Guengerich (2001). "Minipig cytochrome P450 3A, 2A and 2C enzymes have similar properties to human analogs." BMC Pharmacol **1**: 11.
Stein, H., L. Lagos and G. Casas (2016). "Nutritional value of feed ingredients of plant origin fed to pigs." Anim. Feed Sci. Technol. **218**: 33-69.
Stephen, A. M. and J. Cummings (1979). "Water-holding by dietary fibre in vitro and its relationship to faecal output in man." Gut **20**(8): 722-729.
Suenderhauf, C. and N. Parrott (2013). "A physiologically based pharmacokinetic model of the minipig: data compilation and model implementation." Pharm Res **30**(1): 1-15.
Sun, C., L. Chen and Z. Shen (2019). "Mechanisms of gastrointestinal microflora on drug metabolism in clinical practice." Saudi Pharm J **27**(8): 1146-1156.
Sunesen, V. H., B. L. Pedersen, H. G. Kristensen and A. Mullertz (2005). "In vivo in vitro correlations for a poorly soluble drug, danazol, using the flow-through dissolution method with biorelevant dissolution media." Eur J Pharm Sci **24**(4): 305-313.
Sutter, H. M., J. L. Riond and M. Wanner (1993). "Comparative pharmacokinetics of aditoprim in milk-fed and conventionally fed calves of different ages." Res Vet Sci **54**(1): 86-93.
Sutter, H. M. and M. Wanner (1990). "[Feed preparation and pharmacokinetics of chlortetracycline in piglets]." Schweiz Arch Tierheilkd **132**(4): 175-181.
Swaisland, H. C., R. P. Smith, A. Laight, D. J. Kerr, M. Ranson, C. H. Wilder-Smith and T. Duvauchelle (2005). "Single-dose clinical pharmacokinetic studies of gefitinib." Clin Pharmacokinet **44**(11): 1165-1177.
Talattof, A., J. C. Price and G. L. Amidon (2016). "Gastrointestinal motility variation and implications for plasma level variation: oral drug products." Mol. Pharm. **13**(2): 557-567.
Tang, H. and M. Mayersohn (2018). "Porcine Prediction of Pharmacokinetic Parameters in People: A Pig in a Poke?" Drug Metab Dispos **46**(11): 1712-1724.
Tang, H., Y. Pak and M. Mayersohn (2004). "Protein expression pattern of P-glycoprotein along the gastrointestinal tract of the Yucatan micropig." J Biochem Mol Toxicol **18**(1): 18-22.
Toutain, P. L., A. A. Ferran, A. Bousquet-Melou, L. Pelligand and P. Lees (2016). "Veterinary Medicine Needs New Green Antimicrobial Drugs." Front Microbiol **7**: 1196.
Traynham, T. L., D. J. Myers, A. L. Carriquiry and L. A. Johnson (2007). "Evaluation of water-holding capacity for wheat-soy flour blends." J Amer Oil Chem Soc **84**(2): 151-155.
Van Boeckel, T. P., C. Brower, M. Gilbert, B. T. Grenfell, S. A. Levin, T. P. Robinson, A. Teillant and R. Laxminarayan (2015). "Global trends in antimicrobial use in food animals." Proc Natl Acad Sci U S A **112**(18): 5649-5654.
van der Meulen, J., J. T. van der Werf and A. Kijlstra (2007). "Questionnaire survey of disease prevalence and veterinary treatments in organic layer husbandry in the Netherlands." Tijdschr Diergeneeskd **132**(8): 292-295.
Van Duijkeren, E., B. G. Kessels, M. M. Sloet van Oldruitenborgh-Oosterbaan, H. J. Breukink, A. G. Vulto and A. S. van Miert (1996). "In vitro and in vivo binding of trimethoprim and

sulphachlorpyridazine to equine food and digesta and their stability in caecal contents." J Vet Pharmacol Ther **19**(4): 281-287.
Van Leeuwen, Van Gelder, de Leeuw and van der Klis (2006). "An Animal Model to Study Digesta Passage in Different Compartments of the Gastro-Intestinal Tract (GIT) as Affected by Dietary Composition." Curr Nutr Food Sci. **2**: 97-105.
Vandael, F., M. E. Filippitzi, J. Dewulf, E. Daeseleire, M. Eeckhout, M. Devreese and S. Croubels (2019). "Oral group medication in pig production: characterising medicated feed and drinking water systems." Vet Rec **185**(13): 405.
von Rosenvinge, E. C. and J. P. Raufman (2010). "Gastrointestinal peptides and regulation of gastric acid secretion." Curr Opin Endocrinol Diabetes Obes **17**(1): 40-43.
Wang, L., H. Yang, C. Zhang, Y. Mo and X. Lu (2008). "Determination of oxytetracycline, tetracycline and chloramphenicol antibiotics in animal feeds using subcritical water extraction and high performance liquid chromatography." Anal Chim Acta **619**(1): 54-58.
Wang, R., R. Wei, M. Chen and T. Wang (2010). "A new, simple and rapid HPLC method for determination of chlortetracycline in pig solid manure." ITAL J ANIM SCI **9**(2): e37.
Wanner, M., G. Nietlispach and H. M. Sutter (1990). "Influence of citric acid and calcium on the bioavailability of orally administered oxytetracycline in piglets." Deutsche Tierärztliche Wochenschrift **97**(12): 515-518.
Watanabe, K., S. Yamashita, K. Furuno, H. Kawasaki and Y. Gomita (1995). "Metabolism of omeprazole by gut flora in rats." J Pharm Sci **84**(4): 516-517.
Weinberg, E. D. (1957). "The mutual effects of antimicrobial compounds and metallic cations." Bacteriol Rev. **21**(1): 46.
Westfall, P. H. (1997). "Multiple Testing of General Contrasts Using Logical Constraints and Correlations." J AM STAT ASSOC **92**(437): 299-306.
White, J. R. and F. L. Pearce (1982). "Characterization of chlortetracycline (aureomycin) as a calcium ionophore." Biochemistry **21**(24): 6309-6312.
Willis, J. V., M. J. Kendall, R. M. Flinn, D. P. Thornhill and P. G. Welling (1979). "The pharmacokinetics of diclofenac sodium following intravenous and oral administration." Eur J Clin Pharmacol **16**(6): 405-410.
Wilson, G. D., D. G. Harvey and C. R. Snook (1972). "A review of factors affecting blood biochemistry in the pig." Br Vet J **128**(12): 596-610.
Woyengo, T., E. Beltranena and R. Zijlstra (2014). "Nonruminant nutrition symposium: Controlling feed cost by including alternative ingredients into pig diets: A review." J. Anim. Sci. **92**(4): 1293-1305.
Yang, C. S., P. G. Welling, G. R. Wilkinson, D. G. Bailey and C. S. Lieber (1995). Dietary Effects on Drug Metabolism. Pharmacological Sciences: Perspectives for Research and Therapy in the Late 1990s, Springer**:** 177-185.
Yu, D. K., A. T. Elvin, B. Morrill, L. S. Eichmeier, R. C. Lanman, M. B. Lanman and D. H. Giesing (1990). "Effect of food coadministration on 5-aminosalicylic acid oral suspension bioavailability." Clin Pharmacol Ther **48**(1): 26-33.
Yu, L. X., G. L. Amidon, J. E. Polli, H. Zhao, M. U. Mehta, D. P. Conner, V. P. Shah, L. J. Lesko, M. L. Chen, V. H. Lee and A. S. Hussain (2002). "Biopharmaceutics classification system: the scientific basis for biowaiver extensions." Pharm Res **19**(7): 921-925.
Zhang, H. Y. L. (2004). "Dissolution Testing for Solid Oral Drug Products: Theoretical Considerations." Am Pharm Rev **7**(5): 26-31.
Zhang, W., S. Xiao and D. U. Ahn (2013). "Protein oxidation: basic principles and implications for meat quality." Crit Rev Food Sci Nutr. **53**(11): 1191-1201.

Zhou, R., P. Moench, C. Heran, X. Lu, N. Mathias, T. N. Faria, D. A. Wall, M. A. Hussain, R. L. Smith and D. Sun (2005). "pH-dependent dissolution in vitro and absorption in vivo of weakly basic drugs: development of a canine model." Pharm Res **22**(2): 188-192.

Appendix 1

Table 1 A. Estimated adjustment values for multiple comparison of the soaking fluid effect on WHC

Effect		estimate	SE	DDL	F value	Pr > |t|	P aj.	Aj. Lo 95%C.I.	Aj. Hi 95%C.I.
feedstuff*solvent	**DDGS vs. MBM in W**	-0.32	0.048	24	-6.76	<0.0001	<0.0001	-0.4964	-0.1621
feedstuff*solvent	**DDGS vs. SBM in W**	0.97	0.073	24	13.41	<0.0001	<0.0001	0.7281	1.2295
feedstuff*solvent	**DDGS vs. gC in W**	-0.57	0.040	24	-14.19	<0.0001	<0.0001	-0.7149	-0.4363
feedstuff*solvent	**DDGS vs. gR in W**	-0.65	0.050	24	-12.92	<0.0001	<0.0001	-0.8284	-0.4805
feedstuff*solvent	**DDGS vs. gW in W**	-0.39	0.043	24	-8.88	<0.0001	<0.0001	-0.5408	-0.2391
feedstuff*solvent	**MBM vs. SBM in W**	1.30	0.075	24	17.43	<0.0001	<0.0001	1.0502	1.5658
feedstuff*solvent	**MBM vs. gC in W**	-0.24	0.044	24	-5.58	<0.0001	0.0002	-0.3980	-0.09466
feedstuff*solvent	**MBM vs. gR in W**	-0.32	0.053	24	-6.07	<0.0001	<0.0001	-0.5092	-0.1412
feedstuff*solvent	**MBM vs. gW in W**	-0.06	0.047	24	-1.28	0.2114	0.5850	-0.2231	0.1017
feedstuff*solvent	**SBM vs. gC in W**	-1.55	0.070	24	-22.18	<0.0001	<0.0001	-1.7950	-1.3137
feedstuff*solvent	**SBM vs. gR in W**	-1.63	0.076	24	-21.39	<0.0001	<0.0001	-1.8954	-1.3710
feedstuff*solvent	**SBM vs. gW in W**	-1.37	0.072	24	-18.99	<0.0001	<0.0001	-1.6163	-1.1212
feedstuff*solvent	**gC vs. gR in W**	-0.07	0.046	24	-1.70	0.1018	0.4201	-0.2380	0.08028
feedstuff*solvent	**gC vs. gW in W**	0.18	0.038	24	4.77	<0.0001	0.0010	0.05210	0.3191
feedstuff*solvent	**gR vs. gW in W**	0.26	0.049	24	5.36	<0.0001	0.0002	0.09512	0.4338
feedstuff*solvent	**DDGS vs. MBM in SGF**	-0.79	0.048	24	-16.41	<0.0001	<0.0001	-0.9660	-0.6317
feedstuff*solvent	**DDGS vs. SBM in SGF**	1.08	0.073	24	14.86	<0.0001	<0.0001	0.8341	1.3355
feedstuff*solvent	**DDGS vs. gC in SGF**	-0.63	0.040	24	-15.54	<00001	<0.0001	-0.7696	-0.4910
feedstuff*solvent	**DDGS vs. gR in SGF**	-0.80	0.050	24	-15.84	<0.0001	<0.0001	-0.9761	-0.6282
feedstuff*solvent	**DDGS vs. gW in SGF**	-0.58	0.043	24	-13.36	<0.0001	<0.0001	-0.7379	-0.4362
feedstuff*solvent	**MBM vs. SBM in SGF**	1.88	0.075	24	25.09	<0.0001	<0.0001	1.6259	2.1414
feedstuff*solvent	**MBM vs. gC in SGF**	0.16	0.044	24	3.82	0.0008	0.0080	0.01693	0.3203
feedstuff*solvent	**MBM vs. gR in SGF**	-0.003	0.053	24	-0.06	0.9515	0.9515	-0.1873	0.1807
feedstuff*solvent	**MBM vs. gW in SGF**	0.21	0.047	24	4.48	0.0002	0.0019	0.04949	0.3742
feedstuff*solvent	**SBM vs. gC in SGF**	-1.71	0.070	24	-24.47	<0.0001	<0.0001	-1.9557	-1.4744
feedstuff*solvent	**SBM vs. gR in SGF**	-1.88	0.077	24	-24.71	<0.0001	<0.0001	-2.1492	-1.6248
feedstuff*solvent	**SBM vs. gW in SGF**	-1.67	0.072	24	-23.19	<0.0001	<0.0001	-1.9193	-1.4243
feedstuff*solvent	**gC vs. gR in SGF**	-0.17	0.046	24	-3.71	0.0011	0.0096	-0.3310	-0.01278
feedstuff*solvent	**gC vs. gW in SGF**	0.043	0.038	24	1.11	0.2770	0.5924	-0.09028	0.1768
feedstuff*solvent	**gR vs. gW in SGF**	0.21	0.049	24	4.36	0.0002	0.0022	0.04581	0.3845
feedstuff*solvent	**W vs. SGF in DDGS**	0.10	0.045	24	2.39	0.0253	0.1784	-0.04764	0.2644
feedstuff*solvent	**W vs. SGF in MBM**	-0.36	0.051	24	-6.99	<0.0001	<0.0001	-0.5389	-0.1837
feedstuff*solvent	**W vs. SGF in SBM**	0.21	0.092	24	2.31	0.0296	0.1857	-0.1040	0.5327
feedstuff*solvent	**W vs. SGF in gC**	0.053	0.035	24	1.53	0.1386	0.4856	-0.06663	0.1740
feedstuff*solvent	**W vs. SGF in gR**	-0.04	0.055	24	-0.71	0.4838	0.7017	-0.2296	0.1508
feedstuff*solvent	**W vs. SGF in gW**	-0.09	0.042	24	-2.09	0.0471	0.2454	-0.2343	0.05685

Appendix 2

Table 2 A. Estimated adjustment values for multiple comparison of the particle sizes effect on WHC

	Estimate	SE	DDL	F Value	Pr > |t|	P aj.	Aj. Lo 95%C.I.	Aj. Hi 95%C.I.
linear SBM vs. gC	-1.1518	0.3476	79	-3.31	0.0014	0.0094	-2.1195	-0.1840
linear SBM vs. gR	-0.7703	0.3479	79	-2.21	0.0297	0.1049	-1.7390	0.1984
linear SBM vs. gW	1.0065	0.2030	79	4.96	<.0001	<.0001	0.4413	1.5718
linear SBM vs. DDGS	0.2312	0.3453	79	0.67	0.5051	0.7554	-0.7302	1.1925
linear gC vs. gR	0.3815	0.4098	79	0.93	0.3547	0.6159	-0.7594	1.5223
linear gC vs. gW	2.1583	0.2983	79	7.23	<.0001	<.0001	1.3277	2.9889
linear gC vs. DDGS	1.3829	0.4104	79	3.37	0.0012	0.0081	0.2403	2.5256
linear gR vs. gW	1.7768	0.2986	79	5.95	<.0001	<.0001	0.9454	2.6082
linear gR vs. DDGS	1.0015	0.4107	79	2.44	0.0170	0.0731	-0.1420	2.1449
linear gW vs. DDGS	-0.7753	0.2994	79	-2.59	0.0114	0.0568	-1.6090	0.05829
quadr. gC vs. gR	-0.1211	0.3843	79	-0.32	0.7535	0.7554	-1.1911	0.9489
quadr. gC vs. DDGS	0.7552	0.3848	79	1.96	0.0532	0.1447	-0.3162	1.8266
quadr. gR vs. DDGS	0.8763	0.3850	79	2.28	0.0256	0.1043	-0.1956	1.9482

Appendix 3

Table 3 A. Estimated adjustment values for multiple comparison of the soaking time effect on WHC

	Estimate	SE	DDL	Pr > |t|	P aj.	Aj. Lo 95%C.I.	Aj. Hi 95%C.I.
Slope DDGS vs. MBM	-0.00363	0.005758	60	0.5310	0.9870	-0.02054	0.01329
Slope DDGS vs. SBM	-0.00216	0.005758	60	0.7095	0.9987	-0.01907	0.01476
Slope DDGS vs. gC	-0.00181	0.005758	60	0.7549	0.9995	-0.01872	0.01511
Slope DDGS vs. gR	-0.00154	0.005758	60	0.7902	0.9995	-0.01845	0.01538
Slope DDGS vs. gW	-0.00224	0.005758	60	0.6986	0.9984	-0.01916	0.01467
Slope MBM vs SBM	0.001473	0.005758	60	0.7989	0.9995	-0.01544	0.01839
Slope MBM vs gC	0.001823	0.005758	60	0.7527	0.9995	-0.01509	0.01874
Slope MBM vs gR	0.002090	0.005758	60	0.7179	0.9987	-0.01482	0.01900
Slope MBM vs GW	0.001388	0.005758	60	0.8103	0.9995	-0.01553	0.01830
Slope SBM vs gC	0.000350	0.005758	60	0.9518	0.9997	-0.01656	0.01726
Slope SBM vs gR	0.000616	0.005758	60	0.9151	0.9995	-0.01630	0.01753
Slope SBM vs gW	-0.00009	0.005758	60	0.9883	0.9997	-0.01700	0.01683
Slope gC vs gR	0.000267	0.005758	60	0.9632	0.9997	-0.01665	0.01718
Slope gC vs gW	-0.00043	0.005758	60	0.9401	0.9996	-0.01735	0.01648
Slope gR vs gW	-0.00070	0.005758	60	0.9035	0.9995	-0.01762	0.01621

Appendix 4

Figure 1A. poster presented in the virtual event of Le Porc Show 2020