THE INTERACTION OF ACID-PRESERVATION OF WHEAT OR BARLEY, WITH OR WITHOUT ENZYMES, AND PARTICLE SIZE ON WEANLING PIG PERFORMANCE AND GUT HEALTH

A Thesis Submitted to the College of

Graduate and Postdoctoral Studies

In Partial Fulfillment of the Requirements

For the Degree of Doctor of Philosophy

In the Department of Animal and Poultry Science

University of Saskatchewan

Saskatoon

By

DANILO J. MARCOS SOTTO, JR.

PERMISSION TO USE

In presenting this thesis/dissertation in partial fulfillment of the requirements for a Postgraduate degree from the University of Saskatchewan, I agree that the Libraries of this University may make it freely available for inspection. I further agree that permission for copying of this thesis/dissertation in any manner, in whole or in part, for scholarly purposes may be granted by the professor or professors who supervised my thesis/dissertation work or, in their absence, by the Head of the Department or the Dean of the College in which my thesis work was done. It is understood that any copying or publication or use of this thesis/dissertation or parts thereof for financial gain shall not be allowed without my written permission. It is also understood that due recognition shall be given to me and to the University of Saskatchewan in any scholarly use which may be made of any material in my thesis/dissertation.

DISCLAIMER

Reference in this thesis/dissertation to any specific commercial products, process, or service by trade name, trademark, manufacturer, or otherwise, does not constitute or imply its endorsement, recommendation, or favoring by the University of Saskatchewan. The views and opinions of the author expressed herein do not state or reflect those of the University of Saskatchewan and shall not be used for advertising or product endorsement purposes.

Requests for permission to copy or to make other uses of materials in this thesis/dissertation in whole or part should be addressed to:

Head of the Department of Animal and Poultry Science
University of Saskatchewan
6D34 Agriculture Bldg., 51 Campus Dr.
Saskatoon, Saskatchewan S7N 5A8 Canada

OR

Dean
College of Graduate and Postdoctoral Studies
University of Saskatchewan
116 Thorvaldson Building, 110 Science Place
Saskatoon, Saskatchewan S7N 5C9 Canada

ABSTRACT

Low-quality high-moisture grains may be preserved by acidification as an alternative to artificial drying. Because weanling pig diets are commonly acidified to help the weaning transition, these series of experiments were conducted to determine whether the benefits of acidification are maintained when acid-preserved high moisture grains are fed. The interaction with particle size and enzyme activity was also investigated because acidification and particle size both influence gastric pH and thus enzyme activity.

In Chapter 2, bench-scale trials were conducted to determine the effect of acid preservation of high moisture wheat or barley (20% moisture) using propionic acid (Prop) or a phosphoric-acid-based organic-inorganic acid blend (OIB) at low or high concentrations, with or without enzymes (phytase, carbohydrases and protease) on grain quality and estimates of phosphorus and nitrogen availability. The absence of visible mould growth in any of the treatments throughout the 153-day trial indicates that OIB is as effective as Prop in preserving high moisture wheat or barley. A pH of below 5 was maintained in wheat and barley using high concentration of Prop (7.5 g kg^{-1}) up to 153 d, and high concentration of OIB (7 g kg^{-1}) up to 14 d. Acid binding capacity of the high moisture wheat or barley preserved using OIB were lower compared to Prop. Protein dispersibility index, an estimate of N availability, was improved with the addition of enzyme in Prop-preserved wheat and barley. Available P was improved in grains preserved with Prop.

In Chapter 3, a nursery and a metabolism trial were conducted to determine the efficacy of feeding acid-preserved high moisture wheat (APW), with or without enzymes, and the interaction with particle size (Fine or Coarse) on weanling pig (21 d) performance and gut health. Average daily gain (ADG), daily feed intake (ADFI), and feed efficiency (G:F) of pigs fed diets

with acid preserved wheat (APW) were comparable ($P > 0.10$) to pigs fed acidified diets (AD). Acidification, enzyme supplementation or fine grinding improved ($P < 0.05$) ADG and G:F with no evidence of interaction with mode of acidification. Phosphorus digestibility was improved with either OIB or enzyme supplementation. Energy digestibility was comparable in pigs fed Fine or Coarse APW but decreased in Coarse compared to Fine when fed AD. Treatment had no effect on markers of gut health ($P > 0.10$).

Because of the differences in chemical composition of wheat and barley, the same study was conducted using barley in Chapter 4. In the barley study, treatment had no effect on ADG, ADFI or G:F during phase 1 ($P < 0.05$). During phase 2, ADG was higher in pigs fed diets with acid-preserved barley (APB) than those fed AD. Feed intake and G:F were comparable in pigs fed diets with APB or AD. Enzyme supplementation increased ($P < 0.05$) ash (mineral) digestibility while dry matter and energy digestibility were increased ($P < 0.01$) in pigs fed Coarse compared to Fine when fed as APW but not AD. Similar to the wheat trial, treatment had no effect on markers of gut health ($P > 0.10$).

Overall, these observations indicate that feeding acid preserved high moisture grains may be an alternative to direct diet acidification for weanling pigs. The comparable nutrient digestibility of Fine and Coarse when fed as APW but not AD suggests improvement in digestibility in APW. Conversely, nutrient digestibility was improved when APB was fed Coarse compared to Fine. These improvements suggest that fine grinding may not be required when acid preserved grains are used. Economic analysis shows that feeding acid-preserved high-moisture grains may improve income by $1.73 (wheat-based diet) to $2.38 (barley-based diet) per market pig considering costs of acidification, grinding, and savings accruing from avoiding the cost of grain drying.

ACKNOWLEDGEMENTS

Obtaining this PhD degree would not be possible without the help of the following:

Dr. Denise Beaulieu for her supervision and care, patience, understanding, and support throughout my program. This PhD would not have been completed without her guidance, for which, I am forever grateful and indebted. Dr. Tom Scott for believing in my capabilities and taking me in as his student. My committee chairs Drs. Tim Mutsvangwa and Ryan Brook for keeping things in check and making sure I am on-track with my program. My committee members namely Dr. Peiqiang Yu for imparting his expertise in statistics; Dr. Lope Tabil for his processing advise and generosity in letting us use the facilities of the BioEngineering laboratory; Dr. Rex Newkirk for joining my committee on short notice and sharing his knowledge on grain processing and chemistry.

Alberta Crop Improvement Development Fund and Swine Innovation Porc for providing the funding for my project. Malachy Young and Neil Campbell of Gowans Feed Consulting for providing student funding.

Mr. Lee Whittington and the Management of the Prairie Swine Centre Inc. for taking me in as a graduate student and for allowing me to conduct my research in their facility. Staff Doug, Tatjana, Karu, Christine, Atta, and fellow students Michael, Khalil, Dakota and Luke for their help in conducting these experiments.

John Smillie and Scott Bishoff for trusting me to work at the CFRC and for accommodating all the grain pre-treatment and feed requirements for my project. Enkra Darambazar, Natalia Rudnitskaya and Kim Pastl for their assistance in conducting various laboratory analyses.

DEDICATION

This thesis is dedicated to my father, Danilo A. Sotto, to whom I promised that I would obtain a PhD. My mother Idaya M. Sotto and sisters Jing and Amory who supported me in all my goals and aspirations.

I also dedicate this thesis to my wife, Vanessa for single-handedly managing the household, taking care of myself and our children, and bearing with my frequent unavailability. To our children Joaquin and Bettina for their understanding and keeping me inspired and sane during this PhD. I love you all.

TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

LIST OF EQUATIONS

LIST OF ABBREVIATIONS

ABC	Acid binding capacity
AD	Acidified diets
ADF	Acid detergent fiber
ADG	Average daily gain
ADFI	Average daily feed intake
AIA	Acid insoluble ash
AID	Apparent ileal digestibility
AOAC	Association of Official Analytical Chemists
AOCS	American Oil Chemists' Society
aP	Available phsophorus
APB	Acid-preserved barley
APW	Acid-preserved wheat
ASAE	American Society of Agricultural Engineers
ASTM	American Standard Test Method
ATTD	Apparent total tract digestibility
A_w	Water activity
BUF	Buffering capacity
BW	Body weight
d_{gw}	Geometric mean particle size diameter
Ca	Calcium
CDN	Claudin
cDNA	Complementary deoxy ribonucleic acid

CFIA	Canadian Food Inspection Agency
CF	Crude fiber
CFRC	Canadian Feed Research Centre
CFU	Colony forming units
Cl^-	Chloride
CO_2	Carbon dioxide
CP	Crude protein
d	Day
DM	Dry matter
DNA	Deoxy ribonucleic acid
DON	Deoxynivalenol
E. coli	Escherichia coli.
EE	Ether extract
Enz	With enzymes
ETEC	Enterotoxigenic *Escherichia coli.*
FABP	Fatty acid binding protein
FCGBP	FC gamma binding protein
FCR	Feed conversion ratio
FID	Flame ionization detector
Fig.	Figure
GC	Gas chromatography
GE	Gross energy
G:F	Gain to feed ratio

GIT	Gastro-intestinal tract
HCl	Hydrochloric acid
H. suis	*Helicobacter suis*
HPLC	High-performance liquid chromatography
IAP	Intestinal alkaline phosphatase
IFN	Interferon
IL	Interleukin
INF	Inverted formin
KDF	Potassium diformate
kg	Kilogram
kWh	Kilowatt hour
KSU	Kansas State University
L	Liter
LA	Lactic acid
L:M	Lactulose:mannitol ratio
m	Meter
MB	Manitoba
Mg	Magnesium
Min	Mineral
MOA	Mode of acid addition
MUC	Mucin
MW	Molecular weight
N	Nitrogen

NH_3 N	Ammonia nitrogen
NC	Negative control
NDF	Neutral detergent fiber
NoEnz	No enzymes
NSP	Non-starch polysaccharide
NE	Net energy
NRC	National Research Council
O_2	Oxygen
OCLN	Occludin
OIB	Organic-inorganic acid blend
OM	Organic matter
OTU	Operational taxonomical unit
P	Phosphorus
PC	Positive control
PCNA	Proliferating cellular nuclear antigen
pH	Power of hydrogen
phyP	Phytate phosphorus
PWDS	Post-weaning diarrhea syndrome
PCR	Polymerase chain reaction
qPCR	Quantitative polymerase chain reaction
RELM	Resistin-like molecules
RNA	Ribonucleic acid
SAS	Statistical Analysis Software

SCFA	Short chain fatty acid
s_{gw}	Log normal standard deviation
SKCS	Single kernel characterization system
TNF	Tumor necrosis factor
TGF	Transforming growth factor
µL	Microliter
µm	Micrometer
Vit	Vitamin
Zn	Zinc
ZO	Zona occludin

INTRODUCTION

Feed is the single most expensive variable cost of pork production, representing about 65% of total cost (Woyengo et al. 2014). Wheat and barley are the main source of feed energy in animal diets in Western Canada. Therefore, methods that improve the feeding value of wheat or barley will reduce overall cost of pork production in Western Canada.

Wheat and barley are either harvested at, or dried to, less than 15% moisture for prolonged storage (Hackl et al. 2010). Drying, however, increases grain cost due to the use of fuel, power and specialized-drying structures. Harvesting and storage of grains at high-moisture may reduce grain cost by eliminating costs associated with drying. Compared to harvesting dry mature grains, harvesting at high moisture provides grain farmers the additional benefit of harvesting approximately 12 d earlier, and up to 16.7% higher dry matter (DM) yield per acre compared to mature grains because of fewer losses during harvest (Mc Lelland 2008). High-moisture grains may be ensiled in air-tight structures to arrest grain respiration and prevent the growth of aerobic organisms that can cause spoilage. This method, however, requires a large capital investment. Chemical preservation using acids allows for prolonged storage in aerobic conditions without the need for specialized structures (Lynch et al. 1975). Acids have antimicrobial properties that help prevent the proliferation of storage pests (moulds, bacteria and insects), even at high-moisture conditions. For example, high-moisture corn (~24%) treated with propionic acid was stored without deterioration for over 8 months (Lynch et al. 1975). When included in rations for growing-finishing pigs, performance was equal to dry corn (Young et al. 1970).

Acidifiers are added to diets of weanling pigs because newly weaned pigs are not able to maintain a low gastric pH due to insufficient secretion of gastric hydrochloric acid (HCl; Tung and Pettigrew 2006; Kil et al. 2011). This has negative consequences on growth and health status post-weaning because a low pH is required to activate enzymes for the digestion of nutrients and to stop the proliferation of ingested pathogens. Protein digestion starts in the stomach and is catalyzed by the enzyme pepsin, the active form of proenzyme pepsinogen. The optimum pH for the conversion of pepsinogen to pepsin is pH 2.0. Activation declines rapidly beyond 3.5, impairing protein digestion (Lawlor et al. 2005). The low gastric secretion of HCl coupled with the physical and social stresses of weaning result in a post-weaning growth lag which is characterized by reduced growth rates and increased incidence of scouring. Adding organic (citric, formic, fumaric, propionic, etc) and inorganic (phosphoric) acids to the diet of weanling pigs improves growth performance (Ravindran and Kornegay 1993; Partanen and Mroz 1999; Kil et al. 2011; Suiranrayna and Ramana 2015). An exact mode of action has not been identified (Jacela et al. 2009) however, the proposed mechanisms include reduction of digesta pH, modification of gut microflora and enhanced secretion of pancreatic enzymes (Partanen and Mroz 1999; Pettigrew 2006; Kil et al. 2011; Suiranrayna and Ramana 2015). On average, dietary acid supplementation increases the growth rates of pigs by 12% and 6% for the 0 to 2 and 0 to 4 weeks post-weaning, respectively (reviewed by Tung and Pettigrew 2006). Because of their anti-microbial action, organic acids have been proposed as potential replacements to antibiotics in animal production (de Lange 2010). For example, post-weaning diarrhea syndrome (PWDS), mainly caused by enterotoxigenic *E. coli* (ETEC), was successfully controlled using organic acids, especially lactic acid (LA; Tsiloyiannis et al. 2001). Acidification of weanling pig diets is not a new concept, however the presentation of acid via acid-preserved high-moisture grain as an

alternative to direct diet acidification to our knowledge, has not been studied. Whether the benefits of diet acidification will be realized when acid-preserved wheat or barley are fed to weanling pigs instead of a directly acidified diet is not known and needs to be investigated because this will provide producers another tool to utilize low-quality, high-moisture grains with potential savings.

Because of chemical and structural differences between wheat and barely grains, there may be differences in piglet response when either acid-preserved wheat or barley is fed to weanling pigs. Starch, protein and phytins may be hydrolized in both grains by acidification and high moisture storage but the extent of starch hydrolysis in barley may be lower than that in wheat because the barley is hulled and may hamper the penetration of the acid. However, a greater response may be observed in barley due to the the high fiber content which may may be hydrolyzed by the acid. The addition of exogenous enzymes may also elicit a different response from either grain due to the differences in non-starch polysaccharides (NSP) present between the wheat (arabinoxylans) and barley (β-glucans). Between the two, β-glucans are more soluble in water and may be better hydrolyzed by the added enzymes. Exogenous enzymes have been used since the 1950's and have been shown to play a role in the hydrolysis of anti-nutritional factors such as phytin and NSP, thereby enhancing nutrient digestibility and utilization, and reducing nutrient excretion (Adeola and Cowieson 2011). Phytases and carbohydrases are exogenous enzymes added to pig diets specifically to degrade phytins and NSPs to improve the availability of phosphorus (P) and energy respectively. Phytase and carbohydrase require moisture and/or a low pH for optimal activity. The presence of these conditions when high-moisture grains are acidified for storage could therefore enhance exogenous enzyme activity and "pre-digest" the grain prior to feeding. For example, phytate P (phyP) degradation and P availability was

increased by soaking grains possessing intrinsic phytase activities in water (Columbus et al. 2010) with further increase when steeped with phytase (Niven et al. 2007). The preservation of high-moisture (30%) rye and triticale with propionic acid resulted in increased in-vitro starch and protein digestibility compared to dry grains (Czarnecka et al. 1991). Poulsen et al. (2012) fed high-moisture air-tight stored wheat (83% DM) and barley (85.2% DM) to finishing pigs and reported improvements in P and crude protein (CP) digestibility by 12 and 4%, respectively, compared to pigs fed dry-stored grains. Limited studies have been conducted to investigate the potential interaction of acids and exogenous enzymes to improve nutrient digestibility. Jongbloed et al (2000) reported synergism between formic acid and phytase on apparent total tract digestibility (ATTD) of ash, P and magnesium in growing pigs. Another study by Omogbenigun et al. (2003) reported improved in-vitro phytate hydrolysis of corn-soybean meal-based weanling pig diets when organic acids were added to a diet with microbial phytase. When fed to weanling pigs, supplementation with microbial phytase and organic acids improved P utilization and reduced P excretion. However, these experiments (Jongbloed et al. 2000; Omogbenigun et al. 2003) used dry grains.

Grains are ground prior to feed production as this improves pig performance in all stages of the production cycle. In first parity sows, reducing the corn particle size from 1,200 to 400 µm increased litter weight gain by 11% (Wondra et al. 1995). Average daily gain (ADG) increased by 9.7% and G:F improved by 11.6% when particle size of a wheat-based diet was reduced from 1,430 µm to 680 µm in the weanling pig (Mavromichalis et al. 2000). Gain:feed (G:F) was improved by 6% in weanling pigs when corn particle size was reduced from 865 to 339 µm (Rojas and Stein 2015). Wondra et al. (1995) reported that G:F improved by 8% in the finishing pig when corn particle size was reduced from 1,000 µm to 400 µm (diet d_{gw} from 1,017 to 517

μm). Overall, these improvements are attributed to increased surface area of the feed particles, allowing more interaction with enzymes in the digestive system of the pig. In addition to improved animal performance, the improved digestibility results in decreased manure and nutrient excretion, addressing environmental issues from waste generated from pig production systems (Wondra et al. 1995a-d). Although the effects of grinding on pig performance are well documented, the vast majority of the studies were conducted using either corn-soybean meal-based diets or dry grains. We suspect that these results may not be applicable to diets used in Western Canada due to different fiber types and concentration in wheat and barley. High fiber content makes a grain more difficult to grind (Heiman 2005). In a recent study by Lamicchane and Scott (2015), particle size of ground barley was 46% larger than wheat and 58% larger than corn even though ground using the same screen size (3.2mm) in a hammer mill. Compared to barley, wheat results in a finer particle size after grinding possibly because it has no hull to dampen the grinding forces applied on the grain.

Although reducing particle size improves nutrient digestibility for swine, there may be negative consequences for gut health. Feeding finely ground diets to pigs has been identified as a predisposing factor in the development of gastric ulcers (Melnichouk 2002; Friendship 2004). A coarser grind resulted in lower incidence of gastric ulcers, improved intestinal morphology and modified bacterial populations to favor the growth of LA bacteria and reduced *Salmonella sp*. and coliform populations (Mikkelsen et al. 2004). Pigs fed a coarsely ground diet had similar growth performance as those fed a finely-ground, formic acid-supplemented diet (Canibe et al. 2005). The effect of feeding coarsely ground diets on reducing digesta pH and modifying the microbial populations is similar to the proposed mode of action with dietary acid supplementation but with differences in location within the gut. Diet acidification effects are

predominantly in the proximal gastro-intestinal tract (GIT; Kil et al. 2011) whereas studies by Mikkelsen et al. (2004) suggests the effects of coarsely ground diets may be observed in the proximal and distal sections of the GIT. It was proposed that the reduction in gastric pH resulted from a longer retention time of coarsely ground diets in the stomach, favouring the proliferation of LA bacteria. Feeding coarsely ground diets reduced gastric pH and increased the concentration of short chain fatty acids (SCFA) in the caecum and colon in pigs (Nielsen and Ingvartsen, 2000; Mikkelsen et al. 2004). When diets were coarsely ground, the amount of undigested starch entering the caecum and colon was increased. This could be used as a substrate for bacterial fermentation into the SCFAs, butyrate, acetate and propionate.

The combination of coarse particle size and dietary acids successfully controlled the prevalence of *Salmonella sp* in pigs. Weanling pigs fed a coarsely ground diet (53% > 1.4 mm) in combination with potassium diformate resulted in reduced *Salmonella* excretion rate, shorter *Salmonella* shedding period and reduced translocation of *Salmonella* within the infected piglets compared to pigs fed a finely ground diet (11% > 1.4mm; Papenbrock et al. 2005). Visscher et al. (2009) demonstrated a reduction in *Salmonella sp.* prevalence when coarsely ground diets supplemented with formic and propionic acids were fed to growing-finishing pigs compared to feeding finely ground diets supplemented with the organic acids.

The power and fuel cost of drying grain, estimated to be \$10 to \$12 t^{-1}, may be eliminated by preservation of high-moisture grains with acids (Alberta Agriculture, 2015; from 20% to 15% moisture content). However, the energy required to grind high-moisture grains is higher than dry grains (Goransson and Ogle 1984; Heimann 2005; Probst et al. 2013). Optimizing the particle size of acidified high-moisture grains should therefore consider grinding cost and the potential improvements in animal performance and gut health. If grinding acid-preserved high-moisture

grains coarsely results in similar performance as a finely ground artificially dried grain, the anticipated increase in grinding cost of high-moisture grains may be avoided. Moreover, the potential improvements in gastro-intestinal health with acidification, particle size modification or their interaction may mitigate the effects of removing in-feed antibiotics and allow pork producers to market pork from animals fed diets without antibiotics. Additional benefits to grain farmers are earlier harvest time and up to 16.7% increase in DM yield compared to harvesting mature grains due to reduction of losses related to harvesting dry grains (Mc Lelland, 2008).

The overall objective of these series of experiments was to determine whether the benefits of diet acidification on weanling pig performance and gut health are maintained when acid-preserved high-moisture wheat or barley are fed. Storage at high moisture and low pH may potentially improve nutrient release due to activation of enzymes; therefore, our second objective was to determine if there is an interaction with enzyme supplementation. Because particle size affects gastric pH, nutrient digestibility and SCFA production in the hind gut, the third objective was to determine if there is an interaction between acid-preserved high-moisture grain and particle size on animal performance and gut health.

CHAPTER 1. REVIEW OF LITERATURE

Low quality grains or grain by-products regarded as unfit for human food production or consumption are used by the swine industry as feed ingredients. Wheat and barley are harvested at less than 15% moisture for prolonged storage. However, inclement weather conditions sometimes do not allow for harvesting at below 15% moisture (Hackl et al. 2010). Alternative to artificial drying, low-quality high-moisture grains may be preserved by acidification. To enhance the feeding value of low-quality high-moisture grains, three technologies with the potential to improve weanling pig performance, nutrient digestibility and gut health are explored in this thesis. These technologies are:

1) organic and inorganic acids as preservatives for high-moisture grains or diet acidifiers,
2) exogenous enzymes to improve nutrient digestibility, and
3) particle size modification (grinding) to improve animal performance and nutrient digestibility.

The potential for synergisms among these technologies was of specific interest.

1.1 Acid-preserved high-moisture grains

Moisture content of wheat and barley must be less than 15% for prolonged storage without deterioration in quality (Buchanan-Smith et al. 1999; Hackl et al. 2010). When harvested at high-moisture, artificial drying is employed, however, due to the requirements for fuel, power, and specialized-drying structures, this increases cost. Drying cost, estimated at $10 to $12 per tonne may be eliminated by storing grains at high-moisture (Alberta Agriculture and Forestry 2017; Available: http://www1.agric.gov.ab.ca/$department/ deptdocs.nsf/all/faq7453).

Harvesting at high-moisture may be an attractive option for producers who grow their own grains because it provides the additional benefit of harvesting 12 d earlier than conventional methods, and 16.7% higher DM yield compared to mature field-dried barley because of fewer field losses during harvest (McLelland 2008; Sauer and Burroughs 1974).

As an alternative to artificial drying, high-moisture grains may be stored in air-tight containers to arrest grain respiration and prevent the growth of aerobic organisms that cause spoilage (Ton Nu et al. 2015). Advantages of feeding high-moisture stored grains to swine have been noted. For example, feeding finishing pigs high-moisture wheat and barley stored in oxygen-limiting containers had improved digestibility of crude protein (CP) and P (Poulsen et al. 2012). The authors attributed these improvements to enhanced enzyme activity in the high-moisture air-tight-stored grains, most notably endogenous phytase (Poulsen et al 2012). When barley and triticale were supplemented with a combination of phytase, xylanase, β-glucanase and protease enzymes during high-moisture air-tight storage (29% moisture for 49 d) phytate P (phyP) was reduced, and the solubility of P and nitrogen (N) was increased (Ton Nu et al. 2015). Similarly, Niven et al. (2007) demonstrated improved in-vitro P release when corn was steeped in water (24 h at 21 °C) with phytase added at the rate of 1.2 g kg^{-1} DM and soaking corn with phytase for 24 h before feeding improved digestibility of DM, CP and Ca (Columbus et al. 2010).

Another method to preserve high-moisture grains is to modify the atmosphere such that CO_2 levels increase (Paster et al. 1991). Aside from limiting the amount of oxygen, CO_2 is a potent gas that inhibits mould growth and toxin production. Paster et al. (1991) demonstrated that the growth of zearalenone was prevented at 20% CO_2 levels even though the O_2 level was also 20%. However, high-moisture storage in oxygen limiting containers require large capital

investment for specialized air-tight structures and microbial activity resumes when the grain is removed from storage which limits batch size (Jones et al. 1975). Alternatively, preservation of high-moisture grains using mould inhibiting chemicals allows for prolonged storage in aerobic conditions using existing storage facilities.

An ideal grain preservative must have good anti-microbial activity, be cost-effective, non-toxic, non-corrosive and easy to use (Sauer and Burroughs 1974; Lin and Chen 1995). The feed industry uses organic acids as an inexpensive and effective tool to control mould growth (Higgins and Brinkhaus 1999). Organic acids used commercially for preservation of high-moisture grains include acetic, propionic, isobutyric, formic, benzoic, sorbic, lactic or combination of these acids (Sauer and Burroughs 1974; Lin and Chen 1995; Higgins and Brinkhaus 1999). Studies have also shown that phosphoric acid, an inorganic acid, is an effective feed preservative inhibiting the growth of fungi *Aspergillus, Fusarium* and *Penicillium* with the added benefit of supplementing P into the diet (Schoenherr 1994; Lin and Chen 1995). Propionic acid and the mixture of propionic and acetic acid enabled storage of high-moisture corn (~24%) for over 8 months (Lynch et al. 1975; Young et al. 1970) and when fed to pigs, feeding value of the acid-preserved corn was comparable to the dry ground corn (Young 1970; Forsyth 1975; Lynch et al. 1975; Buchanan-Smith et al. 2003). In a study by Czarnecka et al. (1991), preserving high-moisture (30%) rye and triticale with propionic acid for 2 months increased in-vitro starch and protein digestibility relative to the dry grains.

1.2 Acidifiers and pig performance

The stomach pH of newly weaned pigs is often higher than that of mature pigs (Partnen and Mroz 1999) because of insufficient secretion of HCl (Manners 1976; Cranwell and Moughan 1989). Stomach pH decreases to that observed in mature pigs 3 to 4 weeks post-weaning

(Cranwell and Moughan 1989). This has negative consequences on growth and health status immediately post-weaning because a low pH is needed to activate enzymes required for the digestion of nutrients and to limit the proliferation of ingested pathogens (Mroz 2001). Protein digestion starts in the stomach, catalyzed by pepsin, the active form of the proenzyme pepsinogen. The optimum pH for the conversion of pepsinogen to pepsin is 2.0. Conversion declines rapidly beyond 3.5, impairing protein digestion (Lawlor et al. 2005). The low gastric secretion of HCl in young pigs, coupled with the physical and social stresses of weaning may result in a "post-weaning lag" characterized by reduced growth and increased incidence of scouring (Ravindran and Kornegay 1993). Acidifiers are added to diets of newly weaned pigs to address these gastric pH issues (Kil et al. 2011). In a meta-review by Tung and Pettigrew (2006), organic acids added to weanling pig diets improved growth rates of pigs by 12% and 6% for the 0 to 2 and 0 to 4 weeks post-weaning, respectively (Tung and Pettigrew 2006). Although older pigs can maintain a gastric pH of 2, there is evidence that the addition of acid elicits a performance response. In the same review, Tung and Pettigrew (2006) observed a tendency of decreasing magnitude of growth rate improvement as the pig matured. Similarly, an older meta-review by Partanen (2001) suggested that performance response to organic acid supplementation varied from 3.7 to 11.9% improvement in ADG and 3.9 to 4.8% improvement in G:F compared to a non-acidified control, depending on the type of acid and amount added to the diet. For example, the addition of citric acid at 5 to 25 g kg^{-1} improved ADG and G:F of weanling pigs by 3.6 and 4.8% respectively. When 3 to 18 g kg^{-1} formic acid was added to weanling pig diets, improvements of 6.4, 10.6 and 3.9% were observed for ADFI, ADG, and G:F respectively (Partanen 2001). In the same review, 5 to 25 g kg^{-1} fumaric acid resulted in improvements of 4.5 and 4.3% for ADG and G:F respectively, while 4 to 24 g kg^{-1} potassium diformate improved

ADFI, ADG and G:F by 7.7, 11.9 and 3.9%, respectively (Partanen 2001) and 1.5 to 10 g kg^{-1} of 60% propionic acid improved weight gain and feed intake (Ravindran and Kornegay 1993).

Organic acids have been suggested as substitutes for in-feed antibiotics (Pettigrew 2006; Stein and Kil 2006; de Lange et al. 2010; Heo et al. 2012). However, the addition of benzoic acid (3 g kg^{-1}) to a simple corn-soy diet for weanling pigs had no effect on weanling pig performance compared to the those fed the diet without the acid, and these pigs actually performed poorer than those receiving a diet containing an antibiotic (Tylan 10) and animal and milk by-products (Wang et al. 2018). In contrast, benzoic acid added at 5 g kg^{-1} to a corn-wheat-soybean meal diet for six weeks improved overall G:F of pigs compared to those fed the same diet without antibiotic, and was similar to pigs fed a diet with chlortetracycline HCl at 220 mg kg^{-1} and tiamulin at 31.2 mg kg^{-1} (Kiarie et al. 2018). Weanling pig performance was not affected when a coated sodium butyrate preparation (30% purity) was added to a corn-soybean meal weanling pig diet containing fish meal and whey at 1 g kg^{-1} but diarrhea was significantly reduced compared to those fed without the acid (Fang et al. 2013).

Compared to organic acids, inorganic acids are inexpensive and may be as effective as organic acids in improving animal performance (Schoenherr 1994). However, similar to what was observed with organic acids, the results in studies using inorganic acids vary. In a review by Kil et al. (2011), the use of HCl at 0.5 and 1.0 g kg^{-1} improved ADG by 9.7 and 23% respectively, ADFI by 2.1 and 15.7%, and G:F by 7.5 and 5.7% compared to the control. Conversely, Roth and Kirchgessner (1998) reported that phosphoric acid at 0.85 to 3.55% of the diet had no effect on weanling pig performance, while supplementation of HCl at 1.4% and 3% of the diet reduced feed intake and consequently, daily weight gain. A depression in feed intake and growth was observed following the supplementation of the diet of weanling pigs with HCl at

2, 3 and 14 g kg^{-1}. This was attributed to the disruption in electrolyte balance brought about by the influx of Cl^- from the addition of HCl (Ravindran and Kornegay 1995). Ravindran and Kornegay (1993) suggested that when organic acids are combined, optimum pH is maintained throughout the GIT because of the differences in pKa among acids and thus an ability to dissociate over a wide range of pHs. Supplementing weanling pig diets with a combination of the SCFAs; fumaric, citric, and malic, and the medium chain fatty acids capric and caprylic acids at 0.1%, 0.2% and 0.4% resulted in linear improvements in ADG and G:F (Upadhaya et al. 2016). Similarly, supplementation of a citric and sorbic acid blend improved overall ADG of weanling pigs relative to pigs fed the control (Grilli et al. 2010). However, when a combination of butanoic, fumaric and benzoic acids were supplemented, ADG and feed efficiency were only numerically improved compared to pigs fed the control diet (Li et al. 2008). A blend of organic and inorganic acidifiers has gained attention due to lower cost and potential synergism (Tung and Pettigrew 2006; Kil et al. 2014; Ahmed et al. 2014). However, data from the study of Omogbenigun et al. (2003) who evaluated the use of an organic-inorganic acid blend (3.5 g kg^{-1} of the diet) and microbial phytase (500 U kg^{-1}), suggested that the addition of the acid blend did not improve growth performance, although it did potentiate the effect of phytase.

1.3 Proposed modes of action of acids

Proposed mechanisms for the response to acid supplementation include: 1) reduction in gastric pH resulting in improvement in nutrient digestion through activation of proteolytic enzymes and reduced gastric emptying rate, 2) inhibition of microbial growth resulting in modulation of microbial population, and 3) providing nutrients preferred by the cells of the GIT (Ravindran and Kornegay 1993; Partanen and Mroz 1999; Tung and Pettigrew 2006; de Lange et al. 2010; Kil et al. 2011).

1.3.1 *Acids, gastric pH and nutrient digestibility*

Insufficient amounts of HCl are secreted into the stomachs of newly weaned pigs and thus gastric pH is higher than observed in the adult pig (Manners 1976). The inability of the piglet to maintain a low gastric pH post-weaning has negative consequences on growth and gut health (Mosenthin 1998). Primarily because a low pH is required to inhibit the proliferation of ingested pathogens, and for activation of the proenzyme pepsinogen into pepsin, the main enzyme for protein digestion (Mikkelsen et al. 2002).

Protein digestion starts in the stomach, catalyzed by pepsin, the active form of the proenzyme pepsinogen. The optimum pH for the conversion of pepsinogen to pepsin is 2.0 and activation declines rapidly beyond 3.5, thus impairing protein digestion (Lawlor et al. 2005). Gastric emptying rate is also influenced by gastric pH; a lower pH prolongs retention time in the stomach, allowing for longer time for protein hydrolysis (Partanen and Mroz 1999).

Several studies have been conducted to determine the effect of acid supplementation of piglet diets on DM, organic matter, CP, P, and energy digestibility. For example, both organic matter and energy digestibility improved by 1 and 2% with supplementation of 5 or 10 g kg^{-1} citric acid, respectively (Broz and Shulze 1987), but there was no effect on ATTD of CP when added at 10 and 20 g kg^{-1} (Falkowski and Aherne 1984). Conversely, supplementing a complex diet with formic acid at 6 to 24 g kg^{-1} diet increased ATTD of CP by 2.6 to 4.4% in weaned pigs (6 to 14 kg BW). Energy digestibility was improved by 1.9 and 2.2% at higher inclusion levels of 18 and 24 g kg^{-1} diet (review by Partanen an Mroz 1999). Improvements in ATTD of CP by 2, 1.7, and 1.3% respectively were also observed when formic acid (12.5 g kg^{-1}), Ca formate (18 g kg^{-1}), and sodium formate (18 g kg^{-1}) were added to diets of weanling pigs (6 to 14 kg BW, Partanen and Mroz 1999). The improvement in CP digestibility in response to supplementation

of formic acids or its salts decreases with age (Partanen and Mroz 1999), however Jongbloed et al. (2000) reported improvement in ATTD of DM, OM, ash, Ca and P in growing pigs when diets were supplemented with lactic (16 and 32 g kg^{-1}) and formic (8 and 16 g kg^{-1}) acids. While Gabert and Sauer (1994) concluded that adding propionic acid at 0.3 to 2% of weanling pig diet does not affect apparent total tract digestibility (ATTD) of DM, organic matter (OM), CP and gross energy (GE), Partanen and Mroz (1999) calculated in a meta analysis, increased ATTD of DM and ash with propionic acid supplementation. Fumaric acid supplementation (20 g kg^{-1} diet) of a corn-soy diet resulted in numerical increases in apparent ileal digestibility (AID) of CP and amino acids (Giesting and Easter 1991). In growing pigs, propionic acid added at 20 g kg^{-1} to a barley-soybean meal-wheat bran-beet pulp diet (Mosenthin et al. 1992) and lactic acic added at 30 g kg^{-1} to a corn-soybean meal diet (LA; Kemme et al. 1999;) improved AID of indispensable amino acids (Arg, His, Leu, Ile, Met, Thr, Phe and Val). Mroz et al. (1997) reported significant improvement in AID of several essential and non-essential amino acids by 3 to 6% following dietary supplementation of formic, fumaric and n-butyric acids. In contrast, AID of amino acids was not affected in weanling pigs when semi-purified diets with fish meal (Gabert et al. 1995) were supplemented with 10 g kg^{-1} formic acid and a corn-soybean meal diet was supplemented with fumaric acid at 15 or 30 g kg^{-1} (Gabert and Sauer 1994). The studies with weaned pigs used either simple cereal-soybean diets or semi-purified fishmeal diets while those in the grower trials were either corn-soybean meal or complex by-product-based diets. Results from these studies suggest lesser improvement in nutrient digestibility can be expected with acid supplementation when highly digestible ingredients are used in the diet (Partanen and Mroz 1999). Furthermore, a lower response to acidification was observed in diets with milk products. The lowered response in the presence of milk products may be attributed to the high buffering capacity of these

ingredients, and also because lactose can be fermented in the GIT of the pig into LA making the acidifier unnecessary (Ravindran and Kornegay 1995).

Supplementation of the diet with organic acids may improve absorption and retention of minerals. For example, in a review by Partanen and Mroz (1999), Ca, P, Mg and Zn balance were improved by 14, 13, 21 and 43% respectively, when a weanling pig diet was supplemented with 20 g kg^{-1} diet of fumaric acid and zinc deficiency symptoms were reduced when diets with suboptimal levels of Zn were supplemented with 15 g kg^{-1} citric acid. However, in growing pigs fed diets with benzoic acid at 0, 10 or 20 g kg^{-1} for 21 d, the concentration of ash in the femur and Ca content of the ash decreased linearly with increasing acid, indicating changes in bone resorption of Ca to buffer increased absorption of the acid (Sauer et al. 2008). Bone erosion in rats fed acidic diets was attributed to the use of Ca carbonate from its skeleton as a buffer to decreased physiological pH (Petito and Evans 1984).

Several studies have been conducted to investigate the interaction of acids and exogenous enzymes on nutrient digestibility. Jongbloed et al (2000) reported a positive synergy between formic acid and phytase on ATTD of ash, P and Mg in growing pigs. Conversely, Omogbenigun et al. (2003) reported improved P digestibility of a corn-soybean meal-based weanling pig diet when microbial phytase added to a negative control and was similar when to when both microbial phytase and organic acid were present.

1.3.2 Acids and GIT microbial populations

Review articles by Ravindran and Kornegay (1993), Partanen and Mroz (1999), Tung and Pettigrew (2006), and Kil et al. (2011) suggest that dietary acids modulate gut microbial populations by preventing the proliferation of pathogenic bacteria and providing optimum conditions for beneficial bacteria through the reduction of gastric pH. Short chain fatty acids (C1

to C7) naturally occur in plant and animal tissues, and in the pig, are formed through microbial fermentation of carbohydrates, mainly in the large intestine (Lee et al. 2007). Organic acidifiers may have direct antimicrobial effects on pH-sensitive harmful bacteria such as coliforms, *Clostridia*, *Salmonella* and *Listeria*, without affecting the growth of non-pH sensitive bacteria like *Lactobacilli* and *Bifidobacteria* (Gauthier 2002). Essential to their anti-microbial function is their pKa value, which is the pH at which 50% of the acid is undissociated. The undissociated form of the organic acid can diffuse freely through cell membranes into the cytoplasm which is maintained at pH 7 (Fig 1.1).

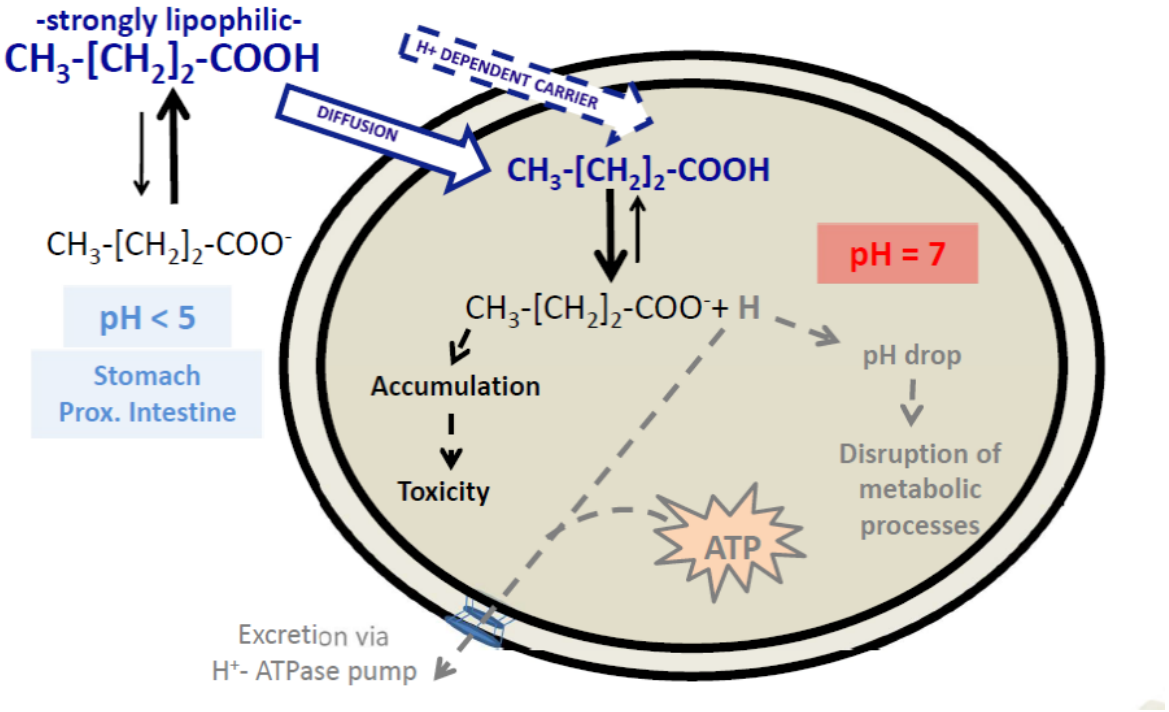

Figure 1.1 Mechanism for anti-microbial properties of organic acids (adapted with permission from Sol 2018).

Inside the bacteria, organic acids can dissociate into H^+ and anions and reduce cellular pH. Because pH-sensitive bacteria do not tolerate large differences in internal and external pH, the H^+-ATPase pump is activated to normalize internal pH. This mechanism is energy consuming and can eventually inhibit bacterial proliferation (Gauthier 2002). Disruption of cellular pH also suppresses the pH-sensitive enzymes with further negative consequences to microbial metabolism (Partanen and Mroz 1999; Suiryanrayna and Ramana 2015). Conversely, inorganic acids, even undissociated, do not penetrate bacterial cell walls, (Gauthier 2002). Increasing chain length, degree of unsaturation and pKa values of SCFA improve anti-microbial efficacy against gram negative bacteria (Partanen and Mroz 1999). Because of their anti-microbial action, organic acids have been proposed as potential alternatives for antibiotics used as growth-promotants in animal production (Pettigrew 2006; Stein and Kil 2006; de Lange et al. 2010; Heo et al. 2012). Studies reviewed by Ravindran and Kornegay (1993) showed that dietary supplementation of organic acids favoured the growth of *Lactobacilli* with consequent reduction in coliforms in the GIT. Aside from the reduction of pH by acidification, the growth of *Lactobacilli* inhibits the colonization and proliferation of E. coli by secreting hydrogen peroxide and blocking intestinal receptors where *E. coli* may attach. Post-weaning diarrhea syndrome (PWDS) mainly caused by enterotoxigenic *E. coli* (ETEC) can be controlled by organic acids, especially LA (Tsiloyiannis et al. 2001). In a review by Kil et al (2011), *Lactobacilli* counts in the small and large intestine were slightly reduced when dietary acidifiers were added although effects on coliform or *E. coli* counts were variable.

1.3.3 Acids as nutrient sources

Some organic acids used as dietary acidifiers are SCFA, which are products of microbial carbohydrate fermentation in the pig GIT and are readily metabolized by the body. It is well

known that SCFA (acetic, propionic, and butyric acid) influence intestinal function and intestinal morphology as indicated by crypt depth and villi height (Lupton and Kurtz 1993; Sakata et al. 1995; Partanen and Mroz 1999). While butyric acid is the preferred energy source of colonocytes and intestinal epithelial cells, other acidifiers such as fumaric acid, may also be used as energy sources by the intestinal epithelial cells (Ravindran and Kornegay 1993; Partanen and Mroz 1999; Kil et al. 2011).

Aside from being a dietary acidifier the inorganic acid, phosphoric acid, can serve as a P source (Schoenherr 1994; Lin and Chen 1995). For example, in a study by Lin and Chen (1995) who evaluated the anti-mould efficacy of phosphoric acid (85%) at 1 and 5 g kg^{-1} diet, the P contribution would be 0.27 and 1.36 g kg^{-1} diet, respectively (molecular weight of H_3PO_4 = 97.99 g mol^{-1}; P is 32% of MW).

1.4 Particle size reduction or grinding

1.4.1 Equipment

Grains and other ingredients are typically ground prior to feed production. The improvements in performance are attributed to the increased surface area as grain or diet particle size decreases, allowing for better action of digestive enzymes with nutrients in the feed. It is particularly important in swine feeding because pigs do not chew thoroughly, and unlike birds, pigs have no gizzard to reduce the particle size of ingested feed (Flis et al. 2014). To reduce grain or diet particle size, the feed industry and on-farm mills commonly use hammer (Fig. 1.2A) or roller mills (Fig. 1.2B).

In a hammer mill, particle size reduction results from impact between the slow-moving grains and the fast-moving hammers (Heimann 2005). Particles with the appropriate size exit the

grinding chamber through the holes in the screen. Larger particles that remain in the grinding chamber are further ground between the hammers and screen surface as they move along the grinding chamber (Heimann 2005).

The roller mill has pairs of cylindrical corrugated rollers that rotate in opposite directions. A roller is fixed in the frame and the corresponding roller is adjusted to set the gap. Fineness of grind can be controlled by gap width and the choice of corrugated rollers. Rollers with coarse grooves will produce larger particle sized-products and rolls with fine grooves will produce a finely ground finished product. Mills typically use a roll speed differential of 1.2:1 to 2:1 which means one roll is rotating 1.2 to 2 times faster than the other. If used without speed differential, grains can be cracked, crimped or flaked using low roll speeds (Heimann 2005).

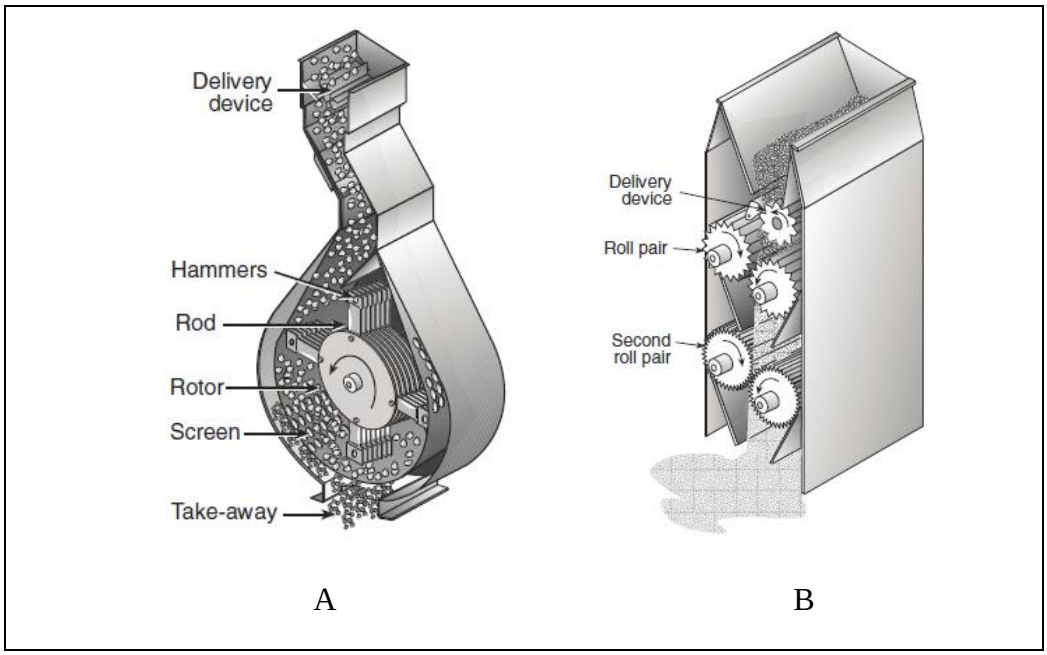

Figure 1.2. Basic design of a hammer (A) and roller (B) mill. (adapted from Koch 2002)

Compared to roller mills, hammer mills are easier to operate, equipment and maintenance costs are lower, and they have the capacity to grind a wider variety of feedstuffs (Hancock and Behnke 2001). However, hammer mills are noisier and produce more dust, which is a health and safety hazard to mill workers as it could result in an explosion. Hammer mills therefore, are typically enclosed in an explosion proof room. Roller mills have higher throughput than hammer mills, producing 15 to 40% more tons per hour and result in a more uniform particle size, (Heimann 2005). A uniform particle size reduces segregation during transport and bridging in the bins and feeders (Groesbeck et al. 2006). The roller mill operates at low speed, produces minimal noise, heat and dusts, resulting in a lower health and explosion risk.

1.4.2 Measuring particle size

The most common method for determining particle size and distribution of dry ground feed materials is by sieving. Particle size is often reported descriptively as coarse, medium, fine, or quantitatively as % coarse, fine or % below or above a specific diameter. This lack of a definitive particle size determination makes it difficult to compare results from different studies. The American Society of Agricultural Engineers (ASAE; now known as American Society of Agricultural and Biological Engineers, ASABE) devised a test procedure approved as an American National Standard in 1997 (Baker and Herman 2002), to standardize particle size determination in ground feed materials. The method requires the use of 13 sieves in a stack (arranged in order of ascending aperture size with a pan at the bottom), a sieve shaker to facilitate flow of materials through the screens, and a balance to weigh the materials retained on each screen. Ground samples (typically 100 grams) are loaded onto the stack of sieves and run through the sieve shaker for 10 minutes. The weight of sample fractions retained on each screen is then measured and used to calculate the mean geometric diameter (d_{gw}), geometric standard

deviation (s_{gw}), surface area per gram and number of particles per gram, assuming a log-normal distribution of the ground material (Wurth et al. 2010).

Different sieve shakers such as Ro-Tap (W.S. Tyler, Ohio, USA), Retsch (Retsch GmbH, Haan, Germany) and others may be used to separate the particles into size fractions and Wurth et al. (2010) reported that particle size readings depended on shaker manufacturer. For example, particle size results from a Retsch sieve shaker were consistently lower with a higher standard deviation compared to a Tyler Ro-Tap sieve shaker. Wurth et al. (2010) reported that the use of sieve agitators (balls and brushes) resulted in lower mean particle size (by 13%) and increased standard deviation by 15% compared to those without sieve agitators. In addition, increasing sieving time to 15 minutes resulted in a lower particle size (by 7%) and a slightly higher standard deviation (by 7%) compared to 10 minutes sieving time. The use of dispersion agents such as Cab-o-sil or Supernat 22-S resulted in reduced mean particle size (by ~11%) compared to those not using any dispersing agent (Wurth et al. 2010).

1.5 Particle size distribution, standard deviation, surface area and shape

Standard deviation or particle size distribution measures the uniformity of particle size of the ground material and is expressed as s_{gw}. Primarily due to the method by which hammer mills reduce particle size (i.e. impact), hammer milling of grains results in a wider particle size distribution, normally ranging from 2.5 to 3.5%, than roller mills where the particle size distribution is typically 2.0 to 2.5% (Heimann 2005). This is important because while gastric ulcers in pigs are associated with fine particles, there is evidence that particle size distribution is also important. For example, Wondra et al. (1995b) evaluated the effect of particle size uniformity of a corn-soybean meal-based diet on finishing pigs and saw a trend of decreasing stomach keratinization as s_{gw} decreased. There were no differences in animal performance

among the different diet s_{gw}, although apparent nutrient digestibility increased with increasing particle size uniformity (decreasing s_{gw}). Fine particles also affected flowability, resulting in feed handling problems such as bridging in the bins and feeders. Decreasing particle size increases angle of repose (a measure of slope required for the grain to flow), moreover, the increase was greater in corn that was hammer rather than roller milled (Groesbeck et al. 2006). The interaction between particle size and grinder was attributed to the lower s_{gw} of roller compared to hammer milled corn resulting in the lower angle of repose when ground using a roller mill (Groesbeck et al. 2006).

The type of grinder may also affect particle shape and animal performance. For example, feeding broiler chickens mash diets using roller milled corn improved body weights and feed conversion ratio (FCR) at 21 and 47 d when compared to those fed hammer-milled corn (Reece et al. 1985). Aside from the differences in particle size and size distribution, the authors noted that particles from hammer milled corn appeared spherical with smoother edges than roller milled corn. In finishing pigs, the digestibility of DM, N and GE was higher in those fed diets with roller milled corn compared to those with hammer milled corn ground to the same particle size (Wondra et al. 1995d). It was suggested that the spherical shape of hammer milled corn makes it less susceptible to enzyme attack.

The most important factor affecting hammer mill grinding throughput and grinding cost is the grain's moisture content (Probst et al. 2013). High-moisture grains are much tougher and require more energy for particle size reduction using a hammer mill (Heimann 2005; Probst et al. 2013) which may further increase grinding cost (Goransson and Ogle 1984). In a study conducted by Probst et al. (2013) using corn with moisture contents from 10.39 to 19.64%, total energy consumed (kWh) increased with increasing moisture content but geometric mean particle

size diameter (d_{gw}), throughput ($kg\,h^{-1}$), and flowability (angle of repose) were not affected. Dust and fines were reduced when corn with a higher moisture content was ground.

1.6 Effects of grinding on pig performance and gut health

1.6.1 Animal performance

Grinding grain/feed reduces the particle size and increases the surface area allowing for greater interaction with the digestive enzymes in the pig's GIT (Rojas and Stein 2015). Studies have consistently demonstrated improvements in pig performance, especially feed efficiency, due to particle size reduction (Flis et al. 2014).

Reducing the particle size of a weanling pig wheat-based diet from 1,430 µm to 680 µm increased ADG by 9.7% and G:F by 11.6% (Mavromichalis et al. 2000). Similarly, reducing the particle size of corn from 865 to 339 µm improved G:F in weanling pigs by 6% (Rojas and Stein 2015). Healy et al. (1994) reported a linear increase in ADG and G:F of pigs 14 d post-weaning fed corn or sorghum as particle size decreased from 930 to 300 µm. The improvement due to grinding was higher in pigs fed corn than sorghum, suggesting that the type of grain used influences the effect of particle size. While the greatest improvement in feed efficiency (6%) was observed when particle size was reduced from 900 to 500 µm, the authors Rojas and Stein (2015) observed that particle size between 500 and 700 µm resulted in optimal profits.

Comparable results have been observed in finishing pigs. Wondra et al. (1995b) reported that G:F increased by 8% in the finishing pig when the corn particle size was reduced from 1,000 to 400 µm (diet mean geometric diameter, $d_{gw,}$ from 1,017 to 517 µm). The ATTD of DM (linear), N (linear) and GE (quadratic) increased as particle size was reduced. In a study by Rojas and Stein (2015), reducing the particle size of corn (865 to 339 µm) in diets fed to growing-finishing pigs resulted in improved starch digestibility (apparent ileal) and G:F. Gain:feed in

finishing pigs was improved by 8.4% when fed diets containing hammer milled corn at 400 µm compared to those containing 800 µm and apparent digestibility of DM, N and GE improved by 3.5, 8.0, and 5.5 percentage points, respectively (diet d_{gw} from 806 to 558 µm; Wondra et al. 1995d).

In first parity sows, reducing the corn particle size in the lactation diet from 1,200 to 400 µm (diet $d_{gw,}$ from 1,298 to 496 µm) increased litter weight gain by 11% but had no influence on BW, backfat loss or return to estrus (Wondra et al. 1995c). In second parity gestatis, reducing the particle size of corn from 1,200 to 400 µm did not influence sow BW or backfat, and litter performance while DM and N digestibilities were increased by 7 and 10% respectively (Wondra et al. 1995a). Clearly, feed efficiency and nutrient digestibility are improved with reduction in particle size. The implications of these improvements include reductions in total manure excreted into the environment and nutrients wasted (Wondra et al. 1995a; Wondra et al. 1995b; Wondra et al. 1995c; Wondra et al. 1995d).

1.6.2 Grinding and stomach integrity

Gastric ulcer in pigs is an animal welfare issue and can lead to economic losses from sudden deaths (Friendship 2004). Chronic gastritis and ulcers of the pars oesophagea in pigs have been associated with a spiral shaped, gram negative bacterium called *Helicobacter suis* (Vermoote et al. 2011). It is the most prevalent non-*Helicobacter pylori* species in humans (de Cooman et al. 2013) and is associated with gastritis, peptic ulcers and gastric mucosa-associated lymphoid tissue lymphoma (Vermoote et al. 2011). Pork products (minced pork) have tested positive for *Helicobacter suis* with contamination most likely coming from the slaughterhouse (de Cooman et al. 2013). *Helicobacter suis* is a zoonotic bacterium that can be transmitted to humans via consumption of pork and its products, thus it is considered a public health risk.

Grinding improves nutrient digestibility and animal performance, however finely ground diets are associated with damaged stomach mucosa, specifically in the non-glandular region surrounding the pars oesophagea (Flis et al. 2014). For example, reducing the particle size of corn from 1,200 to 400 µm increased severity of stomach lesions in the sow (Wondra et al. 1995a; Wondra et al.1995c) and finishing pigs (Wondra et al. 1995b) while in other studies with diets containing corn and hard sorghum, finer particle size resulted in stomach keratinization in weanling (Healy et al. 1994) and finishing pigs (Wondra et al. 1995b; Rojas and Stein 2015), and sows (Wondra et al. 1995a; Wondra et al. 1995c). It can therefore be suggested that diets with fine particle size predispose the pig to gastric ulcers, or some form of gastric lesion.

In contrast, feeding pigs coarsely ground diets improves gastric health. Growing pigs (24 to 110 kg body weight) fed coarsely ground diets had lower *Helicobacter suis* activity in the stomach compared to finely ground diets (Millet et al. 2012). A study by Mikkelsen et al. (2004) in growing pigs (33 kg) showed that pigs fed coarsely ground meal (non-pelleted) had fewer gastric ulcerations compared to those fed finely ground, pelleted diets. Nielsen and Ingvartsen (2000) also reported lower gastric lesion scores in growing pigs (25 kg) fed coarse non- pelleted diets using either rolled barley or wheat. The exact mechanism by which coarsely ground diets protect the mucosal lining of the stomach is not known (Flis et al. 2014) but studies by Nielsen and Ingvartsen (2000) and Mikkelsen et al. (2004) showed that low gastric lesion scores coincided with the firmness and high DM of gastric contents in pigs fed coarsely ground meal. Coarsely ground diets also reduce stomach passage rate, allowing more time for the microbiota to proliferate, as evidenced by increased total anaerobic bacteria and LA bacteria populations, higher concentration of organic acids, and reduced pH in the stomach (Mikkelsen et al. 2004). It was also observed that stomach contents from coarsely ground meal diets had a higher

sedimentation percentage (non-separation of fluid and sediment), higher water holding capacity and a "porridge-like" consistency (Mikkelsen et al. 2004). Hills et al. (2001) suggested that food borne pathogens thrive in the water-rich domains formed in the the matrix microstructure of a porous medium. The physical characteristics of the gastric contents and longer retention time observed by Mikkelsen et al. (2004) could have provided the optimum conditions for the microbiota to proliferate.

Cereals vary in fibre type and amount, which may influence the incidence of gastric ulcers in pigs. Feeding corn and wheat predisposed pigs to gastric ulcers more than a barley-based diet (Johansen et al. 1996; Knudsen 1997 as cited by Nielsen and Ingvartsen 2000). Millet et al. (2012) fed high or low fiber diets ground either fine or coarse to growing-finishing pigs (starting weight of 24 kg) and reported that a coarsely ground, high fiber diet had the lowest lesion scores in the pars oesophagea compared to other treatment combinations. Similarly, the addition of unground sunflower hulls to finely ground diets reduced the occurrence of gastric lesions (Dirkzwager et al. 1998). However, Grosse Liesner et al. (2009) reported that that coarsely ground meal (25% > 2 mm and 29%< 0.4 mm; wheat and barley based) were better at preventing gastric ulcers, and that increasing fiber (42 to 54 g kg^{-1} DM) using lignocellulose did not show any protective effect against ulcers.

The type of grinder used, regardless of particle size and fiber content, may also influence the stomach integrity of pigs. For example, in the study by Nielsen and Ingvartsen (2000), pigs fed diets based on wheat had higher gastric lesion scores than those fed diets based on barley ground to the same particle size using a hammer mill. Conversely, low gastric lesions were observed for both wheat and barley ground to the same particle size using a roller mill. Similarly, pigs fed corn-based diets ground using a roller had lower gastric lesions than pigs fed diets

ground with a hammer mill to the same particle size. This may be attributed to the differences in s_{gw} of particles ground using either roller or hammer mill. Particles from grains ground using the roller typically have a narrower sg_w compared to those ground using hammer mill (Heimann 2005).

Regardless of grain particle size, pelleting consistently results in a deterioration of gastric health, probably because pelleting serves as a secondary grinding process, increasing the proportion of fine particles (< 0.4mm) by 50% (Grosse Liesner et al. 2009), and reducing the proportion of coarse particles (> 1mm) by 9% (barley based) or 14% (wheat based) (Nielsen and Ingvartsen 2000). The percentage of very fine particles (< 0.4 mm) in pelleted diets could negate the prophylactic effect of the large particle size fractions (pre-pelleting) on gastric lesions, and thus should be considered instead of average particle size when assessing diet risk for gastric ulcers (Grosse Liesner et al. 2009; Cappai et al. 2013). The threshold for the proportion of very fine particles before macroscopic lesions develop is probably between 29% and 36% (Grosse Liesner et al. 2009). The heat involved in pelleting increases starch gelatinization, consequently increasing digesta viscosity and promoting the contact between acid chyme and the non-glandular region of the stomach (Cappai et al. 2013)

1.6.3 Grinding and gut microbiota of pigs

Grinding results in a modification of the bacterial population in the gastro intestinal tract of the pig (Mikelsen et al. 2004). In growing pigs (33 kg initial body weight), feeding coarsely-ground, non-pelleted diets resulted in higher total anaerobic bacteria in the stomach compared to those fed finely ground meal or pelleted diets (Mikelsen et al. 2004). Feeding pigs coarsely ground diets (pelleted or meal) increased LA bacterial populations in the stomach, while yeast count was increased by feeding pelleted diets (coarse or fine). Pigs fed coarsely ground diets had

fewer coliform bacteria in the distal small intestine, caecum and mid colon compared to those fed finely ground diets. Conversely, feeding pigs finely ground pelleted diets resulted in higher total anaerobic bacteria and LA bacteria in the mid colon and caecum respectively (Mikkelsen et al. 2004).

Higher anaerobic and LA bacteria populations result in increased concentration of organic acids, especially LA, and reduced pH in the stomach of pigs fed coarse ground diets protecting it from pathogenic bacteria like *Salmonella* and *E. coli.* (Mikkelsen et al. 2004). This allows the stomach to act as a barrier, protecting the pig from enteric diseases, by killing or reducing the number of pathogenic bacteria before they enter the intestinal tract. The longer retention time of the gastric contents, higher water binding capacity and porridge-like consistency of the chyme from feeding coarse diets to pigs, provides optimum conditions for proliferation of the beneficial bacterial population (Mikkelsen et al. 2004)

1.6.4 Grinding and the production of short chain fatty acids (SCFA)

The bacterial fermentation of starch (specifically resistant starch) and fiber produces SCFA, primarily acetate, butyrate, and propionate. The concentration and rate at which they are produced is dictated by microbial species and population, substrate, and gut transit time (Wong et al. 2006). Short chain fatty acids are efficiently absorbed (90-95%, Wong et al. 2006), primarily by the colonocytes of the large intestine (Imoto and Namioka 1978). These may be used as an energy source, contributing up to 15% of the metabolizable energy (ME) requirement of growing pigs and 30% in gestating sows (Dierick et al. 1989; Varel and Yen 1997; Zijsltra et al. 2012). Acetate increases colonic blood flow and enhances ileal motility, while butyrate is the preferred energy substrate of colonocytes, has a role in cell differentiation (Scheppach 1994) and prevents *Salmonella* from invading the epithelial cells of the intestines (Gantois et al. 2006).

Propionic acid stimulates colonic motility and contractile response of middle and distal segments of the colon in rats (Yajima and Sakata 1986). It is oxidized via the citric-acid pathway for energy and a small portion of the propionate is converted into lactate by the epithelial cells (Partanen and Mroz 1999). Residual propionate is metabolized in the liver and used as a gluconeogenic precursor (Jha 2010).

Total fecal SCFA, were increased in pigs fed coarsely ground diets (6 mm screen, estimated d_{gw} 881 µm) compared to those fed fine (3 mm screen, estimated d_{gw} 631 µm) due to increased concentrations of propionate and butyrate (Callan et al. 2007). Rojas and Stein (2015) reported a linear increase in the concentration of acetate, butyrate and propionate in caecal contents as dg_w of corn increased from 339 to 865 µm. Mikkelsen et al. (2004) showed that acetic, propionic, butyric and lactic acids in the stomach and butyrate concentration in the caecum were increased by feeding pigs coarsely ground diets. Increased starch content in the hindgut was observed with coarsely ground diets, increasing the substrate for the microflora in the caecum and large intestine of the pig for fermentation (Brunsgaard 1998; Kamphues et al. 2007). In a study conducted on 4-week old pigs, increasing the amount of resistant starch (using potato starch) increased production of SCFA in the colon, especially butyrate. This resulted in increased colonic weight, coinciding with higher crypt depth, an indication of epithelial cell growth (Hedemann and Knudsen 2007).

1.6.5 Grinding and intestinal morphology and histology

Diet particle size affects morphology, epithelial cell proliferation, production and composition of mucins in the gastro intestinal tract. Hedemann et al. (2005) reported higher relative stomach weight (7.83 vs 7.32 g kg^{-1} BW) in growing pigs (33 kg) fed coarse rather than fine diets (wheat and barley-based). In weaned piglets (10 kg), feeding coarsely ground un-

pelleted diets resulted in significantly heavier stomachs than finely ground, pelleted diets (7.13 vs 5.17 g kg^{-1} BW). These effects were attributed to the firmness of stomach contents and increased retention time. Similarly, stomach weight of pigs fed roller milled diets was 9% heavier compared to that of pigs fed hammer-milled diets and was attributed to firm stomach digesta and reduced gastric emptying rate possibly resulting from coarser particle size of roller compared to hammer milled diets (880 vs 495 µm; Nielsen and Ingvartsen 2000). When pigs were fed fine diets, the small intestine was heavier and longer compared to those fed coarse, attributed to higher amounts of accessible starch for enzymatic digestion compared to those fed coarsely ground diets, possibly providing more energy for use by epithelial cells of the small intestine (Hedemann et al. 2005). While caecal weight was higher in pigs fed coarse non-pelleted diets in the study of Hedemann et al. (2005), this finding does not agree with the earlier works of Brunsgaard (1998) who reported that neither grinding nor cereal type had an effect on caecal weight. Similarly, weight of the colon was not affected by grinding (Brunsgaard 1998; Hedemann et al. 2005). Apart from health reasons, morphological changes in the gastro intestinal tract must be considered in feeding strategies because of possible effects on dressing weight and increased maintenance requirements. Rojas and Stein (2015) reported a linear increase in viscera weight (primarily attributed to the weight of the intestines) and a corresponding linear decrease in dressing percentage (80.2 to 79.3%) of pigs as particle size of corn increased from 339 to 865 µm (2.52 to 3.01 kg).

Epithelial cell proliferation stimulated by feeding pigs coarsely ground diets was attributed to increased butyrate production and the role of this SCFA in cell differentiation and nourishing the colonic mucosa. Pigs fed coarse non-pelleted diets had longer villi (527 vs 442 µm), 20% greater villus area and increased crypt depth in the medial section of the small

intestine than pigs fed coarse pelleted diets (Hedemann et al 2005). Similarly, there was a significant increase in crypt width in the duodenum, jejunum, and caecum in pigs fed coarse meal compared to those fed fine pelleted diets (Callies et al. 2012). Crypt volume (39,607 vs 35,418 μm^2) and crypt depth (566 vs 527 µm) were significantly increased in the colon of pigs fed coarse diets (Brunsgaard 1999).

The mucus layer, comprised of secretory products of intestinal goblet cells, is the initial barrier in the host's innate defense against enteric diseases (Kim and Ho 2010). It traps commensal and pathogenic bacteria, which are eventually eliminated by peristalsis and prevented from reaching the epithelial surface (Kim and Ho 2010). The mucus layer is composed of neutral or acidic mucin glycoproteins. Neutral mucins promote *Salmonella* infection because they contain mannose residues that serve as a binding site for *Salmonella typhimurium* (Vimal 2000), while the acid mucins prevent entry and adherence of pathogenic bacteria to enterocytes because they are less degradable than neutral mucins. Pigs fed non-pelleted diets had a larger staining area for neutral mucin (41%), acidic mucin (46%) and sulfomucin (33%) on the villi of the distal small intestine than those fed pelleted diets. Type of diet, however, did not affect the mucin staining characteristics of the crypts of the caecum and colon (Hedemann et al. 2005) Conversely, Callies et al. (2012) reported significant increases in crypt width (an indirect measure of mucin secretion) in the duodenum, jejunum, and caecum in the small intestine of pigs fed coarse, compared with those fed fine pelleted diets.

1.7 Interaction of acid supplementation and particle size

Although reducing particle size improves nutrient digestibility in pigs, there may be negative consequences for gut health. Feeding finely ground diets is suggested to be a predisposing factor in the development of gastric ulcers in pigs (Melnichouk 2002; Friendship

2004). However, except for the effect of fine particles on stomach ulceration, there has been little work on other aspects of particle size and gut health. Investigations by Dirkzwagger et al. (1998), Nielsen and Ingvartsen (2000), Mikkelsen et al. (2004), Grosse-Liesner et al. (2009), Millet et al. (2012), and Cappai et al. (2013) suggest that feeding a coarsely ground grain/diet was correlated with a lower incidence of gastric ulcers. Several studies have also shown improvements in intestinal morphology (Brunsgaard 1998; Hedeman et al. 2005) and modified fermentation metabolites (Mikkelsen et al. 2004; Kamphues et al. 2007; Rojas and Stein 2015). Pigs fed a coarsely ground diet had similar growth performance as those fed the finely ground, formic acid-supplemented diet (Canibe et al. 2005). The effect of feeding coarsely ground diets on reducing digesta pH and modifying the microbial populations is similar to the proposed mode of action with dietary acid supplementation but with regional differences. Diet acidification effects are observed primarily in the proximal GIT (Kil et al. 2011), whereas the effects of coarsely ground diets are observed in the proximal and distal sections of the GIT. Feeding coarsely ground diets reduced gastric pH and increased the concentration of SCFA in the caecum and colon in pigs (Nielsen and Ingvartsen 2000; Mikkelsen et al. 2004). Although not measured, the reduction in gastric pH was attributed to longer retention time of coarsely ground diets in the stomach favouring the proliferation of LA bacteria. When diets were coarsely ground, the amount of undigested starch entering the caecum and colon was increased. This starch could be used as a substrate for bacterial fermentation into SCFA.

1.8 Summary and conclusions

Diet acidification improves animal performance and nutrient digestibility by reducing gastric pH such that a) enzymes for protein digestion are activated, and b) gastric emptying rate is reduced, exposing the digesta to further proteolysis. Because of the antimicrobial properties of

acids, they may be used as an alternative to antibiotic growth promotants. The response to acidification is affected by the age of the pig (higher response in younger pigs), acid (differences in pKa), and level of acid used, ingredients used in the diet (buffering capacity) and the current performance and health status of the herd. The anti-microbial properties of acids also make them ideal as preservatives of high-moisture grains when harvesting below 15% moisture is not possible or desirable. There are a number of studies showing the efficacy of organic acids in preserving high moisture grains but very limited for an organic-inorganic acid blend. Furthermore, to our knowledge, the efficacy of presenting an acidifier via the acid-preserved grain as an alternative to direct acidification of weanling pig diet is not known and requires examination due to potential economic benefits to the producer and grain farmer. The low pH and high-moisture conditions during storage of acid-preserved high-moisture grains provide the environment required by most enzymes for optimum activity and thus, endogenous and exogenous enzymes may pre-digest the nutrients in the grains. Most particle size studies are conducted using corn or dry grains and it is not known if particle size affects nutrient utilization from high moisture grains. Grinding grains improve pig performance however grinding too finely predisposes pigs to gastric ulcers. The mechanism by which coarse diets improve gut health is similar to acids, i.e. reduced gastric emptying rate provides more time for LA bacteria to proliferate and produce LA and reduce gastric pH. Furthermore, the coarsely ground grain increases the amount of starch that enters the hind gut and influence the SCFA profile from bacterial fermentation. The cost of grinding high-moisture grains may be offset with the elimination of costs associated to drying, the benefits of acidification in pig performance and nutrient digestibility, and potential improvements in nutrient digestibility in acid-preserved high-moisture grains. Therefore, it is possible that, grinding acid-preserved high moisture grains finely

may not be required to achieve the same level of performance as a conventional acidified diet, with the additional benefit of improving the gut health of the pig.

CHAPTER 2. ACID PRESERVATION OF HIGH MOISTURE WHEAT OR BARLEY, WITH OR WITHOUT ENZYMES, AND ITS EFFECT ON GRAIN pH, ACID BINDING AND BUFFERING CAPACITY, AMMONIA NITROGEN AND ESTIMATES OF NITROGEN AND PHOSPHORUS AVAILABILITY

This paper will be submitted to Animal Feed Science and Technology for publication.

ABSTRACT

Two studies were conducted to determine the effect of preserving high moisture wheat or barley with propionic acid (Prop) or a phosphoric acid-based organic-inorganic acid mixture (OIB) at two concentrations (Low or High), with (Enz) or without (NoEnz) enzymes (phytase, carbohydrases and protease) on grain quality and estimates of N and P availability. Treatments were in a 2 (Prop or OIB) x 2 (Low or High) x 2 (NoEnz or Enz) factorial arrangement utilizing a completely randomized design repeated in 4 times (d 0, 4, 14, and 153). Each treatment combination was replicated four times. Barley (Exp. 1) or wheat (Exp. 2) were reconstituted to 20% moisture, acidified and added with enzymes. Immediately after acidification, d 0 samples were bagged and stored at -20 °C. For each treatment combination, about 1.5 kg treated grain were lightly packed into two 1.8 L-capacity, wide-mouth mason jars. Jars were opened at the designated time point, mixed and sampled, pH determined, then stored at -20 °C.

In both trials, mould growth was inhibited by both Prop and OIB. In Exp. 1, barley pH of 5 and below was maintained in High Prop up to d 153 or High OIB up to 14 but not 153 d. In Prop, acid binding capacity was higher on d 0 compared to d 153 which was not observed in OIB (acid by day; $P < 0.01$). Protein dispersibility index (PDI) as an estimate of N availability, was lower in High compared to Low OIB but not Prop (acid by concentration, $P < 0.01$). In OIB-preserved barley, Enz had lower PDI than NoEnz, while the opposite was observed for

Prop (acid by enzyme; $P < 0.01$). At d 0, OIB-preserved barley had higher PDI than those preserved Prop which was not observed at d 153 (acid by day; $P < 0.01$). On d 0, Low had higher PDI compared to High acid concentration while the opposite was observed on d 153 (concentration by day, $P < 0.01$). Available P (aP) was higher at d 0 compared to d 135 in Prop but not OIB (acid by day interaction, $P < 0.05$). In Exp. 2, regardless of concentration, a pH of below 5 was maintained up to d 153 using Prop, but up to 14 and not 153 d using OIB. Higher ABC was observed in Prop compared to OIB ($P < 0.01$). Higher PDI was observed in High compared to Low Prop which was not observed in OIB (acid by concentration; $P <0.01$). When using Prop, PDI was higher in Enz compared to NoEnz, which was not observed in OIB (acid by enzyme, $P < 0.01$). When using Prop, aP was higher in Enz compared to NoEnz in High but not Low. Conversely, the supplementation of enzyme in the OIB treated grains regardless of concentration had no effect on aP (acid by concentration by enzyme; $P<0.05$). In conclusion, OIB inhibited mould growth in high-moisture wheat or barley as effectively as Prop. The effect of acid-preservation, with or without enzyme, storage time or their interaction on the availability of N and P differed between barley and wheat.

INTRODUCTION

Wheat and barley are typically harvested at moisture content below 15% to maintain their quality during storage (Hackl 2010). When this is not possible, artificial drying can be employed, but due to power and fuel costs, in addition to investments for specialized drying structures, costs are higher. High-moisture, air-tight storage of grains is an alternative to artificial drying and improves nutrient availability for pigs compared to dry storage. In a study conducted by Ton Nu et al. (2015), high-moisture, air-tight storage of barley and rye reduced phytate P (phyP) and

increased the solubility of P and nitrogen (N) compared to grains that were stored dry. In the same study, the addition of an enzyme combination (phytase, carbohydrases, and protease) further increased phyP degradation and solubility of P and N compared to high-moisture, air-tight storage alone. Poulsen et al. (2012) fed wheat and barley stored under high-moisture, air-tight conditions for 6 months without inorganic P and microbial phytase to finishing pigs and reported improvements in P and crude protein (CP) digestibility by 12 and 4%, respectively compared to pigs fed dry-stored grains. However, investments in specialized air tight structures are required and microbial activity resumes upon removal from storage which limits batch size during feed production (Jones et al. 1975). Alternatively, high-moisture grains may be preserved by acidification and this can be done using existing grain storage facilities. When fed to pigs, propionic acid-preserved high-moisture corn had similar or improved animal performance compared to feeding the dry grain (Jones et al. 1970; Lynch et al. 1975). In a study by Czarnecka et al. (1991), the addition of propionic acid (1%) to high-moisture rye and triticale during storage prevented mould growth and improved in-vitro digestibility of starch

Acidification of weanling pig diets is practiced to help manage challenges at weaning. In a meta-analysis of several studies, it was demonstrated that acidified diets improve weanling pig growth rate by 6 to 12 % (Tung and Pettigrew 2006). While a mode of action has not been conclusively identified, several investigators suggest 1) reduction in gastric pH, thereby activating digestive enzymes, and 2) inhibition of the growth of pathogenic microorganisms (Partanen 2001; Tung and Pettigrew 2006). Most enzymes require moisture and a low pH for optimum activity. For example, microbial phytase activity peaks at pH 2.5 and 5.5 (Simons et al. 1990), thus the addition of enzymes during acid preservation of high moisture grains may be a viable strategy to improve feeding value by further enhancing nutrient release. Individually, the

acidification and the addition of enzyme during high-moisture storage of grains have been shown to improve nutrient release, however there is limited information on when acids and enzymes are combined during high-moisture storage. Furthermore, while propionic acid can effectively prevent mould growth, it is corrosive and expensive, thus there is interest in alternatives. In a study by Lin and Chen (1994), phosphoric acid, an inorganic acid, at 5 g kg^{-1} was as effective as propionic acid in inhibiting the growth of the fungus *Aspergillus* extracted from poultry feed.

2.1 Objectives

The objective of this study was to determine the effect of acidification of high moisture wheat or barley using either propionic acid or an organic-inorganic acid blend (phosphoric, lactic, malic and citric acids) at low or high concentrations, with or without enzymes on:

1) grain quality as estimated by visible mould growth, grain pH and ammonia N
2) capacity to neutralize acid as estimated by acid binding and buffering capacities, and
3) protein dispersibility index or available P as estimates of N or P availability

2.2 Hypothesis

These trials were conducted with the hypothesis that:

1) an organic-inorganic acid blend will prevent visible mould growth as effectively as propionic acid,
2) increasing the concentration of both acids will increase the availabililty of N or P in acid-preserved high moisture wheat or barley,
3) because of the low pH and high-moisture conditions during storage, the addition of exogenous enzyme will further improve nutrient release.

MATERIALS AND METHODS

2.3 Grains

Wheat (var. Utmost) and barley (var. Austenson) purchased from farms around North Battleford, Saskatchewan were analyzed for moisture content (method 930.15; 130 ˚C for 2 hours; AOAC 2005) and then reconstituted with the appropriate amount of distilled water to increase moisture to 20 %.

2.4 Acids and enzyme

A commercial dietary acidifier composed of 30 to 50% phosphoric acid, 0.1 to 1% lactic, 5 to 10% citric and 1 to 5% malic acid (MaxiCid, Canadian BioSystems, Canada) and propionic acid (99%, Anachemia, Montreal, Quebec, Canada) were used. Jones et al. (1974) recommends propionic acid (Prop) be added at 5 to 8 g kg^{-1} for grain with moisture content of 22%. The manufacturer's recommended inclusion rate for the organic-inorgainc acid blend (OIB) was 1 to 3.5 g kg^{-1} of complete feed. In the current study, OIB was added at 3.5 (1 x) and 7 g kg^{-1} of grain (2 x), while the propionic acid was added at 5 (1 x) and 7.5 g kg^{-1} (1.5 x). A combination of phytase and multi-carbohydrase enzymes with a recommended inclusion rate of 250 g to 1000 g t^{-1} of complete feed (Superzyme Plus, Canadian BioSystems, Calgary, Alberta, Canada) was used. A dosage of 500 mg kg^{-1} of grain supplied 1500 FYT phytase, 1000 XYL xylanase, 250 GLU glucanase, 137.5 INV invertase, 350 HUT protease, 750 CMC cellulase, 3500 FAA amylase and 20 MAN mannanase per kg of grain.

2.5 Experimental design

Separate experiments were conducted for wheat or barley. Treatments, arranged as a 2 x 2 x 2 x 4 factorial included main effects of acids (OIB or Prop), acid concentrations (low or

high), enzyme addition (0 or 500 g t^{-1}) at 4 storage times (0, 4, 14, and 153 d) for a total of 32 treatment combinations. Incubation jars were assigned to 1 of 32 treatments in a completely randomized design with four incubation jars per treatment for a total of 128 incubation jars per experiment.

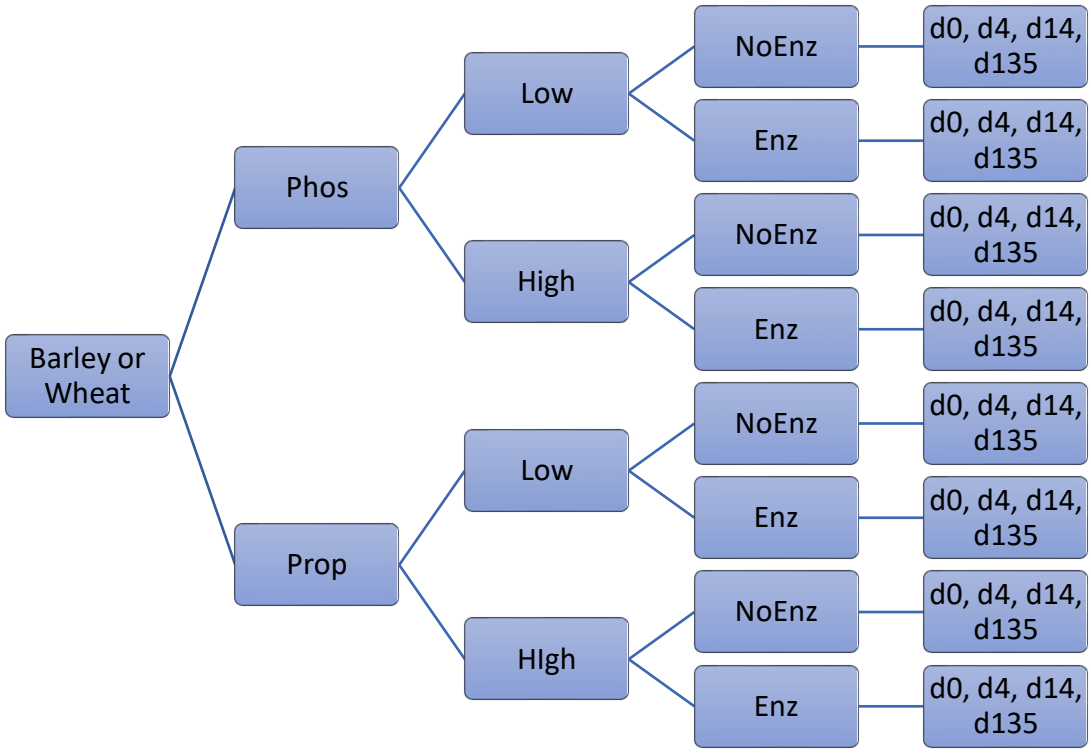

Fig. 2.1 Schematic of the experimental design for the in-vitro trial for either barley or wheat. Treatments, 2 (OIB or Prop) x 2 (High or Low) x 2 (Enz or NoEnz) x 4 storage times were in a factorial arrangement for a total of 32 treatment combinations. Each treatment combination was replicated 4 times for a total of 128 incubation jars (32 x 4) for each trial.

2.6 Reconstitution and acidification

Wheat (12.46% moisture) and barley (11.31% moisture) were reconstituted to 20% moisture by combining 25 kg of wheat or barley with 2.72 kg or 2.30 kg of distilled water, respectively and mixing for 20 minutes in a 40 kg capacity Hobart mixer (Hobart Food

Equipment Canada, Toronto, Ontario Canada). Where required, the enzyme product was added prior to addition of water (enzyme add rate of 500 g t^{-1}) to ensure homogenous mixing. The mixture was allowed to rest for 20 minutes to allow penetration of water into the grain. The appropriate acids were added to the reconstituted grains followed by mixing for another 10 minutes. The acid blend was added at either 3.5 (low) or 7 mL kg^{-1} (high) and Prop at 5 (low) or 7.5 mL kg^{-1} (high). About 1.5 kg of the mixture was loosely packed in appropriately labelled wide mouth 1.89 L-capacity mason jars (Ball Corporation, Broomfield, CO, USA) with the lids closed prior to storage in a room where temperature was maintained at 22 °C.

2.7 Sample and data collection

Grain samples collected immediately after final mixing represented time point d 0. Separate jars were opened at the other designated time points. Jar contents were mixed and about 250 g of sample were collected. An aliquot was analyzed for pH and the remainder was stored in double zip-lock bags at -20 ˚C until chemical analysis.

2.8 Analyses

2.8.1 Grain pH

Grain pH was analyzed at d 0, 4, 14 and 153. Briefly, 5 g of grain was mixed with 30 mL of deionized water, mixed and allowed to sit for 5 min before measuring the pH (Oakton pH 110 Series, Eutech Instruments, Thermo Fisher Scientific, Waltham, Massachusetts, USA).

2.8.2 Ammonia nitrogen

Ammonia N (NH_3 N) concentration was measured on samples collected on d 0 and d 153. Ammonia N was determined by spectrophotometry using a procedure adapted from Fawcett and

Scott (1960). Briefly, 2.5 g of the grain sample were mixed with 20 mL distilled water in a 50 ml screw cap culture tube that had been previously ashed at 500 ˚C for 1 hour to ensure it was clean prior to use. The tube was capped, shaken briefly and incubated overnight at 5 ˚C. After incubation, samples were briefly shaken before centrifuging at 500 g for 5 min. Twenty µL of supernatant was pipetted into 16 × 100 mm glass tubes in duplicate, followed by 2 mL of Na phenate, 3 mL of 0.01% Na nitroprusside and 3 mL 0.02 N Na hypochlorite, in that specific order, and covered with paraffin. Tubes were vortexed briefly, inverted several times then covered with aluminium foil and incubated in the dark at 20 °C for 1 hour. The resulting blue color was measured at 600 nm (Spectronic Helios Gamma UV-Vis Spectrophotometer, Thermo Scientific Waltham, Massachusetts, USA)

2.8.3 Acid binding and buffering capacity

Acid binding (ABC) and buffering capacity (BUF) were measured according to the methods of Jasaitis et al. (1997). Briefly, the initial pH of the grain was measured by suspending 0.5g of the sample in 50 ml deionized water, and then titrated with 0.1 N HCl until pH 4. Acid binding capacity (mEq H^+ kg^{-1}) was calculated by multiplying the total amount of acid used by the normality of the acid. Buffering capacity was calculated by dividing the ABC by the difference of the sample's initial pH and pH 4.

2.8.4 Protein dispersability index

Protein dispersability index (PDI) was determined using method Ba 11-65, (AOCS, 2017). Grains were ground to pass through a 1 mm screen (Retsch, model ZM1, Newton, PA, USA). Twenty grams of ground grain were blended with 300 mL of distilled water (25 ˚C) for 10 min in a Hamilton Beach Blender (model Drink Master 65250, Glen Allen, Virginia, USA) at

8,500 rpm. The slurry was allowed to settle for 10 mins, and the decantate was then centrifuged at 600 g for 10 min. The supernatant was aliquoted into 15 ml duplicates and analyzed for N using Kjeldahl method (method 984.13; AOAC 2005) along with the original un-blended grain sample. Protein dispersability index was calculated as the ratio between water-dispersible protein and total sample protein, expressed in percent.

2.8.5 Total, phytate and available phosphorus

Grains were ground to pass through a 1 mm screen (Retsch, model ZM1, Newton, PA, USA). Total P was analyzed in duplicate following dry ashing (method 965.17; AOAC 2005). The ash was reacted with ammonium vanadate-ammonium molybdate reagent giving a yellow color. The resulting color intensity was read at 400 nm (Spectronic Helios Gamma UV-Vis Spectrophotometer, Thermo Scientific Waltham, Massachusetts, USA). Phytate P (phyP) was analyzed in duplicate using Wade reagent/colorimetric method as described by Gao et al. (2007). Briefly, phyP from the sample was extracted in 2.4% (0.64 N) HCl acid and reacted with Wade reagent before measurement of absorbance of color at 500 nm (Spectronic Helios Gamma UV-Vis Spectrophotometer, Thermo Scientific Waltham, Massachusetts, USA). Available P was determined as the difference between total P and phyP. Available P for the grains treated with low or high concentration of OIB was corrected for the P contribution from the acid

2.8.6 Statistical analysis

The pH data was analyzed as a completely randomized design with repeated measures using a 2 (acid) x 2 (concentration) x 2 (enzyme) factorial treatment structure and 4 time points (d 0, d 4, d 14, and d 153) using Proc Mixed of SAS (SAS 9.4; SAS Institute, Cary, NC). Compund symmetry was used as the variance structure because it had the lowest AIC and BIC

among those tested. For NH_3 N levels, PDI and aP, treatments were repeated in 2 time points (d 0 and d 153). The model included the fixed effects of acid, concentration, enzyme, days in storage and their interactions. The statistical model used is as follows:

$$Yjklm = \mu + \alpha_j + ß_k + \gamma_l + \delta_m + (\alpha ß)_{jk} + (\alpha\gamma)_{jl} + (\alpha\delta)_{jm} + (ß\gamma)_{kl} + (ß\delta)_{km} + (\gamma\delta)_{lm} + (\alpha ß\gamma)_{jkl} + (\alpha ß\delta)_{jkm} + (ß\gamma\delta)_{klm} + (\alpha ß\gamma\delta)_{jklm} + \varepsilon_{jklm};$$

where Y is the parameter, μ the overall mean, α the fixed effect of the j^{th} acid, β the fixed effect of the k^{th} concentration, γ the fixed effect of l^{th} enzyme, δ the fixed effect of m^{th} time, $(\alpha ß)_{jk}$ is the interaction between acid and concentration, and $(\alpha\gamma)_{jl}$ is the interaction between acid and enzyme, $(\alpha\delta)_{jm}$ is the interaction between acid and time, $(ß\gamma)_{kl}$ is the interaction between concentration and enzyme, $(ß\delta)_{km}$ is the interaction between concentration and time, $(\gamma\delta)_{lm}$ is the interaction between enzyme and time, $(\alpha ß\gamma)_{jkl}$ is the interaction between acid, concentration and enzyme, $(\alpha ß\delta)_{jkm}$ is the interaction between acid, concentration and time, $(ß\gamma\delta)_{klm}$ is the interaction between concentration, enzyme and time, $(\alpha ß\gamma\delta)_{jklm}$ is the interaction of acid, concentration, enzyme and time, and ε_{ijklm} is the random error term.

For acid binding and acid buffering capacity, only the treatment jars containing acidified grains without enzymes were analyzed. Data were analyzed as a completely randomized design with a 2 (acid) x 2 (concentration) x 2 (time) factorial arrangement of treatment using Proc Mixed of SAS (SAS 9.4; SAS Institute, Cary, NC).

The model used was:

$Yjkl = \mu + \alpha_j + ß_k + \delta_l + (\alpha ß)_{jk} + (\alpha\delta)_{jl} + (ß\delta)_{kl} + (\alpha ß\delta)_{jkl} + \varepsilon_{jkl}$;

where Y is the parameter, μ the overall mean, α the fixed effect of the j^{th} acid, β the fixed effect of the k^{th} concentration, δ the fixed effect of l^{th} time, $(\alpha ß)_{jk}$ is the interaction between acid and concentration, $(\alpha\delta)_{jl}$ is the interaction between acid and time, $(ß\delta)_{kl}$ is the interaction between concentration and time, $(\alpha ß\delta)_{jkl}$ is the interaction between acid, concentration and time, and ε_{ijkl} is the random error term.

All residual error data were analyzed for normality of distribution using Proc Univariate in SAS (SAS 9.4, SAS Institute, Cary, NC) before subjecting to ANOVA. Means were separated using Tukey's method. In all cases, a P value of < 0.05 was declared significant. If $P > 0.05$ but < 0.10, a tendency was declared.

RESULTS

2.9 Barley

2.9.1 Mould growth

Visual inspection of the high-moisture barley showed no signs of mould growth regardless of treatment or storage times.

2.9.2 pH

The effects of acidifier, concentration, and enzyme addition on pH of barley are shown in Table 2.1.

There was a 4-way interaction among type of acid, concentration, enzyme and day on

pH of high-moisture barley ($P < 0.01$). On d 0, there were significant differences between with or without enzyme supplementation, which was not observed on d 4, 14, or 153 ($P < 0.05$). On d 0, pH from the Prop preserved grains ranged from 2.44 to 2.77, with the lowest pH measured on the Prop-High-Enz treatment. Conversely, the pH of grains preserved using OIB ranged from pH 4.35 to 5.31 on d 0 with the lowest pH measured on the OIB-High-NoEnz treatment. Except for OIB-Low-NoEnz, there was a decrease in pH with OIB from d 0 to d 4 ($P < 0.05$) followed by increases on d 14 and d 153 ($P < 0.05$). In contrast, the pH of all treatments with Prop increased from d 0 to d 4, decreased on d 14 and increased by d 153 ($P < 0.05$).

2.9.3 Ammonia nitrogen

The effect of acid, acid concentration and enzyme on NH_3 N levels of high-moisture barley during storage is presented in Table 2.2.

There was a 4-way interaction among type of acid, concentration, enzyme and day on NH_3 N levels of barley ($P < 0.01$). In the high and low OIB treatments, NH_3 N levels increased from d 0 to 153 in the plus enzyme treatments ($P < 0.05$) but decreased ($P < 0.05$) or were similar from d 0 to 153 when no enzyme was included. Conversely, when propionic acid was used, NH_3 N levels remained unchanged from d 0 to 153, regardless of acid level or enzyme ($P > 0.10$).

2.9.4 Acid binding capacity and buffering capacity

The effect of acid and concentration on ABC and BUF of high-moisture barley during storage is presented in Table 2.3.

There was an interaction between acid and day for ABC ($P < 0.01$). Acid binding capacity was similar on d 0 and 153 for OIB-treated grains (32.95 and 32.65 mEq H^+ kg^{-1},

respectively) but decreased from d 0 to d 153 when prop was used (57.11 and 49.93 mEq H^+ kg^{-1}, respectively).

Buffering capacity increased from 40.11 to 53.62 mEq H^+ kg^{-1} when the concentration of Prop was increased. Similarly, the BUF of OIB increased (18.81 to 22.25 mEq H^+ kg^{-1}) when concentration increased, however was lower than Prop regardless of concentration (acid by concentration, $P < 0.01$)

2.9.5 Protein dispersibility index

The PDI of high-moisture barley preserved with either Prop or OIB at low or high concentrations, with or without enzyme is presented in Table 2.4.

The PDI was reduced at the higher concentration of OIB, but not Prop, regardless of enzyme addition or storage (acid by concentration, $P < 0.01$). There was a decrease in the PDI when the multi-enzyme was added in OIB treated barley, while the opposite trend was seen when Prop was used (acid by enzyme, $P < 0.01$).

The PDI was reduced by 20% from d 0 to d 153 when OIB was used, but only by 12% when Prop was used. OIB treated grains had higher PDI on d 0 compared to Prop (23.77 vs 21.57 %) but did not differ on d 153 (19.0 vs 18.97 %; acid by day, $P < 0.01$).

The PDI was lower in high compared to low acid concentration (21.95 vs 23.39 %) regardless of acid at d 0, while the opposite trend was observed on d 153 (19.35 vs 18.62; concentration by day; $P < 0.01$).

2.9.6 Available P

The effect of acid, acid concentration and enzyme on aP of high-moisture barley during storage is presented in Table 2.5.

There was an increase in aP of barley treated with Prop from d 0 to d 135 which was not observed in OIB (acid by day interaction, $P < 0.05$).

Available P increased from 21.54 to 25.29 (% of total P) with the inclusion of enzyme (enzyme $P < 0.01$) and from 22.53 to 24.29 (% of total P) with high acid concentration (concentration, $P < 0.05$).

2.10 Wheat

2.10.1 Mould growth

There was no visible mould growth in high-moisture wheat regardless of treatment or storage.

2.10.2 pH

The effect of acid, concentration and enzyme on the pH of high-moisture wheat during storage is presented in Table 2.6.

Similar to observed in the barley trial, there was an interaction among type of acid, acid concentration, enzyme and day on pH of grains ($P < 0.01$). Lower pH was measured in grains preserved using Prop on d 0, with the lowest pH observed from Prop-Low-Enz, Prop-High-NoEnz and Prop-High-Enz treatments and the highest pH observed with OIB-Low-NoEnz. The pH of all treatments using Prop had increased by d 4, followed by a decrease in pH at d 14, and again increasing at d 153 ($P < 0.05$). The pH of OIB-treated wheat decreased on d 4 ($P < 0.05$) except for OIB-High-Enz. The pH of OIB-treated wheat remained the same from d 4 to 14 ($P > 0.10$), except for OIB-Low-NoEnz where an increase in pH was observed. Similar to grains treated with Prop, the pH of treatments with OIB increased from d 14 to 153 ($P < 0.05$). Following 153 d of storage the lowest pH was observed when wheat was treated with the high

level of Prop, regardless of enzyme ($P < 0.05$).

2.10.3 Ammonia nitrogen

There was a 3-way interaction among acid type, concentration and enzyme for NH_3 N levels in wheat (Table 2.7). When OIB was used to preserve the wheat, NH_3 N levels decreased on both d 0 and 153 with enzyme addition, compared to no enzyme. This effect was not observed when the wheat was preserved with Prop (acid by enzyme by day, $P < 0.01$).

Averaged across days, NH_3 N decreased by about 40% with the addition of enzyme and OIB was the acid. Conversely, NH_3 N was similar when enzyme was added to Prop at low levels (0.074 vs 0.069) and increased by 12% when used at high concentrations (0.074 vs 0.083) acid by concentration by enzyme, $P < 0.01$).

2.10.4 Acid binding and buffering capacity

Acid binding capacity of high-moisture wheat preserved using propionic acid was higher compared to those preserved using OIB (38.91 vs 25.71 mEq H^+ kg^{-1}; $P < 0.01$; Table 2.8)

In contrast to when OIB was used, wheat preserved with a high concentration of Prop had higher BUF compared when the low concentration was used (56.16 vs 40.48 mEq H^+ kg^{-1}) (acid by concentration, $P < 0.05$).

When Prop was used to preserve wheat, BUF capacity increased (44.22 to 52.42 mEq H^+ kg^{-1}) from d 0 to 153. The opposite effect was observed when OIB was used (22.59 to 18.54 mEq H^+ kg^{-1}; acid by day, $P < 0.01$, Table 2.8).

2.10.5 Protein dispersibility index

The effect of acidifier, concentration, and enzyme on PDI of high-moisture wheat during storage is presented in Table 2.9.

There was a 2-way interaction between the acid type and concentration on PDI % ($P < 0.01$). Increasing the concentration of Prop resulted in higher PDI. Conversely, there was no effect of concentration on % PDI when OIB was used ($P > 0.10$).

There was also an interaction between type of acid and enzyme on PDI % ($P < 0.01$). A higher PDI was observed in Prop with enzyme compared to without, which was not observed when OIB was used.

2.10.6 Available P

The effect of acidifier, concentration and enzyme on aP of high-moisture wheat during storage is presented on Table 2.10.

Available P increased (13.48 to 20.88 % of total P) when the enzyme mixture was added to the high concentration of Prop which was not observed with the low. Conversely, the supplementation of enzyme in the OIB treated grains regardless of concentration had no effect on aP (acid by concentration by enzyme; $P < 0.05$).

DISCUSSION

The use of organic acids as preservatives depends on their ability to kill or reduce growth of microorganisms that cause spoilage. The efficacy of organic acids as anti-microbials is determined by their ability to depress pH and by their pKa, defined as the pH at which 50% of the acid is in an undissociated form (Partanen 2001). By passive diffusion, the undissociated form of acids (non-ionised and lipophilic) freely pass through the cell membrane of the microbe (Gauthier 2002). The cytoplasm is maintained close to pH 7, therefore, once inside, the organic acid dissociates, reducing the pH and disrupting enzyme function and nutrient transport systems (Luek et al. 1980 as cited by Partanen and Mroz 1999). The most effective organic acids as

preservatives, therefore, are those with a high pKa (Foegeding and Busta 1991 as cited by Partanen and Mroz 1999). On the other hand, inorganic acids are unable to penetrate the cell membrane, whether in undissociated or dissociated form (Gauthier 2002) and function solely by maintaining a low pH.

The absence of observable mould growths in all treatments across the different time points indicate the efficacy of the acid blend (OIB) and propionic acid (Prop) in the current experiment as preservatives. This was expected because the pH of the high-moisture barley and wheat was reduced upon acidification, and, except for LA, the pH level was lower than the pKa of the organic acids used in this experiment. The current experiment used propionic acid, an organic acid, and a blend of organic and inorganic acids. Propionic acid has a pKa of 4.87 (O'Neil 2006). The acid blend contained up to 50% phosphoric acid (inorganic acid) which has 3 pKa's, 2.15, 7.09 and 12.32 (O'Neil 2006). The organic acids in the mixture (and their pKa) were LA (3.86), citric acid (3.13, 4.76, and 6.4), and malic acid (3.40 and 5.11). An environmental pH lower than the acid's pKa would mean that most of the acids are in undissociated form, and therefore can function as anti-microbials. The efficacy of propionic acid in controlling moulds has been extensively studied, however, very little is known about the use of phosphoric acid as a mould inhibitor. In the work of Lin and Chen (1995), phosphoric acid (85%) and propionic acid (99%) at 1 g kg^{-1} inhibited the growth of *Penicillium* extracted from poultry diets. At 5 g kg^{-1}, both acids inhibited the growth of *Aspergillus.* Conversely, propionic acid at 1 g kg^{-1} inhibited the growth of *Fusarium*, while phosphoric acid at 5 g kg^{-1} was only partially effective. In the current experiment, both propionic acid and the acid mixture prevented the visible growth of moulds during storage.

Metabolites arising from anaerobic fermentation includes organic acids and NH_3 N, and

the changes in pH or NH_3 N levels during storage are indicative of changes in fermentation patterns brought about by microbial activity in anaerobic conditions (Baron et al. 1986; Wardynski et al. 1993; Sebastian 1996). These parameters are indicators of the efficacy of the acidifiers for inhibiting anaerobic microbes. Although the high-moisture grains were loosely packed in a sealed jar with about an inch of headspace, the conditions may have changed from aerobic to anaerobic during the course of storage due to grain respiration. A study by Weinberg et al. (2008) showed that in hermetically sealed non-acidified corn, the immediate atmosphere was modified from high O_2 to high CO_2 levels due to respiration of moulds, insects and grain. In the same experiment, when corn was reconstituted to 22% or 20% moisture, the concentration of O_2 was totally replaced by CO_2 within 12 and 48 hours, respectively.

The low pH when using propionic acid on either the high-moisture barley (2.44 to 2.77) or wheat (2.01 to 2.65) at d 0 was not expected because the pH of all the acids in the acid blend (phosphoric, pH 1.6; lactic, pH 2.4; citric, pH 2.2; malic, pH 2.2) were lower than propionic acid (pH 3.0) (Lawlor et al. 2005). After 4 d, the pH of the barley or wheat using propionic acid had increased to levels similar to the pH of high-moisture barley or wheat when OIB was used. This may be a reflection of the relative differences in absorption rate of the acids by the grain, with OIB being absorbed quicker than Prop. On d 0, the acidified grains were immediately sampled and analyzed for pH after mixing and it is possible that most of the Prop remained on the surface of the grain during the analysis resulting in a lower pH compared to OIB. Propionic acid is characterized as “oily” (Papatsiros et al. 2012) therefore its entry into the grain may have been affected by the already wet grain surface. Dietary acidifiers are used to lower stomach pH below pH 5 and this is because: 1) most organic acids with anti-microbial activities have a pKa of 3 to 5, and 2) enzymes require this pH level for optimal activity (Papatsiros and Billinis

2012). In the barley trial, the grain pH increased from d 0 to d 153 in all treatments, all having a final pH of above pH 5 except for barley with high concentration of Prop. A pH of less than 5 was achieved using the high concentration of Prop and OIB until d 153 and d 14, respectively. In the wheat trial, the pH of all treatments increased from d 0 to d 153, with only the Prop treatments having a final pH of below 5. In OIB-treated grains, a pH below 5 was maintained only until d 14. Overall, the increase in pH during storage suggests that fermentation did not take place with the use of the acids. Organic acids such as lactic and acetic are typically produced during fermentation with consequent reductions in pH; which was not observed in these trials. Furthermore, these results suggest that when using the acid blend as preservative for high-moisture grains, a high concentration should be used, and storage should be shorter than 153 d.

The efficacy of additives to maintain NH_3 N levels was used by Allen and Stevenson (1975) as a criterion to evaluate the general effectiveness of various preservatives added to wet brewer's grains (23% DM). Ammonia N is produced by the deamination and decarboxylation of amino acids by proteolytic clostridia or saprophytic fungi (Allen and Stevenson 1975). Breirem and Ulvesli (1960), as reviewed by Allen and Stevenson 1975, suggested that clostridia populations are easily controlled at pH 4.0 to 4.2. In the current experiment with high-moisture barley, the NH_3 N levels in the propionic acid treatments did not increase from d 0 to d 153. Conversely, there was an increase in NH_3 N level from d 0 to d 153 when OIB was used, in conjunction with the enzyme. The enzyme mixture used contained proteases, and it is possible that the amino acids liberated were used as substrate for proteolytic bacteria in the OIB treatments. Although no microbial analysis was conducted, these results indicate that Prop controlled the growth of proteolytic bacteria better than OIB. In a study by Livingstone et al.

(1971), the use of propionic acid at 1.3% (13 g kg^{-1}) inhibited the growth of aerobic and anaerobic bacteria on high-moisture barley (21 % moisture) stored for 4 months. The use of propionic acid at 5.0 and 7.5 g kg^{-1} in the current barley trial resulted in a pH of less than 3 upon application. In contrast, the pH using OIB was ≥ pH 4.35. The increase in NH_3 N in OIB treated grains but not in Prop can therefore be attributed to reduction in pH due to Prop upon application. In contrast, in the wheat trial, the NH_3 N level increased from d 0 to d 153 when propionic acid was added at high concentration, with the highest NH_3 N level observed when the enzyme mixture was added. Similarly, the increased NH_3 N levels in treatments using OIB, except for the low concentration without enzyme, suggests anaerobic microbial activity in these treatments during storage.

Protein dispersibility index is the proportion of CP that is solubilized in water after high speed blending (AOCS 2017). It is comparable to the term "soluble nitrogen" commonly used in earlier literature which was reportedly composed of about 50% free amino acids and a small amount of ammonium ions (Phillip et al. 1985; Baron et al. 1986; Buchanan-Smith and Smith 2003). In the feed industry, this parameter has been used to evaluate protein quality of raw materials, i.e. a higher PDI means better protein quality (Iwe et al. 2001). Soluble N content of ensiled ground corn (30% moisture) may be as high as 40% (Philip et al. 1985; Baron et al. 1986). This is generally attributed to microbial or grain-based proteolytic enzymes (Buchanan-Smith and Smith 2003). Studies however, have reported solubilization of N and release of NH_3 even with the addition of mould and yeast inhibiting agents (Wardynski et al. 1993; Sebastian et al. 1996; Buchanan-Smith and Smith 2003) and Prigge et al. (1976) suggested that solubility of nitrogen in ensiled corn is affected by pH rather than bacterial fermentation. The higher PDI of OIB treated barley at low concentration compared to the high concentration was not expected

due to the lower pH resulting from the high concentration at all time points during storage. Although pH was reduced with the higher concentration of Prop, there were no differences in PDI observed between the two concentrations suggesting that pH was not correlated with PDI. The weak negative correlation ($r= -0.30$; $P < 0.10$) between pH and PDI in the current barley experiment may be due to the hulls. It is possible that encapsulation by the hulls serves as a barrier for the action of the exogenous proteases. The higher PDI of grains preserved using OIB compared to Prop at d 0, but not d 153 could be due to the quicker uptake of OIB compared to Prop. In wheat, there was a strong negative correlation ($r= -0.87$; $P < 0.01$) between pH and PDI. The higher PDI with high Prop concentration compared to low can be attributed to the lower pH with the high concentration compared to low concentration of Prop (pH 3.92 vs 4.18). Proteases are active at low pH and are inactive above pH 6 (Crevieu-Gabriel et al. 1999). Conversely, the PDI of OIB was unaffected by concentration of the acid although the pH of the High was lower than the Low acid concentration (pH 4.42 vs 4.77). This may be because the pH of the OIB treated grains, regardless of concentration, were higher than the pH required for optimum protease activity. Hashimoto et al. (1973) reported that the optimal pH for a fungal protease was pH 2 or 3.0. The higher PDI in Prop with enzymes could also be due to its lower pH compared to without enzyme (pH 3.44 vs 3.54), potentiating the effect of the exogenous protease. For the OIB, the pH of with or without enzyme was comparable (pH 4.89 vs 4.94) and both were higher than the optimum pH required by proteases.

Acid binding capacity (ABC) is defined as the amount of acid (mEq H^+) required to reduce the pH of 1 kg of sample to pH 4. Buffering capacity is the ABC divided by the difference of the initial pH and pH 4. This is the amount of acid required to produce a unit change in pH of sample (Jasaitis et al. 1997; Lawlor et al. 2005). The use of ingredients with

lower ABC or BUF is a practice to manipulate stomach acidity (Lawlor et al. 2005). The choice of ingredients for weanling pig diets should therefore consider ABC because of the inherent difficulty of maintaining an acidic condition in the stomach (Batonon-Alavo et al. 2016). The determination of ABC requires the analysis of the initial pH prior to titration with 0.1 N HCl until pH 4. It is important to note that the initial pH used in the current experiments for ABC determination was from the re-analysis of the frozen d 0 samples. It was initially reported that the pH of Prop-preserved grains at d 0 ranged from 2.44 to 2.77 for the barley trial, and 2.01 to 2.65 for the wheat trial. The re-analyzed pH for the d 0 samples of Prop-preserved barley for ABC determination ranged from pH 4.94 to 5.42, and for the wheat trial pH 4.62 to 5.46. This information also supports the assumption that the low pH of the d 0 samples measured upon acidification was due to the slower uptake of Prop by the grain. The ABC of barley in the current experiment ranged from 30.54 to 57.70 mEq H^+. In the wheat trial, the ABC ranged from 22.56 to 41.24 mEq H^+. These values were lower compared to those reported by Lawlor et al. (2005) of 113 and 108 mEq H^+ for barley and wheat, respectively. This was expected because the starting pH for the acid-preserved barley (pH 4.94 to 5.42) or wheat (pH 4.62 to 5.46) were lower compared to the untreated barley or wheat pH of 6.6 and 6.9, respectively, as reported by Lawlor et al. (2005). The ABC of the OIB-treated barley did not change from d 0 to d 153. In both time points, ABC of barley preserved with OIB was lower compared to those preserved using Prop. This can be attributed to the ABC of the acids used in the mixture (i.e. phosphoric acid, -8,858; LA, -5,079; malic, -7,214 and citric acid, -5,605 meq H+ kg^{-1}) which are 3 to 6 times lower than propionic acid (-1,358 meq H+ kg^{-1}; Lawlor et al. 2005). The decrease in ABC of propionic acid-preserved barley after storage can be attributed to the decrease in pH of Prop treated grains from d 0 to d 153 (pH 5.24 vs 5.09; based on reanalyzed

samples for ABC and BUF determination).

In the wheat trial, the higher ABC of the high-moisture wheat preserved using Prop compared to OIB can be explained by the ABC of Prop compared to the acids in the OIB mixture. Barley pH was considerably higher than wheat, probably because of the higher buffering capacity of the untreated barley compared to untreated wheat. This agrees with reports by Jasaitis et al. (1997) and Lawlor et al. (2005) who reported that ABC and BUF were about 5 and 16% higher respectively in barley than wheat.

Available P for the grains treated with low or high concentration of OIB was corrected for the P contribution from the acid. In the barley trial, the use of Prop improved aP after storage regardless of concentration and enzyme addition while OIB did not. It was earlier suggested that OIB had a quicker uptake compared to Prop, possibly hydrolyzing phyP at d 0. Conversely, the delay in the uptake of Prop resulted in lower aP which eventually increased over time as the acid penetrated the grain. The increase in aP with increasing acid concentration was possibly due to the lower pH in the high concentration which better solubilized phyP, while the improvement in aP with the addition of the enzyme mixture can be attributed to the presence of phytase in the enzyme mix. In wheat, aP increased by 42% (13.79 to 20.52 % of total P) when the enzyme was added to Prop at high concentration and could be attributed to the very low pH (pH 3.33) which possibly hydrolyzed phyP. Similar to the barley trial, the addition of the enzyme mixture improved aP regardless of acid, concentration and storage time, which can be attributed to the presence of phytase in the enzyme mix.

SUMMARY AND CONCLUSIONS

Propionic acid (Prop) and OIB inhibited the growth of moulds when used as a preservative for high-moisture barley or wheat as indicated by the absence of visible mould growths in all treatments. An overall increase in pH over time in both acid-preserved high-moisture wheat and barley suggest that anaerobic fermentation was arrested during storage. Levels of NH_3 N indicate that Prop was better than OIB in controlling anaerobic proteolytic bacteria in barley or wheat. The pH of the high-moisture barley was maintained below pH 5 with the high concentration of OIB or Prop (regardless of enzyme addition), however the maximum length of storage when using OIB appears shorter than 153 d. In high moisture wheat, a pH of below 5 was maintaind for 153 d with Prop, and 14 d using OIB. High-moisture wheat or barley preserved using OIB may be better at maintaining a lower pH in weanling pigs, compared to Prop as indicated by their lower acid binding and buffering capacity. Increasing the concentration of acid (regardless of acid type) improved the availability of P but not N (PDI) in barley. In wheat, high concentration of acid (Prop) increased N availability but not aP. The addition of enzymes to Prop-treated barley improved availability of N, while aP was improved with the addition of enzymes with or without acids. The addition of enzymes in Prop-treated wheat improved the availability of P (with high Prop concentration) and N.

TABLES

Table 2.1. Effect of acidifier, concentration and enzyme on pH of high-moisture barley during storage.

Acid	OIB				Prop			
Concentration	Low		High		Low		High	
Enzyme	NoEnz	Enz	NoEnz	Enz	NoEnz	Enz	NoEnz	Enz
Day								
0	5.02fghi	5.31c	4.35n	5.00ghij	2.77p	2.58q	2.6q	2.44r
4	5.06efgh	5.09defg	3.81o	3.89o	5.18de	5.19d	4.92ijk	4.97ghij
14	5.61b	5.53b	4.83kl	4.95hijk	4.9jkl	4.78l	4.65m	4.64m
153	5.77a	5.83a	5.51b	5.52b	5.13def	5.14de	4.96hij	4.95hij

	SEM	P value
Acid	0.004	<0.01
Concentration	0.004	<0.01
Enz	0.004	<0.01
Day	0.008	<0.01

Acid*Concentration	0.006	<0.01
Concentration*Enz	0.006	<0.01
Acid*Concentration*Enz	0.006	<0.01
Acid*Day	0.011	<0.01
Concentration*Day	0.011	<0.01
Enz*Day	0.011	<0.01
Acid*Concentration*Day	0.015	<0.01
Concentration*Enz*Day	0.015	<0.01
Acid*Concentration*Enz*Day	0.022	<0.01

Note: OIB, phosphoric acid; Prop, propionic acid; Enz, with enzyme; NoEnz, no enzyme; SEM standard error of mean. Means without a common letter are different ($P < 0.05$).

Table 2. 2. Effect of acidifier, concentration and enzyme addition on NH_3 N levels of high-moisture barley during storage.

Acid	OIB				Prop			
Concentration	Low		High		Low		High	
Enzyme	NoEnz	Enz	NoEnz	Enz	NoEnz	Enz	NoEnz	Enz
Day	*mg/ dL*							
0	0.12a	0.06g	0.12a	0.06g	0.10cd	0.06g	0.10d	0.07fg
153	0.11bc	0.07ef	0.11ab	0.08e	0.10cd	0.06g	0.10cd	0.07fg

	SEM	P value
Acid	0.001	<0.01
Concentration	0.001	<0.01
Enz	0.001	<0.01
Day	0.001	<0.01
Acid*Concentration	0.001	0.09
Acid*Enz	0.001	<0.01
Acid*Day	0.001	0.61
Concentration*Enz	0.001	0.03

Concentration*Day	0.001	<0.01
Acid*Concentration*Enz	0.001	0.01
Acid*Concentration*Day	0.001	0.57
Acid*Enz*Day	0.001	<0.01
Concentration*Enz*Day	0.001	0.44
Acid*Concentration*Enz*Day	0.002	<0.01

Note: OIB, organic-inorganic acid blend; Prop, propionic acid; Enz, with enzyme; NoEnz, no enzyme; SEM standard error of mean.

Means without a common letter are different ($P < 0.05$).

Table 2.3. Effect of acidifier and concentration on acid binding (ABC) and buffering capacity (BUF) of high-moisture barley during storage.

Acid	OIB		Prop	
Concentration	Low	High	Low	High
Day				
	Acid Binding Capacity (mEq H^+ kg^{-1} grain)			
0	30.54	35.36	56.53	57.7
153	32.59	32.71	50.26	49.6
	Buffering Capacity (mEq H^+ kg^{-1} grain)			
0	19.64	24.01	39.85	54.19
153	17.97	20.45	40.37	53.05

	ABC		BUF	
	SEM	P value	SEM	P value
Acid	0.868	<0.01	1.218	<0.01
Concentration	0.868	0.30	1.218	<0.01
Day	0.868	0.02	1.218	0.08
Acid*Concentration	1.228	0.39	1.722	<0.01
Acid*Day	1.228	0.02	1.722	0.15
Acid*Concentration*Day	1.737	0.39	2.436	0.50

Note: OIB, organic-inorganic acid blend; Prop, propionic acid; SEM standard error of mean.

Table 2.4. Effect of acidifier, concentration and enzyme addition on protein dispersability index (PDI) of high-moisture barley during storage.

Acid	OIB				Prop			
Concentration	Low		High		Low		High	
Enzyme	NoEnz	Enz	NoEnz	Enz	NoEnz	Enz	NoEnz	Enz
Day	*(% of total CP)*							
0	25.42	24.6	22.79	22.29	21.69	21.87	20.97	21.75
153	19.58	18.5	19.11	18.79	17.65	18.74	19.22	20.26

	SEM	P value
Acid	0.107	<0.01
Concentration	0.107	0.03
Enz	0.107	0.76
Day	0.107	<0.01
Acid*Concentration	0.151	<0.01
Acid*Enz	0.151	<0.01
Acid*Day	0.151	<0.01

Concentration*Enz	0.151	0.20
Concentration*Day	0.151	<0.01
Acid*Concentration*Enz	0.214	0.66
Acid*Concentration*Day	0.214	0.49
Acid*Enz*Day	0.214	0.32
Concentration*Enz*Day	0.214	0.85
Acid*Concentration*Enz*Day	0.302	0.38

Note: OIB, organic-inorganic acid blend; Prop, propionic acid; Enz, with enzyme; NoEnz, no enzyme; SEM standard error of mean.

Table 2.5. Effect of acidifier, concentration and enzyme addition on available P (aP) of high-moisture barley during storage.

	OIB				Prop			
	Low		High		Low		High	
	NoEnz	Enz	NoEnz	Enz	NoEnz	Enz	NoEnz	Enz
Day	*% of total P*							
0	21.20	23.17	20.51	25.18	17.56	22.17	19.98	24.76
153	20.26	26.70	22.78	26.44	25.18	24.00	24.83	29.89

	SEM	P value
Acid	0.546	0.73
Concentration	0.546	0.04
Enz	0.546	<0.01
Day	0.546	<0.01
Acid*Concentration	0.772	0.28
Acid*Enz	0.772	0.58
Acid*Day	0.772	<0.05
Concentration*Enz	0.772	0.32

Concentration*Day	0.772	0.81
Acid*Concentration*Enz	1.092	0.31
Acid*Concentration*Day	1.092	0.95
Acid*Enz*Day	1.092	0.17
Concentration*Enz*Day	1.092	0.93
Acid*Concentration*Enz*Day	1.544	0.08

Note: OIB, organic-inorganic acid blend; Prop, propionic acid; Enz, with enzyme; NoEnz, no enzyme; SEM standard error of mean.

Table 2.6. Effect of acidifier, concentration and enzyme addition on pH of high-moisture wheat during storage.

Acid	OIB				Prop			
Concentration	Low		High		Low		High	
Enzyme	NoEnz	Enz	NoEnz	Enz	NoEnz	Enz	NoEnz	Enz
Day								
0	4.99c	4.44hi	3.85m	4.13l	2.65O	2.16p	2.01p	2.05p
4	3.58n	4.70defg	4.17kl	4.13l	4.97C	4.94c	4.74def	4.52gh
14	4.62fg	4.67efg	4.20jkl	4.20kl	4.63fg	4.38hij	4.41hi	4.33ijk
153	5.60a	5.57a	5.31b	5.43ab	4.84cde	4.85cd	4.65fg	4.68defg

	SEM	P value
Acid	0.009	<0.01
Concentration	0.009	<0.01
Enz	0.009	<0.01
Acid*Concentration	0.013	<0.01
Concentration*Enz	0.013	<0.01
Acid*Concentration*Enz	0.018	<0.01

Day	0.012	<0.01
Acid*Day	0.016	<0.01
Concentration*Day	0.016	<0.01
Enz*Day	0.016	<0.01
Acid*Concentration*Day	0.023	<0.01
Concentration*Enz*Day	0.023	<0.01
Acid*Concentration*Enz*Day	0.033	<0.01

Note: OIB, organic-inorganic acid blend; Prop, propionic acid; Enz, with enzyme; NoEnz, no enzyme; SEM standard error of mean.

Means without a common letter are different ($P < 0.05$).

Table 2.7. Effect of acidifier, concentration and enzyme addition on NH_3 N levels of high-moisture wheat during storage.

Acid	OIB				Prop			
Concentration	Low		High		Low		High	
Enzyme	NoEnz	Enz	NoEnz	Enz	NoEnz	Enz	NoEnz	Enz
Day	*mg dL^{-1}*							
0	0.120	0.066	0.124	0.066	0.068	0.066	0.066	0.075
153	0.128	0.086	0.141	0.100	0.080	0.071	0.081	0.093

	SEM	P value
Acid	0.0009	<0.01
Concentration	0.0009	<0.01
Enz	0.0009	<0.01
Day	0.0009	<0.01
Acid*Concentration	0.0013	0.98
Acid*Enz	0.0013	<0.01
Acid*Day	0.0013	0.01
Concentration*Enz	0.0013	0.02

Concentration*Day	0.0013	<0.01
Acid*Concentration* Enz	0.0018	0.01
Acid*Concentration*Day	0.0018	0.61
Acid*Enz*Day	0.0018	<0.01
Concentration*Enz*Day	0.0018	0.18
Acid*Concentration* Enz *Day	0.0026	0.60

Note: OIB, organic-inorganic acid blend; Prop, propionic acid; Enz, with enzyme; NoEnz, no enzyme; SEM standard error of mean. Means without a common letter are different ($P < 0.05$).

Table 2.8. Effect of acidifier and concentration on acid binding (ABC) and buffering capacity (BUF) of high-moisture wheat during storage.

Acid	OIB		Prop	
Concentration	Low	High	Low	High
Day	*Acid Binding Capacity (mEq H^+ kg^{-1} grain)*			
0	27.62	22.56	40.42	41.24
153	24.78	27.89	36.99	36.99
	Buffering Capacity (mEq H^+ kg^{-1} grain)			
0	19.55	25.635	36.255	52.19
153	16.73	20.355	44.714	60.136

	ABC		BUF	
	SEM	P value	SEM	P value
Acid	1.691	<0.01	1.396	<0.01
Concentration	1.691	0.91	1.396	<0.01
Day	1.691	0.60	1.396	0.32
Acid*Concentration	2.392	0.78	1.974	0.03
Acid*Day	2.392	0.32	1.974	0.01
Concentration*Day	2.392	0.46	1.974	0.72
Acid*Concentration*Day	3.383	0.51	2.791	0.90

Note: OIB, organic-inorganic acid blend; Prop, propionic acid; Enz, with enzyme; NoEnz, no enzyme; SEM standard error of mean

Table 2.9. Effect of acidifier, concentration and enzyme addition on protein dispersability index (PDI) of high-moisture wheat during storage.

Acid	OIB				Prop			
Concentration	Low		High		Low		High	
Enzyme	NoEnz	Enz	NoEnz	Enz	NoEnz	Enz	NoEnz	Enz
Day	*% of total CP*							
0	25.90	24.94	25.41	25.22	25.87	26.55	26.81	28.36
153	22.49	22.10	22.74	22.05	22.63	23.29	23.79	24.17

	SEM	P value
Acid	0.107	<0.01
Concentration	0.107	<0.01
Enz	0.107	0.41
Day	0.107	<0.01
Acid*Concentration	0.151	<0.01
Acid*Enz	0.151	<0.01
Acid*Day	0.151	0.20

Concentration*Enz	0.151	0.40
Concentration*Day	0.151	0.81
Acid*Concentration*Enz	0.214	0.91
Acid*Concentration*Day	0.214	0.37
Acid*Enz*Day	0.214	0.32
Concentration*Enz*Day	0.214	0.09
Acid*Concentration*Enz*Day	0.303	0.31

Note: OIB, organic-inorganic acid blend; Prop, propionic acid; Enz, with enzyme; NoEnz, no enzyme; SEM standard error of mean.

Table 2.10. Effect of acidifier, concentration, and enzyme addition on available P of high-moisture wheat during storage.

Acid	OIB				Prop			
Concentration	Low		High		Low		High	
Enzyme	NoEnz	Enz	NoEnz	Enz	NoEnz	Enz	NoEnz	Enz
Day	*% of total P*							
0	15.40	16.63	13.95	15.89	14.24	13.17	13.48	20.88
153	15.48	18.38	12.02	17.83	17.16	14.30	14.10	20.05

	SEM	P value
Acid	0.475	0.74
Concentation	0.475	0.53
Enz	0.475	<0.01
Day	0.475	0.31
Acid*Concentration	0.671	0.01
Acid*Enz	0.671	0.65
Acid*Day	0.671	0.71
Concentration*Enz	0.671	<0.01

Concnetration*Day	0.671	0.27
Acid*Concentration*Enz	0.949	0.02
Acid*Concentration*Day	0.949	0.66
Acid*Enz*Day	0.949	0.12
Concentration*Enz*Day	0.949	0.64
Acid*Concentration*Enz*Day	1.099	0.73

Note: Phos, phosphoric acid; Prop, propionic acid; NoEnz, no enzyme; Enz, with enzyme; SEM standard error of mean.

Means without a common letter are different ($P < 0.05$).

CHAPTER 3. THE INTERACTION OF ACID-PRESERVATION OF WHEAT WITH OR WITHOUT ENZYMES AND PARTICLE SIZE ON THE PERFORMANCE AND GUT HEALTH OF WEANLING PIGS

Presented in part at the 2017 ASAS-CSAS Annual Meeting and Trade Show and at the 2019 BANFF Pork Symposium.

Parts of this manuscript will be submitted to the Canadian Journal of Animal Science.

ABSTRACT

Two studies were conducted to determine the efficacy of feeding acid-preserved high-moisture wheat (APW) as an alternative to direct diet acidification (AD) on weanling pig performance (Study 1) and gut health (Study 2). Acid-preserved wheat was prepared by reconstituting wheat to 20% moisture followed by acidification using either propionic (Prop, 99%, 7.5 g kg^{-1}) or an organic-inorganic acid blend (OIB, phosphoric, lactic, malic and citric acids, 7 g kg^{-1}) and an enzyme cocktail (phytase, carbohydrases and protease, 500 g $tonne^{-1}$) added. A subset of the OIB-treated wheat was prepared without the enzyme to allow for comparison of diets with or without enzymes (Enz or NoEnz). Dry wheat and APW were ground using a 2.0 (Fine) or 4.8 (Coarse; dry and OIB-Enz preserved only) mm screen to allow for comparison of particle size. In Study 1, a total of 320 pigs (21 $\pm$ 2 d, 6.78 ± 0.68 kg) were randomly assigned to 80 pens in 4 rooms. Pens were assigned to 1 of 10 treatments in a randomized complete block design with pen as the experimental unit and room as block. Treatments were: NC (no acid and enzyme), PC (no acid, with enzyme), APW-OIB-Enz-Fine, APW-OIB-Enz-Coarse, APW-OIB-NoEnz-Fine, APW-Prop-Enz-Fine, AD-OIB-Enz-Fine, AD-

OIB-Enz-Coarse, AD-OIB-NoEnz-Fine, or AD-Prop-Enz-Fine. Pigs were fed phase 1 diet from d 0 to 7 (weaning, d 0), then phase 2 from d 8 to d 21. Pigs fed diets with APW or AD had comparable ADG, ADFI or G:F ($P > 0.10$) in both growth phases. During phase 2, pigs fed diets with OIB-NoEnz regardless of mode of acidification had higher ADG and G:F compared to NC ($P < 0.05$); pigs fed Prop had higher ADG and G:F than those fed OIB ($P < 0.05$); and pigs fed Fine had higher ADG and G:F compared to those fed Coarse ($P < 0.05$). In Exp 2, the Prop treatments were removed (eight treatments used). A total of 64 barrows (31 ± 2 d, 6.8 kg) were randomly assigned to 1 of 8 treatments, in 8 blocks with 8 pigs per block in a randomized complete block design. Pigs were on treatment diets for 14 d. Pigs were adapted to pen and diet for 8 d followed by fecal collection for 4 d. On d 13, pigs fed the Enz diets only (5 out of 8 treatments) were given a gavage of indigestible sugar markers, lactulose and mannitol, for estimation of intestinal permeability. These pigs were euthanized on d 14 for collection of tissue (mid-jejunum) for histology and analysis of gene markers for barrier function and inflammatory response. Pigs fed diets with APW had comparable DM, energy, ash and P digestibility as those fed AD. Pigs fed Fine had higher ($P < 0.05$) ATTD of energy compared to Coarse in AD but not APW. Pigs fed diets with enzymes (PC), acid blend (AD-OIB-NoEnz-Fine) or their combination (AD-OIB-Enz-Fine) had higher P ATTD compared to those fed NC ($P < 0.05$). Treatment had no effect on markers of gut health ($P > 0.10$). Overall, pigs fed diets with APW had comparable performance as those fed AD without observable effects on gut health markers measured in this experiment. Because performance and markers of gut health were similar in pigs fed diets with APW an AD, it can be concluded that the addition of acids as APW can be alternative route to direct diet acidification. Comparable energy digestibility in pigs fed Fine or Coarse suggests that, grinding finely may not be required when APW is used.

INTRODUCTION

Storage of wheat requires a moisture content of < 15% (Hackl et al. 2010). When harvesting below 15% is not possible, artificial drying is employed. This increases cost due to power, fuel and specialized drying structures. Alternatively, high-moisture grains may be preserved with acids and there is evidence that when this is fed to pigs performance is comparable or better than when piglets are fed dried grains (Xu et al. 2016; Lopes et al. 2017) .

Grains are ground prior to feed production. Particle size reduction improves the performance of pigs in all stages of the pig production cycle. These improvements are attributed to increased surface area as particle size decreases, allowing for better access of digestive enzymes (Rojas and Stein 2015). For example, reducing the particle size of corn from 865 to 339 µm linearly improved G:F from 0.65 to 0.69 in weanling pigs (9.4 kg BW; Rojas and Stein 2015), In finishing pigs, reducing particle size of corn from 1000 to 400 µm improved G:F by 8% (linear $P < 0.01$, Wondra et al. 1995). Grinding too fine however, predisposes pigs to gastric ulcers (Friendship 2004). In contrast, feeding pigs coarse diets promotes gut health probably resulting from the reduction in gastric pH due to the production of lactic acid (LA) in the stomach and modification of SCFA concentrations in the hind gut. In a study by Mikkelsen et al. (2002), feeding coarse diets reduced gastric pH by increasing the concentration of organic acids in the stomach, reducing gastric emptying rate and increasing the production of butyric acid in the caecum and colon. In the study by Nielsen and Ingvartsen (2000), the high DM and thick consistency of the digesta of pigs fed coarse diets was attributed to reduced gastric emptying rate. This was correlated with reduced gastric lesion scores.

In a meta-analysis by Tung and Pettigrew (2006), it was shown that acidification of diets improved weanling pig growth rates by 12% and 6% at 0 to 2, and 0 to 4 wks post-weaning,

respectively. Weanling pigs secrete insufficient amounts of hydrochloric acid (HCl) into their stomach, and this has negative consequences on nutrient digestibility and gut health. A low pH is required to activate the proenzyme, pepsinogen, into pepsin, the main enzyme of protein digestion. Furthermore, a low pH is required to inhibit the growth and proliferation of pathogenic microorganisms in the stomach and prevent entry into the gastro intestinal tract (GIT; Kil et al. 2011; Papatsiros et al. 2012). While an exact mode of action is not yet known (Jacela et al. 2009; Liu et al. 2018), suggestions include a) reduction of gastric pH thereby enhancing nutrient digestibility, and b) inhibition of proliferation of microorganisms. The anti-microbial property of acids is due to lowering of the environmental pH killing pH sensitive microorganisms. For organic acids, it is the undissociated form that kills or inhibits the growth of fungi and bacteria (Ravindran and Kornegay 1993; Partanen and Mroz; 1998; Lambert and Stratford 1999; Gauthier 2002; Tung and Pettigrew 2006; Kil et al. 2015; Liu et al. 2018). Diet acidification improves nutrient digestibility, specifically DM and crude protein (CP). The low gastric pH activates the proenzyme pepsinogen into pepsin resulting in increased protein digestibility (Ravindran and Kornegay 1993; Partanen and Mroz 1999; Partanen 2001; Tung and Pettigrew 2006; Kil et al. 2015; Liu et al. 2018). Furthermore, low stomach pH reduces gastric emptying rate and stimulates pancreatic enzyme secretions, improving digestibility of other nutrients (Partanen and Mroz 1999; Tung and Pettigrew 2006; Kil et al. 2015). Improvements in Ca and P digestibility were attributed to increased solubility of minerals and phyP at low pH and the chelating properties of acidifiers (Partanen and Mroz 1999).

Intestinal morphology is closely associated with absorption of nutrients in the pig (Pluske 2001). Other than inhibiting microbial growth and altering microbial populations in the GIT, organic acids, specifically SCFA, may also influence intestinal morphology and function (Sakata

and Inagaki 2001). Acetate, propionate and butyrate are SCFAs commonly used as dietary acidifiers. These acids are produced in the GIT through microbial fermentation of complex carbohydrates. Acetate increases blood flow in the colon and enhances ileal motility (Flis et al. 2014), while propionate is absorbed and converted into glucose in the liver (Roberfroid 2007; Jha 2010). Butyrate prevents invasion of *Salmonella* (Gantois et al. 2006), is the preferred energy source of cells in the colon and stimulates cell proliferation in crypts of both the colon and small intestine (Scheppach 1994).

Grains are usually ground prior to feed production, and while particle size reduction improves animal performance, grinding too finely predisposes pigs to gastric ulcers. Except for this effect of fine particles on stomach ulcers, there has been little work on other aspects of particle size and gut health. Nielsen and Ingvartsen (2000) reported firmer digesta consistency and lower incidence of gastric ulcers in pigs (25 kg BW) fed coarse diets. Mikkelsen et al. (2004) demonstrated that feeding pigs (33 kg BW) coarse diets increased concentration of organic acids (LA) in the stomach resulting in reduced gastric pH. The increased concentration of LA was correlated with higher death rates of *Salmonella* (in-vitro). Furthermore, pigs fed the coarse diet had higher concentration of butyric acid in the caecum and colon; and lower counts of coliform bacteria in the distal small intestine, caecum and mid-colon compared to those fed finely ground diets (Mikkelsen et al. 2004). This is possibly due to the increased amount of undigested starch entering the caecum and colon with coarsely ground diets, subsequently used as a substrate for bacterial fermentation (Sakata and Setoyama 1995).

The reduction in digesta pH and modifying microbial populations when feeding coarsely ground diets is similar to the proposed mode of action with dietary acid supplementation, but with regional differences in the GIT. Supplementation of acids and coarse diets may therefore be

complementary strategies for improving weanling pig performance and gut health. Diet acidification effects are typically observed in the proximal GIT (Metzler and Mosenthin 2007; Luckstadt and Mellor 2011; Kil et al. 2015) while coarsely ground diets affect gastric pH and SCFA production in the hind gut (Mikkelsen et al. 2002). Several studies have investigated the interaction of particle size and diet acidification in pigs. Papenbrock et al. (2005), in two separate experiments, reported that the combination of potassium diformate (KDF) and coarsely ground diets resulted in reduced *Salmonella* excretion rate (% of pigs that tested positive for *Salmonella* in rectal swabs) when compared to pigs fed either fine (experiment 1) or coarse (experiment 2) non-acidified diets. However, because excretion rate of *Salmonella* was also reduced in coarse plus KDF when compared to non-acidified coarse diet, the reduction was attributed more to the KDF. Between the two trials, a greater magnitude of decrease in *Salmonella* excretion rate was observed when coarse plus KDF was compared to pigs fed fine non-acidified diet (37.8% $P < 0.01$ vs 15 %, $P < 0.05$) suggesting synergy between coarse diets and KDF supplementation. Canibe et al. (2005) fed pigs (27 kg BW) non-acidified coarse or fine diets, or fine diets with formic acid (18 g kg^{-1}) and reported higher concentration of LA and increased microbial diversity in the stomach of pigs fed coarse compared to those fed fine diets regardless of acid inclusion. Feeding coarse diets increased total anaerobic and LA bacteria in the stomach and distal small intestine and decreased the number of eneterobacteria in the caecum (Canibe et al. 2005).

The response to organic acids depends on the age of the animal, type and level of acid used, diet ingredients and their buffering capacity, and animal performance (Ravindran and Kornegay 1993; Heo et al. 2012). There is very little information on the effect of phosphoric acid-based organic-inorganic acid blend weanling pig performance and gut health. Whether the

benefits of direct diet acidification on performance and gut health of weanling pigs are maintained if the acid is presented as acid-preserved wheat is not known. Because particle size also influences gastric pH and SCFA production, the interaction of diet acidification and particle size needs further investigation. Results from these studies may provide another tool for producers to utilize low quality high-moisture grains and to avoid costs associated with grain drying. Furthermore, this may be a valuable tool to improve performance and gut health of weanling pigs, especially important with the mandated reduction in the use of in-feed antibiotics.

3.1 Objectives

1) To determine the effect of feeding an organic-inorganic acid blend (OIB) or propionic acid (Prop)-preserved high-moisture wheat, with or without exogenous enzymes and its interaction with particle size on the performance of weanling pigs.
2) To determine the effect of OIB-preserved high-moisture wheat, with or without exogenous enzymes and its interaction with particle size on nutrient digestibility, pH of digesta along the GIT, indicators of gut health, microbial populations and hind-gut fermentation profile (SCFA and LA).

3.2 Hypotheses

1) Pigs fed diets with acid-preserved high-moisture wheat will have the same performance as pigs fed acidified diets.
2) Because grains are stored at high-moisture and low pH conditions, endogenous and exogenous enzyme activity will be enhanced, thus nutrient digestibility will be improved in pigs fed acidified high-moisture wheat.
3) Coarse grinding acidified high-moisture wheat will not be detrimental to weanling

pig performance due to improvements in nutrient digestibility.

4) Because coarse diets reduce gastric pH and alter SCFA production in the hind gut, gut health status of pigs fed coarse will improve compared to those fed fine and will be better realized when APB is used.

MATERIALS AND METHODS

3.3 Animal care

All animal procedures adhered to the animal care protocol (Animal Use Protocol No. 20150054) approved by the University of Saskatchewan Committee on Animal Care and Supply for compliance with the Guide to the Care and Use of Experimental Animals by the Canadian Council on Animal Care (CCAC 2009).

3.4 Grains, acids and enzymes

Wheat (var. Utmost) was sourced from a farm in North Battleford, Saskatchewan, Canada and was analyzed for moisture (AOAC 930.15; 130C for 2 h). This was used to determine the amount of distilled water to be added to the grains for reconstitution to 20% moisture. The acids used in these experiments were either propionic acid (Prop; 99%, Anachemia, Montreal, Quebec, Canada) or a commercial organic-inorganic acid blend (OIB) composed of 30 to 50% phosphoric acid, 0.1 to 1% lactic, 5 to 10% citric and 1 to 5% malic acid (Maxi-Cid, Canadian BioSystems, Canada). The commercially available enzyme used was a combination of phytase and multi-carbohydrase enzymes (Superzyme Plus, Canadian BioSystems, Calgary, Alberta, Canada).

3.5 Reconstitution and acidification

Figure 3.2 illustrates the protocol employed for this experiment. Briefly, wheat was reconstituted to 20% moisture and preserved in polyethylene barrels using either Prop or OIB prior to grinding and use in the production of the appropriate treatment diets as outlined in Table 3.1. For each treatment, 250 kg of wheat (12.7% moisture) was mixed with ~23 kg of distilled water for reconstitution to 20% moisture. Water was hand mixed with the grain and the mixture was allowed to sit for 10 min to allow penetration of water into the grains. This was followed by mixing for 20 min using a 200 kg-capacity twin-shaft ribbon and paddle mixer (Scott 363, Scott Equipment, MN, USA). The enzyme was added (500 g t^{-1}; 125 g of enzyme premixed in 500 g of ground wheat as carrier) to the appropriate treatments two min into mixing, followed by the acid. Propionic acid or OIB was added at the rate of 7.0 or 7.5 kg t^{-1} respectively, to the required treatments. Because of the capacity of the mixer, reconstitution was done in 2 batches per treatment (125 kg per batch).

3.6 Storage and corrosion rate

Acidified wheat was stored for 34 d in sealed polyethylene barrels. Carbon steel and galvanized steel coupons (Corrpro, Aegion Corporation, Canada) were embedded in the treated grains to allow for the estimation of the corrosive effects of acids on storage bins by measuring the loss in coupon weight as described in ASTM (method G1-03, 2011). Steel coupons were retrieved prior to feed production and cleaned with a solution composed of HCl (Fisher Scientific), antimony trioxide (Fisher Scientific), stannous chloride (Fisher Scientific), acetone (Fisher Scientific) and distilled water to remove the corroded portion, and then weighed. Weight

loss was the basis for corrosion rate (mils yr^{-1}) of the acids and was calculated using the equation:

$$Corrosion\ rate\ (mil\ year^{-1}) = \frac{K \times W}{A \times T \times D} \quad \text{Eq 3. 1}$$

where, K is a constant (3.45×10^6), W, weight loss in grams; A is the exposed surface area, cm^2; T is time of exposure in hours; and D is density in g cm^{-3} of the coupon.

3.7 Grain grinding, power consumption and throughput

To simulate feed manufacturing practices in on-farm mills, only the grain was ground. After storage, wheat was ground in a hammer mill (G.J. Vis VISHM2014) finely using 2.0 mm screens or coarsely using 4.8 mm screens. For each treatment, wheat was ground in batches of 100 kg to allow for duplication when measuring grinding throughput, motor load and power consumption. Motor load was recorded from the Repete system (Repete Corporation, Wisconsin, USA) while the hammer mill was running idle, and throughout the duration of grain grinding with an interval of 10 s to allow for calculation of total power consumption (kWh). The exact weight of the grain prior to grinding and the time it took to grind this amount was recorded to estimate throughput in tonnes per hour (t h^{-1}).

3.8 Diets

Formulations for phase 1 and 2 experimental diets are outlined in Table 3.2 and Table 3.3, respectively. Experimental diets were formulated to meet or exceed NRC (2012) recommendations for 6 to 13 kg pigs and were provided in 2 phases. Phase 1 diets were fed from exp d 0 to 7 (d 0 is the 21-d weaning age; estimated body weight of 6 to 8 kg). Phase 2 diets were given to pigs from exp d 8 to 21 (estimated body weight of 8 to 13 kg). For treatments using

acid-preserved grains, ingredients were adjusted on a DM basis to account for differences in moisture content between the control grain and acid-preserved grains. Phase 2 diets contained an indigestible marker Celite (4 g kg^{-1} feed as fed basis; Celite 545, Celite Corporation, Lompoc CA, USA) as source of acid insoluble ash (AIA) to allow for estimation of apparent total tract digestibility (ATTD) of nutrients. Diets were devoid of in-feed antibiotics and animal by-products except for phase 1 where whey permeate was added as source of lactose.

Thirty-five kg of each treatment of the phase 1 diets were produced using a 40-kg capacity Hobart mixer (Hobart Food Equipment Canada, Toronto, Ontario Canada) at the Prairie Swine Centre Inc. Two hundred fifty kg of each treatment of the phase 2 diets were mixed at the Canadian Feed Research Centre using a 500 kg-capacity twin-shaft paddle mixer (SLHSJ1, UAS-Muyang). The enzyme was added at the rate of 500 g t^{-1} of feed and was pre-mixed in 500 g of ground wheat as carrier and added to appropriate treatments after 20 seconds of dry mixing, followed by the appropriate type and amount of acid for acidified diet treatments. Total mixing time for each diet was 90 s.

To determine if the treatments had a potential for a carry-over effect on performance, a common commercial diet (Master Feeds, Saskatoon, SK, Canada) was given to all pigs from experiment d 22 to 35 (body weight from 13 to 25 kg).

3.9 Sampling and data collection

3.9.1 Grinding data

A video for each grinding batch was recorded using a screen capture utility (BandiCam, Bandicam Company, Seoul, Korea). The console of the Repete system provided information on

running motor load (% of motor capacity) and actual grain weight. The video of the running time was used to estimate grinding time.

3.9.2 Grain and diet

Grain samples were collected immediately after reconstitution by grab sampling. At the end of the 34-d storage period, samples were collected from the barrels for determination of pH, mould count and chemical analysis with a sampling probe. Ground grain samples were collected during bagging, and then pooled for analysis of particle and handling characteristics. Diet samples were collected at the start of each experimental block and pooled at the end of the experiment. All grain and diet samples were double bagged to minimize moisture loss, and then stored in -20˚C prior to analysis.

EXPERIMENT 1

This experiment was conducted to determine the effect of feeding acid-preserved wheat, with or without enzymes, and its interaction with particle size on weanling pig performance.

3.10 Experimental design

The experiment was conducted at the Prairie Swine Centre Inc. (Saskatoon, SK, Canada). A total of 320 pigs (Camborough Plus females × C337 sires; PIC Canada Ltd., Winnipeg, Manitoba, Canada), were weaned at 21 ± 2 d (6.78 ± 0.64 kg body weight) and assigned to 1 of 80 pens in 4 rooms with 1 room started per week. Four pigs were housed per 123 cm long x 100 cm wide pen with plastic-covered concrete fully slatted floors. Pens were randomly assigned to 1 of 10 treatments in a randomized complete block design (8 pens per treatment) with the pen as the experimental unit and room as block. Room temperature, initially 28˚C, was gradually reduced by 2˚C for 4 weeks until 20˚C. Humidity was maintained at ~40% and a 12h-12h light-dark lighting program was implemented. No antibiotics were fed or administered during the study.

Treatments used in this experiment are summarized in Table 3.1. The pigs were on treatment diets for 21 d followed by a common diet for 14 d. The rooms were cleaned and disinfected 3 d prior to the transfer of animals. Feed and water were provided ad libitum. Feeders and pigs were checked at 0830 and 1500 h daily.

3.11 Sampling and data collection

3.11.1 Body weights and daily weight gain

Pigs were weighed individually at the start of the experiment (d 0) and at the end of every growth phase (d 7, 21, and 35). The average weight of all pigs in each pen was used for estimation of daily weight gain. Average daily gain (ADG) for a phase was calculated by dividing the difference between the initial and final body weight by the number of d the pigs were on that phase.

$$\text{ADG (g d}^{-1}\text{)} = \text{(Final weight – Initial weight) / days on feed} \qquad \text{Eq 3. 2}$$

3.11.2 Feed disappearance

The amount of feed offered, and the amount of feed left in the feeders when changing from phase 1 to phase 2 were weighed to allow for the calculation of feed disappearance to estimate average daily feed intake (ADFI). Daily feed disappearance was calculated by dividing the difference between total feed offered and feed left by the number of days in that stage.

$$\text{ADFI (g d}^{-1}\text{)} = \text{[Total feed offered (g) – Total feed left (g)] / days on feed} \qquad \text{Eq 3. 3}$$

3.11.3 Market weights

On d 35 (pig age 56), 80 pigs were ear-tagged, transferred to the grower-finishing rooms where they were housed together with the other pigs in the experiment, and fed the same commercial diet until approximately one week before market age (4 barrows and 4 gilts per treatment). Tagged pigs were weighed one week before market age and ADG was calculated and used to estimate the market weight at 165 d of age, the average market age of the pigs in the

barn. Estimated weight at 165 d of age and ADG were used to determine the potential for long-term treatment carry-over effect on the performance of pigs post nursery stage.

3.12 Analyses and calculations

3.12.1 Grinding power consumption, throughput and grinding cost

The hammer mill motor (NIMA Premium Efficiency Model, WEG Industries, Jaragua do Sul, Brazil) used in this experiment had a rated capacity of 37.3 kW (50 HP) with an efficiency of 94.1%. Power consumption while the motor was running idle and during grinding was estimated by multiplying their respective motor load with the rated capacity (kW) and motor efficiency. Gross power consumption during grinding was calculated as:

$$\text{Power consumption} = \sum P_i t_i \qquad \text{Eq 3. 4}$$

where P is the power draw (kW) and t is time interval (s) then expressed as kWh.

Net power consumption due to grinding was estimated by deducting the idle power consumption from the gross power consumption during grinding.

Specific power consumption (kWh t^{-1}) was estimated by dividing the net power consumed (kWh) during grinding by the weight of the grain (t) that was ground. Grinding cost was calculated by multiplying the specific power consumption with the power cost of $0.116 per kWh (current cost at the Canadian Feed Research Centre, North Battleford; 2018). Throughput was calculated by dividing the actual amount of grain (kg) and the time it took to grind (seconds), expressed in t h^{-1}.

3.12.2 Chemical analyses of grains and diets

Proximate, Ca, and P analyses of grains and diets were conducted at the Central Testing Laboratories in Winnipeg, Manitoba. Proximate analysis included DM (AOAC 930.15), N

(AOAC 990.03), crude fibre (AOCS Ba6a-05), crude fat (AOCS Am 5-04) and ash (AOAC 923.03). Calcium and P were analyzed using AOAC, 968.08, 935.13A and 985.01. Acid detergent fiber (ANKOM 08-16-06) and NDF (ANKOM 08-16-06) were also measured. Because there was mould growth after storage in the OIB-preserved wheat, obviously contaminated grains were removed prior to grinding. Ground samples from the OIB and Prop preserved grains, and control wheat were analyzed for mycotoxin profile (Prairie Diagnostics Services, Saskatoon, SK). Mycotoxin levels were below maximum allowable levels for swine as recommended by CFIA (Available: http://www.inspection.gc.ca/animals/feeds/regulatory-guidance/rg-8/eng/1347383943203/1347384015909?chap=1) and were considered safe to use in these trials.

Grain and diets were analyzed for pH using the method described by Radecki et al. (1988). Briefly, 5 g of grain or diet were mixed with 10 ml of deionized water and allowed to stand for 10 minutes with periodic shaking prior to pH measurement using a pH meter (Oakton pH 110 Series, Eutech Instruments, Thermo Fisher Scientific, Waltham, Massachusetts, USA).

3.12.3 Particle size analysis

Frozen grain samples were thawed to room temperature prior to analysis. Particle size of the ground grain were analyzed in duplicate using the procedure outlined by ASAE (S319.4 2012) but using a stack of 11 sieves instead of 13 as the rotary sieve shaker can accommodate only 12 stacks, receiving pan included. The sieves US 6 and US 270 were not included, since less than 5% of the sample are retained on these sieves. The stack included the 11 sieves and a receiving pan. The 11 sieves used had hole diameters of 2,380 µm (US 8), 1,680 µm (US 10), 1,191 µm (US 14), 841 µm (US 20), 594 µm (US 28), 500 µm (US 35), 297 µm (US 48), 212 µm

(US 65), 149 µm (US 100), 103 µm (US 150), 74 µm (US 200). Sieves were stacked in order of decreasing screen size, with largest sieve size on top and a receiving pan at the bottom. One hundred grams of a sample was dispensed on the top of the stack and shaken in the rotary sieve shaker for of 10 min (Rotary Lab Sifter, Hoskin Scientific, Canada). The mass remaining on each sieve was weighed and used to calculate geometric mean diameter (dg_w) and particle size standard deviation (sg_w) using the equations described in ASAE (2012).

3.12.4 Angle of repose, coefficient of friction and bulk density

Angle of repose, coefficient of friction and bulk density were analyzed at the BioProcessing Engineering laboratory at the University of Saskatchewan. All samples were analyzed in triplicate.

Emptying angle of repose was determined to characterize the flowability of the ground grain as it exits the bins. It was measured using a clear plastic cylinder with a height of 210 mm and diameter of 145 mm. The cylinder has a false floor 60 mm from the bottom, with a 25.4 mm-diameter hole in the center where the sample was drained. Five hundred grams of sample was loaded in the vessel, leveled, and allowed to drain through the hole in the false floor forming a funnel shaped crater. The vessel was tapped gently if the sample did not drain. The shortest and farthest distance between the crater edge and the drain, and their respective height were measured, averaged, and used to calculate the angle of repose using eq. 3.4.

$$\text{Angle of repose} = \tan^{-1} [\text{opposite (height of crater)} / \text{adjacent (drain to crater)}] \quad \text{Eq 3. 5}$$

Coefficient of friction was measured using an inclining platform with a steel surface to simulate bin and feeder surfaces. A 2 x 2 x 1-inch (l x w x h) wooden frame was filled with the sample, levelled and the excess removed. The frame was slightly lifted to ensure only the sample

touched the steel surface. The platform was inclined slowly (1 full turn of crank shaft in 6 seconds) and the angle (θ) at which the sample started to move along the steel surface was recorded and used to compute for the coefficient of friction using eq. 3.5.

Coefficient of friction = tan θ Eq 3. 6

Bulk density was determined using a Cox funnel, a 0.5 L cup and a wooden striker following the procedure for determining test weight outlined by Canadian Grain Commission (www.grainscanada.gc.ca, accessed: 24 July 2018). Briefly, 500 grams of sample was dropped in the Cox funnel to standardize the flow of the material to the cup then levelled using the wooden striker with three equal zigzag motions. The sample in the cup was weighed and bulk density was computed using eq. 3.6. Bulk density was reported in kg m^{-3} (1 kg m^{-3}= 0.001 kg L^{-1}).

Bulk density (kg L^{-1}) = weight sample (kg) / 0.5 L Eq 3. 7

3.13 Statistical analyses

All residual error data were checked for normality of distribution by Shapiro-Wilk test using Proc Univariate in SAS (SAS 9.4; SAS Institute, Cary, NC). Data were transformed accordingly when $P < 0.05$. Reported P-values were derived from the transformed data, and the least square means were from the untransformed data.

Grinding characteristics and ground grain particle and handling characteristics were analyzed as a completely randomized design using Proc Mixed in SAS with the model stated below:

$$Y_i = \mu + \alpha_i + \varepsilon_i$$

where Y is the parameter to be tested, μ the mean, α_i the effect of treatment and ε_i is the experimental error.

Animal performance parameters were analyzed as a randomized complete block design with the fixed effect of treatment (10 treatments) and random effect of block (4 blocks) using the statistical model:

$$Yij=\mu + \rho_i + \alpha_j + \varepsilon_{ij}$$

where Y is the parameter, μ the overall mean, ρ_i the random effect of the i^{th} block, α_j the fixed effect of the j^{th} treatment, ε_{ij}=error term associated to the j^{th} treatment and i^{th} block.

Data were discussed relative to a protected and an unprotected F-test (Barnette and McLean 1998) using single degree of freedom contrasts to compare treatments and interactions of interest (Marini 2003). These are:

- Effect of acidification
    - Diet 1 vs diet 5 and diet 9 (NC vs diets with OIB, without enzyme)
    - Diet 2 vs diet 3 and diet 7 (PC vs diets with OIB, with enzyme)
- Type of acid and interaction with MOA
    - Diet 3 and 7 vs diet 6 and 10
- Mode of acid addition
    - Diet 3, 4, 5, 6 vs. diet 7,8,9,10 (all APW vs. all AD)
- Effect of enzyme and interaction with MOA
    - Diet 1 vs diet 2 (NC vs PC)
    - Diet 3 and 7 vs diet 5 and 9
- Effect of particle size and interaction with MOA
    - Diet 3 and 7 vs diet 4 and 8

P-values for d 7 G:F and d 22-35 ADFI were derived from square root transformation of data prior to ANOVA. Means were separated using Tukey's test. In all cases, $P < 0.05$ was considered significant, with values between 0.05 and 0.10 a tendency

RESULTS

Chemical analyses of the grains are presented in Table 3.4. Mould was observed at the top of the barrel in OIB, but not Prop-preserved wheat. Mycotoxin levels from all grain samples were either below maximum allowable limits, or not detected (Table 3.5)

Chemical analyses for phase 1 and phase 2 diets are presented in Table 3.6 and Table 3.7, respectively. Dry matter of the ground, acid-preserved high-moisture wheat (APW) ranged from 830.5 to 836.5 g kg^{-1} grain (wet basis; w.b.), while the control dry wheat had 882.5 g kg^{-1}. On average, the DM of diets with dry control wheat was higher than diets using APW by 15.1 and 16.0 g kg^{-1} of diet (w.b.) for phases 1 and 2 respectively. The pH of the acid-preserved high-moisture wheat was lower than the untreated control and the pH of the Prop-preserved wheat was lower than those preserved using OIB. The pH of all the phase 1 diets ranged from 5.52 to 5.56. In phase 2 diets, the pH of the control diets ranged from 5.88 to 6.15 and were higher than the diets with acid. In AD treatments, pH ranged from 5.37 to 5.62, while the pH in diets with APW ranged from 5.34 to 5.67. In both groups, diets with Prop had the lowest diet pH.

3.14 Particle and handling characteristics of ground wheat, and corrosion rate of steel coupons

The grinding characteristics of the whole wheat and particle and handling characteristics of the ground wheat used in this study are presented in Table 3.8.

Finely-ground APW had the lowest grinding throughput while the highest was observed with the coarsely-ground dry wheat ($P < 0.01$). Throughput of finely-ground dry wheat was intermediate and was not different from coarsely-ground OIB-preserved high-moisture wheat.

The power consumption of finely-ground APW were higher than either finely or coarsely ground dry wheat, while the coarsely ground APW had similar power consumption with finely ground dry wheat and higher than coarsely ground dry wheat ($P < 0.01$). However, finely-ground dry wheat had the same grinding cost as coarsely ground APW. Grinding cost followed the trend in power consumption wherein the grinding costs of finely-ground APW were higher compared the finely ground dry wheat, and the grinding cost of coarse APW was higher than dry wheat ground coarsely ($P < 0.01$). Finely ground dry wheat had the same grinding cost as the APW ground coarse.

The mean geometric particle size (d_{gw}) of grains ground using 4.8 mm screen were higher than grains ground using 2.0 mm screen ($P < 0.01$). Bulk density was highest in coarsely ground dry wheat followed by finely ground dry wheat. The bulk density of the finely ground high-moisture grains was lower than the dry ground wheat regardless of particle size. Coarsely ground dry wheat and APW had the lowest angle of repose while the highest was observed from finely ground dry wheat. Angle of repose of finely ground APW was intermediate. Coefficient of friction in coarsely ground dry wheat was similar to coarsely ground APW. The highest coefficient of friction was observed with finely ground dry wheat, but this was not different from finely ground APW.

Corrosion rates of carbon and galvanized steel coupons exposed to either OIB or Prop are presented in Table 3.9. Propionic acid was more corrosive compared to OIB on carbon but not galvanized steel ($P < 0.01$).

3.15 Animal performance

Growth rates, feed intake and feed efficiency of pigs fed control diets, diets with acid-preserved wheat (APW), or acidified diets (AD) are presented in Table 3.10. P-values for treatment contrasts and main treatment effects are shown in Table 3.11.

Growth rate was unaffected by treatment during phase 1 ($P = 0.77$). Pigs fed the positive control (PC) had improved feed intake compared to the negative control (NC; $P < 0.05$).

During phase 2 (d 8 to 21), pigs fed AD-Prop-Enz-Fine had the highest ADG and were higher than pigs fed the NC, and Coarse diets (APW-OIB-Coarse-Enz and AD-OIB-Coarse-Enz), but was similar to those fed PC, APW-OIB-Fine-Enz, APW-OIB-Fine-NoEnz, APW-Prop-Fine-Enz, AD-OIB-Fine-Enz and AD-OIB-Fine-NoEnz ($P < 0.01$). Contrasts demonstrated that pigs fed PC had higher growth rate compared to pigs fed NC (effect of enzyme, $P < 0.01$); pigs fed OIB-NoEnz regardless of MOA had higher ADG compared to pigs fed NC (effect of OIB, $P < 0.01$); pigs fed Prop regardless of MOA had higher ADG compared to those fed OIB (OIB vs Prop, $P < 0.05$); and pigs fed Fine PS had higher ADG compared to those fed Coarse (effect of PS, $P < 0.05$). Pigs fed diets with the acid-preserved wheat had similar ADG compared to pigs fed AD containing control wheat.

There were no differences in ADFI among treatments during phase 2 ($P = 0.74$).

Feed efficiency followed the same trend as growth rates. Pigs fed AD-Prop-Fine-Enz had higher G:F compared to pigs fed NC or Coarse diets (APW-OIB-Coarse-Enz, AD-OIB-Coarse-Enz) and APW-OIB-Fine-Enz, but were similar when compared to pigs fed PC, APW-OIB-Fine-NoEnz, APW-Prop-Fine-Enz, and AD-OIB-Fine-NoEnz ($P < 0.01$). The contrasts showed that pigs fed PC had higher G:F compared to pigs fed NC (enzyme, $P < 0.01$); pigs fed OIB-NoEnz regardless of MOA had better G:F compared to pigs fed NC (OIB, $P < 0.01$); pigs fed Prop had

higher G:F compared to those fed OIB (OIB vs Prop, $P < 0.05$); and pigs fed Fine had higher G:F compared to those fed coarse (particle size, $P < 0.05$).

During phase 3, pigs were fed a common commercial diet to determine the potential for a carryover effect of treatments from phases 1 and 2. In addition, 8 pigs from each treatment were tagged after d 35 and weighed at market to determine possible long-term carryover effects on growth rate. Treatment had no effect on growth rate, feed intake or G:F of pigs during d 22 to 35 ($P = 0.91$). Similarly, treatments had no effect on ADG from d 36 to market ($P = 0.97$) or market weight ($P = 0.92$).

DISCUSSION

Acidification of high-moisture grains is an alternative to artificial drying to preserve quality during storage. In this study, wheat was reconstituted to 20% moisture and preserved using either Prop or OIB (phosphoric, lactic, citric and malic acids) and stored for 34 d. Mould growth in the OIB-preserved grains when stored in barrels was not expected as there were no indications of mould growths when either of the acids were used in an in-vitro jar experiment conducted previously. It is possible that there was more oxygen in the barrels compared to the jars, supporting mould growth. In the in-vitro experiments, incubation jars were opened only at the allocated time point preventing the re-introduction of oxygen into the system. Conversely, when acid-preserved grains were stored in barrels, grain samples were collected weekly from each barrel, allowing repeated oxygen re-introduction. No efforts were made to exclude oxygen as one of the advantages of using acidification as a preservation method is that there is neither a need for airtight structures, nor to remove oxygen from the storage system (Lynch et al. 1975; McLelland 2008; Jokiniemi et al. 2014). These observations suggest that in the current

experiment, the OIB was not able to arrest microbial activity during storage of high-moisture wheat and requires further investigation. The limitation of our method is that dry wheat was reconstituted to achieve high-moisture prior to acidification, as opposed to grain that was harvested at high-moisture. Moisture distribution between the two types of grain may be different; moisture in the reconstituted grain may be concentrated on the surface of the grains, whereas grains harvested at high-moisture, the moisture is bound within the grain. Mould count for the OIB preserved wheat was about 7000 CFU g^{-1}, lower than what is considered safe for forages (500,000 CFU g^{-1}; Adams et al. 1993). Mycotoxin levels were either undetectable or below maximum acceptable limits.

Aside from the acid's ability to reduce pH, the anti-microbial function of an organic acid depends on its pKa, which is defined as the pH at which 50% of the acids are undissociated. The undissociated form of the organic acid can freely pass through the cell membrane of bacteria and moulds into the cytoplasm which is maintained at pH 7. Once inside, the acid dissociates and reduces the pH of the cytoplasm, inhibiting the action of pH dependent enzymes. Furthermore, as a response to the drop in pH, the microbial cell eliminates excess protons by activating the H^+-ATPase pump which is energy consuming. Also, the buildup of the anions inside the cell is thought to be toxic. Combined, these mechanisms inhibit the growth and proliferation of the microorganisms. When the environmental pH is lower than the acid's pKa, most of the acids are in an un-dissociated form, therefore antimicrobial ability increases. The pH of the wheat preserved with Prop in the current experiment was 4.85 and was slightly lower than 4.87 which is the pKa of Prop (O'Neil 2006). Therefore at least 50 % of Prop was in an undissociated form which could explain why mould growth was not observed. The acid components of OIB (and their pKas) were: Phosphoric acid (2.15, 7.09 and 12.32); LA (3.86); citric acid (3.13, 4.76, and

6.4); and malic acid (3.40 and 5.11; O'Neil 2006). The pH of wheat preserved with OIB ranged from pH of 5.68 to 5.72. It is possible that due to the high pH of the grains using OIB, more of the organic acids were in dissociated form and were not able to function as an anti-microbial, hence the mould growth.

Prior to feed production, dry and high-moisture wheat were ground through a hammer mill using either a 2.0- or 4.8-mm screen, and the cost of grinding, particle characteristics and flowability of the ground grains were determined. Probst et al. (2013) suggested that moisture content is the most important property of a material that affects the ease of grinding and cost when using a hammer mill. They reported that although grinding throughput was not affected, total energy consumption was 214% higher in corn with 20% compared to 15% moisture. The higher power consumption and lower throughput was attributed to longer retention of high-moisture corn in the grinding chamber of the hammer mill which reduced rotor speed due to loading and friction (Probst et al. 2013). An increase in moisture in grains such as corn and wheat increases plasticity and viscosity, making it difficult and required more time to grind (Dziki and Laskowski 2005; Lupu et al. 2016). In the current study, APW had a higher grinding cost than dry wheat because of a lower throughput and higher power consumption. The power consumption of the high-moisture wheat was higher than the dry wheat by 96 and 84% when ground through a 2.0 mm or 4.8 mm screen respectively, agreeing with Probst et al. (2013). However, the grinding throughput in the current study was reduced by 46 and 26% when ground fine or coarse, respectively, which was not observed with high-moisture corn in the study of Probst et al. (2013). However, the latter was conducted under laboratory conditions, grinding 500 g of corn grains through a 1.6 mm screen, and a 1.49 kW motor. The current trial was conducted under conditions closer to industrial-scale; used 100 kg of wheat grains, ground though either a

2.0 or a 4.8 mm screen using a 37.29 kW motor.

Similar to Probst et al. (2013) the mean geometric particle size (d_{gw}) in the current experiment did not differ between control wheat and APW when ground using the same screen size. The current experiment showed similar s_{gw} between the APW and dry control wheat while a significant reduction in s_{gw} was noted with increased moisture content in their experiment. It is possible that because the ground dry wheat samples had been frozen, condensation within the bag may have formed during thawing, wetting the grains. Probst et al. (2013) attributed the narrower s_{gw} in the 20% moisture ground corn to the reduction in the amount of dust generated during grinding and amount of unground corn compared to those with 15% moisture (Probst et al. 2013).

Bulk density was highest in dry wheat that was ground coarsely followed by dry wheat that was ground finely and may be attributed the higher s_{gw} in the coarsely ground compared to the finely ground dry wheat. Higher s_{gw} means more coarse and fine particles in the mix. It is possible that fine particles were able to fill the air spaces between larger sized-wheat particles. The bulk density of the finely ground high-moisture grains was lower than the dry ground wheat regardless of particle size. Moisture is less dense than the DM component in the grain (Stroshine 2004) thus ground APW had lower bulk density compared to the ground dry wheat. Moisture may negatively affect flowability of grains due to the formation of interparticle bonds that increase cohesion and adhesion of particles (Moreyra and Peleg 1981; Yan and Barbosa-Canovas 1997). However, in the current experiment, flowabilty, as measured by emptying angle of repose and coefficient for friction, was not affected by moisture content. Similarly, Probst et al. (2013) reported that moisture content did not affect the angle of repose or coefficient of friction in ground corn while Chowdhury et al. (2001), reported a non-linear increase in angle of repose

with increased moisture content of chickpea, a pulse grain commonly used in Bangladesh. Fiber is associated with water holding capacity (Robertson and Eastwood 1980) therefore the difference in these observations is possibly due to the higher fiber content of chickpea compared to corn (NDF 22.28 vs 12.20; www.Feedipedia.org, accessed: 24 July 2018), and the different moisture contents used in these experiments. Probst et al. (2013) used a moisture of 10 to 20% in corn, while Chowdhury et al. (2001) used 10 to 31 % in chickpea.

Tung and Pettigrew (2006) reviewed 50 studies investigating the supplementation of organic acids on performance of pigs at 0 to 2 weeks and 78 studies at 0 to 4 weeks post-weaning. Out of the 50 studies for 0 to 2 weeks post-weaning, 72% reported positive improvements in growth rate, 24% reported a negative response, and 2% reported equal growth rates compared to the control. On average, acidifiers in weanling pig diets improved weanling pig growth rates by 12 % during the first 2 weeks after weaning, and 6 % during 0 to 4 weeks postweaning (Tung and Pettigrew 2006). Although the exact mode of action for this improvement has not been identified (Jacela et al. 2009; Liu et al. 2018), proposed modes of action include: 1) reduction in pH of the digesta resulting in the activation of digestive enzymes and a slower gastric emptying rate consequently increasing nutrient digestibility, and 2) direct killing of pathogenic bacteria, modifying microbial populations (Ravindran and Kornegay 1993; Partanen and Mroz 1999; Suiryanrayna and Ramana 2015).

In the current study, the ADG of pigs fed control diets, diets with APW and AD were similar during the first 7 d post-weaning. This can be attributed to the presence of lactose in all phase 1 diets given during this period. Apart from lactose being more digestible than carbohydrates of plant origin, it is also fermented into LA decreasing pH in the stomach of the pig (Maner et al. 1962; Wilson and Leibholz 1981; Ravindran and Kornegay 1993). Therefore,

minimal growth promoting responses may be expected from organic acid supplementation in milk-based diets (Giesting et al. 1991; Weeden et al. 1991; Metzler and Mosenthin 2009). In contrast to pigs fed the NC, the higher ADFI of pigs fed the PC suggest that the addition of the enzyme mix improved ADFI in pigs 0 to 7 d post-weaning in a diet not supplemented with an acidifier.

Feeding pigs diets acidified with either OIB or Prop improved ADG (in Prop) and G:F (in both OIB and Prop) compared to those fed the negative control during phase 2. Similarly, improvements in ADG and G:F were observed with the addition of enzyme in the diet (NC vs PC). The improvement in ADG and G:F with the use of Prop agrees with Gabert and Sauer (1994) who reviewed studies investigating organic acid supplementation of weanling pig diets. They reported an improvement of 13.8 % in ADG when Prop was added at 1 % of the diet, and 7.8% ($P < 0.05$) in G:F when added at 2 % compared to piglets fed a non-acidified control. The improvement in G:F in pigs fed OIB does not agree with Metzler and Mosenthin (2007) who reported that phosphoric acid did not improve growth rate or feed conversion of pigs even though there was a reduction in gastric pH. The improvements observed with the use of acids or enzyme are exclusive as these were not observed when comparing the PC to wheat-based diets where acid and enzymes were both present.

Comparing the two acids, pigs fed Prop had higher growth rates and feed efficiency compared to pigs fed OIB regardless of mode of acid addition. Except for a non-peer reviewed study comparing Ca propionate and a phosphoric acid-based acidifier (Digestocarp: phosphoric, citric and fumaric acid blend) in pigs from 5 to 9 weeks of age (Schutte and de Jong 1988 as cited by Peris and Calafat 2001), to our knowledge, there is no other comparison in the literature of propionic acid to phosphoric acid-based blend on performance of weanling pigs. Contrary to

the current study, Schutte and de Jong (1988), reported that the phosphoric acid blend resulted in 2.9% higher weight gain, with similar feed intake and feed:gain (F:G) ratio compared to Ca propionate.

Feeding weanling pigs a finely ground diet improved growth rates and G:F compared to those fed coarse diets during phase 2. The improvement in ADG and G:F of pigs fed AD-Prop-Fine-Enz compared to the OIB-Coarse-Enz regardless of mode of addition can be attributed more to particle size than the acid, the enzyme or their combination. This is based on the observation that pigs fed AD-Prop-Fine-Enz had similar ADG and G:F as OIB-Fine-Enz regardless of mode of addition.

The improved growth rates and G:F when pigs were fed finely ground diets, suggests improvement in nutrient digestibility in the fine diets, possibly due to the increase in surface area with finer particle size. Treatment had no effect on ADFI, however, growth rates were improved in AD-Prop-Fine Enz compared to NC and the coarse ground diets (APW-OIB-Coarse-Enz and AD-OIB-Coarse-Enz) and could be an indication of improvements in nutrient digestibility.

The similar ADG, ADFI and GF on d 22 to 35 indicates that treatments had no carry-over effect on performance of nursery pigs. Market weights and growth rates were likewise similar among treatments, indicating that treatments during phase 1 and 2 had no long-term effect on performance of pigs.

SUMMARY AND CONCLUSIONS

In summary, propionic acid may be more effective as a preservative compared to the organic-inorganic acid blend for high-moisture grains. However, this needs further investigation

because the grains used in the current experiment were reconstituted, and the efficacy of the OIB may be different when the grains are harvested at high-moisture. In tough or high-moisture grains, moisture will be bound within the grain, whereas moisture in reconstituted grains may be unbound to grain constituents and concentrated on the surface of the grain. Both acids were equally corrosive to galvanized steel, but propionic acid was more corrosive than phosphoric acid on carbon steel. In the current experiment, despite storing the grains in a cool, dry environment, mould was observed, but mycotoxin levels remained low. Potential for mould development if the high-moisture grains were stored in bins and conditions similar to what can be found in barns requires further investigation.

The use of acid-preserved high-moisture wheat may increase grinding cost by \$0.86 t^{-1} (84%; Coarse) or \$0.89 t^{-1} (97%; Fine) compared to dry wheat ground using the same screen and this should be considered prior to using acid-preserved high-moisture grains. Flowability was not affected by moisture content but was affected by particle size. Acids (Prop and OIB) improved weanling pig performance with no indications of synergism with the exogenous enzyme. Because the pigs fed acidified diets had the same performance as pigs fed diets with APW, we can conclude that the benefits of diet acidification are maintained when APW is used, and that fine grinding may provide better performance compared to coarse.

FIGURES

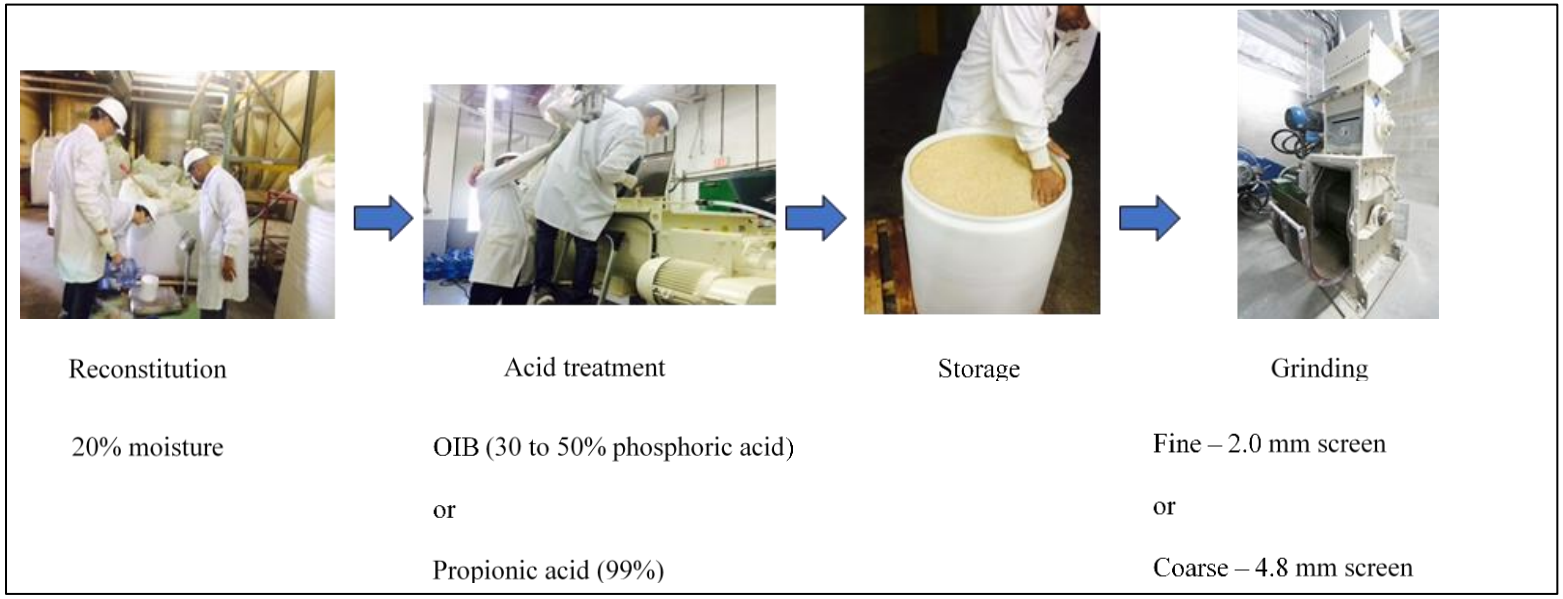

Figure 3.2. Summary of grain pre-treatment protocol.

TABLES

Table 3.1. Treatment description of diets in the wheat experiments.

Treatments	Application	Acid	Enzyme	Particle size (screen)
T1. Negative Control, NC	None	None	NoEnz	Fine (2.0 mm)
T2. Positive Control, PC	None	None	Enz	Fine (2.0 mm)
T3. APW-OIB-Fine-Enz	Acid-preserved wheat, APW	Phosphoric	Enz	Fine (2.0 mm)
T4. APW-OIB-Fine-Enz	Acid-preserved wheat, APW	Phosphoric	Enz	Coarse (4.8 mm)
T5. APW-OIB-Fine-NoEnz	Acid-preserved wheat, APW	Phosphoric	NoEnz	Fine (2.0 mm)
T6. APW-Prop-Fine-Enz	Acid-preserved wheat, APW	Propionic	Enz	Fine (2.0 mm)
T7. AD-OIB-Fine-Enz	Acidified diet, AD	Phosphoric	Enz	Fine (2.0 mm)
T8. AD-OIB-Coarse-Enz	Acidified diet, AD	Phosphoric	Enz	Coarse (4.8 mm)
T9. AD-OIB-Fine-NoEnz	Acidified diet, AD	Phosphoric	NoEnz	Fine (2.0 mm)
T10. AD-Prop-Fine-Enz	Acidified diet, AD	Propionic	Enz	Fine (2.0 mm)

Table 3.2. Ingredient composition of phase 1 wheat-based starter diets.

Treatments	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
MOA	Control[a]		Acid-preserved Wheat, APW[b]				Acidified diet, AD			
Acid[c]	(-)	(-)	OIB			Prop	OIB			Prop
Enzyme	NoEnz	Enz	Enz	Enz	NoEnz	Enz	Enz	Enz	NoEnz	Enz
Particle size[d]	Fine	Fine	Fine	Coarse	Fine	Fine	Fine	Coarse	Fine	Fine
					g kg^{-1} as fed basis					
Wheat	438.8		454.3				438.8			
Soybean meal	216.9		210.9				216.9			
Whey permeate	117.6		114.4				117.7			
Canola meal	100.0		97.2				100.0			
Peas	50.0		48.6				50.0			
Canola oil	26.8		26.0				26.8			
Limestone	13.9		13.6				13.9			
Dicalcium phosphate	11.5		11.2				11.5			
L-lysine (98.5%)	6.9		6.7				6.9			
Salt	6.6		6.4				6.6			

Celite[f]	4.0		3.9				4.0			
L-threonine (98.5%)	2.3		2.2				2.3			
DL-methionine (99%)	2.1		2.0				2.0			
Vit and min premix[e]	1.5		1.5				1.5			
Choline chloride (60%)	1.1		1.1				1.1			
OIB				3.1		3.1		3.1		
Propionic Acid										3.3
Enzymes[g]		0.2		0.2		0.2				0.2

Note: MOA, mode of acid addition; OIB, organic-inorganic acid blend; Prop, propionic acid; NoEnz without enzyme; Enz with enzyme; HCl, hydrochloric acid; Vit, vitamin; Min, mineral.

[a]Wheat control, DM of 882.5 g kg^{-1}, APW DM 830.5 g kg^{-1}.

[b]Reconstituted to 20% moisture and stored in polyethylene barrels for 34 d. Acid and enzyme were added during reconstitution.

[c]Acid composition: OIB; 30 to 50% phosphoric acid, 0.1 to 1% lactic, 5 to 10% citric and 1 to 5% malic acid (Canadian BioSystems, Calgary, Alberta, Canada). Prop: propionic Acid 99% (Anachemia, Montreal, Quebec, Canada).

[d]Fine particle size, grain was ground using 2.0 mm screen; coarse using 4.8 mm screen.

[e]Per kg of the starter premix: Vit A, 8,000,000 IU; Vit D3, 750,000 IU, Vit E, 35,000 mg; Vit K, 2,500 mg; Vit B1, 1,000 mg; Vit B2, 4,000 mg; Vit B3, 20,000 mg; Vit B5, 12,000 mg; Vit B6, 5,000 mg; Vit B7, 100 mg; Vit B9, 500 mg; Vit B12, 20 mg; Fe, 75,000 mg; Zn, 75,000 mg; Mn, 20,000 mg; Cu, 10,000 mg, Se, 150 mg; I, 500 mg.

[f]Source of acid insoluble ash, Celite Corporation, Lompoc CA, USA.

[g]Superzyme Plus phytase and multi-carbohydrase enzyme (Canadian BioSystems, Calgary, Alberta, Canada).

Table 3.3. Ingredient composition of wheat-based phase 2 starter diets

Treatments	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
MOA	Control[a]		Acid-preserved Wheat, APW[b]				Acidified diet, AD			
Acid[c]	(-)	(-)	OIB			Prop	OIB			Prop
Enzyme	NoEnz	Enz	Enz	Enz	NoEnz	Enz	Enz	Enz	NoEnz	Enz
Particle size[d]	Fine	Fine	Fine	Coarse	Fine	Fine	Fine	Coarse	Fine	Fine
				g kg^{-1} as fed basis						
Wheat	590.6		602.8				590.6			
Soybean meal	188.2		182.6				188.2			
Canola meal	100.0		97.0				100.0			
Peas	50.0		48.5				50.0			
Canola oil	25.3		24.6				25.3			
Limestone	14.4		13.9				14.4			
Salt	8.1		7.9				8.1			
Dicalcium phosphate	9.4		9.1				9.4			

L-lysine HCl (98.5%)	5.4	5.2			5.4	
Celite[e]	4.0	3.9			4.0	
Vit and min premix[f]	1.5	1.5			1.5	
L-threonine (98.5%)	1.4	1.4			1.4	
DL-methionine (99%)	0.9	0.9			0.9	
Choline chloride (60%)	0.9	0.9			0.9	
OIB			4.1	4.1	4.1	
Propionic acid						4.4
Enzymes[g]	0.3		0.3	0.3		0.3

Note: MOA, mode of acid addition; OIB, organic-inorganic acid blend; Prop, propionic acid; NoEnz without enzyme; Enz with enzyme; HCl, hydrochloric acid; Vit, vitamin; Min, mineral.

[a]Wheat control, DM of 882.5 g kg^{-1}, acid-preserved wheat DM 830.5 g kg^{-1}.

[b]Reconstituted to 20% moisture and stored in polyethylene barrels for 34 d. Acid and enzyme were added during reconstitution.

[c]Acid composition: OIB; 30 to 50% phosphoric acid, 0.1 to 1% lactic, 5 to 10% citric and 1 to 5% malic acid (Canadian BioSystems, Calgary, Alberta, Canada). Prop: propionic Acid 99% (Anachemia, Montreal, Quebec, Canada).

[d]Fine particle size, grain was ground using 2.0 mm screen; coarse using 4.8 mm screen.

[e]Source of acid insoluble ash, Celite Corporation, Lompoc CA, USA.

[f]Per kg of the starter premix: Vit A, 8,000,000 IU; Vit D3, 750,000 IU, Vit E, 35,000 mg; Vit K, 2,500 mg; Vit B1, 1,000 mg; Vit B2, 4,000 mg; Vit B3, 20,000 mg; Vit B5, 12,000 mg; Vit B6, 5,000 mg; Vit B7, 100 mg; Vit B9, 500 mg; Vit B12, 20 mg; Fe, 75,000 mg; Zn, 75,000 mg; Mn, 20,000 mg; Cu, 10,000 mg, Se, 150 mg; I, 500 mg.

[g]Superzyme Plus phytase and multi-carbohydrase enzyme (Canadian BioSystems, Calgary, Alberta, Canada).

Table 3.4. Chemical analyses of control or high-moisture wheat preserved for 34 d with either OIB (with or without enzyme) or propionic acid. [a]

Wheat	Control[b]	Acid-preserved wheat, APW		
Acid	None	OIB		Prop
Enzyme	NoEnz	Enz[c]	NoEnz	Enz
pH[d]	6.24	5.72	5.68	4.85
DM (g kg^{-1} wheat)	882.5	830.5	833.1	836.5
CP (g kg^{-1} DM)	160.4	161.7	159.5	163.8
CF (g kg^{-1} DM)	35.3	38.9	37.3	36.5
EE (g kg^{-1} DM)	15.7	13.8	10.3	16.3
Ash (g kg^{-1} DM)	18.7	18.0	19.7	18.8
Ca (g kg^{-1} DM)	0.7	0.7	0.5	0.5
P (g kg^{-1} DM)	4.4	4.5	4.3	4.1
ADF (g kg^{-1} DM)	32.5	34.3	42.0	31.2
NDF (g kg^{-1} DM)	120.7	124.4	126.5	113.6

Note: OIB, organic-inorganic acid blend; Prop, propionic acid; NoEnz, no enzyme; Enz, with enzyme; DM, dry matter; CP, crude protein; CF, crude fiber; EE, ether extract; Ca, calcium; P, phosphorus; ADF, acid detergent fiber; NDF, neutral detergent fiber

[a]Analyzed at the Central Testing Laboratory, Winnipeg, Manitoba (with the exception of pH).

[b]Untreated wheat used for negative and positive controls, and acidified diets.

[c]Acid-preserved wheat used for diet T3 and T4 and differed only in particle size.

[d]Analyzed at the General Animal Nutrition Laboratory, Department of Animal and Poultry Science, University of Saskatchewan.

Table 3.5. Mycotoxin analyses of control or high-moisture wheat with enzyme preserved with either OIB or propionic acid.[a]

Wheat	Control	Acid-preserved wheat, APW	
Acid	None	OIB[c]	Prop
Enzyme	NoEnz	Enz	Enz
		µg kg^{-1}	
Deoxynivalenol	173.8	146.0	116.9
3-Acetyl-deoxynivalenol	25.5	<25.0	<25.0
15-Acetyl-deoxynivalenol	<25.0	<25.0	<25.0
Diacetoxyscirpenol	<25.0	<25.0	<25.0
Nivalenol	35.2	<25.0	<25.0
T-2 Toxin	<25.0	<25.0	<25.0
HT-2 Toxin	<25.0	<25.0	<25.0
α-Zearalenol	<66.0	<66.0	<66.0
β-Zearalenol	<66.0	<66.0	<66.0
Aflatoxin B1	<25.0	<25.0	<25.0
Fumonisin B1	<25.0	<25.0	<25.0
Zearalenone	<25.0	<25.0	<25.0

Note: OIB, phosphoric acid, Prop, propionic acid; NoEnz, no enzyme; Enz, with enzyme; DM, dry matter.

[a]Analyzed at Prairie Diagnostic Services, Saskatoon, SK, Canada using HPLC-MS.

Table 3.6. Chemical analyses of phase 1 control diets, diets containing acid-preserved wheat (APW), or acidified diets (AD).[a]

Treatments	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
MOA	Control		Acid-preserved Wheat, APW[b]				Acidified diet, AD			
Acid[c]	(-)	(-)	OIB			Prop	OIB			Prop
Enzyme[d]	NoEnz	Enz	Enz	Enz	NoEnz	Enz	Enz	Enz	NoEnz	Enz
Particle Size[e]	Fine	Fine	Fine	Coarse	Fine	Fine	Fine	Coarse	Fine	Fine
pH[f]	5.52	5.52	5.52	5.55	5.53	5.54	5.55	5.56	5.55	5.56
DM (g kg^{-1} diet) [f]	889.1	888.1	874.3	868.5	876.1	876.2	887.7	889.7	887.7	891.1
CP (g kg^{-1} DM)	254.5	252.1	262.8	265.9	253.4	242.2	256.6	253.1	252.7	256.5
CF (g kg^{-1} DM)	47.4	47.3	51.7	50.7	53.8	48.0	46.2	49.4	49.6	51.7
EE (g kg^{-1} DM)	45.9	49.4	50.2	46.1	43.0	46.6	48.3	47.0	46.7	45.6
Ash (g kg^{-1} DM)	73.0	72.1	74.9	77.6	75.2	74.8	75.9	77.0	75.5	73.8
Ca (g kg^{-1} DM)	11.2	11.7	11.7	12.4	11.3	12.0	11.7	12.5	12.4	11.0
P (g kg^{-1} DM)	9.0	9.0	9.0	9.6	9.0	8.7	8.9	9.4	9.2	8.6
ADF (g kg^{-1} DM)	49.0	49.3	49.8	57.5	57.1	52.6	47.2	51.5	56.9	61.3

NDF (g kg^{-1} DM)	104.5	111.0	102.5	115.0	115.3	102.6	115.6	113.6	119.2	113.7

Note: MOA, mode of acid addition; DM, dry matter; CP, crude protein; CF, crude fiber; EE, ether extract; Ca, calcium; P, phosphorus; ADF, acid detergent fiber; NDF, neutral detergent fiber.

[a]Analyzed at the Central Testing Laboratory, Winnipeg, Manitoba (with the exception of pH).

[b]Reconstituted to 20% moisture and stored in polyethylene barrels for 34 d. Acid and enzyme were added during reconstitution.

[c]Acid composition: OIB: 30 to 50% phosphoric acid, 0.1 to 1% lactic, 5 to 10% citric and 1 to 5% malic acid (Canadian BioSystems, Calgary, Alberta, Canada). Prop: propionic Acid 99% (Anachemia, Montreal, Quebec, Canada).

[d]Enzyme composition: phytase and multi-carbohydrase enzyme combination.

[e]Fine particle size, grain was ground using 2.0 mm screen; coarse using 4.8 mm screen.

[f]Analyzed at the General Animal Nutrition Laboratory, Dept. of Animal and Poultry Science, University of Saskatchewan, Canada.

Table 3.7. Chemical analyses of phase 2 control diets, diets containing acid-preserved wheat (APW), or acidified diets (AD). [a]

Treatments	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
MOA	Control		Acid-preserved Wheat, APW[b]				Acidified diet, AD			
Acid[c]	(-)	(-)	OIB			Prop	OIB			Prop
Enzyme[d]	NoEnz	Enz	Enz	Enz	NoEnz	Enz	Enz	Enz	NoEnz	Enz
Particle Size[e]	Fine	Fine	Fine	Coarse	Fine	Fine	Fine	Coarse	Fine	Fine
pH[f]	6.15	5.88	5.67	5.61	5.49	5.34	5.59	5.62	5.57	5.37
DM (g kg^{-1} diet) [f]	892.2	890.4	869.21	864.6	868.3	865.9	889.2	885.6	888.6	890.8
CP (g kg^{-1} DM)	249.6	256.6	249.0	261.1	255.3	251.5	248.2	257.4	241.6	282.6
CF (g kg^{-1} DM)	43.0	41.8	39.4	40.8	41.6	39.3	42.7	37.7	39.5	39.5
EE (g kg^{-1} DM)	43.7	43.6	40.0	48.2	48.1	49.3	54.9	43.8	52.4	52.5
Ash (g kg^{-1} DM)	70.8	61.7	67.7	62.9	66.6	62.9	57.7	70.6	61.8	66.8
Ca (g kg^{-1} DM)	11.7	9.2	11.6	10.4	9.9	10.2	8.4	13.3	10.0	10.3
P (g kg^{-1} DM)	7.5	7.4	7.9	8.0	8.3	7.7	7.6	8.2	7.8	7.6
ADF (g kg^{-1} DM)	53.7	51.3	53.2	48.4	53.3	55.9	64.9	47.5	54.5	62.0

NDF (g kg^{-1} DM)	126.1	111.6	117.0	107.2	122.2	121.3	121.6	126.6	113.7	111.2

Note: MOA, mode of acid addition; DM, dry matter; CP, crude protein; CF, crude fiber; EE, ether extract; Ca, calcium; P, phosphorus; ADF, acid detergent fiber; NDF, neutral detergent fiber.

[a]Analyzed at the Central Testing Laboratory, Winnipeg, Manitoba (with the exception of pH).

[b]Reconstituted to 20% moisture and stored in polyethylene barrels for 34 d. Acid and enzyme were added during reconstitution.

[c]Acid composition: OIB; 30 to 50% phosphoric acid, 0.1 to 1% lactic, 5 to 10% citric and 1 to 5% malic acid (Canadian BioSystems, Calgary, Alberta, Canada). Prop: propionic Acid 99% (Anachemia, Montreal, Quebec, Canada).

[d]Enzyme composition: phytase and multi-carbohydrase enzyme combination; Enz with enzyme, NoEnz without enzyme.

[e]Fine particle size, grain was ground using 2.0 mm screen and coarse using 4.8 mm screen.

[f]Analyzed at the General Animal Nutrition Laboratory, Dept. of Animal and Poultry Science, University of Saskatchewan, Canada.

Table 3.8. Grinding cost, particle and handling characteristics of control and acid-preserved high-moisture wheat.

	Dry wheat		Acid-preserved wheat, APW					
Acid	N/A	N/A	OIB			Prop		
Enzyme	N/A	N/A	Enz	Enz	NoEnz	Enz		
Particle size[a]	Fine	Coarse	Fine	Coarse	Fine	Fine	SEM	P value
Grinding properties and cost								
Throughput (t h^{-1})	2.91b	4.44a	1.59c	3.27b	1.58c	1.57c	0.066	<0.01
Power consumption (kWh t^{-1})	7.08b	3.10d	12.75a	5.25c	12.57a	13.48a	0.353	<0.01
Power cost ($ t^{-1})	0.82b	0.36d	1.48a	0.61c	1.46a	1.56a	0.041	<0.01
Particle and handling characteristics								
d_{gw} (µm)	525b	938a	501b	920a	502b	570b	25.8	<0.01
s_{gw} (µm)	2.05b	2.15ab	2.22a	2.23a	2.24a	2.1ab	0.03	0.02
Bulk density (kg m^{-3})	654b	681a	609c	575d	579d	600c	4.0	<0.01
Angle of repose (°)	75a	51d	64bc	54d	56cd	67ab	1.7	<0.01
Coefficient of friction	0.41a	0.36c	0.39abc	0.37bc	0.40ab	0.38abc	0.01	<0.01

Note: OIB, organic-inorganic acid blend; Prop, propionic acid; NoEnz, without enzyme; Enz, with enzyme; d_{gw}, mean geometric

diameter; s_{gw}, particle size standard deviation; SEM, standard error of mean. Means without a common letter within a row are different ($P < 0.05$).

[a]Fine particle size means that wheat was ground using 2.0 mm screen and coarse using 4.8 mm screen.

Table 3.9. Corrosion rate of steel coupons exposed to organic-inorganic acid blend or propionic acid.

Acid	Coupon type	Average corrosion rate (mils yr^{-1})
OIB	Carbon steel	0.15c
OIB	Galvanized steel	7.37a
Prop	Carbon steel	2.93b
Prop	Galvanized steel	7.61a
SEM		0.291
P value		
Acid		<0.01
Coupon		<0.01
Acid x coupon		<0.01

Note: OIB, organic-inorganic acid blend; Prop, propionic acid; SEM, standard error of the mean.

Means without a common letter within a column are different ($P < 0.05$).

Table 3.10. Performance of weanling pigs fed either control, diets with acid-preserved wheat (AD), or acidified diets (AD) where wheat was ground either finely or coarsely.[a]

Treatments	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10		
MOA	NC	PC	Acid-preserved wheat, APW				Acidified diet, AD					
Acid	(-)	(-)	OIB			Prop	OIB			Prop		
Enzyme	NoEnz	Enz	Enz	Enz	NoEnz	Enz	Enz	Enz	NoEnz	Enz		
Particle size	Fine	Fine	Fine	Coarse	Fine	Fine	Fine	Coarse	Fine	Fine	SEM	P value
					0 to 7 d							
ADG (g d^{-1})	14	20	6	2	11	4	18	-11	-8	10	14.3	0.77
ADFI (g d^{-1})	61	82	71	68	72	72	72	63	75	71	7.9	0.59
G:F	0.12	0.14	0.01	-0.04	0.07	-0.02	0.09	0.09	-0.79	0.05	0.3	0.48
					8 to 21 d							
ADG (g d^{-1})	202b	257ab	245ab	221b	235ab	260ab	244ab	199b	247ab	294a	22.5	<0.01
ADFI (g d^{-1})	308	327	333	322	304	328	305	292	316	334	26.4	0.74
G:F	0.66c	0.78abc	0.74bc	0.69bc	0.77abc	0.80ab	0.80ab	0.70bc	0.78abc	0.88a	0.032	<0.01
					22 to 35 d[b]							

ADG (g d^{-1})	614	601	620	618	633	613	614	606	635	637	21.7	0.91
ADFI (g d^{-1})	792	797	771	807	802	822	760	800	786	816	50.3	0.80
G:F	0.78	0.77	0.81	0.78	0.80	0.75	0.83	0.77	0.82	0.79	0.050	0.16
					36 to 144 d[c]							
ABW (kg)	130.3	134.2	136.0	133.6	136.3	132.6	135.6	131.7	134.3	130.3	3.57	0.92
ADG (g d^{-1})	1.03	1.04	1.07	1.05	1.07	1.03	1.07	1.04	1.05	1.07	0.03	0.97

Note: MOA, mode of acid addition; NoEnz, without enzyme; Enz, with enzyme; ADG, average daily gain; ADFI, average daily feed intake; G:F, feed efficiency; ABW, average body weight; SEM, standard error of mean. Means without a common letter within a row are different ($P < 0.05$).

[a]Means were calculated from 8 pens per treatment.

[b]Day 22 to 35 data used d 21 average body weight as a covariate.

[c]Average body weight and ADG were average of 8 pigs per treatment except for T10 where 1 pig died. Pig age: 56 to 165 d.

P values of d 0 to 7 G:F and d 22 to 35 ADFI were based on square root transformation of data.

Table 3.11. P values of treatment contrasts.

	Acid		Type of Acid		Enzyme		Particle Size	MOA	Interactions		
	PC vs OIB-Enz	PC vs Prop-Enz	OIB vs Prop	NC vs OIB-NoEnz	NC vs PC	OIB-Enz vs OIB-NoEnz	OIB-Fine vs OIB-Coarse	APW vs AD	MOA x acid type	MOA x Enz	MOA x PS
					0-7 d						
ADG	0.55	0.34	0.66	0.76	0.76	0.83	0.15	0.95	0.78	0.52	0.27
ADFI	0.19	0.20	0.99	0.14	**0.03**	0.83	0.34	0.93	0.88	0.88	0.65
G:F	0.22	0.22	0.97	0.29	0.74	0.68	0.81	0.10	0.85	0.22	0.81
					8-21 d						
ADG	0.47	0.25	**0.02**	**<0.01**	**0.01**	0.82	**0.02**	0.58	0.24	0.66	0.44
ADFI	0.71	0.86	0.50	0.90	0.44	0.63	0.49	0.43	0.32	0.25	0.98
G:F	0.66	0.11	**0.01**	**<0.01**	**<0.01**	0.86	**0.01**	0.051	0.66	0.31	0.39
					22-35 d						
ADG	0.49	0.29	0.65	0.38	0.64	0.35	0.80	0.86	0.42	0.82	0.87
ADFI	0.29	0.50	**0.04**	0.99	0.94	0.23	0.16	0.51	0.96	0.92	0.87
G:F	0.04	0.98	**0.01**	0.29	0.58	0.65	**0.04**	0.23	0.53	0.74	0.63
					36-144 d						

ABW	0.72	0.96	0.71	0.24	0.42	0.89	0.36	0.95	0.55	0.82	0.83
ADG	0.46	0.87	0.49	0.34	0.70	0.78	0.47	0.90	0.60	0.69	0.84

Note: MOA, mode of acid addition; PS, particle size; ABW, average body weight; ADG, average daily gain; ADFI, average daily feed intake; G:F, feed efficiency; SEM standard error of mean.

EXPERIMENT 2

The objective of experiment 2 was to determine the effect of feeding OIB-preserved wheat, with or without enzyme, and its interaction with particle size on nutrient digestibility and gut health of weanling pigs.

3.16 Experimental design

A total of 64 barrows (Camborough Plus females × C337 sires; PIC Canada Ltd., Winnipeg, Manitoba, Canada) pre-selected based on age and weight at weaning (21 ± 2 d, 5.5 to 6.5 kg BW) were used. Because the experimental room could only accommodate 8 pigs at a time, the experiment was started in 8 successive batches. Each batch of pigs was housed together in the nursery rooms and fed a commercial diet designed for newly weaned piglets for 10 d. The initial room temperature of 28 ºC was reduced by 2 ˚C weekly until the pigs were transferred to experimental rooms. Humidity in the nursery rooms was maintained at ~40% and a 12-12 h light-dark lighting program was implemented.

At 31 d of age (6.88 ± 0.5 kg BW), pigs were transferred to the experimental room where they could be housed individually. They were randomly assigned to 1 of 8 treatments in a randomized complete block design with pig as the experimental unit, and batch as block (8 treatments, 8 pigs per treatment started in 8 batches). The pigs were housed individually in pens 150 cm long x 75 cm wide with polyvinylchloride walls 2.29 cm thick and plastic-coated slatted floors. Pigs were adapted to housing and the phase 2 diets for 8 d, followed by 4 d of fecal collection. To facilitate adaptation to the pen, a sticker with a mirror-like surface (30.48 x 30.48 cm) was attached on the wall of each pen to provide an illusion of a companion (Enrichment for individually housed weanling pigs), and toys (PVC pipes or plastic balls) were offered as

enrichment. Feed was offered to provide 3 x maintenance energy requirement (197 kcal/kg ME x $BW^{0.60}$, NRC 2012) and clean water was provided *ad libitum*. Daily feed allocation was divided into two equal amounts, given at 08:30 and 15:30 h. Feed refusal, if any, was collected daily from d 5 until the end of fecal collection (d 12). Refusals were collected prior to morning feeding, weighed and deducted from daily feed offered to estimate daily feed disappearance. The room was maintained at 24 ˚C, ~40% humidity, and 12-12 h light-dark lighting program during the entire experimental period.

3.17 Experimental diets

For this experiment, only the controls and OIB-containing phase 2 diets were used (8 of the 10 treatments in Table 3.1). Although effective, Prop was more corrosive and pungent than the OIB treatment. Furthermore, there is limited information on the use of phosphoric acid-based OIB as a preservative and a diet acidifier and therefore warrants further investigation.

Diets were formulated to meet or exceed NRC (2012) recommendations for pigs 8 to 13 kg BW and to be isocaloric and isonitrogenous. The ingredients in diets using APW were adjusted on a DM basis to account for the differences in DM between the control and high-moisture grain. Diets contained Celite (Celite 545- Celite Corporation, Lompoc CA, USA) added at 4 g kg^{-1} diet as a source of acid-insoluble ash (AIA), an indigestible marker used to estimate the apparent total tract digestibility of nutrients using the indigestible marker method described by Adeola (2001).

3.18 Sample and data collection

3.18.1 Fecal collection

A fecal collection system (customized Velcro rings with a 10 lb plastic bag) was glued (OSTO-BOND, Montreal Ostomy, Quebec, Canada) around the anus of the pig according to the procedure by Van Kleef et al. (1994). Feces were collected twice a day. However, because most of the pigs had transient diarrhea during the 4-d collection period, firm fecal samples were selected and used to estimate ATTD of nutrients and energy using AIA as an indigestible marker as described by Adeola (2001). All fecal samples were stored immediately at -20 ˚C.

3.18.2 Intestinal permeability (lactulose and mannitol gavage)

On d 13, pigs in 5 of the 8 treatment groups (5 treatments x 8 pigs per treatment = 40 pigs) were given the indigestible sugar markers by oral gavage based on the methods of Wijtten et al (2011), Zhang et al (2000) and Kansagra et al (2003). Treatments were selected to evaluate the effect of feeding acid-preserved high-moisture wheat and its interaction with particle size on intestinal permeability and gut health: T2) positive control, T3) APW-OIB-Enz-Fine, T4) APW-OIB-Enz-Coarse, T7) AD-OIB-Enz-Fine and T8) AD-OIB-Enz-Coarse. On d 14, the same pigs were euthanized to allow for collection of digesta and tissue samples. The remaining 24 pigs were returned to the Prairie Swine Centre commercial herd.

The sugar marker solution was prepared to provide 500 mg of lactulose and 100 mg of mannitol per ml; and was administered at 1 ml kg^{-1} of the pig's body weight. Briefly, the sugar solution was prepared by dissolving 374.81 ml of lactulose solution (667 g ml^{-1} SANIS Lactulose Solution USP, Sanis Health Inc, Ontario, Canada) and 51.02 g mannitol (D-mannitol 95%, Sigma Aldrich) in distilled water to reach a total volume of 500 ml in a volumetric flask.

Feed was withdrawn 2 h (0630 h) before the gavage and returned 2 h post gavage. Pig weights at the end of the fecal collection period (d 12, day prior to gavage) were used as basis for the amount of sugar solution administered. Pigs were administered the gavage by intubation using a feeding tube (size 14FR, MED-RX, Canadian Hospital Specialties, Ltd., Oakville, Ontario, Canada) modified to ~46 cm in length and attached to a 12 ml syringe. The syringe was flushed with 10 ml of distilled water to ensure that no sugar solution remained. Blood was collected by jugular venipuncture into serum Vacutainer tubes (BD Vacutainer, BD, Missisagua, ON, Canada) prior to gavage to serve as baseline lactulose and mannitol levels, and exactly 2 h post gavage. The tubes were spun at 830 x g for 10 minutes and serum was collected and stored at -20 ˚C prior to analysis.

3.18.3 Euthanasia, tissue and digesta collection

Pigs were euthanized on d 14 (44 d of age) one hour after the morning feeding using captive bolt. A mid-line abdominal incision allowed the collection of gastro-intestinal organs. The 1st and last metre of the small intestine were assumed to be duodenum and ileum, respectively with the jejunum between. A section of the mid-jejunum (5 cm) was collected, cut open lengthwise and placed in 10 % formalin for histological measurements. Another section from the mid-jejunum was placed in sterile plastic bags (3” x 5”, Fisherbrand, Fisher Scientific, Hampton, New Hampshire, USA) and immediately put on dry ice, then stored at -80 ˚C prior to analysis of genetic markers of barrier function. Digesta from the stomach, duodenum, jejunum, ileum, caecum and mid-colon were collected for determination of pH. A subset of digesta samples from caecum and mid-colon were collected in 2 ml cryovials and immediately placed in dry ice then stored in -80 ˚C prior to analysis of microbial populations and SCFA and LA.

3.19 Analyses and calculations

3.19.1 Apparent total tract digestibility of DM, energy, ash and phosphorus

Analysis of diet and fecal DM, energy, ash (AOAC 923.03), P (AOAC 965.17) and acid insoluble ash (AOAC 920.08) were conducted at the General Nutrition Laboratory of the Department of Animal and Poultry Science at the University of Saskatchewan. Diet DM was determined with AOAC 930.15 (135 ˚C for 2 h). Feces collected from each pig were thawed, pooled and homogenized. Fecal DM was determined using the two-step drying method described by Goering and Van Soest (1970). Briefly, partial DM of the feces was first determined by drying in a force-draft oven at 55 ˚C for 48 h or until there was no observed change in weight, and then ground to pass through 1 mm screen using a Retsch Mill (model ZM1, Newton, PA, USA) prior to analysis of final DM. Laboratory DM was determined by further drying two grams of the partially dried feces at 135 ˚C for 2 h (AOAC 930.15). Final DM was calculated using eq 3.7.

$$\text{Final DM}, \% \ = \% \text{ Partial DM x } \frac{\% \text{ Laboratory DM}}{100} \qquad \text{Eq 3.8}$$

Diet and feces (0.5 to 0.7 g, pelleted) were analyzed for gross energy using an isoperibol bomb calorimeter (model 6400, Parr Instrument Co., Moline, Illinois, USA) with benzoic acid as standard.

Apparent total tract digestibility was calculated using the equation:

$$\text{ATTD}, \% = 100 - \left[\left(\frac{\% \text{ MD x } \%\text{NF}}{\%\text{MF x } \%\text{ND}}\right) \text{x } 100\right] \qquad \text{Eq 3. 9}$$

where M_D is the amount of marker in the diet, M_F is the amount of marker in feces, N_F is the amount of nutrient in the feces and N_D is the amount of nutrient in the diet.

3.19.2 Digesta pH

Immediately after collection, digesta samples from the stomach, small intestine (duodenum, jejunum, ileum), caecum and colon were analyzed for pH according to the method described by Risley et al. (1993) with a slight modification. Briefly, each sample was homogenized and 1 g of digesta was mixed with 9 ml deionized water (1:9 w/v) and allowed to stand for 5 minutes with periodic shaking (instead of magnetic stirrer) prior to measurement using a pH meter (Oakton pH 110 series, Eutech Instruments, Thermo Fisher Scientific,Waltham, Massachusetts, USA). Readings were recorded in duplicate.

3.19.3 Intestinal permeability (lactulose and mannitol)

Frozen samples were thawed on ice. An aliquot (600 µL) was filtered through 0.45 µm cutoff ultrafiltration spin columns (Millipore, Sigma) by spinning at 2500 x g for 2 minutes at 4 °C. Serum lactulose and mannitol were analyzed at the National Research Council (Saskatoon, SK, Canada) using ion chromatography techniques based on the procedure by Cabrera (2013), and Hurum and Rohrer (2016). Analysis was performed on a Dionex ICS-3000 ion chromatography system using Chromeleon software (version 6.80 SR10, build 2818). The system consisted of an autosampler, dual pump and detector / chromatography (DC) modules, with the DC containing the analytical column, guard column, and high-performance anion exchange–pulsed amperometric detector (HPAE-PAD), all held at 30 °C. The columns used were a Dionex CarboPac MA1 4 x 50 mm guard followed by a Dionex CarboPac MA1 BioLC Analytical 4 x 250 mm column. The mobile phase was 480 mM NaOH at a flow rate of 0.4 mL min^{-1}. The detector was programmed to run a standard quadratic waveform and quantified using

the calibration curves run with the samples to yield the amount of analyte present (μg mL^{-1}). Standards and blanks were prepared and analyzed with each batch of serum samples.

3.19.4 Histology

Sections of the jejunum were fixed in 10% neutral buffered formalin and processed for paraffin embedding and staining with hematoxylin and eosin (H&E stain; 0.5 um thick, 3 sections per slide) at Prairie Diagnostic Services (Saskatoon, SK). Slides were viewed under a light microscope and photomicrographs were acquired at 10 x magnification using the imaging software Axio Vision LE64 v 4.9.1 (Carl Zeiss AG, Oberkochen, Germany) running a high-resolution digital camera (AxioCamMR, Carl Zeiss AG, Oberkochen, Germany). A demarcation line was drawn perpendicular to the villi to estimate the junction of the villi and crypt was used as reference point for measurement. Between fifteen to twenty intact villi and crypt were measured per pig.

3.19.5 RNA extraction, reverse transcription, and PCR

Frozen jejunal samples were thawed on ice for 30 min. A cross section (250 mg) was homogenized in 5 mL cold guanidine isothiocyanate and β-mercaptoethanol-based lysis buffer (QIAGEN, Hilden, Germany) in a 15 ml conical tube (VWR, Radnor, Pennsylvania, USA) using a handheld homogenizer (POWER GEN 125, Fisher Scientific, Hampton, New Hampshire, USA). This was done in pulses to prevent the lysate from heating and denaturing the unstable RNA. The lysate was allowed to stand on ice for 30 min to dissipate the froth formed during homogenization. Between samples, the homogenizer probe was washed, in nuclease free water, 70% ethanol, and then again nuclease free water to prevent cross contamination.

RNA was extracted using RNEasy Mini Kit according to manufacturer's procedures (QIAGEN, Hilden, Germany). Briefly, 1 mL aliquot of the lysate was spun at 16,000 x g for 3 min, and 600 µL supernatant was transferred to 1.5 ml Eppendorf tubes. Ethanol (70%) was added to the sample at a 1:1 ratio (v/v), mixed and centrifuged in filter columns at 8,000 g for 30 seconds. The membrane bound RNA was sequentially washed and centrifuged (8,000 g for 30 seconds) with 700 µL of RW1 wash buffer (reduced concentration of guanidine thiocyanate, QIAGEN, Hilden, Germany) and 500 µL of RPE wash buffer (contains ethanol, QIAGEN, Hilden, Germany) twice. The RNA bound to the filter membrane was eluted by pipetting 30 µL of nuclease free water onto the membrane and spinning at 8,000 x g for one minute. Samples were tested for RNA yield (nucleotide concentration) and purity by measuring optical density at A_{260} and A_{280} using the Nano Drop 2000 (Thermo Fisher Scientific, Waltham, Massachusetts, USA). Samples used for reverse transcription had a minimum nucleotide concentration of 300 ng μL^{-1} and $A_{260/280}$ of 1.86.

Reverse transcription was conducted using Applied Biosystems High Capacity cDNA Reverse Transcription kit (Thermo Fisher Scientific, Waltham, Massachusetts, USA). The total reverse transcription reaction mixture was 20 µL and consisted of 2 µL 10 x RT buffer, 0.8 µL 25 x dNTP (100 mM), 2 µL 10 x RT random primers, 1 µL of Multiscribe Reverse Transcriptase (50 U μL^{-1}), and 14.2 µL RNA (1,000 ng total RNA). Reverse transcription was performed on a thermal cycler (C1000 Touch, Bio Rad Hercules, California, USA) with the following temperature conditions: 10 min at 25˚C, 120 min at 37˚C and 5 min at 85˚C.

Real time PCR was conducted on a CFX96 Real-Time PCR Detection System (Bio-Rad, Hercules, California, USA) using EvaGreen PCR supermix (SSOFast Evagreen Supermix, Bio-Rad, Hercules, California, USA). The total reaction mixture per well was 20 µL; composed of 10

µL EvaGreen PCR supermix, 0.8 µL of forward primer (10 uM), 0.8 µL reverse primer (10 uM), 2 µL of cDNA (diluted 1/200) and 6.4 µL nuclease free water. Amplification was performed in Bio-Rad optical 96-well reaction plates. The PCR parameters were: 30 s at 95 ˚C to activate the enzyme, 5 s at 95 ˚C for denaturation, followed by 40 cycles of 5 s at 55 ˚C to 60 ˚C for annealing/extension. This was followed by melt curve analysis to evaluate the specificity of each reaction well for all samples in a run. The following conditions were implemented: 1 min at 95 ˚C, 5 s at 55 ˚C, and 5 s at 95 ˚C. The presence of a single PCR product from each reaction was confirmed by a single melting peak. No template control (NTC) and nuclease free water was amplified in each plate to address the potential for contamination.

A standard curve was created by the amplification of 5-fold serial dilution of pooled cDNA samples (200 ng μL^{-1}, 40 ng μL^{-1}, 8 ng μL^{-1}, 1.6 ng μL^{-1}, 0.32 ng μL^{-1}, 0.64 ng μL^{-1}) by real time PCR using gene specific primers. Threshold cycles were plotted against cDNA template concentration and the data was fit to a straight line. A linear regression coefficient of 0.99 indicated acceptable standard curves, and the slope was used to calculate amplification efficiency using Eq. 3.10.

$$\text{Efficiency} = \left[10^{-1/\text{slope})}\right] - 1 \qquad \text{Eq 3. 10}$$

Samples were analyzed in duplicate and cycle threshold was adjusted for each plate. Starting cDNA template quantities for each sample were estimated using the linear regression equation derived from the standard curve. Starting quantities of each sample were normalized using the geometric mean of starting quantities of reference genes β-Actin and RPL19.

3.19.6 Short chain fatty acids and lactic acid

Samples were thawed overnight in a 5 ˚C chiller. Short chain fatty acids and LA analysis used gas chromatography techniques based on the procedure by Khorasani et al. (1993) and Lenahan et al. (2010). Briefly, digesta samples were diluted with 25% metaphosphoric acid at a 2:1 ratio (w/v) for caecal and 1:1 (w/v) for colonic digesta samples followed by vortexing. Samples were centrifuged twice at 12,000 g for 10 min. Supernatant was collected and again centrifuged at 16,000 g for 10 minutes once , or twice, if samples remained turbid. The supernatant was aliquoted into 600 µL duplicates. The internal standard, isocaproic acid (containing 4.56 µmol mL^{-1} isocaproic acid in 0.15 mol L^{-1} oxalic acid) was added to each replicate, vortexed, then samples were filtered through 0.45 µm PVDA filter (Fisher Scientific, Hampton, New Hampshire, USA) into a 2 ml glass sample vial (Agilent Technologies, Santa Clara, California, USA). The SCFA and LA in caecal and colonic digesta samples were determined on an Agilent 6890 gas chromatogram equipped with a flame ionization detector (Agilent Technologies, Santa Clara, California, USA) using a capillary column ZB-FFAP (30 m length x 0.32 mm x 0.25 µm film thickness; ZEBRON, Phenomenex, Torrance, California, USA). The initial oven temperature was set at 90 ˚C with a hold time of 0.1 min, followed by the 1^{st} ramp; 10 ˚C min^{-1} increase until 170 ˚C and hold time of 0.1 min and the 2^{nd} ramp; 20 ˚C min^{-1} up to 230 ˚C with a hold time of 2.0 min. Hydrogen gas was used for the FID and helium gas was used as carrier.

3.19.7 Microbial populations

Colonic digesta microbiome was determined by a commercial lab (Microbiome Insight, University of British Columbia-Life Sciences Center, Vancouver, BC, Canada) Samples were

initially placed into a MoBio PowerMag Soil DNA Isolation Bead Plate and DNA was extracted on a KingFisher robot. Bacterial 16S rRNA genes were PCR-amplified with dual-barcoded primers targeting the V4 region, as per the protocol of Kozich et al. (2013). Amplicons were sequenced with an Illumina MiSeq using the 250-bp paired-end kit (v.2). Sequences were denoised, taxonomically classified using Greengenes (v. 13_8) as the reference database, and clustered into 97%-similarity operational taxonomic units (OTUs) with the mothur software package (Schloss et al. 2009), following the recommended procedure (https://www.mothur.org/wiki/MiSeq_SOP; accessed Nov 2017). The potential for contamination was addressed by co-sequencing DNA amplified from specimens and from four each of template-free controls and extraction kit reagents, processed the same way as the specimens. Operational taxonomic units were considered contaminants and consequently removed if their mean abundance in controls reached or exceeded 25% of their mean abundance in specimens.

3.20 Statistics

Residual error data were checked for normality of distribution by Shapiro-Wilk test using Proc Univariate in SAS (SAS 9.4; SAS Institute, Cary, NC). If $P < 0.05$, data were transformed accordingly and subjected to one-way ANOVA using the mixed procedure of SAS. P-values reported are from transformed data and least square means were from untransformed data.

Animal performance and ATTD were analyzed as a randomized complete block design with the fixed effect of treatment (8 treatments with 8 pigs per treatment) and random effect of batch/block (8 batches or blocks). The model used was:

$$Yij=\mu + \rho_i + \alpha_j + \varepsilon_{ij}$$

where Y is the parameter, μ the overall mean, ρ_i the random effect of the i^{th} block, α_j the fixed effect of the j^{th} treatment, ε_{ij}=error term associated to the j^{th} treatment and i^{th} block.

Gut health measurements (intestinal permeability, digesta pH, histology measurements, SCFA, LA, and expression of genetic markers) were analyzed similar to performance and ATTD except with five treatments.

Means were separated using Tukey's Honest Significant Difference (HSD) test. In all cases, $P \leq 0.05$ was considered significant, with values between 0.05 and 0.10 a tendency.

Data were discussed relative to a protected and an unprotected F-test (Barnette and McLean 1998) using single degree of freedom contrasts to compare treatments and interactions of interest (Marini 2003).

Colonic digesta samples were analyzed for microbial populations by Microbiome Insights (University of British Columbia-Life Sciences Center, Vancouver, BC, Canada) which also performed the statistical evaluation. Alpha diversity was estimated with the Shannon index on raw OTU abundance tables after filtering out contaminants. The significance of diversity differences was tested with an ANOVA. Differenctial abundance testing was conducted using DESeq2 package. All analyses were conducted in the R environment (https://www.r-project.org/; accessed: 5 July 2018).

RESULTS

3.21 Performance

Overall, treatment had no effect on growth rate or feed efficiency of pigs (Table 3.12; $P > 0.10$). Comparing the enzyme treatments, pigs fed Enz had higher ADG compared to those fed NoEnz ($P < 0.10$; Table 3.13).

3.22 Apparent total tract digestibility

Apparent total tract digestibility (ATTD) of DM, energy, ash and P are presented in Table 3.14. When fed AD, pigs fed Fine had higher digestibility of DM ($P < 0.10$), energy ($P < 0.05$) and ash ($P < 0.10$) than those fed Coarse and was not observed when diets with APW were fed. Phosphorus digestibility in pigs fed AD-Phos-Enz-Fine and AD-Phos-NoEnz-Fine was higher compared to pigs fed NC ($P < 0.05$). Pigs fed PC had higher P digestibility compared to pigs fed NC ($P < 0.01$) and, pigs fed diets with OIB without enzyme regardless of MOA had higher P digestibility compared to those fed PC ($P < 0.01$).

3.23 Gut health

3.23.1 Intestinal permeability, jejunum histology and genetic markers of barrier function and immune response

Our interest was on the interaction of acid-preservation of wheat and particle size on gut health measures of weanling pigs, therefore only the pigs fed diets with the enzymes were selected to allow us to evaluate the main effects of acidification, particle size and their interaction (5 out of 8 treatments used in the digestibility trial). Measurements were taken from 8 pigs per treatment (5 treatments x 8 pigs = 40 pigs total).

Intestinal permeability as estimated by the lactulose to mannitol ratio before, and 2 h post gavage are presented on Table 3.16. Treatment had no effect on intestinal permeability, however there was a decrease in L:M ratio 2 h post gavage (0.085 to 0.028 for 0 and +2 h respectively, $P < 0.01$).

At d 14, the same pigs given the gavage were euthanized to allow for the collection of digesta and tissue samples from the GIT. Jejunum histology and expression of genetic markers for barrier function, inflammatory and anti-inflammatory response, cellular proliferation and maturity are presented in Table 3.17 and Table 3.18 respectively. Target genes and the primers used to analyze for the genetic markers, regression coefficient, slope and reaction efficiency are presented in Table 3.19. Treatment had no effect on villi height, crypt depth and villi:crypt ratio in the mid jejunum, or the expression of the targeted gene markers.

3.23.2 Digesta pH

The pH of digesta collected from the different sections of the GIT are presented in Table 3.20. Treatment had no effect on pH of digesta from the stomach, jejunum, caecum and colon. Comparing mode of acid addition, pigs fed APW had lower ($P < 0.01$) duodenal digesta pH compared to pigs fed AD. Pigs fed the diets with APW regardless of particle size, had a higher ($P < 0.05$) ileal digesta pH compared to pigs fed the PC and the AD-Coarse. Comparing particle size, pigs fed the Coarse diet had a tendency to have lower ($P < 0.10$) jejunal pH than pigs fed Fine. Comparing mode of acid addition, pigs fed AD had lower ($P < 0.01$) ileal pH compared to pigs fed APW.

3.23.3 Short chain fatty acids and lactic acid

Caecal SCFA and LA concentration and molar proportion of individual SCFA relative to total SCFA and are presented in Table 3.21 and Table 3.22. Colonic SCFA and LA concentration and molar proportion of SCFA are outlined in Table 3.23 and Table 3.24 respectively. Treatment had no effect on total SCFA and LA concentration and molar proportions of the individual SCFA in the caecum ($P > 0.10$). Acetic acid (% of total SCFA) in pigs fed APW-Fine was higher than pigs fed AD-Coarse ($P < 0.05$). Comparing MOA, % acetic acid was higher in pigs fed APW than those fed AD ($P < 0.05$). Butyric and valeric acid (as % of SCFA) were higher in pigs fed AD compared to those fed APW ($P < 0.10$). In the colon, the concentration and molar proportion of valeric acid from pigs fed the AD was higher compared to pigs fed APW ($P < 0.05$).

3.23.4 Microbial Populations

The diversity of microbial populations was not different among treatment groups (Figure 3.4. Microbial population diversity in colonic digesta samples from pigs fed either PC, diets with APW or acidified diets, where wheat was ground Fine or Coarse. Differential abundance testing identified a single low-abundance bacterial OTU (*Prevotella*) that had higher counts in pigs fed APW-Fine and was depleted in pigs fed APW-Coarse (Figure 3.4, $P < 0.01$).

DISCUSSION

Acidification is an alternative storage method to artificial drying of high-moisture grains. Moreover, diet acidification improves weanling pig performance by reducing gastric pH thereby improving nutrient digestibility and inhibiting growth of pathogenic microorganisms (Liu et al. 2018). Blends of organic and inorganic acids are of interest as dietary acidifiers due to lower cost

and proposed synergy in lowering gut pH and efficacy at controlling pathogenic microorganisms (Tung and Pettigrew 2006; Kil et al. 2014; Ahmed et al. 2014).

Acidification of weanling pig diets improves nutrient, specifically DM and CP, digestibility (reviewed by Partanen and Mroz 1999; Tung and Pettigrew 2006). It is generally accepted that the addition of organic acids to weanling pig diets reduces gastric pH, which activates the proenzyme pepsinogen into pepsin resulting in increased protein digestibility. Moreover, a low stomach pH reduces gastric emptying rate and stimulate pancreatic secretions, further improving nutrient digestibility (Partanen and Mroz 1999; Tung and Pettigrew 2006, Kil et al. 2015). Several studies have also reported improvements in Ca and P digestibility due to increased solubility and the chelating properties of organic acidifiers (Partanen and Mroz 1999).

In the current study, P ATTD was improved by direct diet addition of OIB (AD-NoEnz-Fine vs NC), enzymes (PC vs NC) and their combination (AD-Enz-Fine vs NC) to a diet devoid of both; and was similar to when diets containing APW were fed. It has been demonstrated that phytase activity is enhanced in the presence of organic acids (LA and formic acid); improvements are attributed to the lower stomach pH increasing the solubility of phyP and P (Jongbloed et al. 1995; Blank et al. 2012), and through chelation of minerals, increasing absorption (Ravindran and Kornegay 1993). However, in the current study, OIB and the exogenous enzymes did not have a synergistic effect on P ATTD in weanling pigs as indicated by similar P ATTD of APW-Enz-Fine and AD-Enz-Fine and the PC (Table 3.14). The lack of a difference in gastric digesta pH of pigs on these treatments possibly explains the absence of synergy between OIB and enzymes (Table 3.20). In pigs fed AD, there was a reduction in DM, energy and ash ATTD using coarse compared to fine wheat. Because grinding coarsely did not reduce DM, energy and ash ATTD in APW fed pigs, this suggests that there was improvement in

energy and mineral utilization in APW as hypothesized. However, OIB and enzyme supplementation independently improved ATTD of P and no synergism was noted between OIB and enzyme on P ATTD as had been hypothesized. On average, feeding pigs diets with APW resulted in similar nutrient digestibility as feeding AD. Grinding coarsely resulted in lower ATTD of DM, energy and ash in untreated wheat, but not APW. To our knowledge, there is very limited literature on the effect of an organic-inorganic acid blend on nutrient digestibility for weanling pigs. This is important information because previous reviews have highlighted the potential of using a blend of organic and inorganic acids for economic reasons and enhancement in efficacy of organic acids. Inorganic acids are cheaper than organic acids and effectively reduce pH due to their low acid binding and buffering capacity (Kil et al., 2011). The synergy between organic and inorganic acids is because inorganic acids lower environmental pH effectively, maintaining the organic acids in undissociated form such that lower concentrations are required for anti-microbial action (Kil et al. 2011; Che et al. 2012).

For gut health determination, we euthanized pigs that were given only the enzyme diets (5 treatments out of 8) to give focus to the main treatment effects of mode of acid addition and particle size and their interaction. In the meta-analysis conducted by Tung and Pettigrew (2006), supplementation of organic acids effectively reduced the diet pH, however a consequent reduction in gastric pH was not always observed. In the current experiment, there was no effect of mode of acid addition, particle size or their interaction on the digesta pH in the stomach. This however, does not agree with Mikkelsen et al. (2002) who reported a reduction in gastric pH when growing pigs (33 kg BW) were fed coarse rather than fine barley-wheat-soy-based diet. The stomach digesta pH in the current study ranged from 3.92 to 4.24, higher than pH values reported by Mikkelsen et al. (2002) of 3.38 to 4.19. The difference between the pH values from

Mikkelsen et al. (2002) and the current trial was possibly because the latter used older pigs (33 kg initial body weight) which were able to maintain a lower gastric pH. Additionally, the presence of barley in their (Mikkelsen et al. 2002) diets may have resulted in a slower gastric emptying rate.

The digesta pH from the duodenum, jejunum, and ileum from the current experiment agrees with normal pH values for pigs reported by van der Klis and Jansman (2002). Similar to stomach pH, treatments had no effect on the pH of digesta from duodenum, jejunum, caecum and mid-colon. Our observations on the pH of the digesta from the stomach, duodenum and jejunum agree with the study of Li et al. (2008) who fed a dry organic acid blend of 2-hydroxy-4-(methylthio) butanoic acid, fumaric acid and benzoic acid at 0.5 and 1% to weanling pigs and observed no changes in digesta pH in these locations. In the current experiment, a higher ileal digesta pH in pigs fed the APW was observed compared to pigs fed either the PC or AD-Coarse diets. Czarneka et al. (1991) observed increased in-vitro starch digestibility in propionic acid (1% w/w) preserved rye and triticale. It is possible that the more digestible DM (94.42%) in pigs fed diets with APW, than those fed PC (93.98%) or AD-coarse (93.41%) resulted in less substrate for microbial fermentation at the ileum. The ileum has the highest microbial population in the small intestine and more undigested nutrients entering this region could be used as a substrate for fermentation in pigs fed PC or AD-coarse, possibly resulting in higher organic acid production consequently reducing pH. In agreement with the studies conducted by Mikkelsen et al. (2002), there were no differences in the pH between fine or coarse diets in the pH of the small intestine, caecum or colon. To our knowledge, the effect of feeding acid-preserved high-moisture wheat with different particle size on digesta pH from different regions of the piglet GIT has not

been reported. Our results indicate the potential for an enhanced rate of starch digestion with acidified high-moisture wheat.

The mode of acid addition or particle size did not affect intestinal structure and barrier function in the jejunum of pigs, nor were interactions observed. The lack of significance on the ratio of serum L:M in pigs among treatments indicate that intestinal permeability was not altered. This result is corroborated by measurements of villous height, crypt depth and villi:crypt ratio, and expression of genetic markers of barrier function (MUC2, CDN4, OCLN and ZO1), cellular proliferation (PCNA) and epithelial cell maturity (IAP) in the mid jejunum. The decrease in L:M ratio 2 h post-gavage indicates increased uptake of mannitol relative to lactulose. Mannitol crosses the mucosa primarily via the paracellular route (Wijtten et al. 2011). The increased uptake of mannitol may not necessarily mean that paracellular barrier function is compromised but rather a normal absorptive response to the presence of the sugar or an increased concentration in the luminal contents. Because of the size of the molecule, mannitol is absorbed 2 x as fast as lactulose (182 vs 342 Da; Bjarnson et al. 1995). Transcellular permeability also did not seem to be compromised because inflammatory (IL-8, IL-1β, and TNF-α) and anti-inflammatory response (IL-10) was not evident. The lack of differences in these cytokines also suggest that the integrity of the mucosa was not altered regardless of treatment, which is supported by our observation on villi height, crypt depth, and villi:crypt ratio.

Total and individual SCFA and LA production in the caecum and colon were not affected by mode of acid addition, particle size or their interaction. However, the higher concentration and molar proportion of valeric acid in pigs fed AD compared to those fed APW for both caecal and colonic contents may be an indication of increased protein fermentation in the hind gut brought about by the influx of undigested protein from the small intestine of the pigs fed AD

(Rajesh and Berrocoso 2016). Although not measured, this suggests improved N digestibility in pigs fed diets with APW compared to those fed AD. Using an in-vitro technique, Iyayi and Adeola (2015) fermented ileal digesta samples from pigs fed graded levels of wheat bran using fecal slurry from the same pigs as inoculant. The authors reported an increase in molar proportion of acetic acid from 68 to 74 % as wheat bran increased from 100 g kg^{-1} to 300 g kg^{-1} and attributed the increase to the arabinoxylan in the digesta. In the current trial, the higher molar proportion of acetic acid (as % of total SCFA) in pigs fed APW-Fine compared to AD-Coarse in caecum ($P < 0.05$) and colon (numerical) is possibly related to the higher digestibility of DM and energy in pigs fed APW-Fine. It should be noted that there was an interaction between MOA and PS wherein the Coarse diet had lower DM, energy and ash digestibility compared to Fine in pigs fed AD. In the current trial, it is likely that more starch and protein were digested and absorbed in the small intestine of pigs fed APW-Fine as indicated by the higher energy and DM ATTD, the concentration of arabinoxylan and other fibrous components in the digesta that entered the caecum and colon may be relatively higher compared to pigs fed AD-Coarse. This is supported in part by the results of the microbial population analysis of colonic contents in the current study. *Prevotella* are saccharolytic bacteria strongly associated with fiber rich diets and produce acetate and succinic acids as end products (Downes et al. 2007). Although microbial population diversity in the colon did not differ among treatments, higher counts of the genus *Prevotella* were observed in pigs fed APW-Fine compared to pigs fed the other treatments. There was a single high point sample in APW-Fine which could have pulled the mean bacterial count higher than the other treatments. However, because there was no biological reason why it should be treated as an outlier, it was considered a real number. Higher counts of *Prevotella* in pigs fed APW-Fine could explain why the molar proportion of acetic acid in the colon was higher (ns; P

> 0.10) than pigs fed the other diets. Assuming the microbial populations are similar in the caecum and colon, this could also explain why acetic acid was higher in pigs fed APW-Fine compared to the other treatments.

SUMMARY AND CONCLUSIONS

In summary, the addition of enzyme or acid in wheat-based diets independently improved P digestibility (vs NC) but not when both were present (vs PC). In acidified diets, pigs fed coarse had lower DM, energy and ash digestibility than pigs fed Fine, however, this was not observed in pigs fed diets with APW. Treatment had no effect on gut health parameters (jejunum histology and gene expression of genetic markers for barrier function, inflammatory and anti-inflammatory response and cellular proliferation and maturity). Digesta pH from the ileum of pigs fed APW was higher than pigs fed AD and PC. Total SCFA and LA in the caecum and colon did not differ among treatments. However, concentration and molar proportion of valeric acid was higher in pigs fed AD compared to those fed APW, and molar proportion of acetic acid (as % of total SCFA) was higher in the caecum of pigs fed APW-Fine compared to those fed AD-Coarse. Although the low abundance OTU *Prevotella* differed among treatments, no overall treatment effect was noted on microbial population diversity in the colon.

In conclusion, nutrient digestibility was similar when pigs were fed acid-preserved wheat or acidified diets. When feeding diets using acid-preserved high-moisture wheat, grinding finely may not be necessary. The improvements in nutrient digestibility in pigs fed APW alter the composition of digesta entering the hind gut, with subsequent modification in the microbial and SCFA profile. Feeding OIB either through APW or AD did not alter gut health measurements.

FIGURES

Figure 3.3. Enrichment for individually housed weanling pigs**.**

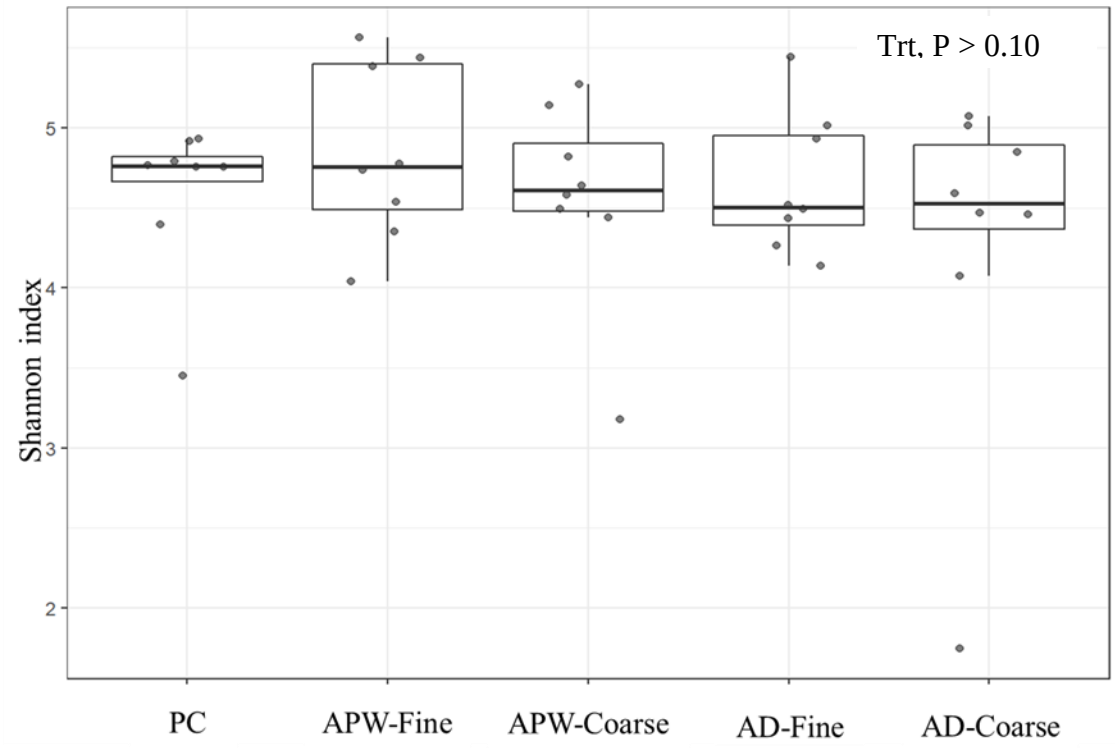

Figure 3.4. Microbial population diversity in colonic digesta samples from pigs fed either PC, diets with APW or acidified diets, where wheat was ground Fine or Coarse.

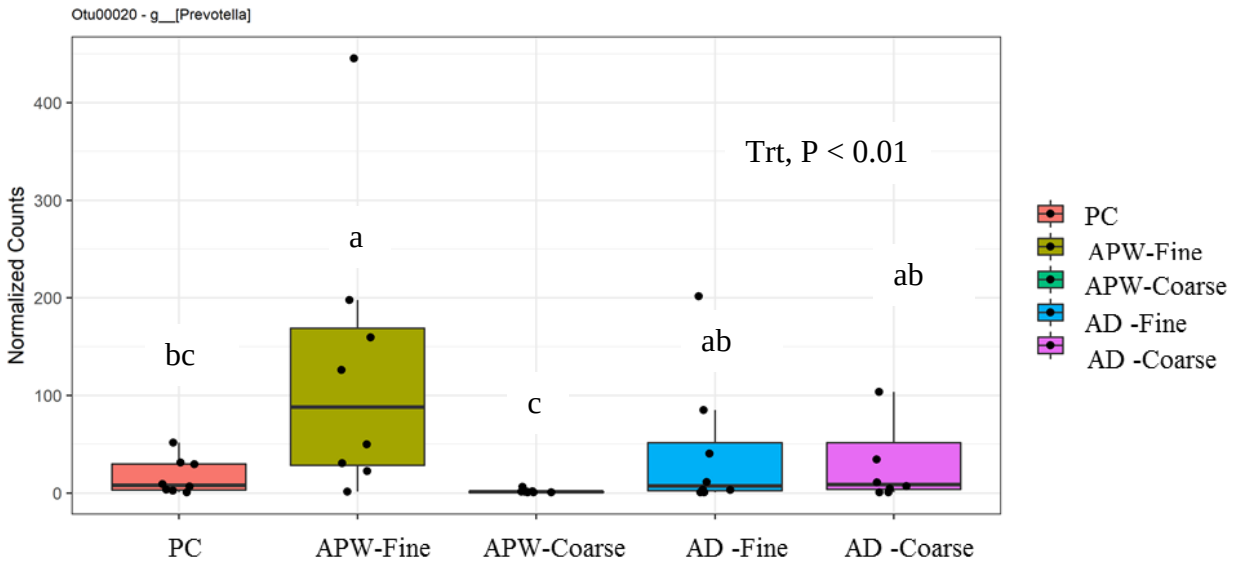

Figure 3.5. Differential abundance testing of low-abundance bacterial OTU *Prevotella* in colonic digesta samples from pigs fed either PC, diets with APW or acidified diets, where wheat was ground either Fine or Coarse

TABLES

Table 3.12. Performance of weanling pigs fed the control, diets with acid-preserved wheat (APW), or acidified diets (AD) where wheat was ground either finely or coarsely.[a]

Treatments	T1	T2	T3	T4	T5	T7	T8	T9		
MOA	NC	PC	Acid-preserved wheat, APW			Acidified diet, AD				
Acid	(-)	(-)	OIB	OIB	OIB	OIB	OIB	OIB		
Enzyme	NoEnz	Enz	Enz	Enz	NoEnz	Enz	Enz	NoEnz		
Particle size	Fine	Fine	Fine	Coarse	Fine	Fine	Coarse	Fine	SEM	P Value
ADG (g d^{-1})	374	419	448	435	297	368	368	326	61.3	0.45
ADFI (g d^{-1})	522	507	527	504	439	462	474	410	63.4	0.66
G:F	0.72	0.81	0.83	0.86	0.66	0.79	0.81	0.79	0.073	0.44

Note: MOA, mode of acid addition; NC, negative control; PC, positive control; OIB, organic-inorganic acid blend; PS, particle size; ADG, average daily gain; ADFI, average daily feed intake; G:F feed efficiency, Enz with enzyme, NoEnz without enzyme.

[a]Calculated from 8 pigs per treatment after feeding their respective diets for 12 d. Data represents d 5 to 12 of the experiment using body weight on d 5 as covariate.

T**able 3.13**. P values of pre-planned contrasts.

				Acid		MOA	Enzyme		PS		
Item	PC vs other	PC vs APW	PC vs AD	NC vs OIB-NoEnz	PC vs OIB-Enz	APW vs AD	NC vs PC	OIB-Enz vs OIB-NoEnz	OIB-Fine vs OIB-Coarse	MOA x Enz	MOA x PS
ADG	0.47	0.71	0.30	0.35	0.86	0.32	0.57	**0.07**	0.89	0.33	0.89
ADFI	0.49	0.77	0.30	0.13	0.82	0.29	0.84	0.17	0.90	0.73	0.72
G:F	0.74	0.72	0.78	0.95	0.96	0.86	0.30	0.17	0.67	0.22	0.89

Note: MOA, mode of acid addition; PS, particle size; ADG, average daily gain; ADFI, average daily feed intake; G:F feed efficiency.

Table 3.14. Apparent total tract digestibility of nutrients in weanling pigs fed control, diets with acid-preserved wheat (APW), or acidified diets (AD) where wheat was ground either finely or coarsely.[a]

Treatments	T1	T2	T3	T4	T5	T7	T8	T9		
MOA	NC	PC	Acid-preserved wheat, APW			Acidified diet, AD				
Acid	(-)	(-)	OIB	OIB	OIB	OIB	OIB	OIB		
Enzyme	NoEnz	Enz	Enz	Enz	NoEnz	Enz	Enz	NoEnz		
Particle size	Fine	Fine	Fine	Coarse	Fine	Fine	Coarse	Fine	SEM	P Value
					Diet Nutrient Analyses[b]					
DM (g kg^{-1} diet)	892.25	890.38	869.21	864.56	868.26	889.19	885.56	888.57		
GE (Mcal kg^{-1} DM)	4.46	4.54	4.49	4.50	4.50	4.65	4.46	4.51		
Ash (g kg^{-1} DM)	7.57	6.72	7.02	6.16	6.88	6.72	6.26	6.41		
P (g kg^{-1} DM)	0.69	0.75	0.73	0.68	0.75	0.70	0.67	0.72		
					Digestibility (%)[c]					
DM	93.56	93.98	94.24	94.61	93.88	94.44	93.41	94.50	0.385	0.21
GE	78.93	79.78	79.82	80.54	79.84	81.92	77.83	81.75	1.088	0.11
Ash	52.66	54.83	54.50	56.26	55.17	59.48	54.13	56.19	2.245	0.47
P	38.28b	51.86a	46.87ab	47.68ab	47.17ab	52.65a	46.23ab	51.12a	3.018	0.01

Note: MOA, mode of acid addition; NC, negative control; PC, positive control; OIB, organic-inorganic acid blend; NoEnz, no enzyme; Enz, with enzyme; SEM, standard error of mean; DM, dry matter; GE, gross energy; P, phosphorus. Means without a common letter within a row are different ($P < 0.05$).

[a]Average of eight pigs per treatment.

[b]Analyzed in duplicate at the General Nutrition Laboratory of the Department of Animal and Poultry Science, University of Saskatchewan.

[c]Calculated from acid insoluble ash and nutrient analyses of diets and feces.

Table 3.15. P values of pre-planned contrasts.

				Acid		MOA	Enzyme		PS		
Item	PC vs other	PC vs APW	PC vs AD	NC vs OIB-NoEnz	PC vs OIB-Enz	APW vs AD	NC vs PC	OIB-Enz vs OIB-NoEnz	T3T7 vs T4T8	MOA x Enz	MOA x PS
DM	0.65	0.52	0.83	0.21	0.41	0.55	0.41	0.53	0.35	0.71	**0.06**
GE	0.65	0.81	0.54	0.14	0.39	0.59	0.55	0.96	0.10	0.95	**0.02**
Ash	0.62	0.84	0.47	0.24	0.39	0.46	0.46	0.51	0.38	0.32	**0.09**
P	0.26	0.14	0.52	**<0.01**	0.52	0.24	**<0.01**	0.75	0.30	0.69	0.18

Note: PC, positive control; APW, acid-preserved wheat; AD, acidified diet; MOA, mode of acid addition; Enz, with enzyme; PS, particle size; GE, gross energy; P, phosphorus.

Table 3.16. Serum lactulose:mannitol ratio of weanling pigs fed diets with positive control, acid-preserved wheat (APW), or acidified diets (AD) where wheat was ground either finely or coarsely.[a]

Treatment	T2	T3	T4	T7	T8						
MOA	PC	Acid-preserved wheat, APW		Acidified diet, AD							
Acid	None	OIB	OIB	OIB	OIB						
Enzyme	Enz	Enz	Enz	Enz	Enz	Treatment		Time		Treatment*time	
PS	Fine	Fine	Coarse	Fine	Coarse	SEM	P value	SEM	P value	SEM	P value
0 Hours	0.172	0.068	0.049	0.043	0.095	0.0179	0.92	0.030	**<0.01**	0.044	0.36
+2 Hours	0.002	0.050	0.002	0.087	0.001						

Note: MOA, mode of acid addition; PC, positive control; OIB, organic-inorganic acid blend; Enz, with enzyme; PS, particle size; SEM, standard error of mean.

[a]Average of serum samples collected from 6 pigs per treatment.

P values were generated from log transformed data.

Table 3.17. Histology of mid-jejunum of weanling pigs fed with positive control, acid-preserved wheat (APW), or acidified diets (AD) where wheat was ground either finely or coarsely.[a]

Treatment	T2	T3	T4	T7	T8								
MOA	PC	Acid-preserved wheat, APW		Acidified diet, AD									
Acid	None	OIB	OIB	OIB	OIB			Treatment contrasts					
Enzyme	Enz	Enz	Enz	Enz	Enz								
Particle size	Fine	Fine	Coarse	Fine	Coarse	SEM	P value	PC vs other	PC vs APW	PC vs AD	MOA	PS	MOA x PS
Villous height, µm	448	395	417	406	403	38.2	0.88	0.32	0.37	0.35	0.97	0.79	0.74
Crypt depth, µm	300	314	305	295	310	25.0	0.98	0.83	0.76	0.93	0.77	0.88	0.61
Villous:crypt	1.54	1.28	1.46	1.40	1.37	0.168	0.86	0.42	0.43	0.49	0.91	0.66	0.53

Note: MOA, mode of acid addition; PC, positive control; OIB, organic-inorganic acid blend; PS; particle size; Enz with enzyme; SEM, standard error of mean.

[a]Measured from 15 to 20 well formed villi and crypt per pig for each pig. Data presented are average of 8 pigs per treatment.

Table 3.18. Expression of genetic markers indicative of barrier function, inflammatory and anti-inflammatory response, cellular proliferation and maturity in the mid-jejunum of weanling pigs fed positive control, diets with acid-preserved wheat (APW), or acidified diets (AD) where barley was ground either finely or coarsely.[a]

Treatment	T2	T3	T4	T7	T8								
MOA	PC	Acid-preserved wheat, APW		Acidified diet, AD									
Acid	None	OIB	OIB	OIB	OIB			Treatment contrasts					
Enzyme	Enz	Enz	Enz	Enz	Enz								
Particle size	Fine	Fine	Coarse	Fine	Coarse	SEM	P value	PC vs other	PC vs APW	PC vs AD	MOA	PS	MOA x PS
MUC2	1.19	0.65	0.62	0.88	0.78	0.250	0.68	0.35	0.21	0.64	0.34	0.66	0.62
CDN	1.23	0.95	1.06	1.47	1.24	0.189	0.88	0.84	0.60	0.88	0.40	0.82	0.55
OCLN	1.20	1.10	0.97	1.25	1.10	0.262	1.00	0.88	0.91	0.88	0.96	0.90	0.94
ZO1	1.00	0.74	0.78	1.11	0.85	0.203	0.98	0.94	0.90	0.99	0.89	0.57	0.75
IL-1β[b]	1.77	1.53	1.26	1.62	1.81	0.653	0.71	0.78	0.86	0.74	0.87	0.37	0.35
TNF-α[c]	1.48	0.95	0.87	1.79	1.82	0.434	0.51	0.86	0.47	0.68	0.16	0.30	0.60
IL-8[d]	1.47	1.18	0.64	1.53	1.47	0.388	0.74	0.87	0.82	0.59	0.35	0.80	0.30
IL-10[e]	1.58	1.09	1.04	1.62	1.29	0.497	0.92	0.40	0.36	0.55	0.67	0.88	0.96

IAP	1.14	0.95	1.23	1.53	1.38	0.254	0.85	1.00	0.79	0.80	0.53	0.95	0.33
PCNA	0.86	1.23	0.65	1.33	1.39	0.313	0.43	0.69	0.97	0.50	0.44	0.53	0.11

Note: MOA, mode of acid addition; PC, positive control; OIB, organic-inorganic acid blend; Enz, with enzymes; SEM, standard error of mean; MUC2, mucin 2; CDN4, claudin 4; OCLN, occludin; ZO-1, zona occludin 1; IL-1β, interleukin 1β; TNF-α, tumor necrosis factor α; IAP, intestinal alkaline phosphatase; PCNA, proliferating cellular nuclear antigen.

[a]Analyzed from mid-jejunal samples of 8 pigs per treatment.

P values were generated from log transformed data.

[b]Means based on the following n per treatment; T2 n=7; T3, n=6; T4, n=6; T7, n=8; T8, n=7. Highest SEM was reported.

[c]Means based on the following n per treatment; T2 n=8; T3, n=7; T4, n=7; T7, n=8; T8, n=7. Highest SEM was reported.

[d]Means based on the following n per treatment; T2 n=8; T3, n=8; T4, n=7; T7, n=8; T8, n=8. Highest SEM was reported.

[e]Means based on the following n per treatment; T2 n=4; T3, n=4; T4, n=7; T7, n=4; T8, n=2). Highest SEM was reported.

Table 3.19. Target genes and the primers used.

Target	Forward (5' to 3')	Reverse (5' to 3')	T_A (°c)	Eff (%)	R^2	Reference/NCBI/EST
		Reference Genes				
ACTβ	CACGCCATCCTGCGTCTGGA	AGCACCGTGTTGGCGTAGAG	63	116	0.998	Nygard et al. (2007)
RPL19	AACTCCCGTCAGCAGATCC	AGTACCCTTCCGCTTACCG	60	119	0.996	Meurens et al. (2009a)
		Markers of Barrier Function				
MUC2	ACCCGCACTACGTCACCTTC	GGCAGGACACCTGGTCATTG	62	111	0.991	BX671371
CDN4	CAACTGCGTGGATGATGAGA	CCAGGGGATTGTAGAAGTCG	60	106	0.998	Pasternak et al. 2015
OCLN	GAGTACATGGCTGCTGCTGA	TTTGCTCTTCAACTGCTTGC	60	111	0.980	Alizadeh et al. 2015
ZO-1	ACGGCGAAGGTAATTCAGTG	CTTCTCGGTTTGGTGGTCTG	60	120	0.985	XM_003353439.2
		Markers of Inflammatory Response				
IL8	TCCTGCTTTCTGCAGCTCTC	GGGTGGAAAGGTGTGGAATG	62	120	0.985	Meurens et al. 2009b
IL-1β	AGAAGAGCCCATCGTCCTTG	GAGAGCCTTCAGCTCATGTG	62	111	0.997	Meurens et al. (2009b)
TNFα	CCAATGGCAGAGTGGGTATG	TGAAGAGGACCTGGGAGTAG	60	108	0.996	Meurens et al. 2009b
		Marker of anti-inflammatory response				
IL10	CCATGGAAGTGGTCCGCCAA	GCCCAGGTAGCCATGGATC	55	104	0.984	Willing, 2007
		Cellular Maturity and Proliferation				

IAP	CTAAAGGGGCAGATGAATGG	CACCTGTCTGTCCACGTTGT	60	100	0.996	Lackeyram et al. 2015
PCNA	TACGCTAAGGGCAGAAGATAATG	CTGAGATCTCGGCATATACGTG	58	105	0.988	Willing and Van Kessel, 2007

Note: ACTβ, β-actin; RPL19, ribosomal protein L19; MUC2, mucin 2; CDN4, claudin 4; OCLN, occludin; ZO-1, zona occludin 1; IL-1β, interleukin 1β; TNF-α, tumor necrosis factor α; IAP, intestinal alkaline phosphatase; PCNA, proliferating cellular nuclear antigen.

Table 3.20. Digesta pH in different locations of the gastro-intestinal tract of weanling pigs fed the positive control, diets with acid-preserved (APW), or acidified diets (AD) where wheat was ground either finely or coarsely.[a]

Treatment	T2	T3	T4	T7	T8								
MOA	PC	Acid-preserved wheat, APW		Acidified diet, AD									
Acid	None	OIB	OIB	OIB	OIB			Treatment contrasts					
Enzyme	Enz	Enz	Enz	Enz	Enz								
Particle size	Fine	Fine	Coarse	Fine	Coarse	SEM	P value	PC vs other	PC vs APW	PC vs AD	MOA	PS	MOA x PS
Stomach	4.24	3.97	4.11	3.92	3.97	0.274	0.92	0.43	0.55	0.40	0.76	0.70	0.90
Duodenum[b]	6.08	5.86	5.73	6.17	6.05	0.185	0.30	0.46	0.15	0.89	**0.08**	0.45	0.97
Jejunum	6.29	6.54	6.36	6.48	6.28	0.133	0.53	0.40	0.33	0.59	0.59	0.15	0.89
Ileum	6.82b	7.56a	7.51a	6.98ab	6.61b	0.236	**0.03**	0.20	**0.02**	0.93	**<0.01**	0.40	0.50
Caecum	5.87	6.14	6.02	6.07	5.89	0.163	0.67	0.34	0.26	0.54	0.52	0.34	0.83
Colon	6.76	6.97	6.79	6.74	6.70	0.140	0.68	0.82	0.50	0.79	0.25	0.41	0.62

Note: MOA, mode of acid addition; PC, positive control; Enz with enzyme; SEM, standard error of mean. Means without a common letter within a row are different ($P < 0.05$).

[a]Average of samples collected from 8 pigs per treatment.

[b]Means were based on number of pigs where duodenal digesta samples were collected (T2, n=7; T3, n=5; T4, n=7; T7, n=6; T8, n=5). Highest SEM was reported.

Table 3.21. Concentration of short chain fatty acids (SCFA) and lactic acid (LA) in caecal digesta of weanling pigs fed positive control, diets with acid-preserved wheat (APW), or acidified diets (AD) where wheat was ground either finely or coarsely.

Treatment	T2	T3	T4	T7	T8								
MOA	PC	Acid-preserved wheat, APW		Acidified diet, AD									
Acid	None	OIB	OIB	OIB	OIB			Treatment contrasts					
Enzyme	Enz	Enz	Enz	Enz	Enz								
Particle size	Fine	Fine	Coarse	Fine	Coarse	SEM	P value	PC vs other	PC vs APW	PC vs AD	MOA	PS	MOA x PS
		µmol g^{-1} caecal digesta											
Acetic	79.14	75.74	83.94	76.14	73.92	6.561	0.84	0.82	0.93	0.61	0.47	0.65	0.43
Propionic	58.92	47.48	59.19	57.09	55.69	5.391	0.54	0.51	0.40	0.70	0.57	0.35	0.23
Isobutyric	0.22	0.19	0.09	0.17	0.17	0.090	0.94	0.69	0.61	0.83	0.71	0.63	0.62
Butyric	19.35	14.47	16.80	17.69	18.86	2.053	0.47	0.30	0.14	0.67	0.20	0.39	0.78
Isovaleric	0.30	0.34	0.17	0.33	0.27	0.087	0.57	0.79	0.90	0.55	0.38	0.19	0.56
Valeric	1.96	1.51	1.42	2.02	2.25	0.289	0.23	0.63	0.17	0.62	**0.03**	0.81	0.59
Caproic	0.00	0.00	0.16	0.00	0.00	0.049	**0.09**	0.46	0.18	1.00	0.10	0.11	0.10
Lactic[a]	147.40	139.90	169.97	384.10	324.31	37.113	0.83	0.39	0.65	0.28	0.45	0.66	0.93

SCFA, Total	159.88	139.73	161.76	153.44	151.16	12.831	0.76	0.56	0.56	0.63	0.90	0.45	0.35

Note: PC, positive control; MOA, mode of acid addition; OIB, organic-inorganic acid blend; Enz with enzyme; SCFA, short chain fatty acid; SEM, standard error of mean.

P values for isobutyric, isovaleric and caproic acids were derived from square root transformation of data.

[a]Average was based on the number of samples where LA was detected (T2, n=7; T3, n=6; T4, n=7; T7, n=6; T8, n=5). Highest SEM was reported

Table 3.22. Molar proportion of short chain fatty acids (SCFA) in caecal digesta of weanling pigs fed positive control, diets with acid-preserved wheat (APW), or acidified diets (AD) where wheat was ground either finely or coarsely.

Treatment	T2	T3	T4	T7	T8								
MOA	PC	Acid-preserved wheat, APW		Acidified diet, AD									
Acid	None	OIB	OIB	OIB	OIB			Treatment contrasts					
Enzyme	Enz	Enz	Enz	Enz	Enz								
Particle size	Fine	Fine	Coarse	Fine	Coarse	SEM	P value	PC vs other	PC vs APW	PC vs AD	MOA	PS	MOA x PS
			% of total SCFA										
Acetic	49.28[b]	54.26[a]	52.03[ab]	49.89[b]	48.86[b]	1.068	**0.01**	0.11	**0.01**	0.94	**<0.01**	0.13	0.58
Propionic	36.96	33.95	36.35	37.19	36.56	5.391	0.48	0.54	0.29	0.96	0.22	0.52	0.28
Isobutyric	0.16	0.14	0.07	0.10	0.10	0.068	0.95	0.67	0.64	0.74	0.87	0.65	0.64
Butyric	12.09	10.33	10.46	11.24	12.73	0.960	0.28	0.38	0.14	0.93	**0.09**	0.37	0.46
Isovaleric	0.21	0.25	0.12	0.21	0.16	0.070	0.60	0.78	0.98	0.63	0.58	0.16	0.54
Valeric	1.30	1.08	0.87	1.36	1.59	0.217	0.14	0.75	0.21	0.49	**0.02**	0.98	0.24
Caproic	0.00	0.00	0.10	0.00	0.00	0.032	0.13	0.49	0.21	1.00	0.13	0.13	0.13

Note: PC, positive control; MOA, mode of acid addition; Enz with enzyme; SCFA, short chain fatty acid; SEM, standard error of

mean.

P values for isobutyric, isovaleric and valeric acids were derived from square root transformation of data.

Table 3.23. Concentration of short chain fatty acids (SCFA) and lactic acid (LA) in colonic digesta of weanling pigs fed positive control, diets with acid-preserved wheat (APW), or acidified diets (AD) where wheat was ground either finely or coarsely.

Treatment	T2	T3	T4	T7	T8								
MOA	PC	Acid-preserved wheat, APW		Acidified diet, AD									
Acid	None	OIB	OIB	OIB	OIB			Treatment contrasts					
Enzyme	Enz	Enz	Enz	Enz	Enz								
Particle size	Fine	Fine	Coarse	Fine	Coarse	SEM	P value	PC vs other	PC vs APW	PC vs AD	MOA	PS	MOA x PS
				μ*mol* g^{-1} *digesta*									
Acetic	74.98	71.89	77.55	73.85	76.69	4.626	0.86	1.00	0.96	0.95	0.89	0.29	0.72
Propionic	45.80	39.48	48.61	45.19	46.28	4.798	0.74	0.87	0.77	0.99	0.73	0.29	0.41
Isobutyric	1.42	1.32	1.30	1.42	1.43	0.198	0.97	0.63	0.64	0.69	0.92	0.70	0.72
Butyric	19.68	14.40	17.67	17.08	18.85	2.385	0.47	0.35	0.22	0.63	0.36	0.22	0.62
Isovaleric	1.61	1.50	1.38	1.59	1.65	0.248	0.96	0.63	0.52	0.82	0.61	0.74	0.94
Valeric	2.92	2.21	2.44	3.15	3.37	0.516	0.34	0.98	0.38	0.41	**0.04**	0.63	0.91
Caproic	0.06	0.00	0.04	0.05	0.05	0.048	0.90	0.72	0.56	0.94	0.53	0.61	0.61
Lactic[a]	191.26	119.34	123.46	126.50	138.49	69.595	0.93	0.38	0.39	0.44	0.84	0.88	0.94

SCFA, Total	146.49	130.81	149.00	142.32	148.33	11.145	0.72	0.74	0.61	0.93	0.61	0.26	0.56

Note: PC, positive control; MOA, mode of acid addition; Enz with enzyme; SCFA, short chain fatty acid; SEM, standard error of mean.

P values for isobutyric, butyric, isovaleric valeric and caproic acids were derived from square root transformation of data.

[a]Average was based on the number of samples where LA was detected (T2, n=4; T3, n=5; T4, n=5; T7, n=8; T8, n=7). Highest SEM was reported.

Table 3.24. Molar proportion of short chain fatty acids (SCFA) in colonic digesta of weanling pigs fed the positive control, diets with acid-preserved wheat (APW), or acidified diets (AD) where wheat was ground either finely or coarsely.

Treatment	T2	T3	T4	T7	T8								
MOA	PC	Acid-preserved wheat, APW		Acidified diet, AD									
Acid	None	OIB	OIB	OIB	OIB			Treatment contrasts					
Enzyme	Enz	Enz	Enz	Enz	Enz								
Particle size	Fine	Fine	Coarse	Fine	Coarse	SEM	P value	PC vs other	PC vs APW	PC vs AD	MOA	PS	MOA x PS
			as % of total SCFA										
Acetic	52.29	55.59	52.56	52.18	51.87	1.445	0.37	0.64	0.32	0.88	0.17	0.26	0.35
Propionic	30.73	29.60	32.07	31.49	31.31	1.382	0.76	0.80	0.95	0.69	0.68	0.41	0.34
Isobutyric	1.06	1.08	0.93	1.04	0.95	0.172	0.96	0.74	0.79	0.73	0.93	0.48	0.86
Butyric	12.81	10.87	11.84	11.86	12.51	0.893	0.53	0.27	0.16	0.54	0.33	0.34	0.85
Isovaleric	1.22	1.24	0.99	1.17	1.09	0.208	0.92	0.69	0.68	0.75	0.91	0.44	0.69
Valeric	1.85	1.62	1.58	2.22	2.24	0.228	0.11	0.78	0.37	0.17	**0.01**	0.96	0.91
Caproic	0.04	0.00	0.02	0.03	0.03	0.027	0.89	0.69	0.52	0.92	0.51	0.66	0.59

Note: MOA, mode of acid addition; PC, positive control; APW, acid-preserved wheat; AD, acidified diet; OIB, organic-inorganic acid

blend; Enz with enzyme; SEM, standard error of mean; SCFA, short chain fatty acid.

P values for valeric and caproic acids were derived from square root transformation of data.

CHAPTER 4. THE INTERACTION OF ACID-PRESERVATION OF BARLEY, WITH OR WTIHOUT ENZYMES, AND PARTICLE SIZE ON PERFORMANCE AND GUT HEALTH OF WEANLING PIGS

Presented in part at the 2017 ASAS-CSAS Annual Meeting and Trade Show, 2018 BANFF Pork Symposium, and 2018 SaskPork Symposium.

Parts of this manuscript will be submitted to the Canadian Journal of Animal Science.

ABSTRACT

Two experiments were conducted to determine the efficacy of feeding acid preserved high moisture barley (APB) as an alternative to direct diet acidification (AD) on performance (Study 1) and gut health (Study 2) of weanling pigs. Barley was reconstituted to 20% moisture and preserved using a an organic-inorganic acid blend (OIB; phosphoric, lactic, malic and citric acids), with or without enzymes (Enz or NoEnz; phytase, carbohydrases, and protease), then stored in polyethylene barrels for 38 d. Dry barley and APB were ground in a hammer mill using a 2.0 mm screen (Fine). A subset of the dry barley and APB-Enz were ground using a 4.0 mm screen (Coarse) to allow for the comparison of particle size and its interaction with MOA. In Study 1, 256 pigs (21±2 d of age, 6.78±0.68 kg) were assigned to 64 pens in 4 rooms. Each pan was assigned to 1 of 8 treatments in a randomized complete block design. The 8 treatments were: negative control (NC, without acid or enzyme), positive control (PC, without acid, plus enzyme), APB-Enz-Fine, APB-Enz-Coarse, APB-NoEnz-Fine, AD-Enz-Fine, AD-Enz-Coarse, AD-NoEnz-Fine. Diets were fed as phase 1 from 0 to 7 d and phase 2 at 8 to 21 d post-weaning. Fecal samples were collected from each pen at the end of phase 2. In Study 2, 40 barrows (29 ± 2

d, 6.28 ± 0.58 kg) were assigned to 1 of 5 treatments in a randomized complete block design. The treatments, mode of acid addition (APB or AD) and particle size (Fine or Coarse) were in a 2 x 2 factorial arrangement, plus a control. The phase 2 diets used in Study1 were fed for 17 d. On d 16, pigs were gavaged with a solution of the indigestible sugar markers lactulose and mannitol, for estimation of intestinal permeability. Pigs were euthanized on d17 for collection of tissue samples for analysis of gene markers of barrier function and histology (mid-jejunum), and digesta for analysis of pH, concentration of short chain fatty acids and lactic acid, and microbial populations. In Study 1, treatment had no effect on ADG, ADFI or G:F of pigs during phase 1. During phase 2, pigs fed APB had higher ($P < 0.05$) ADG than those fed AD. Daily feed intake and G:F was comparable among pigs fed APB or AD ($P > 0.10$). Pigs fed Coarse had higher ($P < 0.05$) feed intake that those fed Fine regardless of MOA. Dry matter and energy ATTD were higher in pigs fed Coarse compared to Fine when fed APB diets, but not AD (MOA x PS, $P<0.01$). Pigs fed Enz had higher ($P<0.05$) ash digestibility than those fed NoEnz, and pigs fed Coarse had higher ($P<0.05$) ash digestibility compared to those fed Fine. In Study 2, treatment had no overall effect on markers of gut health measured in this experiment. The concentration of acetic acid was higher in pigs fed APB compared to AD ($P<0.05$) and valeric acid was higher in pigs fed Fine compared to Coarse regardless of MOA ($P<0.05$). Overall, the performance and gut health of pigs fed APB was comparable to those fed AD. It can be concluded that feeding APB can be an alternative to AD and fine grinding may not be required when APB is used.

INTRODUCTION

Maintenance of grain quality during storage requires a moisture content less than 15% (Hackl et al. 2010). If harvesting below 15% is not possible, artificial drying is employed, but this increases cost due to fuel, power and specialized drying structures. Barley stored in oxygen-limiting silos had improved feeding value potentially due to the activation of endogenous enzymes (Poulsen et al. 2012; Ton Nu et al. 2015). Alternatively, high-moisture grains may be preserved by acidification (Xu et al. 2016; Lopes et al. 2017). Propionic acid is commonly used to preserve high-moisture grains (McLelland 2008), however in addition to being corrosive, it is pungent and may pose health problems to workers in the mill.

In a meta-review by Tung and Pettigrew (2006), it was shown that diet acidification with organic acids improved weanling pig growth rates by 12% and 6% at 0 to 2, and 0 to 4 weeks post-weaning, respectively. The suggested modes of action include reduction in gastric pH resulting in improvements in nutrient digestibility, and the antimicrobial properties of organic acids. The secretion of hydrochloric acid (HCl) in the stomach of young pigs is insufficient and this negatively impacts nutrient digestibility and gut health. A stomach pH of 2.5 to 3.5 is required to activate the proenzyme, pepsinogen, into pepsin, the main enzyme of protein digestion. Similarly, a low pH is required for peak activity of exogenous microbial phytases (Simons et al. 1990). The reduction in gastric pH with acid supplementation is reported to inhibit the growth and proliferation of pathogenic microorganisms in the stomach and prevent entry further down the gastro-intestinal tract (GIT; Kil et al. 2011; Papatsiros et al. 2012). The pKa of the acid is the pH at which 50% is in undissociated form. The undissociated form of organic acids freely enters the microbial cell where pH is maintained at pH 7. Because the pH is higher than the pKa of organic acids, the acid dissociates, lowering pH and disrupting pH dependent

enzymatic processes altering metabolism (Gauthier 2002). Conversely, inorganic acids effectively reduce digesta pH but are not able to enter the microorganism even in the undissociated form (Gauthier 2002). Short chain fatty acids (SCFA) are organic acids naturally occurring in the GIT of pigs as products of bacterial fermentation of complex carbohydrates, primarily in the hind gut (Lee et al. 2007). Organic acids promoted the growth of *Lactobacilli* and reduced coliform load in the GIT when supplemented to the diets of weanling pigs (Ravindran and Kornegay 1993). Furthermore, SCFAs stimulate epithelial cell proliferation in the small and large intestines (Sakata and Inagaki 2001) thereby improving gut morphology.

Grains are ground prior to feed production. The reduction in particle size improves performance of pigs in all stages of the pig production cycle. The improvement is attributed to the increased surface area as particle size is reduced, providing better access for digestive enzymes. For example, G:F in finishing pigs improved by 8% when the corn particle size was reduced from 1,000 to 400 µm (linear, $P < 0.01$; Wondra et al. 1995). However, grinding too finely predisposes pigs to gastric ulcers (Friendship 2004). In contrast, feeding pigs coarse diets promote gut health. This is thought to be due to the reduction in gastric pH resulting from the production of lactic acid (LA) in the stomach, and to modification of SCFA concentrations in the hind gut. The digesta of pigs fed coarse diets is of a thicker consistency with high DM content, due to the reduced gastric emptying rate. This correlates with reduced gastric lesion scores (Nielsen and Ingvartsen 2000). In a study by Mikkelsen et al. (2002), feeding coarse diets reduced gastric pH by increasing the concentration of organic acids in the stomach, reducing gastric emptying rate and increasing the production of butyric acid in the caecum and colon compared to pigs fed fine diets.

4.1 Objectives

The overall objective of these series of experiments was to determine whether the benefits of diet acidification are maintained when the acids are provided through acidified barley. Information generated will provide producers another tool to utilize low-quality high-moisture barley and avoid costs associated with drying. Specifically, the objectives of these studies were:

1) To determine the effect of feeding an organic-inorganic acid blend (OIB)-preserved high-moisture barley (APB), with or without enzymes and its interaction with particle size on the performance and nutrient digestibility for weanling pigs.
2) To determine the effect of feeding APB and its interaction with particle size on gut health, pH of digesta along the GIT, fermentation products SCFA and LA in the caecum and colon, and microbial population diversity in the colon.

4.2 Hypotheses

These studies were conducted with the following hypotheses:

1) Pigs fed diets with APB would have the same performance as pigs fed an AD.
2) Because APB was stored at high-moisture and low pH conditions, exogenous and endogenous enzyme activities will be enhanced with consequent improvements in nutrient digestibility.
3) Improvements in nutrient digestibility would negate the need to grind APB finely.
4) Because coarse diets reduce gastric pH and alter SCFA in the hind gut, gut health status as estimated by lactulose:mannitol ratio, jejunal intestinal morphology, gene markers of barrier function, inflammatory and anti-inflammatory response, cellular maturity and proliferation, digesta pH and SCFA concentration will improve in pigs

fed coarse diets and would be better realized when APB is used.

MATERIALS AND METHODS

4.3 Animal care

All animal procedures were performed in adherence to the standard animal care protocol (Animal Use Protocol No. 20150054) approved by the University of Saskatchewan Committee on Animal Care and Supply for compliance with the Guide to the Care and Use of Experimental Animals by the Canadian Council on Animal Care (CCAC 2009).

4.4 Experimental diets

The eight dietary treatments used in this experiment are outlined in Table 4.1. Experimental diets were provided in two phases and were formulated to meet or exceed NRC (2012) recommendations for 6 to 13 kg pigs. Phase 1 diets were fed to pigs from experiment d 0 to 7 (estimated BW of 6 to 8 kg). Pigs received phase 2 diets from experiment d 8 to 21 (estimated BW of 8 to 13 kg). The ingredients in diets using acid-preserved barely (APB) were adjusted on a DM basis to account for differences in moisture content between the control barley and APB. In acidified diets (AD), the amount of acid and enzyme added were calculated to match the estimated acid contribution of the APB. Phase 2 diets contained an indigestible marker Celite (4 g kg^{-1} feed as fed basis; Celite 545- Celite Corporation, Lompoc CA, USA) as source of acid insoluble ash (AIA) to allow for the determination of apparent total tract digestibility (ATTD) of DM, energy, ash and P. Diets did not contain antibiotics or animal by-products except for phase 1 which contained whey permeate as a source of lactose. A common commercial diet (Master Feeds, Saskatoon, SK, Canada) was given to all pigs from experiment d

22 to 35 (BW from 13 to 25 kg) to determine if the treatments have a potential for a carry-over effect on pig performance post nursery stage. Formulation for phase 1 and phase 2 diets are summarized in Table 4.2 and Table 4.3, respectively. Phase 1 diets were produced at the Prairie Swine Centre Inc. and phase 2 at the Canadian Feed Research Center (CFRC, North Battleford, Saskatchewan, Canada).

4.5 Grains, acids and enzymes

Barley (var Austenson) was sourced from farms surrounding North Battleford, Saskatchewan, Canada. Barley contained 14.7% moisture (AOAC 930.15). This value was used to determine the amount of water to be added to increase the moisture to 20%. The commercial phosphoric acid-based organic-inorganic acid blend (OIB) was composed of 30-50% phosphoric acid, 0.1 to 1% lactic, 5 to 10% citric and 1 to 5% malic acid (MaxiCid, Canadian BioSystems, Canada). The commercially available enzyme cocktail (Enz) used was a combination of phytase and multi-carbohydrase enzymes (Superzyme Plus, Canadian BioSystems, Calgary, Alberta, Canada). The enzyme was added at 500 mg kg^{-1} of grain, effectively supplying 1500 FYT phytase, 1000 XYL xylanase, 250 GLU glucanase, 137.5 INV invertase, 350 HUT protease, 750 CMC cellulase, 3500 FAA amylase and 20 MAN mannanase per kg of grain.

4.6 Reconstitution, acidification and storage

Two hundred fifty kilograms of barley (14.7% moisture) were mixed with 16.6 kg of distilled water for reconstitution to 20% moisture. A 200 kg-capacity twin-shaft ribbon and paddle mixer (SCOTT 363, Scott Equipment, MN, USA) was used to homogenize the grain, water, acid and enzyme mixture. Because of limitations in the mixer capacity, each treatment was prepared in two batches of 125 kg. Barley and water were initially mixed by hand and

steeped for 10 min to allow for better water penetration into the kernel. These were then transferred to the mixer and mixed for 20 min. The enzyme was added at 500 g t^{-1} (125 g of enzyme premixed in 500 g of ground barley as carrier) after 2 min of mixing, followed by the acid which was added at 7 kg t^{-1} (1.75 kg per treatment). Because the only difference between, T3 and T4 was particle size, the grains were prepared together (total of 500 kg). The acidified barley was stored for 38 d in sealed polyethylene barrels at ambient room temperature.

4.7 Grain grinding, and throughput

Following storage, barley was ground in a hammer mill (G.J. Vis VISHM2014) using either a 2.0- or 4.0-mm screen for the Fine or Coarse particle size, respectively. Each treatment was ground in batches of 100 kg to allow measurements of throughput, power consumption and grinding cost in duplicate. To simulate feed production practices in on-farm mills, only the grain was ground. Motor load (% of motor capacity) was recorded while the hammer mill was running idle, and throughout the duration of grinding with an interval of 10 seconds to allow for calculation of power consumption (kWh). The weight of the grain prior to grinding and the grinding time was recorded to estimate grinding throughput (t h^{-1}).

EXPERIMENT 1.

This study was conducted to determine the effect of feeding acid-preserved barley, with or without enzymes, and its interaction with particle size on performance and nutrient digestibility in weanling pigs.

4.8 Experimental design and animal care

The experiment was conducted at the Prairie Swine Centre Inc. (Saskatoon, SK, Canada). A total of 256 pigs (Camborough Plus females × C337 sires; PIC Canada Ltd., Winnipeg, Manitoba, Canada) were weaned at 21 ± 2 d (6.78 ± 0.68 kg body weight) and assigned to one of 64 pens (123 cm long x 100 cm wide) in four nursery rooms with one room started per week. Each pen was equipped with a self feeder and a nipple drinker. Floors were plastic-covered slats. Pens were randomly assigned to one of eight treatments in a randomized complete block design (8 pens per treatment) with the pen as the experimental unit and room as block. The rooms were cleaned and disinfected 3 d prior to the transfer of animals. Room temperature was maintained at 28 ˚C and gradually reduced by 2 ˚C every week until 20 ˚C. Humidity was maintained at ~40% and a 12-12 h light-dark lighting program was implemented. Feed and water were provided ad libitum. Feeders were checked at daily 0830 and 1500 h for feed availability. The pigs were on experimental diets for 21 d followed by a common diet for 14 d, for a total trial duration of 35 d. There were no antibiotics administered during the study.

4.9 Sample and data collection

4.9.1 Grinding data

A video of the Repete system console was recorded for each grinding batch using a screen capture utility (BandiCam, Bandicam Company, Seoul, Korea). The captured video

provided information on running motor load (% of motor capacity) and actual grain weight. The video running time was used to estimate grinding time.

4.9.2 Grain and diet

Grain samples were collected per batch as it exited the mixer and pooled within treatment. At the end of the 38-d storage period, samples were collected from the barrels using a sampling probe for determination of pH, mould count, mycotoxin and chemical analysis. Ground grain samples were collected during bagging and pooled for particle size and handling characteristics. Diet samples were collected at the start of each experimental block and pooled. All grain and diet samples were double bagged to minimize moisture loss and stored at -20 ˚C until analysis.

4.9.3 Body weights and daily weight gain

Pigs were weighed individually at 0830 h at the start of the experiment (d 0; 21 d of age) and at the end of every growth phase (d 7, 21, and 35). Pigs were not fasted prior to weighing. Average weight of all pigs in each pen was determined to allow for estimation of daily weight gain. Average daily gain was determined by dividing the difference between the initial and final body weight by the number of days the pigs were on that phase (Eq. 4.1)

$$\text{ADG (g d}^{-1}\text{)} = \text{(Final weight – Initial weight) / days on feed} \qquad \text{Eq. 4. 1}$$

4.9.4 Feed disappearance

The amount of feed offered, and the amount of feed left in the feeders at the end of the growth phase was weighed to allow for the calculation of daily feed disappearance to estimate daily feed intake. Daily feed disappearance was calculated by dividing the difference between total feed offered and feed remaining by the number of days in that stage (Eq. 4.2).

$$\text{ADFI (g d}^{-1}\text{)} = \text{[Total feed offered (g) – Feed remaining (g)] / days on feed} \qquad \text{Eq. 4. 2}$$

4.9.5 Fecal collection

Freshly voided feces were collected at the end of phase 2 from at least 2 pigs in each pen by grab sampling. All fecal samples were stored at -20 ˚C until analysis. Dry matter, energy, ash, P, and AIA of the diet and feces were analyzed to estimate ATTD using the indigestible marker method described by Adeola (2001).

4.10 Market weights

Forty-eight pigs (8 treatments x 6 pigs per treatment, 3 barrows and 3 gilts) were ear-tagged on d 35 of the experiment (56 d of age) prior to transfer to the growing-finishing rooms where they were fed a common commercial grower-finisher diet until market. Pigs were weighed approximately 1 week before market and ADG was used to estimate the market weight at 165 d of age, the average market age of the pigs in the barn.

4.11 Analyses

4.11.1 Grains and diets

Proximate, Ca, and P analyses of grains and diets were conducted at the Central Testing Laboratories in Winnipeg, Manitoba. Proximate analysis included measures of DM (AOAC 930.15), N (AOAC 990.03), crude fibre (AOCS Ba6a-05), crude fat (AOCS Am 5-04) and ash (AOAC 923.03). Calcium and P were analyzed using AOAC, 968.08. Acid detergent fiber (ANKOM 08-16-06) and NDF (ANKOM 08-16-06) were also measured. Because there was visible mould growth in the APB, contaminated grains were removed prior to grinding and ground samples of control wheat and APB were analyzed for mycotoxin profile (Prairie Diagnostics Services, Saskatoon, SK). Mycotoxin levels were below maximum allowable levels

for swine as recommended by CFIA (Available: http://www.inspection.gc.ca /animals/feeds/regulatory-guidance/rg-/eng/1347383943203/1347384015909?chap=1).

Grains and diets were analyzed for pH using the method described by Radecki et al. (1988). Briefly, 5 g of grain or diet were mixed with 10 ml of deionized water and allowed to stand for 10 min with periodic shaking prior to pH measurement using a pH meter (Oakton pH 110 Series, Eutech Instruments, Thermo Fisher Scientific, Waltham, Massachusetts, USA).

4.11.2 Grinding power consumption, throughput and grinding cost

The hammer mill motor (NIMA Premium Efficiency Model, WEG Industries, Jaragua do Sul, Brazil) used in this experiment had a rated capacity of 37.3 kW (50 HP) with an efficiency of 94.1%. Power consumption while the motor was running idle and during grinding was estimated by multiplying their respective motor load with the rated capacity (kW) and motor efficiency. Gross power consumption during grinding was calculated as:

$$\text{Power consumption} = \sum P_i t_i, \qquad \text{Eq. 4. 3}$$

where P is the power draw (kW) and t is time interval (s) then expressed as kWh

Net power consumption due to grinding was estimated by deducting the idle power consumption from the gross power consumption during grinding.

Specific power consumption (kWh t^{-1}) was estimated by dividing the net power consumed (kWh) during grinding by the weight of the grain (t) that was ground. Grinding cost was calculated by multiplying the specific power consumption with the power cost of $0.116 per kWh (current cost at the Canadian Feed Research Centre, North Battleford; 2018)

Throughput was calculated by dividing the actual amount of grain (kg) and the time it took to grind (seconds), expressed in t h^{-1}.

4.11.3 Particle size analysis, bulk density, angle of repose and coefficient of friction

Frozen diet samples were thawed to room temperature prior to analysis. Particle size of the ground grain and diets were analyzed using the procedure outlined in ASAE (2012) using a stack of 11 sieves instead of 13, as the rotary sieve shaker can only accommodate 12 stacks, receiving pan included. Sieves US 6 and US 270 were omitted because less than 5% of the sample were retained on these sieves. The 11 sieves used had a hole diameter of 2,380 μm (US 8), 1,680 μm (US 10), 1,191 μm (US 14), 841 μm (US 20), 594 μm (US 28), 500 μm (US 35), 297 μm (US 48), 212 μm (US 65), 149 μm (US 100), 103 μm (US 150), and 74 μm (US 200). Sieves were stacked in order of decreasing opening size with the receiving pan at the bottom. One hundred grams of a sample was dispensed on the top of the stack and shaken for 10 min (Rotary Lab Sifter, Hoskin Scientific, Canada). The sample remaining on each sieve was weighed and used to calculate geometric mean diameter (dg_w) and particle size standard deviation (sg_w) using the equations described in ASAE (2012).

4.11.4 Angle of repose, coefficient of friction and bulk density

Samples were analyzed for emptying angle of repose, coefficient of friction and bulk density in triplicate at the BioProcess Engineering laboratory at the University of Saskatchewan (Saskatchewan, SK, Canada).

Emptying angle of repose provides an estimate of the angle required at the bottom of a bin to initiate flow. It was determined using a clear plastic cylinder 210 mm in height with a diameter of 145 mm. It has a false floor located 60 mm from the bottom with a 25.4 mm-diameter hole in the center from which the sample was allowed to drain. Five hundred grams of sample was loaded in the vessel, leveled, and drained through the hole in the false floor forming a funnel shaped crater. The vessel was tapped gently if the sample did not drain. The shortest and

farthest distance between the crater edge and the drain, and their respective height were measured and averaged. These were used to calculate the angle of repose using the equation:

$$\textbf{Angle of repose} = \textbf{tan}^{-1}\left[\frac{\textbf{opposite (distance from floor hole to edge or crater)}}{\textbf{adjacent (height of crater)}}\right] \quad \text{Eq. 4.4}$$

Coefficient of friction was measured using an inclined platform with a steel surface to simulate bin and feeder surfaces. Sample was loaded in a 5.08 x 5.08 x 2.54 mm (l x w x h) wooden frame, gently levelled and the excess removed. The frame was slightly elevated to ensure only the sample contacted the steel surface. The platform was inclined slowly (1 full turn of crank shaft in 6 s) and the angle (θ) at which the sample started to move along the steel surface was recorded and used to compute for the coefficient of friction using the equation:

$$\text{Coefficient of friction} = \tan\theta \quad \text{Eq. 4. 5}$$

Bulk density was determined using a Cox funnel, a 0.5 L cup and a wooden rod following the procedure outlined by Canadian Grain Commission (www.grainscanada.gc.ca, accessed: 28 July 2018). Briefly, 500 g of sample was dropped into the Cox funnel to standardize the flow of the material to the cup and was levelled using the wooden rod with three equal zigzag motions. The sample in the cup was weighed and bulk density was computed using the equation below. Bulk density was reported in kg m^{-3} (1 kg m^{-3}= 0.001 kg L^{-1}).

$$\text{Bulk density (kg L}^{-1}\text{)} = \text{weight sample (kg)} \div 0.5\ \text{L} \quad \text{Eq. 4. 6}$$

4.11.5 Diet and fecal DM, energy, ash, phosphorus and acid insoluble ash

Analysis of diet and fecal DM, energy, ash (AOAC 923.03), P (AOAC 965.17) and AIA (AOAC 920.08) were conducted at the General Nutrition Laboratory of the Department of

Animal and Poultry Science at the University of Saskatchewan. Diet DM was analyzed using AOAC 930.15 (135 ˚C for 2 h). Feces were thawed, and samples collected from the same pig were pooled and homogenized. Fecal DM was determined using the two-step drying method described by Goering and Van Soest (1970). Briefly, partial DM of the feces was first determined by drying in a force-draft oven at 55 ˚C for 48 h or until there was no observed change in weight. The feces were then ground to pass through 1 mm screen using a Restch Mill (ZM1, Newton, PA, USA) prior to analysis of final DM. Laboratory DM was determined by further drying two grams of the partially dried feces (aoac930.15; 135 ˚C for 2 h). Final DM was calculated using the equation:

$$\textbf{Final dry matter}, \% = \% \textbf{ Partial dry matter x } \frac{\% \textbf{ Laboratory dry matter}}{\textbf{100}} \qquad \text{Eq. 4. 7}$$

Diet and feces were analyzed for GE using an isoperibol bomb calorimeter (model 6400, Parr Instrument Co., Moline, Illinois, USA) with benzoic acid as standard.

Apparent total tract digestibility was calculated using the equation:

$$\text{ATTD}, \% = 100 - \left[\left(\frac{\% \text{ MD x } \%\text{NF}}{\%\text{MF x } \%\text{ND}}\right) \text{x } 100\right] \qquad \text{Eq. 4. 8}$$

where M_D is the amount of marker in the diet, M_F is the amount of marker in feces, N_F is the amount of nutrient in the feces and N_D is the amount of nutrient in the diet.

4.12 Statistical analyses

Residual error data were subjected to the Shapiro-Wilk test using Proc Univariate of SAS (SAS 9.4; SAS Institute, Cary, NC) to check normality of distribution. Data were transformed if $P < 0.05$.

Grain and diet particle and handling characteristics were analyzed as a completely randomized design using the Mixed Procedure of SAS (SAS 9.4; SAS Institute, Cary, NC) using the model:

$$Yi=\mu + \alpha_i + \varepsilon_i$$

where Y is the parameter to be tested, μ the mean, α_i the effect of treatment and ε_i is the experimental error.

Animal performance and ATTD data were analyzed as a randomized complete block using Proc Mixed in SAS with treatment as fixed effect and room as a random block effect. Data were analyzed using the statistical model:

$$Yij=\mu + \rho_i + \alpha_j + \varepsilon_{ij}$$

where Y is the parameter, μ the overall mean, ρ_i the random effect of the i^{th} block, α_j the fixed effect of the j^{th} treatment, ε_{ij}=error term associated to the j^{th} treatment and i^{th} block.

In all cases, $P < 0.05$ was considered significant, with P values between 0.05 and 0.10 a tendency. Means were separated using Tukey option in SAS. Data were discussed relative to a protected and an unprotected F-test (Barnette and McLean, 1999) using single degree of freedom pre-planned contrasts (Marini 2003). These included:

- Effect of acidification
    - Without enzyme (NC vs OIB-NoEnz)
    - With enzyme (NC vs OIB-Enz)
- Effect of enzyme and interaction with MOA
    - Without acid (NC vs PC)
    - With acid (OIB-Enz vs OIB-NoEnz)
- Mode of acid addition

- APB vs. AD

- Effect of particle size and interaction with MOA
  - OIB-Fine vs OIB-Coarse

RESULTS

Chemical and mycotoxin analyses of the grains are presented in Table 4.4 and Table 4.5 respectively. The DM of the ground, acid-preserved high-moisture barley (APB) ranged from 794.1 and 794.3 g kg^{-1} grain, while the control dry barley had 853.0 g kg^{-1}. The pH of the acid-preserved high-moisture barley was lower than the untreated control barley by an average of 0.71 pH units. Mould growth was observed at the top of the barrel of the OIB-preserved barley (870,000 CFU g^{-1}). Mouldy grains were removed from the barrel prior to grinding and samples of the ground control barley and APB were analyzed for mycotoxin levels. Mycotoxin levels were either undetectable or below maximum allowable limits (Table 4.5).

Table 4.6 and Table 4.7 summarize the chemical analyses of phase 1 and phase 2 diets respectively. On average, the DM of diets with dry control barley was higher than diets with APB by 27.73 g kg^{-1} in phase 1 and 28.96 g kg^{-1} in phase 2. The pH of the phase 1 diets ranged from 5.64 to 5.75, while in phase 2 diets, pH ranged from 5.41 to 5.51. In both dietary phases, pH of barley-based diets without acid did not differ from diets with acid regardless mode of addition.

4.13 Grinding cost and particle characteristics

Particle and handling characteristics of ground grains and diets are presented in Table 4.8. The highest grinding throughput was observed in coarsely ground dry barley, followed by coarsely ground APB and finely ground dry barley. The lowest grinding throughput was

observed with finely ground APB ($P < 0.01$). On average, compared to dry untreated barley grinding APB reduced throughput by 15.9% and 25.0% when ground using the 4.0 or the 2.0 mm screen, respectively. Power consumption was highest in finely ground APB and the lowest was from coarsely ground dry barley ($P < 0.01$). Finely ground dry barley and coarsely ground APB were intermediate and did not differ from each other. Compared to the control barley ground using the same screen size, power consumption increased by 80% when high-moisture barley was ground through 2.0 mm screen, and 76% when ground using 4.0 mm screen ($P < 0.01$). Overall, grinding cost increased by \$0.85 t^{-1} when high-moisture barley was ground using the 2.0 mm screen, and \$0.50 t^{-1} when grinding using 4.0 mm screen compared to dry untreated barley ($P < 0.01$) ground using the same screen.

Average particle size of high-moisture barley ground using either 2.0 mm or 4.0 mm was greater compared to the control barley ground using the same screen ($P < 0.01$), while there were no differences in particle size distribution (Table 4.8, $P < 0.10$). Bulk density of the ground high-moisture grains was less than the ground barley control ($P < 0.01$). Emptying angle of repose of APB-Enz-Coarse was lower than APB-Enz-Fine ($P < 0.05$), but both treatments were not different from Fine and Coarse control barley or from APB-Fine. Coefficient of friction of all APB were higher compared to control dry barley, and APB-Fine with or without enzyme, was higher than APB-Coarse ($P < 0.01$).

4.14 Animal performance

Average daily gain, feed intake and G:F ratio are presented in Table 4.9. Although pigs were in generally good health throughout the experiment, there were 5 mortalities (4 pigs died

and 1 euthanized). However, necropsy conducted by Prairie Diagnostic Services (Saskatoon, SK, Canada) indicated that deaths were not related to treatment.

Treatment had no effect on ADG, ADFI or G:F, feed intake or feed efficiency of pigs during phase 1 ($P > 0.10$).

During phase 2, pigs fed APB-Coarse-Enz had higher ADG compared to those fed the controls (NC or PC), fine diets without enzyme regardless of MOA of acid (APB or AD-NoEnz-Fine) or AD-Enz-Fine but not when compared to pigs fed APB-Fine-Enz or AD-Coarse-Enz ($P < 0.05$). Pigs fed AD-Enz-Coarse had higher ADFI compared to pigs fed APB-NoEnz-Fine or AD-NoEnz-Fine but not when compared to pigs fed NC, PC, APB-Enz-Fine, or APB-Enz-Coarse ($P < 0.01$). Pigs fed PC had higher ADFI compared to pigs fed AD-NoEnz-Fine. Feed efficiency of pigs fed APB-Enz-Coarse, APB-Enz-Fine or AD-NoEnz-Fine were higher than those fed NC, PC or AD-Enz-Coarse ($P < 0.05$).

Treatment contrasts showed that pigs fed APB had higher ADG compared to pigs fed AD (Table 4.10, $P < 0.10$) while ADFI or G:F did not differ ($P > 0.10$). Average daily feed intake was higher in pigs fed the PC diet compared to pigs fed APB or AD-NoEnz-Fine ($P < 0.05$) and ADFI of pigs fed AD or APB-Enz-Coarse was higher than pigs fed AD and APB-Enz-Fine ($P < 0.05$). Pigs fed acidified diets with (AD and APB-Enz-Fine) or without enzyme (AD and APB-NoEnz-Fine) tended to have higher G:F compared to pigs fed PC or NC respectively ($P < 0.10$). Pigs fed the combination of acids and enzymes had higher G:F compared to those fed diets devoid of both (NC; $P < 0.10$). In pigs fed APB, G:F tended to be higher when fed coarse compared to fine, which was not observed in pigs fed AD ($P < 0.10$).

A common diet was provided to the pigs during phase 3 to determine the potential for a carry-over effect. During phase 3, ADG of pigs previously fed APB-Enz-Coarse during phases 1

and 2 was higher than pigs fed APB-Enz-Fine, AD-Enz-Coarse and AD-NoEnz-Fine ($P < 0.05$). Treatment had no effect on ADFI during phase 3. Pigs fed APB-Enz-Coarse during phases 1 and 2 had higher G:F compared to pigs fed APB-Enz-Fine, AD-Enz-Coarse and AD-NoEnz-Fine ($P < 0.05$).

Overall, comparing MOA of acid, ADG and G:F of pigs fed diets with APB was higher than pigs fed AD (Table 4.10, $P < 0.05$). Comparing enzyme addition, ADFI of pigs fed PC was higher than pigs fed NC ($P < 0.05$) and regardless of MOA pigs fed NoEnz-Fine were higher than pigs fed Enz-Fine ($P < 0.05$).

4.15 Apparent total tract digestibility

Apparent total tract digestibility (ATTD) of DM, energy, ash and P are presented in Table 4.11. When fed diets with APB, DM and energy ATTD were improved in pigs fed Coarse compared to Fine which was not observed when pigs were fed AD (Table 4.12, $P < 0.01$). Pigs fed APB-Enz-Coarse had higher ATTD of ash when compared to pigs fed NC, APB-NoEnz-Fine or AD-NoEnz-Fine. Comparing enzyme addition, pigs fed diets with Enz had higher ATTD ash compared to those without Enz, ($P < 0.05$). Comparing PS, pigs fed Coarse had higher ATTD of ash compared to those fed Fine ($P < 0.01$). Digestibility of P tended to be higher in pigs fed Coarse compared to those fed Fine ($P = 0.09$).

DISCUSSION

High-moisture grains are prone to mould growth and artificial drying is often employed prior to storage. Alternatively, acids may be used to preserve high-moisture grains (Sauer and Burroughs 1974; Lin and Chen 1995; Higgins and Brinkhaus 1999). For example, Lynch et al

(1975) successfully preserved corn with propionic acid for four months, and when fed to finishing pigs, performance was similar to those fed the dry counterpart.

In pig production, weanling pig diets are typically acidified to help alleviate post-weaning lag, characterized by reduced growth rates and increased incidence of scouring immediately post-weaning (Lawlor et al. 2005). There is interest in the use of organic and inorganic acid blends (OIB) because inorganic acids are cheaper and effectively reduce pH, such that the organic acids remain in an undissociated form, which is required for effective anti-microbial action. However, there is little information on the use of these acids, specifically a phosphoric acid-based OIB, as a dietary acidifier and as a grain preservative.

In the current study, there was visible mould growth (870,000 CFU g^{-1}) in the high-moisture barley after 38 d in storage. This was not expected as mould growth was not observed in a previously conducted bench-scale trial using the phosphoric acid-based OIB to preserve high-moisture barley. In the current study, air was repeatedly re-introduced during weekly grain sampling which could have encouraged the mould growth in grains exposed to air. This contrasts to the bench-scale trial where each incubation jar was only opened once. Furthermore, the pH of the grains after storage were 5.79 (Enz) and 5.76 (NoEnz) and were higher than the pKa of the organic acids in the OIB mixture (LA, 3.86; citric acid, 3.13, 4.76, and 6.4; and malic acid 3.40 and 5.11; O'Niel 2006). Therefore, more of the organic acids would have been dissociated and therefore not functioning as an anti-microbial. These observations suggest that OIB was not able to totally preserve the high-moisture barley in aerobic conditions. However, the current study utilized barley that was reconstituted to 20% moisture. This water is retained in the pores within the grain by capillary forces. This unbound water is measured by water activity and strongly influences microbial activity. Water activity may be higher in reconstituted barley compared to

barley harvested at high-moisture where water is bound within the grain components and may elicit a different response in mould growth when preserved using the OIB.

The higher grinding cost of high-moisture barley was due to the reduction in grinding throughput and increased power consumption and is in agreement with the study of Probst et al (2013) who also reported increased power consumption and grinding cost with increased moisture in corn. Unlike the work of Probst et al. (2013) who saw no effect of moisture on particle size, the particle size of the high-moisture barley was coarser than dry barley that was ground using the same screen size by 133 μm using 2.0 mm screen, and by 210 μm when using the 4.0 mm screen. These observations can be attributed to the effect of moisture on the barley hull, making it more elastic and harder to grind causing particle size to increase. Probably due to the lower density of water than other components of the grain, (Stroshine 1998), ground APB had a lower bulk density than dry ground barley. Particle size of the dry barley did not affect flowability on a steel surface as measured by coefficient of friction. In the APB treatment, Coarse was more flowable than Fine. This can be attributed to the smaller surface area of the Coarse APB in contact with the steel surface compared to Fine.

Treatment had no effect on ADG, ADFI or G:F of newly weaned pigs fed control diets, diets with APB, or AD during phase 1. All the phase 1 diets contained lactose. As suggested by Giesting et al. (1991), Weeden et al. (1991), and Metzler and Mosenthin (2009), minimal growth promoting response can be expected from organic acid supplementation in milk-supplemented diets because lactose is a digestible source of energy and reduces stomach pH when LA is produced during fermentation (Maner et al. 1962; Wilson and Leibholz 1981; Ravindran and Kornegay 1993). However, during this phase, most of the pigs lost weight. The negative G:F in all treatments could be attributed to the negative ADG of pigs in most pens and low ADFI. This

is possibly due to the high inclusion of barley in the diet (413 to 431 g kg^{-1}) which is normally used at 100 to 150 g kg^{-1} in diets for young pigs. Results indicate, that lower levels of APB may be required for younger pigs.

Similar ADG between NC and PC during phase 2 suggests that the addition of enzyme, had no effect on growth rate of pigs fed barley-based diets. Similarly, the addition of OIB in diets without enzyme (NC vs AD-NoEnz-Fine) had no effect on growth rates. However, higher ADG (P = 0.08) was observed in pigs fed diets where both acid and enzymes were present compared to those fed a diet devoid of both. This suggests a synergism between the acid and the enzymes on barley. The commercial enzyme product contained phytases, carbohydrases, and proteases. We do not have information on the individual enzymes used in the commercial enzyme product, however, literature suggests a pH of 2.5 and 5.5 are required for peak activity of exogenous microbial phytases (Simons et al. 1990). Proteases are typically secreted as zymogens and converted into the active enzyme forms at low pH. For example, pepsinogen is quickly converted into pepsin, the main enzyme for protein digestion, at pH 2 to 3.5 and rapidly declines as pH increases. Pepsin is active at a low pH and inactive above pH 6 (Crevieu-Gabriel et al. 1999). In the current experiment the pH of diets with APB ranged from 5.41 to 5.47, and AD from 5.41 to 5.48 and may have potentiated the effect of these enzymes.

Comparing MOA, pigs fed diets with APB had higher growth rates compared to those fed AD (Table 4.10, P = 0.05) and may be attributed to the lower fiber (ADF and NDF) of APB compared to the dry control barley. Acid preserved barley had 26% lower ADF (60 and 49 vs 72 g kg^{-1} DM) and 8% lower NDF (170 and 168 vs 184 g kg^{-1} DM) compared to the control barley which may have resulted in a higher energetic value compared to the control barley.

In pigs fed AD, there was a tendency for higher ADFI in pigs fed Coarse compared to Fine which was not observed in APB (Table 4.10; MOA by PS, P = 0.10). This can be attributed to the improvement in ATTD of DM and energy in pigs fed Coarse compared to Fine observed with the APB. The increase in ADFI of pigs fed Coarse compared to Fine in pigs fed AD could be a compensatory response to the reduced digestibility of nutrients in Coarse barley as this was not accompanied by an improvement in G:F. However, the ATTD of DM and energy in pigs fed AD, regardless of particle size, did not differ and therefore does not support this observation. Digestibility measures the difference between nutrient intake and output but does not give information about the site of digestion or fermentation of DM (i.e. starch) which may affect energetic efficiency of starch use. A coarse particle size may cause a switch from enzymatic digestion to bacterial fermentation or site of starch digestion from the small intestine to the hind gut with consequent reduction in energetic efficiency of the starch (Gerrits et al. 2012; Zjilstra et al. 2012). Possibly, because of the enhanced activity of endogenous and exogenous enzymes at low pH and high-moisture during storage of grains, starch (or DM) may have been easily digested and absorbed in the small intestine of pigs fed APB. Combined with the suggested reduction in flow rate with coarse particle size, this may have further improved ATTD in pigs fed APB-Coarse compared to those fed APB-Fine.

The addition of OIB, regardless of MOA tended to improve G:F in diets with (P = 0.08) or without the enzyme complex (P = 0.09). Enzyme addition improved G:F only in the presence of OIB (OIB-Enz vs NC, P = 0.06) but not when OIB was absent (NC vs PC, P > 0.10). The addition of enzyme in the APB tended to improve G:F compared to when added through AD (P = 0.09). This can be attributed to the hydrolysis of nutrients during storage, suggesting enhanced exogenous enzyme activity when added to OIB-preserved barley. Similar to MOA and PS, there

may have been differences in the site of digestion and absorption between dry and acid-preserved barley.

In the current experiment, the addition of enzyme had no effect on the ATTD of DM, GE, ash or P (NC vs PC). Similarly, the addition of acid did not affect ATTD of DM or GE in weanling pigs. In pigs fed diets with APB, DM and GE digestibility when fed Coarse compared to Fine which was not was not observed in pigs fed AD. The improvement in digestibility of DM and energy in coarsely ground APB can be a combination of improved digestibility of starch (DM) and reduced gastric emptying rate. In an in-vitro study by Czarnecka et al. (1991), propionic acid preservation of high-moisture rye and triticale improved starch amylolytic activity. Furthermore, coarse diets reduce gastric emptying rate in pigs (Maxwell et al. 1970; Regina et al. 1999; Mikkelsen et al 2004). Ash ATTD was improved in pigs fed Coarse diets compared to Fine regardless of MOA of acid but was not affected by the addition of acid or enzyme. There was a tendency for higher ATTD of P in pigs fed Coarse compared to Fine (Table 4.11, P = 0.09). This again could be due to the longer retention time of the digesta in the stomach of pigs when diets are coarse. Overall, the supplementation of acids in weanling pigs presented as APB had similar ATTD of DM, GE, ash and P compared to pigs fed AD and does not fully explain the improvement in the growth of pigs fed APB, suggesting that other mechanisms may have been involved, possibly improvement in gut health with the use of APB. Furthermore, improvements were observed when diets with APB were fed coarse, but not as AD.

SUMMARY AND CONCLUSIONS

The increased grinding cost and reduced flowability of high-moisture barley must be considered when using this method of preservation. Fine grinding dry barley (2.0mm screen) had

the same grinding cost as grinding high-moisture barley coarsely (4.0 mm). The supplementation of barley-based diets with enzyme improved feed intake in pigs, but required the presence of the OIB for improvements in ADG and G:F to be realized. Feeding pigs acid-preserved barley had similar performance and resulted in similar nutrient digestibility compared to their AD counterpart suggesting feeding diets with acidified barley may be an alternative to direct acidification of weanling pig diets. When using APB in the diets, feeding coarse diets had no detrimental effects on the piglets and may even improve nutrient digestibility when compared to fine diets.

TABLES

Table 4.1. Treatment description of diets used in acid-preserved high-moisture barley experiments.

Treatment	Application	Acid	Enzyme	Particle size (screen)
T1. Negative Control	None	None	NoEnz	Fine (2.0 mm)
T2. Positive Control	None	None	Enz	Fine (2.0 mm)
T3. APB-Fine-Enz	Acid-preserved Barley, APB	OIB	Enz	Fine (2.0 mm)
T4. APB-Coarse-Enz	Acid-preserved Barley, APB	OIB	Enz	Coarse (4.0 mm)
T5. APB-Fine-NoEnz	Acid-preserved Barley, APB	OIB	NoEnz	Fine (2.0 mm)
T6. AD-Fine-Enz	Acidified Diet, AD	OIB	Enz	Fine (2.0 mm)
T7. AD-Coarse-Enz	Acidified Diet, AD	OIB	Enz	Coarse (4.0 mm)
T8. AD-Fine-NoEnz	Acidified Diet, AD	OIB	NoEnz	Fine (2.0 mm)

Table 4.2. Ingredient composition of barley-based phase 1 starter diets.[a]

Treatments	T1	T2	T3	T4	T5	T6	T7	T8
MOA	NC	PC	Acid-preserved barley, APB[b]			Acidified diet, AD		
Acid[c]	(-)	(-)	OIB	OIB	OIB	OIB	OIB	OIB
Enzyme	NoEnz	Enz	Enz	Enz	NoEnz	Enz	Enz	NoEnz
Particle size[d]	Fine	Fine	Fine	Coarse	Fine	Fine	Coarse	Fine
				g kg^{-1} as fed basis				
Barley	413.4			431.5			413.4	
Soybean meal	229.4			222.4			229.4	
Whey permeate	117.6			114.0			117.6	
Canola meal	100.0			96.9			100.0	
Peas	50.0			48.4			50.0	
Canola oil	38.4			37.2			38.4	
Dicalcium phosphate	13.1			12.7			13.1	
Limestone	13.2			12.8			13.2	
L-Lysine HCl (98.5%)	6.7			6.5			6.7	
Salt	6.4			6.2			6.4	

Celite[e]	4.0		3.9		4.0	
DL-methionine (99.5%)	2.4		2.3		2.4	
L-threonine (98.5%)	2.4		2.4		2.4	
Vit and min premix[f]	2.0		1.9		2.0	
Choline chloride (60%)	1.1		1.1		1.1	
OIB				2.9	2.9	2.9
Enzyme[g]		0.2		0.2	0.2	

Note: MOA, mode of acid addition; OIB, organic-inorganic acid blend; Prop, propionic acid; NoEnz without enzyme; Enz with enzyme; HCl, hydrochloric acid; Vit, vitamin; Min, mineral.

[a]Barley control, DM of 853 g kg^{-1} and acid-preserved barley DM 794 g $kg^{-1.}$

[b]Reconsitituted to 20% moisture and stored in polyethylene barrels for 34 d. Acid and enzyme were added during reconstitution

[c]Acid composition: OIB; 30-50% phosphoric acid, 0.1 to 1% lactic, 5 to 10% citric and 1 to 5% malic acid (Canadian BioSystems, Calgary, Alberta, Canada).

[d]Fine particle size, grain was ground using 2.0 mm screen; coarse using 4.0 mm screen.

[e]Source of acid insoluble ash, Celite Corporation, Lompoc CA, USA.

[f]Per kg of the starter premix contains: Vit A, 8,000,000 IU; Vit D3, 750,000 IU, Vit E, 35,000 mg; Vit K, 2,500 mg; Vit B1, 1,000 mg; Vit B2, 4,000 mg; Vit B3, 20,000 mg; Vit B5, 12,000 mg; Vit B6, 5,000 mg; Vit B7, 100 mg; Vit B9, 500 mg; Vit B12, 20 mg; Fe, 75,000 mg; Zn, 75,000 mg; Mn, 20,000 mg; Cu, 10,000 mg, Se, 150 mg; I, 500 mg.

[g]Superzyme Plus phytase and multi-carbohydrase enzyme (Canadian BioSystems, Calgary, Alberta, Canada).

Table 4.3. Ingredient composition of barley-based phase 2 starter diets.[a]

Treatments	T1	T2	T3	T4	T5	T6	T7	T8
MOA	NC	PC	Acid-preserved barley, APB[b]			Acidified diet, AD		
Acid[c]	(-)	(-)	OIB	OIB	OIB	OIB	OIB	OIB
Enzyme	NoEnz	Enz	Enz	Enz	NoEnz	Enz	Enz	NoEnz
Particle size[d]	Fine	Fine	Fine	Coarse	Fine	Fine	Coarse	Fine
				g kg^{-1}, as fed basis				
Barley	536.8			554.6			536.8	
Soybean meal	228.5			219.7			228.5	
Canola meal	99.6			95.7			99.6	
Peas	49.8			47.9			49.8	
Canola oil	39.7			38.2			39.7	
Limestone	13.2			12.7			13.2	
Dicalcium phosphate	11.1			10.7			11.1	
Salt	7.9			7.6			7.9	
L-lysine HCl (98%)	4.3			4.1			4.7	
Celite[e]	4.0			3.8			4.0	

Vit and Min premix[f]	2.0		1.9			2.0		
DL-methionine (99%)	1.1		1.0			1.1		
L-threonine (98.5%)	1.2		1.2			1.2		
Choline chloride (60%)	0.9		0.9			0.9		
OIB				3.8		3.8		3.8
Enzyme[g]		0.3		0.3		0.3		

Note: MOA, mode of acid addition; OIB, organic-inorganic acid blend; Prop, propionic acid; NoEnz without enzyme; Enz with enzyme; HCl, hydrochloric acid; Vit, vitamin; Min, mineral.

[a]Barley control, DM of 853 g kg^{-1} and acid-preserved barley DM 794 g $kg^{-1.}$

[b]Reconsitituted to 20% moisture and stored in polyethylene barrels for 38 d. Acid and enzyme were added during reconstitution.

[c]Acid composition: OIB; 30-50% phosphoric acid, 0.1 to 1% lactic, 5 to 10% citric and 1 to 5% malic acid (Canadian BioSystems, Calgary, Alberta, Canada).

[d]Fine particle size, grain was ground using 2.0 mm screen; coarse using 4.0 mm screen.

[e]Source of acid insoluble ash, Celite Corporation, Lompoc CA, USA.

[f]Per kg of the starter premix contains: Vit A, 8,000,000 IU; Vit D3, 750,000 IU, Vit E, 35,000 mg; Vit K, 2,500 mg; Vit B1,

1,000 mg; Vit B2, 4,000 mg; Vit B3, 20,000 mg; Vit B5, 12,000 mg; Vit B6, 5,000 mg; Vit B7, 100 mg; Vit B9, 500 mg; Vit B12, 20 mg; Fe, 75,000 mg; Zn, 75,000 mg; Mn, 20,000 mg; Cu, 10,000 mg, Se, 150 mg; I, 500 mg.

[g]Superzyme Plus phytase and multi-carbohydrase enzyme (Canadian BioSystems, Calgary, Alberta, Canada).

Table 4.4. Chemical analyses of control or high-moisture barley preserved with organi-inorganic acid blend (OIB), with or without enzymes.[a]

Barley	Control[b]	Acid-preserved barley	
Acid	N/A	OIB[c]	OIB
Enzyme	N/A	Enz	NoEnz
pH	6.48	5.79	5.76
DM (g kg^{-1} grain)	853.0	794.3	794.1
CP (g kg^{-1} DM)	118.1	113.0	113.7
CF (g kg^{-1} DM)	46.6	48.4	45.6
EE (g kg^{-1} DM)	30.4	28.9	26.5
Ash (g kg^{-1} DM)	33.9	27.4	27.6
Ca (g kg^{-1} DM)	1.0	0.6	0.6
P (g kg^{-1} DM)	4.2	4.3	4.1
ADF (g kg^{-1} DM)	71.8	59.6	49.0
NDF (g kg^{-1} DM)	184.1	169.6	168.4

Note: OIB, organic-inorganic acid blend; NoEnz, no enzyme; Enz, with enzyme; DM, dry matter; CP, crude protein; CF, crude fiber; EE, ether extract; Ca, calcium; P, phosphorus; ADF, acid detergent fiber; NDF, neutral detergent fiber

[a]Analyzed at the Central Testing Laboratory, Winnipeg, Manitoba (with the exception of pH).

[b]Used for control and acidified diets.

[c]Acid-preserved barley used for diet treatments T3 and T4 where the difference was only on particle size.

Table 4.5. Mycotoxin analyses of control or high-moisture barley with preserved an organic-inorganic acid blend (OIB), with or without enzymes.[a]

Barley	Control	Acid-preserved barley	
Acid	N/A	OIB	OIB
Enzyme	N/A	Enz	NoEnz
		µg kg^{-1}	
Deoxynivalenol	63.20	147.70	112.00
3-Acetyl-deoxynivalenol	25.50	30.00	<25.00
15-Acetyl-deoxynivalenol	<25.00	<25.00	<25.00
Diacetoxyscirpenol	<25.00	<25.00	<25.00
Nivalenol	26.80	49.20	34.70
T-2 Toxin	<25.00	<25.00	<25.00
HT-2 Toxin	<25.00	<25.00	<25.00
α-Zearalenol	<66.00	<66.00	<66.00
β-Zearalenol	<66.00	<66.00	<66.00
Aflatoxin B1	<25.00	<25.00	<25.00
Fumonisin B1	<25.00	<25.00	<25.00
Fumonisin B2	<25.00	<25.00	<25.00
Ochratoxin A	<25.00	<25.00	<25.00
Zearalenone	<25.00	<25.00	<25.00

Note: OIB, organic-inorganic acid blend; Prop, propionic acid; NoEnz, no enzyme; Enz, with enzyme; DM, dry matter

[a]Analyzed at the Prairie Diagnostic Services, Saskatoon, SK, Canada.

Table 4.6. Chemical analyses of phase 1 control diets, diets containing acid-preserved barley (APB), and acidified diets (AD).[a]

Treatments	T1	T2	T3	T4	T5	T6	T7	T8
MOA	NC	PC	Acid-preserved barley[b]			Acidified diet		
Acid[c]	None	None	OIB	OIB	OIB	OIB	OIB	OIB
Enzyme[d]	NoEnz	Enz	Enz	Enz	NoEnz	Enz	Enz	NoEnz
Particle size[e]	Fine	Fine	Fine	Coarse	Fine	Fine	Coarse	Fine
pH[f]	5.74	5.75	5.64	5.71	5.68	5.69	5.71	5.65
DM (g kg^{-1} diet)	882.2	894.5	842.9	864.0	900.4	901.5	902.1	903.5
Crude Protein (g kg^{-1} DM)	236.2	240.5	244.8	235.1	239.4	244.2	231.8	243.8
Crude Fiber, (g kg^{-1} DM)	41.0	38.2	43.9	45.5	40.1	44.5	47.8	46.6
EE (g kg^{-1} DM)	64.4	64.7	63.8	64.4	57.4	64.9	63.2	65.1
Ash (g kg^{-1} DM)	87.2	88.5	92.4	93.5	89.1	86.5	88.5	87.3
Ca (g kg^{-1} DM)	13.4	13.2	14.0	14.2	14.0	12.6	13.0	12.8
P (g kg^{-1} DM)	9.6	9.3	9.7	9.8	9.8	9.4	9.5	9.2
ADF (g kg^{-1} DM)	65.3	78.8	57.3	63.8	61.2	70.6	78.0	63.8
NDF (g kg^{-1} DM)	125.9	97.6	109.5	116.6	122.5	109.7	107.3	112.3

Note: MOA, mode of acid addition; Enz with enzyme, NoEnz without enzyme; DM, dry matter; CP, crude protein; CF, crude fiber; EE, ether extract; Ca, calcium; P, phosphorus; ADF, acid detergent fiber; NDF, neutral detergent fiber.

[a]Analyzed at the Central Testing Laboratory, Winnipeg, Manitoba

[b]Reconstituted to 20% moisture and stored in polyethylene barrels for 38 d. Acid and enzymes were added during reconstitution.

[c]Acid composition: OIB; 30-50% phosphoric acid, 0.1 to 1% lactic, 5 to 10% citric and 1 to 5% malic acid (Canadian BioSystems, Calgary, Alberta, Canada).

[d]Enzyme composition: phytase and multi-carbohydrase enzyme combination

[e]Fine particle size means that grain was ground using 2.0 mm screen and coarse using 4.8 mm screen

[f]Analyzed at the General Animal Nutrition Laboratory, Dept. of Animal and Poultry Science, University of Saskatchewan, Canada

Table 4.7. Chemical analyses of phase 2 control diets, diets containing acid-preserved barley (APB), and acidified diets (AD).[a]

Treatments	T1	T2	T3	T4	T5	T6	T7	T8
MOA	NC	PC	Acid-preserved barley[b]			Acidified diet		
Acid[c]	None	None	OIB	OIB	OIB	OIB	OIB	OIB
Enzyme[d]	NoEnz	Enz	Enz	Enz	NoEnz	Enz	Enz	NoEnz
Particle size[e]	Fine	Fine	Fine	Coarse	Fine	Fine	Coarse	Fine
pH[f]	5.51	5.42	5.47	5.41	5.41	5.41	5.48	5.51
DM (g kg^{-1} diet)	881.4	883.4	853.4	853.6	860.0	884.4	882.8	891.1
CP (g kg^{-1} DM)	209.6	200.7	225.8	223.3	219.2	189.2	221.5	197.9
CF (g kg^{-1} DM)	54.9	59.1	58.1	60.7	56.9	60.5	64.1	60.4
EE (g kg^{-1} DM)	75.1	76.5	68.9	73.4	74.4	79.7	72.6	74.9
Ash (g kg^{-1} DM)	65.0	67.8	70.9	75.3	68.3	72.0	70.5	66.5
Ca (g kg^{-1} DM)	9.1	9.7	9.0	11.1	9.7	11.0	9.5	9.4
P (g kg^{-1} DM)	7.8	8.1	7.9	8.5	8.5	8.5	7.8	8.3
ADF (g kg^{-1} DM)	82.6	89.8	103.3	85.8	80.6	81.4	104.7	104.2

NDF (g kg^{-1} DM)	145.2	153.7	153.8	144.0	147.0	144.5	151.2	150.9

Note: MOA, mode of acid addition; Enz with enzyme, NoEnz without enzyme; DM, dry matter; CP, crude protein; CF, crude fiber; EE, ether extract; Ca, calcium; P, phosphorus; ADF, acid detergent fiber; NDF, neutral detergent fiber.

[a]Analyzed at the Central Testing Laboratory, Winnipeg, Manitoba

[b]Reconstituted to 20% moisture and stored in polyethylene barrels for 38 d. Acid and enzyme were added during reconstitution.

[c]Acid composition: OIB; 30-50% phosphoric acid, 0.1 to 1% lactic, 5 to 10% citric and 1 to 5% malic acid (Canadian BioSystems, Calgary, Alberta, Canada).

[d]Enzyme composition: phytase and multi-carbohydrase enzyme combination.

[e]Fine particle size; grain was ground using 2.0 mm screen and coarse using 4.0 mm screen.

[f]Analyzed at the General Animal Nutrition Laboratory, Dept. of Animal and Poultry Science, University of Saskatchewan, Canada

Table 4.8. Grinding cost, particle and handling characteristics of control and acid-preserved high-moisture barley (APB).

Barley	Control		Acid-preserved barley, APB				
Acid	N/A	N/A	OIB	OIB	OIB		
Enzyme	N/A	N/A	Enz	Enz	NoEnz		
Particle size[A]	Fine	Coarse	Fine	Coarse	Fine	SEM	P value
			Grinding properties and cost				
Throughput (t h^{-1})	2.09c	2.88a	1.58d	2.42b	1.59d	0.021	<0.01
Power consumption (kWh t^{-1})	8.85b	5.85c	13.41a	8.22b	13.86a	0.196	<0.01
Power cost ($ t^{-1})	1.02b	0.68c	1.56a	0.96b	1.61a	0.024	<0.01
			Particle and handling characteristics				
d_{gw} (μm)	597d	877b	771c	1087a	690cd	17.2	<0.01
s_{gw} (μm)	2.11	1.93	1.99	1.92	2.28	0.069	0.06
Bulk density (kg m^{-3})	555a	527b	457c	460c	456c	3.0	<0.01
Angle of repose (˚)	78ab	80ab	90a	70b	85ab	3.6	0.02
Coefficient of friction	0.31c	0.26c	0.54a	0.46b	0.50a	0.010	<0.01

Note: OIB, organic-inorganic acid blend; Prop, propionic acid; NoEnz, without enzyme; Enz, with enzyme; d_{gw}, mean geometric

diameter; s_{gw}, particle size standard deviation; SEM, standard error of mean. Means within a row without a common letter are different ($P < 0.05$).

[A]Fine particle size; grain was ground using 2.0 mm screen and coarse using 4.0 mm screen.

Table 4.9. Performance of weanling pigs fed control, diets with acid-preserved barley (APB), or acidified diets (AD) where barley was ground either finely or coarsely.[a]

Treatments	T1	T2	T3	T4	T5	T6	T7	T8		
MOA	NC	PC	Acid-preserved barley, APB			Acidified diet, AD				
Acid	None	None	OIB	OIB	OIB	OIB	OIB	OIB		
Enzyme	NoEnz	Enz	Enz	Enz	NoEnz	Enz	Enz	NoEnz		
Particle size	Fine	Fine	Fine	Coarse	Fine	Fine	Coarse	Fine	SEM	P value
					0 to 7 d					
ADG (g d^{-1})	-30	-29	-15	-17	-25	-27	-13	-22	14.5	0.82
ADFI (g d^{-1})	56	56	63	58	56	55	68	59	11.6	0.75
G:F	-0.86	-0.71	-0.48	-0.43	-2.32	-2.00	-0.30	-0.42	0.896	0.55
					8 to 21 d					
ADG (g d^{-1})	198c	214bc	246ab	260a	203c	215bc	221abc	204bc	18.0	**0.04**
ADFI (g d^{-1})	369abc	386ab	353abc	367abc	337bc	345abc	422a	292c	30.7	**<0.01**
G:F	0.56b	0.57b	0.70a	0.72a	0.62ab	0.62ab	0.55b	0.69a	0.048	**0.03**
					22 to 35 d[b]					
ADG (g d^{-1})	582abc	620abc	556c	651a	650ab	601abc	566bc	557bc	40.0	**0.03**

ADFI (g d^{-1})	772	834	776	848	833	816	792	801	23.3	0.14
G:F	0.74abc	0.74abc	0.72bc	0.80a	0.77ab	0.74abc	0.71c	0.70c	0.022	**0.01**
				36 to 144 d[c]						
ABW (165 d, kg)	136.0	135.0	130.8	137.1	136.1	132.3	132.3	134.5	0.035	0.90
ADG (g d^{-1})	1.08	1.08	1.04	1.06	1.08	1.04	1.04	1.07	0.048	0.90

Note: MOA, mode of acid addition; PS, particle size; NC, negative control; OIB, organic-inorganic acid blend; NoEnz, without enzyme; Enz, with enzyme; SEM, standard error of mean; ADG, average daily gain; ADFI, average daily feed intake; G:F, feed efficiency. Means within a row without a common letter are different ($P < 0.05$).

[a]Means were calculated from 8 pens per treatment.

[b]Day 22 to 35 data used d 21 average body weight as a covariate.

P value of d 0 to 7 G:F was based on square root transformation of data.

Day 144 ABW and d 36 to 144 ADG were average of 6 pigs per treatment except for T1 and T2 where 1 pig died each.

Table 4.10. P values of treatment contrasts.

	Acidification		Enzyme		Acid and Enz vs				
	w/o enzyme	w/ enzyme	w/o acid	w/ acid	without	MOA	PS	Interactions	
	NC vs OIB NoEnz	PC vs OIB Enz	NC vs PC	OIB-Enz vs OIB-NoEnz	NC vs OIB-Enz	APB vs AD	OIB-Fine vs OIB-Coarse	MOA x Enz	MOA x PS
					0 to 7 d				
ADG	0.56	0.49	0.94	0.83	0.44	0.80	0.50	0.39	0.39
ADFI	0.77	0.61	0.98	0.80	0.63	0.77	0.50	0.33	0.13
G:F	0.63	0.62	0.90	0.88	0.72	0.88	0.26	0.05	0.41
					8 to 21 d				
ADG	0.76	0.36	0.45	0.07	0.08	**0.05**	0.47	0.27	0.79
ADFI	**0.02**	0.11	0.52	0.07	0.39	0.95	**0.02**	0.33	0.10
G:F	**0.08**	**0.09**	0.86	0.86	**0.06**	0.11	0.56	**0.09**	0.28
					22 to 35 d				
ADG	0.34	0.66	0.55	0.31	0.76	**<0.01**	0.61	0.67	0.74
ADFI	0.09	0.18	0.05	0.33	0.38	0.35	0.29	0.10	**0.04**

G:F	1.00	0.75	0.60	0.19	0.39	**0.01**	0.07	0.46	0.36
					36 to 144 d				
ABW	0.82	0.40	0.38	0.96	0.85	0.84	0.87	0.24	0.68
ADG	0.71	0.68	0.33	0.65	0.47	0.87	0.49	0.29	0.75

Note: MOA, mode of acid addition; PS, particle size; NC, negative control; OIB, organic-inorganic acid blend; NoEnz, without enzyme; Enz, with enzyme; ADG, average daily gain; ADFI, average daily feed intake; G:F, feed efficiency.

Table 4.11. Apparent total tract digestibility of nutrients in weanling pigs fed control, diets with acid-preserved barley (APB) or acidified diets (AD) where barley was ground either finely of coarsely.[a]

Treatment	T1	T2	T3	T4	T5	T6	T7	T8		
MOA	NC	PC	Acid-preserved barley, APB			Acidified diet, AD				
Acid	None	None	OIB	OIB	OIB	OIB	OIB	OIB		
Enzyme	Noenz	Enz	Enz	Enz	Noenz	Enz	Enz	Noenz		
Particle size	Fine	Fine	Fine	Coarse	Fine	Fine	Coarse	Fine	SEM	P value
					Chemical analyses[b]					
DM (g kg^{-1} diet)	881.37	883.42	853.41	853.55	860.00	884.42	882.80	891.05		
GE (Mcal kg^{-1} DM)	4.56	4.55	4.59	4.58	4.59	4.61	4.54	4.57		
Ash (g kg^{-1} DM)	64.72	71.24	80.09	73.18	72.08	66.43	74.28	68.67		
P (g kg^{-1} DM)	6.99	7.22	8.26	7.96	7.88	7.04	7.73	7.29		
					Apparent total tract digestibility, ATTD (%)[c]					
DM	90.57ab	90.59ab	88.95b	91.49a	89.75ab	90.86ab	90.37ab	89.56ab	0.487	0.02
GE	75.97ab	76.20ab	72.74b	78.42a	74.26ab	77.27ab	76.62ab	75.46ab	1.082	0.02
Ash	47.38bc	51.82ab	48.97abc	57.99a	44.44c	49.94abc	56.78ab	45.76c	2.258	<0.01
P	41.86	50.18	46.09	57.54	44.33	51.54	53.77	41.93	4.530	0.09

Note: MOA, mode of acid addition; NC, negative control; PC, positive control; NoEnz, without enzyme; Enz, with enzyme; SEM, standard error of mean; DM, dry matter; GE, gross energy; P, phosphorus. Means within a row without a common letter are different ($P < 0.05$).

[a]Average of eight pens per treatment.

[b]Analyzed in duplicate at the General Nutrition Laboratory of the Department of Animal and Poultry Science, University of Saskatchewan.

[c]Calculated from acid insoluble ash and nutrient analyses of diets and feces.

Table 4.12. P-values of contrasts.

	Acidification		Enzyme		Acid and Enz vs without	MOA	Particle size	Interactions	
	w/o enzyme	w/ enzyme	w/o acid	w/ acid					
	NC vs OIB NoEnz	PC vs OIB Enz	NC vs PC	OIB-Enz vs OIB-NoEnz	NC vs OIB-Enz	APB vs AD	OIB-Fine vs OIB-Coarse	MOA x Enz	MOA x PS
DM	0.13	0.26	0.98	0.61	0.27	0.62	**0.04**	**0.04**	**<0.01**
GE	0.40	0.37	0.88	0.89	0.47	0.14	**0.02**	0.13	**<0.01**
Ash	0.38	0.37	0.14	**0.04**	0.43	0.84	**<0.01**	0.93	0.61
P	0.81	0.79	0.16	0.18	0.18	0.95	0.11	0.35	0.28

Note: MOA, mode of acid addition; NC, negative control; PC, positive control; NoEnz, without enzyme; Enz, with enzyme; DM, dry matter; GE, gross energy; P, phosphorus.

EXPERIMENT 2.

This study was conducted to determine the effect of feeding acid-preserved barley, with or without enzymes, and its interaction with particle size on gut health of weanling pigs.

4.16 Experimental design

A total of 40 barrows (Camborough Plus females × C337 sires; PIC Canada Ltd., Winnipeg, Manitoba, Canada) were pre-selected during weaning based on age (21 ± 2 d) and body weight (5.0 to 6.0 kg BW) from the farrowing rooms in three successive batches (15, 10 and 15 pigs for batch 1, 2 and 3 respectively). Pigs in each batch were group-housed and fed a commercial diet for 8 d in the nursery rooms. At 29 d of age (experiment d1, 6.26 ± 0.53kg BW), pigs were randomly assigned to 1 of 5 pens with 2 or 3 pigs in each pen depending on batch size. Pens were randomly assigned to 1 of 5 treatments for adaptation to diet. Pigs were group-housed to minimize the number of days the pigs were in isolation. On d 9 (37 d of age) pigs were weighed and transferred to individual pens and remained on their treatment diets until slaughter (45 d of age). Feed remaining was weighed to allow for estimation of feed disappearance per pig. The pigs were housed individually in 1.5 m long x 1.5 m wide pens with polyvinylchloride walls and plastic-coated slatted floors. Toys (PVC pipes or plastic balls) were provided as enrichment and, a see-through plexi-glass window (0.3 x 0.3m) allowed the pigs to see each other and potentially reduce the stress due to isolation. The pigs were offered the diets to supply three times maintenance energy requirement (110 kcal kg^{-1} DE x $BW^{0.75}$, Adeola 2001). The daily feed allocation was divided into two equal amounts, provided at 0830 and 1530 h. Feed refusal, if any, was collected daily until d 15, weighed and deducted from daily feed offered to estimate daily feed disappearance. The pigs had *ad libitum* access to water and the experimental room was

maintained at 24 ˚C, ~40% humidity, and 12-12 h light-dark lighting program during the entire experiment.

4.17 Experimental diets

For this experiment, only the enzyme containing diets were used (5 of the 8 treatment diets in Table 4.1). Treatments were selected to allow for the evaluation of the effect of feeding APB and its interaction with particle size on intestinal permeability and gut health. Treatments used for this experiment were: T2) positive control, T3) APB-Enz-Fine, T4) APB-Enz-Coarse, T6) AD-Enz-Fine and T7) AD-Enz-Coarse.

4.18 Sample and data collection

4.18.1 Intestinal permeability (lactulose and mannitol gavage)

To determine the effect of acidification of high-moisture barley and its interaction with particle size on intestinal permeability, pigs were given a gavage of insoluble sugar markers on d 16 (44 d of age) based on the methods of Wijtten et al (2011), Zhang et al (2000) and Kansagra et al (2003). Pigs were weighed one day before the gavage (d 15) to allow estimation of the amount of sugar solution administered. The sugar marker solution provided 500 mg of lactulose and 100 mg of mannitol per kg BW. The oral gavage was given by intubation using a feeding tube (size 14FR, MED-RX, Canadian Hospital Specialties, Ltd., Oakville, Ontario, Canada) modified to ~46 cm in length and attached to a 12 ml syringe. The syringe and feeding tube were flushed with 10 ml of distilled water immediately after gavage to ensure that no sugar solution remained in the tube. Feed was withdrawn 2 h (0630 h) before the gavage and returned 2 h post-gavage. Blood was collected by jugular venipuncture into silica spray-coated, red-capped Vacutainer tubes (BD Vacutainer, BD, Mississagua, ON, Canada) before gavage to serve as

baseline, and exactly 2 h post gavage. Blood was spun at 830 g for 10 min and serum was collected and stored in -20 ˚C until analysis.

4.18.2 Euthanasia, tissue and digesta collection

Pigs were euthanized at d 17 (45 d of age) to allow for collection of tissue and digesta samples. One-hour post morning feeding, pigs were weighed, then humanely euthanized using a captive bolt. Gastro-intestinal organs were collected via a mid-line incision in the abdomen. The small intestine was divided into thirds to represent the duodenum, jejunum and ileum. A section of the mid-jejunum (5 cm) was collected, cut open lengthwise and placed in 10% formalin for histological measurements. Another section from the mid-jejunum was placed in sterile plastic bags (3” x 5”, Fisherbrand, Fisher Scientific, Hampton, New Hampshire, USA) and immediately put on dry ice subsequent to storage at -80 ˚C until analysis of gene markers. Digesta from the stomach, mid-duodenum, mid-jejunum, mid-ileum, caecum and mid-colon were collected for determination of pH. A subset of digesta samples from caecum and mid-colon were collected in 2 ml cryovials and immediately placed in dry ice then stored in -80 ˚C until analysis of microbial populations, SCFA and LA.

4.19 Analyses and calculation

4.19.1 Digesta pH

Immediately after collection, digesta samples from the stomach, small intestine (duodenum, jejunum, ileum), caecum and colon were analyzed for pH according to the method described by Risley et al. (1993). Briefly, each sample was homogenized and 1 g of digesta was mixed with 9 ml deionized water (1:9 w/v) and allowed to sit for 5 min with periodic shaking

prior to pH measurement (Oakton pH 110 series, Eutech Instruments, Thermo Fisher Scientific, Waltham, Massachusetts, USA). Readings were recorded in duplicate.

4.19.2 *Lactulose and mannitol*

Frozen samples were thawed on ice. An aliquot (600 µL) was filtered through 0.45 µm cutoff ultrafiltration spin columns (Millipore, Sigma) by spinning at 2500 x g for 2 min at 4 °C. Each filtered sample was split into duplicate HPLC vials, with each vial containing 200 µL of sample and then frozen. Serum lactulose and mannitol were analyzed at the National Research Council (Saskatoon, SK, Canada) using ion chromatography based on the procedure of Cabrera (2013) and Hurum and Rohrer (2016). Briefly, the analysis was performed on a Dionex ICS-3000 ion chromatography system using Chromeleon software (version 6.80 SR10, build 2818). The system consisted of an autosampler, dual pump and detector / chromatography (DC) modules, with the DC containing the analytical column, guard column, and high-performance anion exchange – pulsed amperometric detector (HPAE-PAD), all maintained at 30 °C. The columns used were a Dionex CarboPac MA1 4 x 50 mm guard followed by a Dionex CarboPac MA1 BioLC Analytical 4 x 250 mm column. The mobile phase was 480 mM NaOH at a flow rate of 0.4 mL min^{-1}. The detector was programmed to quantify using the calibration curves run with the samples to yield the amount of analyte present (µg mL^{-1}). Standards and blanks were prepared and analyzed with each batch of serum samples.

4.19.3 Histology

Sections of the jejunum (5 µm thick; 3 per slide) were fixed in 10% neutral buffered formalin and processed for paraffin embedding and staining with hematoxylin and eosin (H&E stain) at Prairie Diagnostic Services (Saskatoon, SK). Slides were viewed under a clinical light microscope and photomicrographs were acquired with 10 x magnification using the imaging

software AxioVision LE64 v 4.9.1 (Carl Zeiss AG, Oberkochen, Germany) running a high-resolution digital camera (AxioCamMR, Carl Zeiss AG, Oberkochen, Germany). A line was drawn to estimate the junction of the villi and crypt and was used as reference point for measurement. Between 15 to 20 intact villi and the corresponding crypt were measured per pig.

4.19.4 RNA extraction, reverse transcription, and PCR

After thawing on ice, a cross section (250 mg) of the mid-jejunum was homogenized in 5 mL cold guanidine isothiocyanate and β-mercaptoethanol-based lysis buffer (QIAGEN, Hilden, Germany) using a handheld homogenizer (POWER GEN 125, Fisher Scientific, Hampton, New Hampshire, USA). The lysate remained on ice for 30 min to dissipate the froth formed during homogenization. The homogenizer probe was washed with nuclease-free water, 70% ethanol, and nuclease-free water to prevent cross contamination of samples.

RNA was extracted using the RNEasy Mini Kit according to manufacturer's procedures (QIAGEN, Hilden, Germany). Briefly, 1 mL aliquot of the lysate was spun at 16,000 g for 3 min, and 600 µL supernatant was transferred to 1.5 ml Eppendorf tubes. Ethanol (70%) was added to the sample at a 1:1 ratio (v/v), mixed, and centrifuged in filter columns at 8,000 g for 30 s. The membrane bound RNA was sequentially washed and centrifuged (8,000 g for 30 s) with 700 µL of RW1 wash buffer (reduced concentration of guanidine thiocyanate, QIAGEN, Hilden, Germany) and 500 µL of RPE wash buffer (contains ethanol, QIAGEN, Hilden, Germany) twice. The RNA bound to the filter membrane was eluted by pipetting 30 µL of nuclease free water onto the membrane and spinning at 8,000 g for one minute. Samples were tested for RNA yield (nucleotide concentration) and purity by measuring optical density at A_{260} and A_{280} using the Nano Drop 2000 (Thermo Fisher Scientific, Waltham, Massachusetts, USA). Samples to be used

for reverse transcription had a minimum nucleotide concentration of 282 ng μL^{-1} and an $A_{260/280}$ of 1.98.

Reverse transcription was conducted using Applied Biosystems High Capacity cDNA Reverse Transcription kit (Thermo Fisher Scientific, Waltham, Massachusetts, USA). The total reverse transcription reaction mixture was 20 µL and consisted of 2 µL 10 x RT buffer, 0.8 µL 25 x dNTP (100 mM), 2 µL 10 x RT random primers, 1 µL of Multiscribe Reverse Transcriptase (50 U μL^{-1}), and 14.2 µL RNA (1,000 ng total RNA). Reverse transcription was performed on a thermal cycler (C1000 Touch, Bio Rad Hercules, California, USA) with the following temperature conditions: 10 min at 25˚C, 120 min at 37˚C and 5 min at 85˚C.

Real time PCR was conducted on a CFX96 Real-Time PCR Detection System (BIO-RAD, Hercules, California, USA) using EvaGreen PCR supermix (SSOFAST EVAGREEN SUPERMIX, BIO-RAD, Hercules, California, USA). The total reaction mixture per well was 20 µL; composed of 10 µL EvaGreen PCR supermix, 0.8 µL of forward, 0.8 µL reverse primers (10 uM), 2 µL of cDNA (diluted 1/200) and 6.4 µL nuclease free water.

Amplification was performed in Bio-Rad optical 96-well reaction plates. The PCR parameters were as follows: 30 s at 95 ˚C to activate the enzyme, 5 s at 95 ˚C for denaturation, followed by 40 cycles of 5 s at 55 ˚C to 60 ˚C for annealing/extension. This was followed by a melt curve analysis to evaluate the specificity of each reaction well for all samples in a run. The following conditions were implemented: 1 min at 95 ˚C, 5 s at 55 ˚C, and 5 s at 95 ˚C. The presence of a single PCR product from each reaction was confirmed by a single melting peak.

A standard curve was created by the amplification of 5-fold serial dilution of pooled cDNA samples (200 ng μL^{-1}, 40 ng μL^{-1}, 8 ng μL^{-1}, 1.6 ng μL^{-1}, 0.32 ng μL^{-1}, 0.64 ng μL^{-1}) by real time

PCR using gene specific primers. Threshold cycles were plotted against cDNA template concentration and the data was fitted to a straight line. A linear regression coefficient of 0.99 indicated acceptable standard curves, and the slope was used to calculate amplification efficiency using the equation:

$$Efficiency = \left[10^{-1/slope)}\right] - 1 \qquad \text{Eq. 4. 9}$$

Samples were analyzed in duplicate and cycle threshold was adjusted for each plate. Starting cDNA template quantities for each sample were estimated using the linear regression equation derived from the standard curve. Starting quantities of each sample were normalized using the average starting quantities of reference genes β-Actin, GAPDH and RPL19.

4.19.5 Short chain fatty acids and lactic acid

Samples were thawed overnight in a 5 ˚C chiller. Short chain fatty acids and LA were analyzed using gas chromatography (GC) based on the procedures of Khorasani et al. (1993) and Lenahan et al. (2010). Briefly, digesta samples were diluted with 25% metaphosphoric acid at a ratio of 2:1 (w/v) for caecal and 1:1 (w/v) for colonic digesta samples and briefly vortexed. Samples were centrifuged twice at 12,000 g for 10 min. Supernatant was collected and again centrifuged at 16,000 g for 10 min. This was repeated if samples remained turbid. The supernatant was aliquoted into 600 µL duplicates. The same amount of internal standard, isocaproic acid (containing 4.56 µmol mL^{-1} isocaproic acid in 0.15 mol L^{-1} oxalic acid) was added to each replicate, vortexed, then samples were filtered through a 0.45 µm PVDA filter (Fisher Scientific, Hampton, New Hampshire, USA) into 2 ml glass GC vials (Agilent Technologies, Santa Clara, California, USA). The SCFA and LA in caecal and colonic digesta samples were determined on an Agilent 6890 GC equipped with a flame ionization detector (Agilent Technologies, Santa Clara, California, USA) using a capillary column ZB-FFAP (30 m

length x 0.32 mm x 0.25 μm film thickness; ZEBRON, Phenomenex, Torrance, California, USA). The initial oven temperature was set at 90 ˚C with a hold time of 0.1 min, followed by the 1st ramp; 10 ˚C min^{-1} increases until 170 ˚C and a hold time of 0.1 min and the 2nd ramp; 20 ˚C min^{-1} up to 230 ˚C with a hold time of 2.0 min. Hydrogen gas was used for the detector and helium was used as the carrier gas.

4.19.6 Microbial populations

Colonic digesta samples were sent to Microbiome Insights (University of British Columbia-Life Sciences Center, Vancouver, BC, Canada) for analysis. Specimens were placed into a MoBio PowerMag Soil DNA Isolation Bead Plate. DNA was extracted following MoBio's instructions on a KingFisher robot. Bacterial 16S rRNA genes were PCR-amplified with dual-barcoded primers targeting the V4 region, as per the protocol of Kozich et al. (2013). Amplicons were sequenced with an Illumina MiSeq using the 250-bp paired-end kit (v.2). Sequences were denoised, taxonomically classified using Greengenes (v. 13_8) as the reference database, and clustered into 97%-similarity operational taxonomic units (OTUs) with the Mothur software package (v. 1.39.5; Schloss et al. 2009), following the recommended procedure (https://www.mothur.org/wiki/MiSeq_SOP; accessed Nov 2017). The potential for contamination was addressed by co-sequencing DNA amplified from specimens and from four each of template-free controls and extraction kit reagents processed the same way as the specimens. Operational taxonomic units were considered putative contaminants (and were removed) if their mean abundance in controls reached or exceeded 25% of their mean abundance in specimens.

4.20 Statistics

All error data were checked for normality of distribution using Shapiro-Wilk test using Proc Univariate of SAS (SAS 9.4; SAS Institute, Cary, NC) prior to subjecting to ANOVA. Data were transformed if $P < 0.05$. Reported P values were derived from the transformed data and the least mean squares derived from the untransformed data.

Data were analyzed as a randomized complete block design with the fixed effect of treatment and random effect of block using Proc Mixed of SAS. The statistical model used was:

$$Yij=\mu + \rho_i + \alpha_j + \varepsilon_{ij}$$

where Y is the parameter, μ the overall mean, ρ_i the random effect of the i^{th} block, α_j the fixed effect of the j^{th} treatment, ε_{ij}=error term associated to the j^{th} treatment and i^{th} block.

Lactulose:mannitol ratio was analyzed similarly, except time was included in the model.

Means were separated using Tukey's Honest Significant Difference (HSD) test. In all cases, $P \leq 0.05$ was considered significant, with P values between 0.05 and 0.10 a tendency. Data were discussed relative to a protected F-test and an unprotected F-test (Barnette and McLean 1999) using single degree of freedom contrasts to compare treatments and interactions of interest (Marini 2003).

Colonic digesta samples were analyzed for microbial populations by Microbiome Insights (University of British Columbia-Life Sciences Center, Vancouver, BC, Canada) which also performed the statistical evaluation. Alpha diversity was estimated with the Shannon index on raw OTU abundance tables after filtering out contaminants. The significance of diversity differences was tested with an ANOVA and differential abundance testing was conducted using

DESeq2 package. All analyses were conducted in the R environment (https://www.r-project.org/; accessed: 5 July 2018).

RESULTS

4.21 Performance

Average daily gain, feed intake and G:F ratio of pigs fed the PC, diets with APB and acidified diets, either Coarse or Fine are presented in Table 4.13. Treatment had no effect on ADG, ADFI or G:F of pigs during the 17 d test ($P > 0.10$).

4.22 Intestinal permeability, jejunum histology and genetic markers of barrier function and immune response

Treatment had no effect on intestinal permeability of weanling pigs estimated using the L:M ratio ($P > 0.10$), however there was a decrease in L:M ratio 2 h post gavage compared to compared to 0 h (time, $P < 0.01$; Table 4.14).

Jejunal histology and the expression of genetic markers for barrier function, inflammatory and anti-inflammatory response, cellular proliferation and maturity are presented in Table 4.15 and Table 4.16, respectively. Target genes and the primers used to analyze for the genetic markers, regression coefficient, slope and reaction efficiency are presented in Table 4.17.

Treatment had no effect on villous height, crypt depth or the villi:crypt ratio. There was also no effect of treatment on expression of genetic markers except for PCNA where pigs fed the APB-Coarse and AD-Fine diets had a higher expression compared to those fed AD-Coarse ($P < 0.05$).

4.23 Digesta pH

Digesta samples from the different sections of the GIT were collected and analyzed for pH. Treatment had no effect on pH of digesta from the stomach, duodenum, jejunum, ileum, caecum or colon (Table 4.18, $P > 0.10$).

4.24 Short chain fatty acids and lactic acid

Caecal and colonic digesta were collected and analyzed for SCFA and LA concentration. Caecal SCFA and LA concentration per gram of digesta, and molar proportion of individual SCFA (% of total SCFA) are presented in Table 4.19 and Table 4.20 respectively. The concentration of SCFA and LA in the colon and the molar proportions of individual SCFA are described in Table 4.21 and Table 4.22. In the caecum, pigs fed diets with APB tended to have higher concentrations of acetic acid compared to pigs fed PC or AD (Table 4.19, $P = 0.06$). This observation was confirmed by contrasts comparing APW to either PC or AD ($P < 0.05$). Comparing the main effect of particle size, pigs fed Fine had higher valeric acid concentration (Table 4.19, $P < 0.05$) and molar proportion (Table 4.20, $P < 0.05$) compared to Coarse. Furthermore, comparing the main effect of MOA, propionic acid (as % of total SCFA) tended to be higher in AD fed pigs compared to those fed APB ($P=0.02$).

Treatment had no effect on the concentration of SCFA in colonic contents except for the concentration of valeric acid, which was higher in pigs fed Fine diets compared to those fed Coarse ($P < 0.05$). Total SCFA was similar across treatments but the contrast comparing the main effect of MOA showed a tendency for APW fed pigs to be higher than PC ($P < 0.08$). Pigs fed Fine diets had higher concentration of LA in the colon compared to those fed Coarse ($P < 0.05$). Pigs fed Coarse regardless of MOA had higher molar proportion of acetic acid than those

fed Fine, while pigs fed Fine diets had increased molar proportions of butyric acid compared to those fed Coarse ($P < 0.05$).

4.25 Microbial Populations

The Alpha diversity of microbial populations were not different among treatment groups (Figure 4.1, $P > 0.10$). However, differential abundance testing showed *Ruminoccoccus* (Figure 4.2) and *Bacteroidetes* (Figure 4.3) were two low abundance taxa found to be different among treatments. *Ruminoccoccus* was apparently depleted in PC and APB-Coarse ($P < 0.01$), whil*e* *Bacteroidetes* was abundant only in PC ($P <0.01$).

DISCUSSION

This study was conducted to test the hypothesis that the benefits of feeding an acidified diet would be maintained when pigs are fed diets containing acid-preserved barley. Furthermore, because a coarse particle size has been shown to promote gut health by influencing gastric pH and the production of SCFA in the hind gut of growing pigs, it was hypothesized that feeding weanling pigs coarse diets will further improve gut health in diets containing acids.

Aside from the absorptive function of intestinal epithelial cells, the single layer of these cells lining the small intestine also serve as a barrier that protects the pig from its external environment. Lactulose (342 Da) and mannitol (182 Da) are indigestible sugars used to assess intestinal barrier function, mainly the paracellular route (Wijtten et al. 2011). An increase in L:M indicates a decrease in intestinal barrier function, while a decrease in L:M suggests improved barrier function. In the current experiment, intestinal permeability as estimated by L:M ratio was similar among pigs before and after the gavage regardless of treatment, suggesting that intestinal permeability was not altered. The decrease in L:M ratio 2 h after the gavage reflects the higher absorbability of mannitol relative to lactulose. Bjarnson et al. (1995) reported that the absorbability of mannitol was attributed to the smaller size of the molecule compared to lactulose (182 vs 342 Da). This is supported by similar villi height, crypt depth or villi:crypt ratio in the mid jejunum of pigs regardless of treatment. Furthermore, the similar abundance of the gene intestinal alkaline phosphatase (IAP), an indicator of epithelial cell maturity in the villi, among treatments suggests that the villi was not damaged in any of the treatments. This assumption is corroborated by the similar expression of inflammatory cytokines (IL-8, IL-1β, and TNF-α) among treatments, and low expression of anti-inflammatory cytokine (IL-10) which was not

amplified in a number of samples. These results agree with Namkung et al. (2004) who fed a corn-based diet supplemented with an acid blend containing phosphoric, acetic, propionic, citric and LA to pigs weaned at 16 to 19 d of age for 14 d also and reported the lack of effect on jejunal histology and inflammatory immune response.

Proliferating cellular nuclear antigen (PCNA) is indicative of increased cell proliferation in the crypt (Willing and Van Kessel, 2007) or an increase in the number of crypts (Sakata and Inagaki, 2001). In the current study, there was a modest positive correlation between the expression of PCNA and crypt depth ($r=0.34$, $P < 0.03$, $n=39$). The expression of PCNA was downregulated in pigs fed AD-Coarse compared to pigs fed APB-Coarse and AD-Fine although there were no differences in crypt depth. This observation may be related to the higher production of LA relative to total SCFA in pigs fed AD-Fine compared to the other treatments. Sakata and Inagaki (2001) reported that SCFAs increase mucosal and submucosal mass and crypt cell numbers while LA does not. The shift of fermentation into LA instead of SCFA occurs when the pH is 6 and below (Sakata and Inagaki, 2001). In the current experiment, the caecal pH of pigs fed AD-Coarse was 5.97 and slightly lower compared to pigs fed APB-Coarse (pH 6.10) or AD-Fine (pH 6.14). In the colon, LA concentration was lower in Coarse diets compared to Fine.

The concentration of SCFA measured from luminal contents represents only about 5% of the total SCFA produced in the hindgut (Sakata and Inagaki 2001) and is a net result of SCFA production and absorption, thus the SCFA measured is the amount that was unabsorbed (Montoya et al. 2016). In the current study, there was no overall effect of treatment on total SCFA but there were treatment effects on the concentration of individual SCFAs in the caecum

and colon. The concentration of acetic acid in the caecum increased when pigs were fed diets with APB compared to those fed PC or AD. This suggests that the digesta entering the caecum of APB fed pigs had relatively higher NSP as these produce acetic acid upon fermentation. This observation may indicate that starch digestion and absorption in the small intestine was improved in the APB compared to dry barley. Comparing particle size, the lower concentration and molar proportion of valeric, higher molar proportion of acetic, and lower molar proportion of butyric acids in the colon of pigs fed Coarse are possibly due to the previously reported higher DM digestibility (Table 4.11) of coarsely ground compared to finely ground barley regardless of MOA of the acids. Lower valeric and butyric acids in the colon suggests lower protein and starch concentrations, respectively of the digesta entering the colon, and a high % acetic acid indicates relatively higher NSPs entering the colon (Jha 2010).

Microbial population diversity was not different among treatments, however, *Ruminococcus* was depleted in pigs fed diets with PC and APB-Coarse, and *Bacteroidetes* abundance was low in pigs fed APB and AD compared to PC. *Ruminococcus* is a cellulolytic bacterium which is abundant in the rumen and may also contribute in the breakdown of plant cell walls in the large intestine of the pig (Varel and Yen 1997). The fermentation products of cellulose by *Ruminococcus flavifaciens* include succinic acid, acetic acid and formic acid (Latham and Wolin 1977). However, in the current experiment, the concentration of acetic acid was not increased in the colon of pigs where this bacterium was found. *Bacteroidetes* is a rod-shaped, anaerobic bacteria than may be pathogenic. Its abundance in pigs fed PC but not in pigs fed diets with APB and AD suggests that the use of acids, regardless of mode of addition, successfully controlled the population of this pathogenic bacteria.

SUMMARY AND CONCLUSIONS

The gut health status of pigs fed AD was similar to those fed diets with APB, indicating that feeding acid-preserved high-moisture barley may be an alternate route to obtain the benefits to gut health observed with direct acid supplementation. Grinding coarsely neither improved the gut health status of pigs, nor had a synergy with acidification to improve gut health. Changes in the SCFA concentrations in the hind gut are reflective of the differences in nutrient digestibility due to MOA, PS or their interaction.

FIGURES

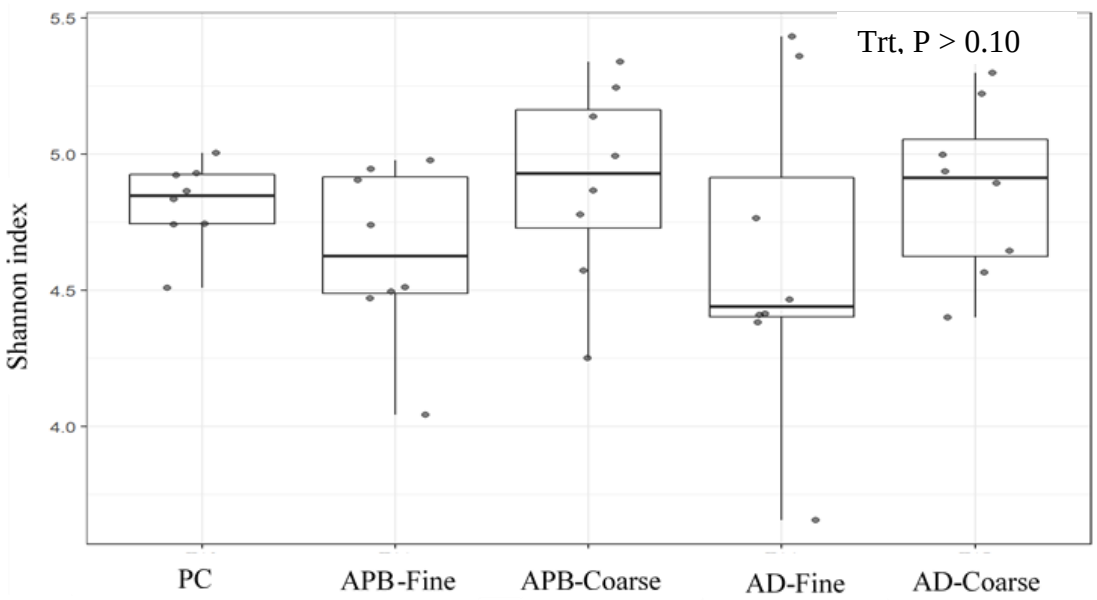

Figure 4.1. Microbial population diversity in colonic digesta samples (Shannon Index) from pigs fed either PC, diets with acid-preserved wheat or acidified diets, where wheat was ground fine or coarse.

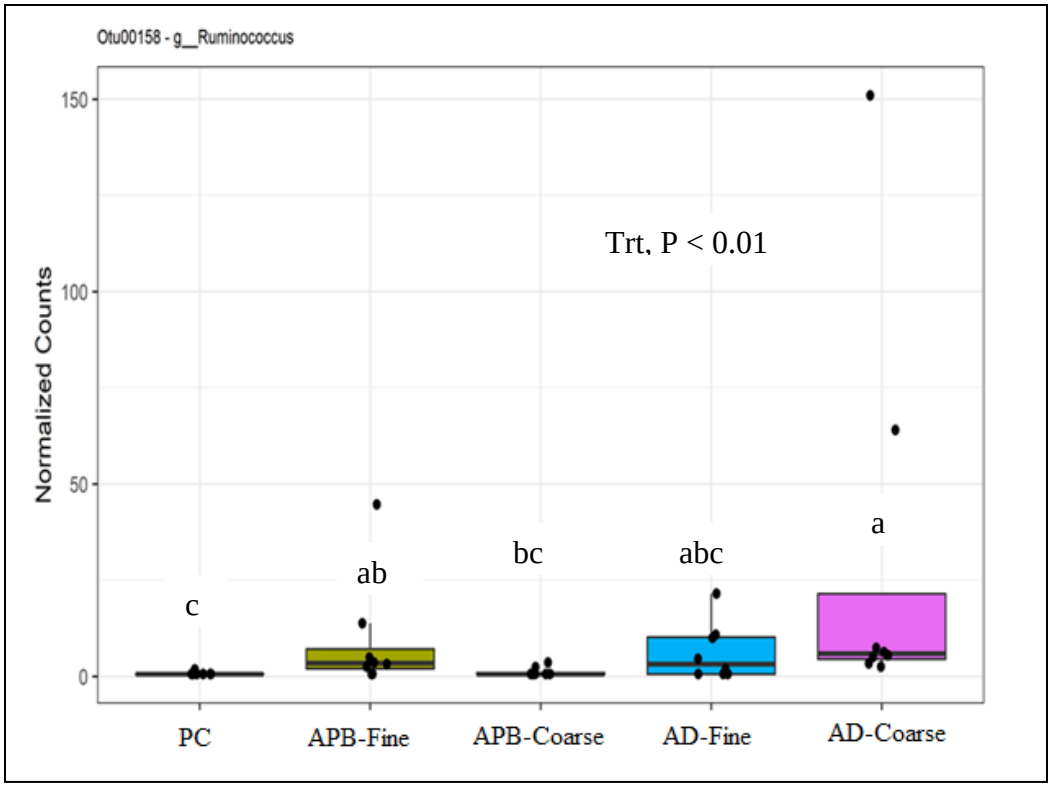

Figure 4.2. Effect of feeding diets with APB and AD on low abundance taxa *Ruminococcus.*

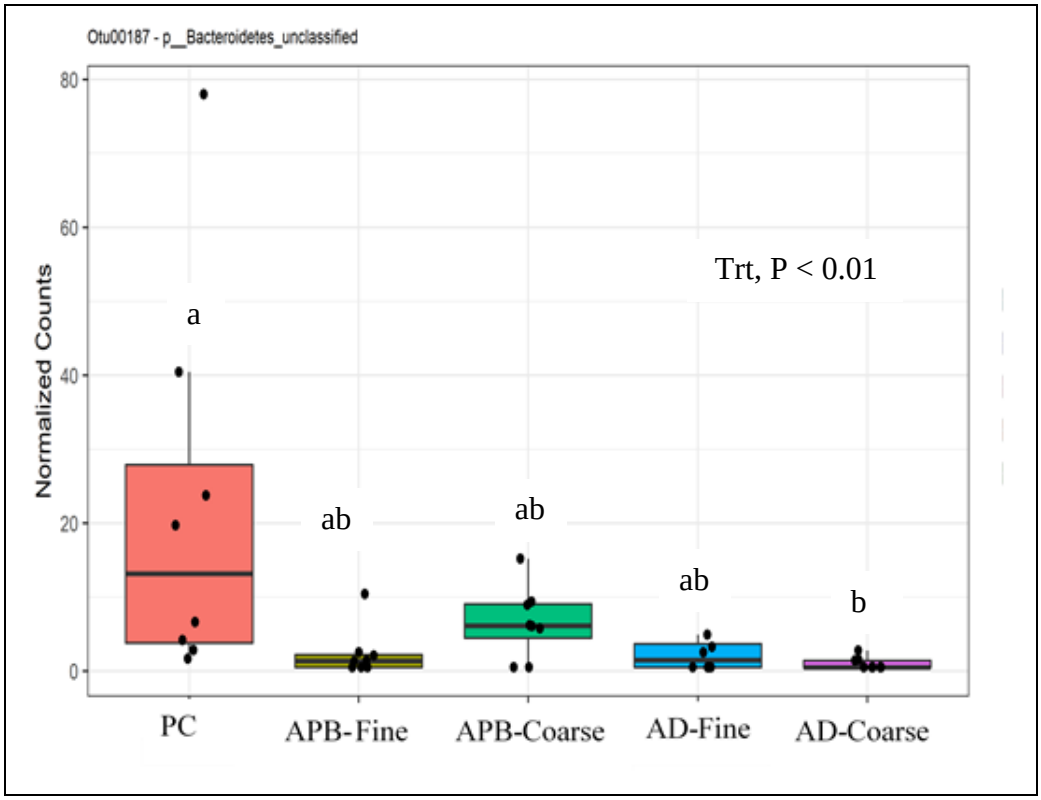

Figure 4.3. Effect of feeding diets with APB and AD on low abundance taxa *Bacteroidetes*

TABLES

Table 4.13. Peformance of weanling pigs fed the positive control, diets with acid-preserved barley (APB), or acidified diets (AD), where barley was ground either finely or coarsely.[a]

Treatment	T10	T11	T12	T14	T15							
Barley	PC	Acid-preserved barley, APB		Acidified diet, AD								
Enzyme	Enz	Enz	Enz	Enz	Enz			Treatment contrasts				
PS	Fine	Fine	Coarse	Fine	Coarse	SEM	P Value	PC vs APB	PC vs AD	MOA	PS	MOA x PS
ADG	226	254	242	235	245	31.3	0.85	0.33	0.52	0.67	0.94	0.55
ADFI	389	373	369	379	378	32.0	0.76	0.19	0.45	0.49	0.86	0.87
G:F	0.57	0.67	0.65	0.62	0.64	0.041	0.35	0.05	0.20	0.38	0.94	0.48

Note: NC, negative control; PC, positive control; MOA, mode of acid addition; PS, particle size; ADG, average daily gain; ADFI, average daily feed intake; G:F feed efficiency, Enz with enzyme, NoEnz without enzyme

[a]Average of 8 pigs per treatment after feeding their respective diets for 14 d.

Table 4.14. Serum concentration of lactulose, mannitol, and ratio of lactulose:mannitol in weanling pigs fed control, diets with acid-preserved barley (APB) or acidified diets (AD) where barley was ground either finely or coarsely.[a]

	Treatment											
Barley	PC	Acid-preserved barley, APB		Acidified diet, AD								
Enzyme	Enz	Enz	Enz	Enz	Enz		Treatment		Time		Treatment x Time	
Particle Size	Fine	Fine	Coarse	Fine	Coarse	Time	SEM	P value	SEM	P value	SEM	P value
				Lactulose (µg mL^{-1})								
0 Hours	0.06	0.06	0.06	0.06	0.06	0.06b	0.581	0.35	0.426	<0.01	0.773	0.35
+2 Hours	0.94	4.56	2.41	0.84	1.40	2.03a						
				Mannitol (µg mL^{-1})								
0 Hours	0.40	0.32	0.12	0.73	0.44	0.40b	3.066	0.31	2.353	<0.01	3.981	0.24
+2 Hours	22.37	16.07	23.46	21.94	17.07	20.29a						
				Lactulose:Mannitol								
0 Hours	1.24	1.25	1.98	0.99	1.24	1.34a	0.256	0.12	0.153	<0.01	0.198	0.37
+2 Hours	0.03	0.30	0.09	0.04	0.15	0.12b						

Note: PC, positive control; Enz, with enzyme; SEM, standard error of mean. Means without a common letter within a column are different ($P < 0.05$).

P values were generated from log transformed data

[a]Means were average of 8 samples per treatment analyzed in duplicate. For samples with non-detected mannitol and lactulose, minimum detectable limits of 0.01 and 0.02 μg μL^{-1}, respectively were used.

Table 4.15. Histology of mid-jejunum in weanling pigs fed the positive control, diets with acid-preserved barley (APB), or acidified diets (AD) where barley was ground either finely or coarsely.[a]

Treatment	T10	T11	T12	T14	T15							
Barley	PC	Acid-preserved barley, APB		Acidified diet, AD								
Enzyme	Enz	Enz	Enz	Enz	Enz			Treatment contrasts				
PS	Fine	Fine	Coarse	Fine	Coarse	SEM	P Value	PC vs APB	PC vs AD	MOA	PS	MOA x PS
Villous height	414	435	377	372	394	34.3	0.58	0.85	0.64	0.73	0.85	0.12
Crypt depth	112	109	104	111	109	3.4	0.45	0.16	0.56	0.32	0.26	0.70
Villi:Crypt	1.71	1.59	1.32	1.45	1.46	0.178	0.53	0.24	0.33	0.81	0.62	0.24

Note: MOA, mode of acid addition; PC, positive control; Enz with enzyme; SEM, standard error of mean

[a]Measured from 15 to 20 intact villi and crypt from jejunum of each pig. Tissue samples were collected from 8 pigs per treatment.

Table 4.16. Expression of genetic markers indicative of barrier function, inflammatory, anti-inflammatory and cellular proliferation and maturity in the mid-jejunum of weanling pigs fed positive control, diets with acid-preserved barley (APB), or acidified diets (AD) where barley was ground either finely or coarsely.[a]

Treatment	T10	T11	T12	T14	T15							
Barley	PC	Acid-preserved barley, APB		Acidified diet, AD								
Enzyme	Enz	Enz	Enz	Enz	Enz			Treatment contrasts				
PS	Fine	Fine	Coarse	Fine	Coarse	SEM	P Value	PC vs APB	PC vs AD	MOA	PS	MOA x PS
MUC2	0.56	0.70	0.72	0.64	0.52	0.099	0.59	0.28	0.88	0.26	0.47	0.48
CDN4	1.21	1.13	0.88	0.88	0.80	0.156	0.21	0.25	0.04	0.25	0.27	0.57
OCLN	1.00	0.78	0.81	0.87	0.70	0.123	0.51	0.17	0.16	0.97	0.55	0.43
ZO1	0.94	0.81	0.85	1.00	0.79	0.133	0.63	0.48	0.81	0.56	0.29	0.38
IL-1β[b]	1.16	0.93	0.84	1.12	0.92	0.387	0.81	0.87	0.65	0.71	0.26	0.84
TNF-α[c]	1.30	0.82	0.89	1.26	1.27	0.374	0.91	0.62	0.82	0.39	0.71	0.88
IL-8[d]	0.82	0.86	0.99	0.70	0.70	0.354	0.98	0.80	0.86	0.62	0.73	0.96
IL-10[e]	0.95	1.25	0.90	0.98	0.59	0.300	0.64	0.65	0.54	0.27	0.16	0.92

IAP	1.19	1.10	0.57	1.04	0.85	0.203	0.13	0.11	0.25	0.53	0.05	0.37
PCNA	0.58ab	0.76ab	0.85a	0.91a	0.48B	0.089	<0.01	0.02	0.37	0.05	0.04	<0.01

Note: PC, positive control; MOA, mode of acid addition; Enz, with enzyme; SEM, standard error of mean; MUC2, mucin 2; CDN4, claudin 4; OCLN, occludin; ZO-1, zona occludin 1; IL-1β, interleukin 1β; TNF-α, tumor necrosis factor α; IAP, intestinal alkaline phosphatase; PCNA, proliferating cellular nuclear antigen. Means without a common letter within a row are significantly different ($P < 0.05$).

P values for MUC2, CDN4, ZO1, IL-1β, TNF-α, IL8, and PCNA were derived after log transformation of data.

[a]Analyzed from mid-jejunal samples of 8 pigs per treatment.

[b]T2 n=8; T3, n=7; T4, n=8; T7, n=8; T8, n=7). Highest SEM was reported.

[c]T2 n=8; T3, n=8; T4, n=8; T7, n=8; T8, n=7). Highest SEM was reported.

[d]T2 n=6; T3, n=8; T4, n=7; T7, n=8; T8, n=5). Highest SEM was reported.

[e]T2 n=6; T3, n=5; T4, n=7; T7, n=6; T8, n=6). Highest SEM was reported.

Table 4.17. Target genes and the primers used.

Target	Forward (5' to 3')	Reverse (5' to 3')	T_A	Eff.	R^2	Reference/NCBI/EST
		Reference Genes				
ACTB	CACGCCATCCTGCGTCTGGA	AGCACCGTGTTGGCGTAGAG	63	100	0.995	Nygard et al. (2007)
GAPDH	CTTCACGACCATGGAGAAGG	CCAAGCAGTTGGTGGTACAG	63	100	0.990	Bruel et al. (2010)
RPL19	AACTCCCGTCAGCAGATCC	AGTACCCTTCCGCTTACCG	60	100	0.990	Meurens et al. (2009a)
		Markers of barrier function				
MUC2	ACCCGCACTACGTCACCTTC	GGCAGGACACCTGGTCATTG	62	100	0.995	BX671371
CDN4	CAACTGCGTGGATGATGAGA	CCAGGGGATTGTAGAAGTCG	60	100	0.998	Pasternak et al. 2015
OCLN	GAGTACATGGCTGCTGCTGA	TTTGCTCTTCAACTGCTTGC	60	115	0.996	Alizadeh et al. 2015
ZO1	ACGGCGAAGGTAATTCAGTG	CTTCTCGGTTTGGTGGTCTG	60	111	0.999	XM_003353439.2
		Markers of inflammatory response				
IL8	TCCTGCTTTCTGCAGCTCTC	GGGTGGAAAGGTGTGGAATG	62	100	0.990	Meurens et al. 2009b
IL-1B	AGAAGAGCCCATCGTCCTTG	GAGAGCCTTCAGCTCATGTG	62	100	0.990	Meurens et al. (2009b)
TNFα	CCAATGGCAGAGTGGGTATG	TGAAGAGGACCTGGGAGTAG	60	96	0.994	Meurens et al. 2009b
		Marker of anti-inflammatory response				
IL10	CCATGGAAGTGGTCCGCCAA	GCCCAGGTAGCCATGGATC	55	105	0.970	Willing, 2007

Cellular maturity and turn-over						
IAP	CTAAAGGGGCAGATGAATGG	CACCTGTCTGTCCACGTTGT	60	95	0.995	Lackeyram et al 2015
PCNA	TACGCTAAGGGCAGAAGATAATGCTGAGATCTCGGCATATACGTG		58	100	0.995	Willing and Van Kessel, 2007

Note: Eff, efficiency; R^2, regression coefficient; MUC2, mucin 2; CDN4, claudin 4; OCLN, occludin; ZO-1, zona occludin 1; IL-1β, interleukin 1β; TNF-α, tumor necrosis factor α; IAP, intestinal alkaline phosphatase; PCNA, proliferating cellular nuclear antigen.

Table 4.18. Digesta pH in different locations of the gastro-intestinal tract (GIT) of pigs fed the positive control, diets with acid-preserved barley (APB), or acidified diets (AD) where barley was ground either finely or coarsely.[a]

Treatment	T10	T11	T12	T14	T15							
Barley	PC	Acid-preserved barley, APB		Acidified diet, AD								
Enzyme	Enz	Enz	Enz	Enz	Enz			Treatment contrasts				
PS	Fine	Fine	Coarse	Fine	Coarse	SEM	P Value	PC vs APB	PC vs AD	MOA	PS	MOA x PS
Stomach	4.29	4.26	4.12	4.40	4.62	0.401	0.84	0.80	0.57	0.31	0.91	0.57
Duodenum[b]	6.02	5.97	5.81	6.00	6.05	0.175	0.85	0.46	0.96	0.41	0.74	0.52
Jejunum	6.94	6.61	6.53	6.58	6.74	0.143	0.25	0.04	0.11	0.49	0.77	0.39
Ileam[c]	7.55	7.04	7.26	7.23	7.41	0.142	0.12	0.02	0.18	0.23	0.15	0.91
Caecum	6.22	5.84	6.10	6.14	5.97	0.164	0.49	0.21	0.39	0.61	0.78	0.19
Colon	6.90	6.55	6.89	6.74	6.81	0.169	0.58	0.38	0.53	0.76	0.23	0.42

Note: MOA, mode of acid addition; PC, positive control; Enz with enzyme; SEM, standard error of mean.

[a]Average of samples collected from 8 pigs per treatment.

[b]Based on number of pigs where duodenal digesta samples were collected (T2, n=6; T3, n=4; T4, n=3; T6, n=3; T7 n=4). Highest

SEM was reported.

[c]Average was based on number of pigs where ileal digesta samples were collected (T2, n=7; T3, n=8; T4, n=8; T6, n=7; T7 n=8).

Highest SEM was reported.

Table 4.19. Concentration of short chain fatty acids (SCFA) and lactic acid (LA) in caecal digesta of weanling pigs fed the positive control, diets with acid-preserved barley (APB), or acidified diets (AD) where barley was ground either finely or coarsely.[a]

Treatment	T10	T11	T12	T14	T15							
Barley	PC	Acid-preserved barley, APB		Acidified diet, AD								
Enzyme	Enz	Enz	Enz	Enz	Enz			Treatment contrasts				
PS	Fine	Fine	Coarse	Fine	Coarse	SEM	P Value	PC vs APB	PC vs AD	MOA	PS	MOA x PS
			µmol g^{-1} digesta									
Acetic	67.78	79.60	81.33	67.72	72.14	4.446	0.06	**0.02**	0.68	**0.02**	0.47	0.75
Propionic	60.29	73.10	65.85	66.10	73.94	5.872	0.37	0.16	0.15	0.96	0.99	0.18
Isobutyric	0.08	0.11	0.30	0.13	0.11	0.114	0.15	0.72	0.89	0.77	0.13	0.12
Butyric	13.74	19.12	16.73	15.49	14.44	1.835	0.28	0.07	0.59	0.12	0.36	0.72
Isovaleric	0.11	0.19	0.48	0.36	0.19	0.153	0.35	0.17	0.11	0.76	0.98	0.20
Valeric	2.18	3.27	2.14	3.30	1.96	0.531	0.20	0.34	0.62	0.59	**0.03**	0.88
Caproic	0.25	0.00	0.13	0.48	0.47	0.168	0.15	0.32	0.26	**0.01**	0.48	0.91
Lactic	251.32	204.70	245.36	177.02	437.74	96.440	0.49	0.83	0.86	0.63	0.09	0.49
SCFA, Total	144.43	175.40	166.96	153.58	163.26	10.418	0.20	**0.03**	0.22	0.22	1.00	0.38

Note: PC, positive control; MOA, mode of acid addition; PS, particle size; SCFA, short chain fatty acid; LA, lactic acid; SEM, standard error of mean.

[a]Samples were obtained from 8 pigs per treatment after receiving their respective diet for 16 d.

P values for isobutyric, valeric, caproic, lactic and total SCFA were derived from square root transformation of data

Table 4.20. Molar proportion of short chain fatty acids (SCFA) in caecal digesta of pigs fed positive control, diets with acid-preserved barley (APB), or acidified diets (AD) where barley was ground either finely or coarsely.[a]

Treatment	T10	T11	T12	T14	T15							
Barley	PC	Acid-preserved barley, APB		Acidified diet, AD								
Enzyme	Enz	Enz	Enz	Enz	Enz			Treatment contrasts				
PS	Fine	Fine	Coarse	Fine	Coarse	SEM	P Value	PC vs APB	PC vs AD	MOA	PS	MOA x PS
			% total SCFA									
Acetic	47.33	45.68	49.04	44.58	44.30	1.794	0.26	0.99	0.17	0.10	0.37	0.29
Propionic	41.38	41.57	39.00	42.81	45.09	1.702	0.11	0.55	0.18	**0.02**	0.89	0.12
Isobutyric	0.05	0.07	0.24	0.10	0.09	0.089	0.08	0.90	0.66	0.38	0.06	0.12
Butyric	9.51	10.73	9.97	9.89	8.91	0.781	0.60	0.38	0.91	0.24	0.28	0.89
Isovaleric	0.08	0.12	0.38	0.25	0.14	0.124	0.35	0.18	0.12	0.78	0.93	0.18
Valeric	1.51	1.82	1.31	2.07	1.20	0.292	0.20	0.80	0.92	0.86	**0.02**	0.60
Caproic	0.15	0.00	0.06	0.30	0.28	0.102	0.13	0.31	0.24	**0.01**	0.53	0.92

Note: PC, positive control; MOA, mode of acid addition; PS, particle size; SCFA, short chain fatty acid; SEM, standard error of

mean.

[a]Samples were obtained from 8 pigs per treatment after receiving their respective diet for 16 d.

P values for isobutyric, valeric, and caproic were derived after square root transformation of data.

Table 4.21. Concentration of short chain fatty acids (SCFA) and lactic acid (LA) in colonic digesta of weanling pigs fed the positive control, diets with acid-preserved barley (APB), or acidified diets (AD) where barley was ground either finely or coarsely.[a]

Treatment	T10	T11	T12	T14	T15							
Barley	PC	Acid-preserved barley, APB		Acidified diet, AD								
Enzyme	Enz	Enz	Enz	Enz	Enz			Treatment contrasts				
PS	Fine	Fine	Coarse	Fine	Coarse	SEM	P Value	PC vs APB	PC vs AD	MOA	PS	MOA x PS
			µmol g^{-1} digesta									
Acetic	65.97	77.63	73.96	64.25	75.44	4.879	0.22	0.11	0.52	0.23	0.45	0.14
Propionic	40.66	57.91	45.17	48.49	50.09	5.470	0.27	0.11	0.21	0.68	0.31	0.20
Isobutyric	1.12	0.98	1.29	0.88	1.34	0.244	0.58	0.95	0.98	0.92	0.10	0.75
Butyric	13.63	19.37	14.37	14.58	14.35	1.640	0.11	0.12	0.68	0.15	0.12	0.15
Isovaleric	1.28	1.18	1.49	1.07	1.49	0.287	0.67	0.90	0.94	0.95	0.14	0.84
Valeric	2.23	3.56	2.38	2.69	2.21	0.376	0.08	0.12	0.63	0.18	**0.03**	0.36
Lactic	114.11	158.09	29.16	155.80	54.85	48.880	0.17	0.68	0.85	0.80	**0.01**	0.76
SCFA, Total	124.89	160.62	138.66	131.96	144.93	11.082	0.22	0.08	0.32	0.32	0.69	0.12

Note: PC, positive control; MOA, mode of acid addition; PS, particle size; SCFA, short chain fatty acid; LA, lactic acid; SEM, standard error of mean.

[a]Samples were obtained from 8 pigs per treatment after receiving their respective diet for 16 d.

P values for isovaleric acid were derived after square root transformation of data.

Table 4.22. Molar proportion of short chain fatty acids (SCFA) in colonic digesta of weanling pigs fed the positive control, diets with acid-preserved barley (APB), or acidified diets (AD) where barley was ground either finely or coarsely.[a]

Treatment	T10	T11	T12	T14	T15							
Barley	PC	Acid-preserved barley, APB		Acidified diet, AD								
Enzyme	Enz	Enz	Enz	Enz	Enz			Treatment contrasts				
PS	Fine	Fine	Coarse	Fine	Coarse	SEM	P Value	PC vs APB	PC vs AD	MOA	PS	MOA x PS
			% total SCFA									
Acetic	53.09	48.84	53.48	49.65	52.63	1.559	0.14	0.32	0.32	0.99	**0.02**	0.60
Propionic	32.35	35.43	32.16	35.84	33.96	1.590	0.36	0.46	0.20	0.49	0.11	0.67
Isobutyric	0.92	0.76	1.03	0.76	0.97	0.228	0.58	0.61	0.73	0.84	0.12	0.71
Butyric	10.85	11.85	10.40	10.86	9.82	0.575	0.18	0.70	0.48	0.18	**0.04**	0.72
Isovaleric	1.06	0.93	1.19	0.91	1.08	0.275	0.77	0.68	0.78	0.87	0.25	0.60
Valeric	1.74	2.20	1.75	1.99	1.55	0.212	0.23	0.31	0.86	0.30	**0.04**	0.95

Note: PC, positive control; MOA, mode of acid addition; PS, particle size; SCFA, short chain fatty acid; LA, lactic acid; SEM, standard error of mean.

[a]Samples were obtained from 8 pigs per treatment after receiving their respective diet for 16 d.

P values for isobutyric, isovaleric and valeric acids were derived from square root transformation of data.

CHAPTER 5. GENERAL DISCUSSION

5.1 Overview

Acidification of high-moisture (>15%) grains is an alternative to artificial drying to preserve grain during storage. Additionally, acidifiers are added to diets of weanling pigs to address nutrient digestibility and gut health issues due to increased gastric pH resulting from low hydrochloric acid (HCl) secretion in young pigs (Partanen and Mroz, 1999; Kil et al. 2011). The suggested modes of action to provide these benefits include: a) reduction of gastric pH resulting in improved nutrient digestibility due to activation of enzymes and reduced gastric emptying rate; and b) improved gut health due to direct killing of pathogenic microorganisms. It is not known whether the benefits observed with direct acidification of weanling pig diets will be observed if acid-preserved high-moisture grains are used in the diets.

Another technology used in pig production is the supplementation of exogenous enzymes such as phytases, carbohydrases or proteases to the diet. For example, high-moisture, air-tight storage of barley, wheat and triticale enhanced endogenous and exogenous enzyme activity improving the digestibility of CP and P in pigs (Poulsen et al., 2012 Ton Nu et al., 2015). High-moisture and low pH are requirements for optimum activity of most enzymes. Therefore, acidification of high-moisture wheat and barley, with exogenous enzymes may be a viable strategy to improve the feeding value of these grains to weanling pigs.

Particle size reduction improves pig performance in all stages of the pig production cycle and thus, grains are ground prior to feed production. However, fine grinding (< 500 μm) predisposes pigs to gastric ulcers. In contrast, feeding pigs coarse diets reduces gastric pH and alters SCFA production in the hind gut, promoting gut health (Mikkelsen et al. 2004). This is

similar to the mode of action of diet acidification, but with regional differences. The benefits of acidification are realized primarily in the proximal GIT (Kil et al. 2011), while studies by Mikkelsen et al. (2004) suggest that the effects of coarse diets are in the proximal and distal GIT.

The interaction between particle size and diet acidification has been investigated in growing pigs (Papenbrock et al. 2005; Visscher et al. 2009) but to our knowledge, not in young pigs. For young pigs, grinding corn finely (from 865 to 339 μm) linearly improved G:F from 0.65 to 0.69 and this was attributed to improved energy digestibility (Rojas and Stein, 2015). Grinding grains that are high-moisture increases grinding cost because of reduction in throughput and increased power consumption. However, because of the action of exogenous enzymes in acid-preserved high-moisture grains, it was hypothesized that nutrient digestibility would improve, eliminating the necessity for fine grinding with a potential to improve gut health.

The use of acids in weanling pig diets is not a novel concept. However, its presentation as an acid-preserved high-moisture grain as an alternative to direct dietary acid supplementation for weanling pigs to our knowledge has not been investigated. If successful, an acidifier could be used as a preservative for high-moisture grains, performance enhancer and an alternative to antibiotics to improve gut health, providing potential savings for the producer. The information generated from this thesis provides producers a tool to utilize low-quality high-moisture grains. In the future, it can be an alternative feeding strategy to reduce cost and improve animal performance.

The overall objective of these series of experiments was to determine whether the benefits of diet acidification with or without enzymes on weanling pig performance and gut health are maintained when acid-preserved high-moisture wheat or barley are fed. Because particle size affects gastric pH and SCFA production in the hind gut, the second objective was to

determine if there is an interaction between acid-preserved high-moisture grain and particle size on animal performance and gut health

5.2 Effect of phosphoric and propionic acid at high or low inclusion, with or without exogenous enzymes on mould growth, fermentation products and estimates of P and CP availability during storage of high-moisture wheat or barley

Two in vitro trials were conducted to determine the effect of acid preservation of high-moisture wheat or barley at low or high acid concentration, with or without enzyme during storage on mould growth, pH, NH_3 N, and estimates of P and CP availability (Chapter 3). Propionic acid is an effective preservative of high-moisture grain (Jones 1975). However, there is very little information on the use of a phosphoric acid-based organic-inorganic acid blend used for direct diet acidification and as a grain preservative. The use of an inorganic-organic acid blend of acidifier is gaining interest due to the synergy between the two types of acid resulting in reduced acidification cost.

One of the key findings in the in vitro studies is that both Prop and OIB (organic-inorganic acid blend) inhibited mould growth in high-moisture wheat or barley as indicated by the absence of visible moulds in any of the treatments during the experiment. Conversely, in the grains used for the in vivo trials, mould growth was observed in the OIB-treated high-moisture wheat and barley. The incubation jars in the in vitro trials were opened only at their allocated time points. In contrast, the grains used in the in vivo trials were stored in polyethylene barrel and were opened weekly for sampling, reintroducing air which may have caused moulds to grow. One of the advantages of acid-preservation is that anaerobic conditions are not necessary (Lynch et al. 1975; McLelland 2008; Jokiniemi et al. 2014). It is possibly because the water in

reconstituted grains is “free”, conversely moisture in tough grains may be bound within the grain and are not free to be used by microorganisms for growth.

Another key finding is that the pH of the high-moisture barley or wheat was maintained below pH 5 in vitro with the high concentration of either Prop or OIB. A pH of less than 5 was desired because most of the endogenous and exogenous enzymes require a low pH for optimum activity. The pKa for most SCFA is around this level, thus improving their effectiveness as an antimicrobial. Results suggest that acid-preserved high-moisture grains should be stored less than 153 d and pH below 5 or lower maintained. High-moisture wheat or barley may be better at maintaining a lower pH because of their lower ABC. Finally, barley and wheat responded differently to acidification and enzyme addition. In barley, the addition of enzyme or acid at high concentration regardless of acid-type increased aP in high-moisture barley suggesting the absence of synergy between enzyme and acid in vitro. Conversely in wheat, the addition of enzymes in grains preserved with high concentration of Prop resulted in increased aP and may be evidence of synergy between enzymes and Prop.

5.3 Effect of acid preservation of high-moisture grains on mould growth, steel corrosion and grinding cost.

In the in vitro trials, there was no visible mould growth in the high-moisture grains preserved with either Prop or OIB regardless of time point and suggesting they are equally as effective in controlling mould growth. However, in the OIB-preserved high-moisture wheat and barley used in the in vivo trials, mould growth was observed during the 34 and 38 d of storage, respectively, possibly due to the reintroduction of air during sampling. To address this concern, we removed the mouldy portion of the grain and sent samples from the remaining grains for

mycotoxin analysis. In the wheat in vivo trials, Prop and OIB were compared which allowed for the comparison of each acid on corrosion of steel coupons representing storage bins and feeders. Propionic acid and the phosphoric acid-based OIB were equally as corrosive on galvanized steel, but Prop was more corrosive on carbon steel than OIB. Other than being extremely pungent and more dangerous to work with, its corrosiveness was another reason why Prop was not included in the succeeding experiments. Storage bins made of galvanized steel may be coated to protect its surface from corrosion.

Using the specific power consumption from the current experiments and power cost of \$0.116 per kWh (CFRC, 2018), grinding high-moisture wheat or barley finely increased grinding cost by \$0.77 or \$0.57 t^{-1} of grain respectively, compared to the dry grain. When ground coarsely, grinding cost increased by \$0.25 t^{-1} in high-moisture wheat, and \$0.28 t^{-1} in high-moisture barley compared to the dry counterpart. The increase in cost when grinding high-moisture grain finely may be addressed by grinding coarsely. For example, grinding high-moisture wheat coarsely resulted in \$0.21 t^{-1} lower grinding cost compared to grinding dry wheat finely and is possibly due to the lower power consumption and higher grinding throughput when grinding coarsely. In barley, the grinding cost of high-moisture barley coarsely was similar to the cost of grinding dry barley finely (\$0.96 vs \$1.02 t^{-1}).

5.4 Interaction of acid-preservation of wheat and particle size, with or without enzymes, on animal performance and gut health

Two trials were conducted to determine the efficacy of feeding APW as an alternative to direct acidification of weanling pig diets, on performance and gut health. In the nursery trial, Prop and OIB were compared, with or without enzyme, ground finely or coarsely. In the

metabolism trial, limitations in the facility required the refinement of the experimental design. Propionic acid treatments were omitted for 3 reasons, 1) the organic acid was pungent, 2) it was more corrosive than OIB, and 3) there is increasing interest in the use of an organic-inorganic acid blend as a preservative and acidifier due to reduced cost and perceived improved efficacy.

Results suggest that Prop may be more effective than OIB at preserving the grains due to mould growth in OIB-preserved grains. There was no observed performance response with supplementation of acids at phase 1; possibly due to the presence of lactose (from whey permeate) in the diet which may produce LA and reduce gastric pH when fermented. During phase 2, pig performance was improved with the supplementation of either Prop or OIB, enzyme or fine particle size individually without any indication of interactions. Comparing the mode of acid addition, feeding pigs with diets containing APW had the same ADG, ADFI and G:F as feeding their AD counterparts. This means that the use of APW may be an alternative to direct diet acidification for weanling pigs.

Apparent total tract digestibility of DM, GE, ash and P was reduced by 1.03, 4.09, 5.35, and 6.42 percentage points respectively when AD was fed coarse. This reduction in digestibility was not observed in pigs fed diets with APW and suggests increased digestibility of energy and nutrients in APW. These improvements mean that fewer costly nutrients are wasted by excretion in the manure.

Gut health measures were studied in pigs fed a subset of treatments (those with enzyme only, thus removing the enzyme comparison) with the objective of determining the potential for an interaction between acidification and particle size. Treatment had no effect on gut health parameters which included, estimates of intestinal permeability, jejunal histology, or expression of genetic markers for barrier function, inflammation or cellular proliferation and maturity in the

jejunum. Total SCFA and LA in the caecum and colon did not differ among treatments. However, concentrations of individual SCFA may have been altered due to changes in DM digestibility in APW. Microbial diversity (Shannon Index) in the colon was not different among treatments, however differential abundance testing identified a low abundance OTU *Prevotella*, a genus of bacteria associated with high fiber diets and acetic acid production was highest in pigs fed APW-Fine and lowest in APW-Coarse. Overall, these observations mean that the same gut health status as direct diet acidification can be achieved when the acid is presented as APW.

5.5 Interaction of acid-preservation of barley and particle size, with or without enzyme, on weanling pig performance and gut health

Experiments similar to the wheat study were conducted using barley. High diet inclusion of barley was used because we expected an effect of acidification and enzyme addition especially on high-moisture barley due to the presence of hulls. This may be the reason why growth rates were low during week 1 of the experiment. However, we saw improvements in the growth rates of pigs with the use of APB compared to those fed AD during phase 2. This disproves the hypothesis that pigs fed diets with APB will have the same performance as those fed AD. Additionally, digestibility of DM and energy was improved in pigs fed diets with coarse APB which was not observed in pigs fed AD. Although not measured, we suspect that the "pre-digestion" of barley during high-moisture and low pH storage, coupled with reduced gastric emptying rate associated with coarse diets could be responsible for these improvements. The addition of enzyme improved ash digestibility regardless of mode of addition and P digestibility tended to improve with coarse diets regardless of mode of acid and enzyme addition. Pigs fed AD had the same gut health status as pigs fed APB and there were no indications of interaction

with particle size. Information on the effect of MOA or PS on SCFA concentrations in the caecum and colon suggests possible dietary modifications necessary to alter its SCFA concentration in the hindgut to improve gut health. The low abundance of bacterium *Bacteroidetes* in pigs fed diets with the OIB compared to the non-acidified PC may be evidence that acid supplementation modulates microbial population. Overall, it appears that the performance advantage of using APB in pigs may be due to the improvements in nutrient digestibility. In this trial, likely because the pigs used were from a herd with a high health status, there was minimal influence on gut health status.

5.6 Comparing wheat and barley results

Results suggest that presenting the acid either as APW or APB may be an alternative to direct acidification of weanling pig diets. In wheat, pigs fed Coarse had similar performance compared to Fine when fed APW while in barley, pigs fed Coarse had improved performance compared to those fed Fine when fed APB. In wheat-based diets, acid or enzymes independently improved weanling pig performance and no synergy was noted. In contrast, enzymes and acid improved weanling pig performance in barley only when both were present. The difference in response between wheat and barley is likely due to the higher fiber content of barley compared to wheat. A greater response was observed in feeding acid preserved high moisture barley relative to acidified diet using dry barley and is possibly due to the reduction of fiber fractions (ADF and NDF) after high moisture and low pH storage which may have increased its energy value relative to the dry control barley. Furthermore, the hydrolysis of fiber probably provided the substrates required by the carbohydrase enzymes present in the enzyme cocktail used in these experiments.

Furthermore, higher fiber content may have further reduced gastric emptying rate, allowing further enzymatic hydrolysis of nutrients.

5.7 Economic analysis

Comparing the cost contribution of drying grains with acid preservation of high-moisture wheat on feed cost per pig, we estimate a net savings of about $1.73 per pig grown to market weight of 130 kg (Appendix 1). This was calculated based on the estimated variable and fixed costs of grain drying or acidification, cost of diet acidification and the cost of grinding dry or high-moisture grains.

We estimate a total of 372 kg feed (124 kg weight gain x 3.0 FCR) required to bring a pig to market and will need about 186 kg dried wheat (86% DM) to produce (assuming 50% inclusion) or 200 kg equivalent high-moisture grain (80% DM). Total cost to dry 200 kg of high-moisture wheat required is estimated at $ 3.99 per pig ($2.56 operating cost + $1.43 fixed cost) assuming current cost of fuel and other inputs. Weanling pig diets are typically acidified, and we estimated a cost of $0.02 per pig based on the acid inclusion and cost. We estimated a grinding cost of $0.07 per pig to be used in the weanling and growing stages based on current power cost. The total cost of drying, acidification, and grinding when using dry grain was $4.08 per pig. When using acidified high-moisture wheat, we estimated a total acidification and grinding cost of $2.34 per pig (acidification cost of $2.22 and a grinding cost of $0.12 per pig).

Using the same model for barley, the cost of using APB till market was estimated to be $2.38 lower per pig when compared to dry barley (Appendix 2).

5.8 Scope and limitations of the thesis

The grains used in the animal trials were reconstituted to contain 20% moisture. For both wheat and barley used in the in vivo trials, mould growth was observed when the phosphoric acid-based OIB was used. Whether mould growth will occur when tough grains (harvested at high-moisture) are preserved with the same acid mixture is a question that cannot be answered by this thesis and should be an area for future research. Similarly, storage under field conditions may be of interest to evaluate the viability of this preservation method for high-moisture grains.

Due to limited funds and because the focus of the studies was nutrient digestibility and pig performance, gene expression and microbiome analysis were limited to one location in the GIT and no regional comparisons were made. Jejunum was chosen for analysis of gene expression because this is where majority of nutrient absorption takes place. Treatment effects may be more apparent in the duodenum as pancreatic secretions serve as a buffer and increase the digesta pH and may have been neutralized by the time the acids reached mid jejunum. Aside from microbial diversity in the colon, microbiota from the stomach and ileum would be potential sites to measure this parameter to determine if there are regional differences in microbial population diversity due to treatment.

5.9 Recommendations for future study

The use of phosphoric acid-based OIB as a grain preservative needs further evaluation. Potentially, a trial utilizing grains harvested at high-moisture (tough grains) rather than reconstituted. Different storage methods or duration, potentially similar to field conditions, need to be explored when using a phosphoric acid-based OIB. Similar studies done on older pigs are suggested in order to determine the interaction of acid-preserved high-moisture grains and

particle size on performance, nutrient digestibility and gut health as this could be an alternate feeding strategy to improve producer profitability.

In vitro and in vivo trials comparing high-moisture air tight stored grains and acid-preserved grains to isolate the effects of acidification and high-moisture storage on fermentation metabolites (ammonia N, pH, SCFA) and changes in PDI and aP would aid our understanding of the importance of these molecules and potential benefits to the growing pig.

5.10 Overall conclusions

The overall objective of these series of experiments was to determine whether the benefits of diet acidification are maintained when acidified, high-moisture grains are fed. Results of this study indicate that feeding acidified high-moisture wheat or barley to weanling pigs gave similar performance to feeding acidified diets. It can therefore be concluded that feeding acid-preserved high-moisture grains can be an alternative to direct diet acidification for weanling pigs with improvements in digestibility of DM, energy, ash or P. Likely due to improvements in digestibility, high-moisture grains are not required to be ground finely for weanling pigs. Taking into account the costs of grain drying (fixed and variable costs), acidification and grinding, the use of acidified high-moisture grains may reduce feed cost by $1.73 (wheat-based diet) to $2.38 (barley-based diet) per market pig due to the avoidance of costs from drying. The implication of this finding is that it provides producers an alternative tool to utilize and improve the feeding value of low quality, high-moisture wheat and barley with a potential to reduce cost by elimination of costs associated to artificial drying. Furthermore, the improvement in digestibility of DM, ash and P may reduce excretion of these nutrients in the manure, addressing environmental pollution originating from pig production systems.

REFERENCES

Abulreesh, Hussein. 2012. *Salmonellae* in the environment. Pages 19-50 in B. Annous, and J.B. Gurtler, eds. *Salmonella*-dsitribution, adaptation, control measures and molecular technologies. Intech Open.

Adams, R.S., Kephart, K.B., Ishler, V.A., Hutchinson, L.J., and Roth, G.W. 1993. Mold and mycotoxin problems in livestock feeding. Pages 16. The Pennsylvania State University, College of Agricultural Sciences and Cooperative Extension Publication, University Park, PA.

Adeola, O. 2001. Digestion and balance techniques in pigs. Pages 903-906 in A.J. Lewis and L.L. Southern, eds. Swine Nutrition. CRC Press, Washington DC.

Ahmed, S.T., Hwang, J.A., Hoon, J., Mun, H.S., and Yang, C.J. 2014. Comparison of single and blend acidifiers as alternative to antibiotics on growth performance, fecal microflora, and humoral immunity in weaned piglets. Asian-Australas. J. Anim. Sci. 27(1):93-100.

Alberta Agriculture and Forestry. 2017. Grain drying-frequently asked questions. [Online]. Available: http://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/faq7453 [13 Jun. 2018].

Allen, W.R., and Stevenson, K.R. 1975. Influence of additives on the ensiling process of wet brewers' grains. Can. J. Anim. Sci. 55(3):391-402.

American Oil Chemists' Society. 2017. Official methods and recommended practices of the AOCS. Champaign, Ill, USA.

ASAE. 2012. Method of determining and expressing fineness of feed materials by sieving. ASAE S319 3.

Association of Official Analytical Chemists. 2005. Official method of analysis. 18th ed. AOAC. Arlington, VA, USA.

Baker, S., and Hermann, T. 2002. Evaluating particle size. MF-2051 Feed Manufacturing, Department of Grain Science and Industry, Kansas State University. 5 pp.

Barnette, J.J., and McLean, M.E. 1998. Protected versus unprotected multiple comparison procedures. 27th annual meeting of the mid-south educational research association. New Orleans, LA. Accessed Feb. 21, 2019. http://eric.ed.gov/PDFS/ED427044.pdf.

Baron, V.S., Stevenson, K.R., and Buchanan-Smith, J.G. 1986. Proteolysis and fermentation of grain-corn ensiled at several moisture levels and under several simulated storage methods. Can. J. Anim. Sci. 66(2):451-461.

Batonon-Alavo, D.I., Bouza, B., Cholet, J.C.G., and Mercier, Y. 2016. A method for determination of the acidifying value of organic acids used in pig diets in the acid binding capacity at pH 4 (BC-4) system. Anim. Feed Sci. Technol. 216:197-203.

Berends, B.R., Urlings, H.A.P., Snijders, J.M.A., and Van Knapen, F. 1996. Identification and quantification of risk factors in animal management and transport regarding *Salmonella* spp. in pigs. Int. J. Food Microbiol. 30(1):37-53.

Betscher, S., Beineke, A., Schönfeld, L., and Kamphues, J. 2010. Effects of diet's physical form (grinding intensity; meal/pellets) on morphological and histological parameters (e.g. ratio of neutral to acid mucins) of the gastrointestinal tract in weaned piglets. Livest. Sci. 134(1–3):149-151.

Breirem, K., and Ulvesli, O. 1960. Ensling methods. Herbage Abstr. 30: 1-8.

Broz, J., and Schulze, J. 1987. Efficacy of citric acid as a feed additive in early weaned piglets. J. Anim.Physiol. Anim. Nut. 58(1-5):215-223.

Brunsgaard, G. 1998. Effects of cereal type and feed particle size on morphological characteristics, epithelial cell proliferation, and lectin binding patterns in the large intestine of pigs. J. Anim. Sci. 76(11):2787-2798.

Buchanan-Smith, J.G., Smith, T.K., and Morris, J.R. 2003. High-moisture Grain and Grain By-Products. Pages 825-854 Silage Science and Technology. American Society of Agronomy, Crop Science Society of America, Soil Science Society of America, Madison, WI.

Cabrera, R.A., Usry, J.L., Arrellano, C., Nogueira, E.T., Kutschenko, M., Moeser, A.J., and Odle, J. 2013. Effects of creep feeding and supplemental glutamine or glutamine plus glutamate (Aminogut) on pre- and post-weaning growth performance and intestinal health of piglets. J. Anim. Sci. Biotech. 4(1):29-41.

Callan, J.J., Garry, B.P., and O'Doherty, J.V. 2007. The effect of expander processing and screen size on nutrient digestibility, growth performance, selected faecal microbial populations and faecal volatile fatty acid concentrations in grower–finisher pigs. Anim. Feed Sci. Technol. 134(3–4):223-234.

Callies, A., Sander, S.J., Verspohl, J., Beineke, A., and Kamphues, J. 2012. Effect of grinding intensity and feed physical form on in vitro adhesion of *Salmonella typhimurium* and mannose residues in intestinal mucus receptors for *Salmonella*e. J. Anim. Sci. 90(Suppl 4):272-274.

Canadian Council on Animal Care. 2009. CCAC guidelines on: the care and use of farm animals in research, teaching and testing. Ottawa, ON, Canada.

Canibe, N., Højberg, O., Højsgaard, S., and Jensen, B.B. 2005. Feed physical form and formic acid addition to the feed affect the gastrointestinal ecology and growth performance of growing pigs. J. Anim. Sci. 83(6):1287-1302.

Cappai, M.G., Picciau, M., and Pinna, W. 2013. Ulcerogenic risk assessment of diets for pigs in relation to gastric lesion prevalence. BMC Vet. Res. 9(1):36-43.

Che, T.M., Adeola, O., Azain, M.J., Carter, S.D., Cromwell, G.L., Hill, G.M., Mahan, D.C., Miller, P.S., and Pettigrew, J.E. 2012. Effect of Dietary Acids on Growth Performance of Nursery Pigs: A Cooperative Study. J. Anim. Sci. 90(12):4408-4413.

Chowdhury, M.M.I., Sarker, R.I., Bala, B.K., and Hossain, M.A. 2001. Physical properties of gram as a function of moisture content. Int. J. Food Prop. 4(2):297-310.

Columbus, D., Niven, S.J., Zhu, C.L., and de Lange, C.F. 2010. Phosphorus utilization in starter pigs fed high-moisture corn-based liquid diets steeped with phytase. J. Anim. Sci. 88(12):3964-3976.

Craik, J.D., and Miller, F.B. 1959. The flow properties of powders under humid conditions. J. Pharm. Phamacol. 10 (Supp):136-143T.

Crevieu-Gabriel, I., Gomez, J., Caffin, J.P., and Carre, B. 1999. Comparison of pig and chicken pepsins for protein hydrolysis. Reprod. Nut., Dev. 39:443-454.

Czarnecka, M., Czarnecki, Z., and Źuromska, M. 1991. Effects of chemical preservation of high-moisture grain on carbohydrates and nitrogen compounds: in vitro experiments. Anim. Feed Sci. and Technol. 34(3-4):343-352.

De Cooman, L., Houf, K., Smet, A., Flahou, B., Ducatelle, R., De Bruyne, E., Pasmans, F., and Haesebrouck, F. 2014. Presence of Helicobacter suis on pork carcasses. Int. J. Food Microbiol. 187:73-76.

de Lange, C.F.M., Pluske, J., Gong, J., and Nyachoti, C.M. 2010. Strategic use of feed ingredients and feed additives to stimulate gut health and development in young pigs. Livest. Sci. 134(1):124-134.

Deplancke, B. and Gaskins, H.R. 2001. Microbial modulation of innate defenses: goblet cells and the intestinal mucus layer. Am. J. Clin. Nutr. 73(6):1131S-1141S.

Dirkzwager, A., Elbers, A.R.W., van der Aar, P.J., and Vos, J.H. 1998. Effect of particle size and addition of sunflower hulls to diets on the occurrence of oesophagogastric lesions and performance in growing-finishing pigs. Livest. Prod. Sci. 56(1):53-60.

Downes, J., Sutcliffe, I.C., Booth, V., and Wade, G.W. 2007. *Prevotella* maculosa sp. nov., isolated from the human oral cavity. Int. J. Systematic Evolutionary Microbiol. 57:2936-2939.

Eisemann, J.H., and van Heugten, E. 2007. Response of pigs to dietary inclusion of formic acid and ammonium formate. J. Anim. Sci. 85(6):1530-1539.

Falkowski, J.F., and Aherne, F.X. 1984. Fumaric and citric acid as feed additives in starter pig nutrition. J. Anim. Sci. 58(4):935-938.

Fang, C.L., Sun, H., Wu, J., Niu, H.H., and Feng, J. 2014. Effects of sodium butyrate on growth performance, haematological and immunological characteristics of weanling piglets. J. Anim. Physiol. Anim. Nutr. 98:665-667.

Fawcett, J.K., and Scott, J.E. 1960. A rapid and precise method for the determination of urea. J. Clin. Pathol. 13(2):156-159.

Flis, M., Sobotka, W., and Purwin, C. 2014. Effect of feed structure on nutrient digestibility, growth performance, and gastrointestinal tract of pigs – A Review. Ann. Anim. Sci. 14(4):757-768.

Forsyth, D.M. 1975. Organic acid-preserved high-moisture corn for swine. J. Anim. Sci 41(3):747-751.

Friendship, R.M. 2004. Gastric ulceration in Swine. J. Swine Health Prod. 12:34-35.

Gabert, V.M., and Sauer, W.C. 1994. The effects of supplementing diets for weanling pigs with organic acids. A review. J. Anim. Feed Sci. 3(2):73-87.

Gabert, V.M., and Sauer, W.C. 1994. The effects of fumaric acid and sodium fumarate supplementation to diets for weanling pigs on amino acid digestibility and volatile fatty acid concentrations and ileal digesta. Anim. Feed Sci. Tech. 53:243-254.

Gabert, V.M., Sauer, W.C., Schmitz, M., Ahrens, F., and Mosenthin, R. 1995. The effect of formic acid and buffering capacity on the ileal digestibilities of amino acids and bacterial populations and metabolites in the small intestine of weanling pigs fed semipurified fish meal diets. Can. J. Anim. Sci. 75(4):615-623.

Gantois, I., Ducatelle, R., Pasmans, F., Haesebrouck, F., Hautefort, I., Thompson, A., Hinton, J., and Van Immerseel, F. 2006. Butyrate specifically down-regulates *Salmonella* pathogenicity island 1 gene expression. Appl. Environ. Microbiol. 72:946-949.

Gao, Y., Shang, C., Maroof, M.A.S., Biyashev, R.M., Grabau, E.A., Kwanyuen, P., Burton, J.W., and Buss, G.R. 2007. A Modified Colorimetric Method for Phytic Acid Analysis in Soybean. Crop Sci. 47:1797-1803.

Gauthier, R. 2002. Intestinal health, the key to productivity (The case of organic acids). Proc. Precongreso Cientifico Avicola IASA. XXVII Convencion ANECA-WPDC, Puerto Vallarta, Jal. Mexico.

Giesting, D.W., and Easter, R.A. 1985. Response of starter pigs to supplementation of corn-soybean meal diets with organic acids. J. Anim. Sci. 60(5):1288-1294.

Goering, H.K., and Van Soest, P.J. 1970. Forage fiber analysis: Apparatus, reagents, procedures and some applications. USDA-ARS Agricultural Handbook 379. Washington DC.

Goodband, R.D., Diederich, W., Dritz, S.S., Tokach, M.D., DeRouchey, J.M., and Nelssen, J.L. 2006. Comparison of particle size analysis of ground grain with or without the use of a flow agent. Swine Day 2006. Kansas State University, Manhatttan.

Göransson, L., and Ogle, R.B. 1985. Anaerobically stored high-moisture cereals for growing pigs. Anim. Feed Sci. Technol. 12(3):159-169.

Grilli, E., Messina, M.R., Tedeschi, M., and Piva, A. 2010. Feeding a microencapsulated blend of organic acids and nature-identical compounds to weaning pigs improved growth performance and intestinal metabolism. Livest. Sci. 133(1):173-175.

Groesbeck, C.N., Goodband, R.D., Tokach, M.D., Nelssen, J.L., Dritz, S.S., and Derouchey, J.M. 2006. Particle size, mill type, and added fat influence angle of repose of ground corn. The Prof. Anim. Sci. 22(2):120-125.

Grosse Liesner, V., Taube, V., Leonhard-Marek, S., Beineke, A., and Kamphues, J. 2009. Integrity of gastric mucosa in reared piglets - effects of physical form of diets (meal/pellets), pre-processing grinding (coarse/fine) and addition of lignocelluloses (0/2.5%). J. Anim. Physiol. Anim. Nutr. 93:373 - 380.

Hackl, W., Pieper, B., Pieper, R., Korn, U., and Zeyner, A. 2010. Effects of ensiling cereal grains (barley, wheat, triticale and rye) on total and pre-caecal digestibility of proximate nutrients and amino acids in pigs. J. Anim. Physiol. Anim. Nutr. 94(6):729-35.

Hancock, J.D., and Behnke, K.C. 2001. Use of ingredient and diet processing technologies (grinding, mixing, pelleting and extruding) to produce quality feeds for pigs. Pages 469-498 in A.J. Lewis and L.L. Southern, eds. Swine Nutrition. CRC Press, Boca Raton, FL.

Hashimoto, H., Iwaasa, T., and Yokotsuka, T. 1973. Some properties of acid protease fro the thermophilic fungus, *Penicillium duponti* K1014. App. Microbiol. 25 (4):578-583.

Healy, B.J., Hancock, J.D., Kennedy, G.A., Bramel-Cox, P.J., Behnke, K.C., and Hines, R.H. 1994. Optimum particle size of corn and hard and soft sorghum for nursery pigs. J. Anim. Sci. 72(9):2227-2236.

Hedemann, M.S., Mikkelsen, L.L., Naughton, P.J., and Jensen, B.B. 2005. Effect of feed particle size and feed processing on morphological characteristics in the small and large intestine of pigs and on adhesion of *Salmonella enterica* serovar *typhimurium* DT12 in the ileum in vitro. J. Anim. Sci. 83(7):1554-1562.

Heiman, M. 2005. Particle size reduction. Pages 108-106 in E.K. Schofield, ed. Feed manufacturing technology. American Feed Industry Association, Arlington, USA.

Heo, J.M., Opapeju, F.O., Pluske, J.R., Kim, J.C., Hampson, D.J., and Nyachoti, C.M. 2012. Gastrointestinal health and function in weaned pigs: a review of feeding strategies to control post-weaning diarrhoea without using in-feed antimicrobial compounds. J Anim. Physiol. Anim. Nut. 97:207-237.

Hurd, H.S., McKean, J.D., Griffith, R.W., Wesley, I.V., and Rostagno, M.H. 2002. *Salmonella enterica* infections in market swine with and without transport and holding. App. Environ. Microbiol. 68(5):2376-2381.

Hurum, D., and Rohrer, J. 2016. Determination of carbohydrates in urine by HPAE-PAD. Thermo Fisher Scientific, Sunnyvale, CA, USA.

Imoto, S., and Namioka, S. 1978. VFA metabolism in the pig. J. Anim. Sci. 47(2):479-487.

Iwe, M.O., van Zuilichem, D.J., Ngoddy, P.O., and Lammers, W. 2001. Amino acid and protein dispersibility index (PDI) of mixtures of extruded soy and sweet potato flours. Lebensm.-Will. u.-Technol. 34:71-75.

Iyayi, E.A., and Adeola, O. 2015. Quantification of short-chain fatty acids and energy production from hindgut fermentation in cannulated pigs fed graded levels of wheat bran. J. Anim. Sci. 93:4781-4787.

Jacobs, B.M., Patience, J.F., Dozier, W.A.III, Stalder, K.J., and Kerr, B.J. 2011. Effects of drying methods on nitrogen and energy concentrations in pig feces and urine, and poultry excreta. J. Anim. Sci. 89:2624-2630.

Jasaitis, D.K., Wohlt, J.E., and Evans, J.L. 1997. Influence of Feed Ion Content on Buffering Capacity of Ruminant Feedstuffs In Vitro. J. Dairy Sci. 70(7):1391-1403.

Jha, R. 2010. Fibre fermentation in the pig intestine: Effect on metabolite production and ntirogen excretion. Ph.D. thesis, University of Saskatchewan, Saskatoon, SK. 150 pp.

Jin, L., Reynolds, L.P., Redmer, D.A., Caton, J.S., and Crenshaw, J.D. 1994. Effects of dietary fiber on intestinal growth, cell proliferation, and morphology in growing pigs. J. Anim. Sci. 72(9):2270-2278.

Jones, G.M., Donefer, E., and Elliot, J.I. 1970. Feeding value for dairy cattle and pigs of high-moisture corn preserved with propionic acid. Can. J. Anim. Sci. 50(3):483-489.

Jongbloed, A.W., Mroz, Z., van der Weij-Jongbloed, R., and Kemme, P.A. 2000. The effects of microbial phytase, organic acids and their interaction in diets for growing pigs. Livest. Prod. Sci. 67(1):113-122.

Kamphues, J., Brüning, I., Papenbrock, S., Möβeler, A., Wolf, P., and Verspohl, J. 2007. Lower grinding intensity of cereals for dietetic effects in piglets. Livest. Sci. 109(1):132-134.

Kansagra, K., Stoll, B., Rognerud, C., Niinikoski, H., Ou, C., Harvey, R., and Burrin, D. 2003. Parenteral nutrition adversely affects gut function in neonatal piglets. Gastrointest. Liver Physiol. 285:G1162-G1170

Kemme, P.A., Jongbloed, A.W., Mroz, Z., Kogut, J., and Beynen, A.C. 1999. Digestibility of nutrients in growing–finishing pigs is affected by Aspergillus niger phytase, phytate and lactic acid levels: 1. Apparent ileal digestibility of amino acids. Livest. Prod. Sci. 58(2):107-117.

Khorasani, G.R., Robinson, P.H., and Kennelly, J.J. 1993. Effects of canola meal treated with acetic acid on rumen degradation and intestinal digestibility in lactating dairy cows. J. Dairy Sci. 76(6):1607-1616.

Kiarie, E., Voth, C., Wey, D., Zhu, C., Vingerhoeds, P., Borucki, S., and Squires, J. 2018. Comparative efficacy of antibiotic growth promoter and benzoic acid on growth performance, nutrient utilization and indices of guty health in nursery pigs fed corn-wheat-soybean meal diet. Can. J. Anim. Sci. [in press]

Kil, D.Y., Piao, L.G., Long, H.F., Lim, J.S., Yun, M.S., Kong, C.S., Ju, W.S., Lee, H.B., and Kim, Y.Y. 2006. Effects of organic or inorganic acid supplementation on growth performance, nutrient digestibility and white blood cell counts in weanling pigs. Asian-Aust. J. Anim. Sci. 19(2):252-261.

Kil, D.Y., Kwon, B.W, and Kim G.B. 2011. Dietary acidifiers in weanling pigs: a review. Rev. Colomb. Cienc. Pecu. 24:231-247.

Kim, Y.S., and Ho, B. 2010. Intestinal goblet cells and mucins in health and disease: Recent insights and progress. Curr. Gastroenterol. Rep. 12:319-330.

Knudsen, K.E.B. 1997. Carbohydrate and lignin contents of plant materials used in animal feeding. Anim. Feed Sci. and Tech. 67(4):319-338.

Kozich, J.J., Westcott, S.L., Baxter, N.T., Highlander, S.K., and Schloss, P.D. 2013. Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the miseq illumina sequencing platform. App. Environ. Microbiol. 79(17):5112-5120.

Lambert, R.J., and Stratford, M. 1999. Weak-acid preservatives: modelling microbial inhibition and response. J. App. Microbiol. 86:157-164.

Lawlor, P.G., Lynch, P.B., Caffrey, P.J., O'Reilly, J.J., and O'Connell, M.K. 2005. Measurements of the acid-binding capacity of ingredients used in pig diets. Irish Vet. J. 58(8):447-452.

Lawrence, T.L.J. 1970. Some effects of including differently processed barley in the diet of the growing pig: 2. In vivo gastric pH changes. Anim. Sci. 12(1):151-163.

Lee, D.N., Liu, S.R., Chen, Y.T., Wang, R.C., Lin, Y.S., and Weng, C.F. 2007. Effects of diets supplemented with organic acids and nucleotdes on growth, immunce responses and digestive tract development in weaned pigs. J. Anim. Physiol. Anim. Nutr. 91:508-518.

Lenahan, M., Kelly, S., Fanning, S., and Bolton, D.J. 2010. The effect of bovine diet on Salmonella survival in synthetic abomasal fluid. J. Appl. Microbiol. 109(6):2060-2068.

Li, E., Dhital, S., and Hasjim, J. 2014. Effects of grain milling on starch structures and flour/starch properties. Starch 66:15-27.

Li, Z., Yi, G., Yin, J., Sun, P., Li, D., and Knight, C. 2008. Effects of organic acids on growth performance, gastrointestinal pH, intestinal microbial populations, and immune response of weaned pigs. Asian Australas. J. Anim. Sci. 21(2):252-261.

Lin, D.C., and Chen, C.T. 1995. Relative antifungal efficacies of phosphoric acid and other compounds on fungi isolated from poultry feed. 217-226 pp.

Livingstone, R.M., Denerley, H., Stewart, C.S., and Elsley, F.W.H. 1971. Moist barley for growing pigs: some effects of storage method and processing. Anim. Sci. 13(3):547-556.

Low, A.G. 1990. Nutritional regulation of gastric secretion, digestion and emptying. Nut. Res. Rev. 3(1):229-252.

Luckstadt, C., and Mellor, S. 2011. The use of organic acids in animal nutrition, with special focus on dietary potassium diformate under European and Austral-Asian conditions. Pages 123-130 Recent advances in animal nutrition, Australia.

Lynch, P.B., Hall, G.E., Hill, L.D., Hatfield, E.E., and Jensen, A.H. 1975. Chemically preserved high-moisture corns in diets for growing-finishing swine. J. Anim. Sci. 40(6):1063-1069.

Mahan, D.C., Newton, E.A., and Cera, K.R. 1996. Effect of supplemental sodium chloride, sodium phosphate, or hydrochloric acid in starter pig diets containing dried whey. J. Anim. Sci. 74(6):1217-1222.

Mahan, D.C., Wiseman, T.D., Weaver, E., and Russell, L. 1999. Effect of supplemental sodium chloride and hydrochloric acid added to initial starter diets containing spray-dried blood plasma and lactose on resulting performance and nitrogen digestibility of 3-week-old weaned pigs. J. Anim. Sci. 77(11):3016-3021.

Manners, M.J. 1976. The development of digestive function in the pig. Proc. Nutr. Soc. 35(1):49-55.

Marini, R.P. 2003. Approaches to analyzing experiments with factorial arrangement plus other treatments. Hort. Sci. 3:117-120.

Mavromichalis, I., Hancock, J.D., Senne, B.W., Gugle, T.L., Kennedy, G.A., Hines, R.H., and Wyatt, C.L. 2000. Enzyme supplementation and particle size of wheat in diets for nursery and finishing pigs. J. Anim. Sci. 78(12):3086-3095.

McLelland, M. 2008. Storage of high-moisture barley. [Online] Available: https://open.alberta.ca/dataset/71caa0b4-e91e-4b78-aedc-66f2f92c2325/resource/d0da48c5-f92c-48b9-8859-104b2a4223a9/download/2008-114-61-1.pdf [13 Jun. 2018].

Melnichouk, S.I. 2002. Mortality associated with gastric ulceration in swine. Can. Vet. J. 43(3):223-2255.

Metzler, B., and Mosenthin, R. 2007. Effects of organic acids on growth performance and nutrient digestibilities in pigs. Pages 39-54 in C. Luckstadt, ed. Acidifiers in animal nutrition-a guide for feed preservation and acidification to promote animal performance. Nottingham University Press, Nottingham

Mikkelsen, L.L., Naughton, P.J., Hedemann, M.S., and Jensen, B.B. 2004. Effects of physical properties of feed on microbial ecology and survival of *Salmonella enterica serovar typhimurium* in the pig gastrointestinal tract. App. Environ. Microbiol. 70(6):3485-3492.

Millet, S., Kumar, S., De Boever, J., Meyns, T., Aluwe, M., De Brabander, D., and Ducatelle, R. 2012. Effect of particle size distribution and dietary crude fibre content on growth performance and gastric mucosa integrity of growing-finishing pigs. Vet. J. 192(3):316-321.

Moreyra, R., and Peleg, M. 2006. Effect of equilibrium water activity on the bulk properties of selected food powders. J. Food Sci. 46(6):1918-1922.

Morrow, W.E.M., and Funk, J. 2001. *Salmonella* as food borne pathogen in pork. Animal science facts. ANS 01-816S. [Online]. Available: https://projects.ncsu.edu/project/swine_extension/publications/factsheets/816s.htm [13 Jun. 2018].

Mosenthin, R. 1998. Physiology of small and large intestine of swine - review. Asian-Australas. J. Anim. Sci. 11(5):608-619.

Naughton, P.J., Mikkelsen, L.L., and Jensen, B.B. 2001. Effects of non-digestible oligosaccharides on *Salmonella enterica* Serovar *typhimurium* and non-pathogenic Escherichia coli in the pig small intestine in vitro. App. Environ. Microbiol. 67(8):3391-3395.

Nielsen, E.K., and Ingvartsen, K.L. 2000. Effect of cereal type, disintegration method and pelleting on stomach content, weight and ulcers and performance in growing pigs. Livest. Prod. Sci. 66(3):271-282.

NRC. 2012. Nutrient requirements of swine. Eleventh ed. National Academic Press, Washington, D. C. 20418 USA.

O'Neil, M. J., (ed.) 2006. The Merck Index: An encyclopedia of chemicals, drugs and biologicals. 14th ed. New Jersey.

Omogbenigun, F.O., Nyachoti, C.M., and Slominski, B.A. 2003. The effect of supplementing microbial phytase and organic acids to a corn-soybean based diet fed to early-weaned pigs. J. Anim. Sci. 81(7):1806-1813.

Papatsiros, V.G., and Billinis, C. 2012. The prophylactic use of acidifiers as antibacterial agenst in swine. Pages 295-310 *in* V. Bobbarala, ed. Antimicrobial agents. InTech.

Papatsiros, V.G., Christodoulopoulos, G., and Filippopoulos, L.C. 2012. The use of organic acids in monogastric animals (swine and rabbits). J. Cell Anim. Biol. 6(10):154-159.

Papenbrock, S., Stemme, K., Amtsberg, G., Verspohl, J., and Kamphues, J. 2005. Investigations on prophylactic effects of coarse feed structure and/or potassium diformate on the microflora in the digestive tract of weaned piglets experimentally infected with *Salmonella derby*. J. Anim. Physiol. Anim. Nutr. 89(3-6):84-87.

Partanen, K. 2001. Organic acids-their efficacy and modes of actions in pigs. Pages 201-217 in A. Piva, K.E. Bach Knudsen, and J.E. Lindberg, eds. Gut environment of pigs. Nottingham University Press, Nottingham, United Kingdom.

Partanen, K.H., and Mroz, Z. 1999. Organic acids for performance enhancement in pig diets. Nutr. Res. Rev. 12(1):117-45.

Peris, S., and Calafat, F. 2018. Acidification and other physiological additives. Pages 63-70 in J. Brufau, ed. Feed manufacturing in the Mediterranean region. Improving safety: From feed to food. Zaragoza : CIHEAM.

Petito, S.L., and Evans, J.L. 1984. Calcium status of the growing rat as affected by diet acidity from ammonium chloride, phosphate and protein. J. Nutr. 114(6):1049-1059.

Pettigrew, J.E. 2006. Reduced use of antibiotic growth promoters in diets fed to weanling pigs: Dietary tools, Part 1. Anim. Biotechnol. 17(2):207-215.

Phillip, L.E., Garino, H.J., Alli, I., and Baker, B.E. 1985. Effects of anhydrous ammonia on amino acid preservation and feeding value of high-moisture ear corn for growing steers. Can. J. Anim. Sci. 65(2):411-417.

Pluske, J.R. 2001. Morphological and functional changes in the small intestine of the newly weaned pig. Pages 1-27 in A. Piva, K.E. Bach KnudsenandJ.E. Lindberg, eds. Gut environment of pigs. Nottingham University Press, Nottingham, United Kingdom.

Pluske, J.R., Hampson, D.J., and Williams, I.H. 1997. Factors influencing the structure and function of the small intestine in the weaned pig: a review. Livest. Prod. Sci. 51(1):215-236.

Poulsen, H.D., Blaabjerg, K., Norgaard, J.V., and Ton Nu, M.A. 2012. High-moisture air-tight storage of barley and wheat improves nutrient digestibility. J. Anim. Sci. 90:242-244.

Prigge, E.C., Johnson, R.R., Owens, F.N., and Williams, D. 1976. Soluble nitrogen and acid production of high-moisture corn. J. Anim. Sci. 42(2):490-496.

Probst, K., Ambrose, K., and Ileleji, K. 2013. The effect of moisture content on the grinding performance of corn and corncobs by hammer milling. Transactions of the ASAE 56(3):1025-1033.

Radcliffe, J.S., Zhang, Z., and Kornegay, E.T. 1998. The effects of microbial phytase, citric acid, and their interaction in a corn-soybean meal-based diet for weanling pigs. J. Anim. Sci. 76(7):1880-1886.

Radecki, V.S., Juhl, R.M., and Miller, R.E. 1988. Fumaric and citric acids as feed additives in starter pig diets: Effect on performance and nutrient balance. J. Anim. Sci. 66(10):2598-2605.

Ravindran, V., and Kornegay, E.T. 1993. Acidification of weaner pig diets: A review. J. Sci. Food Agric. 62:313-322.

Reece, F.N., Lott, B.D., and Deaton, J.W. 1985. The effects of feed form, grinding method, energy level, and gender on broiler performance in a moderate (21 °C) environment. Poult Sci 64(10):1834-1839.

Richert, B.T., and DeRouchey, J.M. 2010. Swine feed processing and manufacturing. National Swine Nutrition Guide. US Pork Center of Excellence. Available: http://porkgateway.org/resource/swine-feed-processing-and-manufacturing/ [13 Jun. 2018]

Risley, C.R., Kornegay, E.T., Lindemann, M.D., Wood, C.M., and Eigel, W.N. 1993. Effect of feeding organic acids on gastrointestinal digesta measurements at various times postweaning in pigs challenged with enterotoxigenic *Escherichia coli*. Can. J. Anim. Sci. 73(4):931-940.

Roberfroid, M. 2007. Prebiotics: The concept revisited. J. Nutr. 137:830S-837S.

Rojas, O.J., and Stein, H.H. 2015a. Effects of reducing the particle size of corn grain on the concentration of digestible and metabolizable energy and on the digestibility of energy and nutrients in corn grain fed to growing pigs. Livest. Sci. 181:187-193.

Rojas, O.J., and Stein, H.H. 2015b. Impact of particle size reduction on feed cost and feed efficiency. Adv. in Pork Prod. 26:226-227.

Sakata, T., Adachi, M., Hashida, M., Sato, N., and Kojima, T. 1995. Effect of n-butyric acid on epithelial cell proliferation of pig colonic mucosa in short-term culture. Dtsch Tierarztl Wochenschr 102(4):163-164.

SAS, 2003. SAS user's guide: Statistics. Version 9.4. SAS Institute Inc., Cary, NC.

Sauer, W., and Burroughs, R. 1974. Efficacy of various chemicals as grain mold inhibitors. Transactions of the ASAE 17:557-559.

Sauer, W., Cervantes, M., Yanez, J., Araiza, B., Murdoch, G., Morales, A., and Zijlstra, R.T. 2008. Effect of dietary inclusion of benzoic acid on mineral balance in growing pigs. Livest. Sci. 122:162-168.

Scheppach, W. 1994. Effects of short chain fatty acids on gut morphology and function. Gut 35(1 Suppl):S35-S38.

Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., Lesniewski, R.A., Oakley, B.B., Parks, D.H., Robinson, C.J., Sahl, J.W., Stres, B., Thallinger, G.G., van Horn, D.J., and Weber, C.F. 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl. Environ. Microbiol. 75(23):7537-7541.

Sebastian, S., Phillip, L.E., Fellner, V., and Idziak, E.S. 1996. Comparative assessment of bacterial inoculation and propionic acid treatment of aerobic stability and microbial populations of ensiled high-moisture ear corn. J. Anim. Sci. 74(2):447-456.

Simons, P.C.M., Versteegh, H.A.J., Jongbloed, A.W., Kemme, P.A., Slump, P., Bos, K.D., Wolters, M.G.E., Beudeker, R.F., and Verschoor, G.J. 1990. Improvement of phosphorus availability by microbial phytase in broilers and pigs. Br. J. Nutr. 64:525-540.

Sol, C. 2018. Organic acids in today's livestock production. Proc. 2018 ANCC Conference. Edmonton, Alberta.

Stein, H.H., and Kil, D.Y. 2006. Reduced use of antibiotic growth promoters in diets fed to weanling pigs: dietary tools, part 2. Anim. Biotechnol. 17(2):217-231.

Straw, M.L., Kornegay, E.T., Evans, J.L., and Wood, C.M. 1991. Effects of dietary pH and phosphorus source on performance, gastrointestinal tract digesta, and bone measurements of weanling pigs. J. Anim. Sci. 69:4496-4504.

Stroshine, R.L. 1998. Physical properties of agricultural materials and food products. Dept. of Agricultural Engineering Purdue Univer. Press, West Lafayette, New York, USA. 287 pp.

Suiryanrayna, M.V.A.N., and Ramana, J.V. 2015. A review of the effects of dietary organic acids fed to swine. J. Anim. Sci. Biotech. 6(1):45-56.

Tester, R.F., Karkalas, J., and Qi, X. 2004. Starch structure and digestibility: Enzyme-substrate relationship. World's Poult. Sci. J. 60:186-195.

Ton Nu, M.A., Blaabjerg, K., Labouriau, R., and Poulsen, H.D. 2015. High-moisture airtight storage of barley and triticale: Effect of moisture level and grain processing on nitrogen and phosphorus solubility. Anim. Feed Sci. Technol. 21:125-137.

Tsiloyiannis, V.K., Kyriakis, S.C., Vlemmas, J., and Sarris, K. 2001. The effect of organic acids on the control of porcine post-weaning diarrhoea. Res. Vet. Sci. 70(3):287-293.

Tsukahara, T., Kishino, E., Inoue, R., Nakanishi, N., Nakayama, K., Ito, T., and Ushida, K. 2013. Correlation between villous height and the disaccharidase activity in the small intestine of piglets from nursing to growing. Anim. Sci. J. 84(1):54-59.

Tung, C.M., and Pettigrew, J.E. 2011. Critical review of acidifiers. Practical ideas to address high feed and production costs. [Online]. Available: https://www.pork.org/wp-content/uploads/2008/09/05-169-PETTIGREW-UofILL.pdf [13 Jun. 2018].

Upadhaya, S.D., Lee, K.Y., and Kim, I.H. 2016. Effect of protected organic acid blends on growth performance, nutrient digestibility and faecal micro flora in growing pigs. J. App. Anim. Res. 44(1):238-242.

van der Klis, J.D., and Jansman, A.J.M.A. 2001. Optimising nutrient digestion, absorption and gut barrier function in monogastrics: reality or illusion? Pages 15-36 in M.C. Block, H.A.

Vahl, C.F.M. de Lange, A.W. van der Braak, G. Hemke, and M. Hessing, eds. Nutrition and health of the gastrointeastinal tract. Wageningen Academic Publishers, Wageningen.

van Kleef, D.J., Deuring, K., and van Leeuwen, P. 1994. A new method of faeces collection in the pig. Lab. Anim. 28:78-79.

Vermoote, M., Vandekerckhove, T.T.M., Flahou, B., Pasmans, F., Smet, A., De Groote, D., Van Criekinge, W., Ducatelle, R., and Haesebrouck, F. 2011. Genome sequence of Helicobacter suis supports its role in gastric pathology. Vet. Res. 42(1):51-60.

Vimal, D.B., Khullar, M., Gupta, S., and Ganguly, N.K. 2000. Intestinal mucins: the binding sites for *Salmonella typhimurium*. Mol. Cell. Biochem. 204(1-2):107-117.

Visscher, C.F., Winter, P., Verspohl, J., Stratmann-Selke, J., Upmann, M., Beyerbach, M., and Kamphues, J. 2009. Effects of feed particle size at dietary presence of added organic acids on caecal parameters and the prevalence of *Salmonella* in fattening pigs on farm and at slaughter. J Anim Physiol Anim Nutr (Berl) 93(4):423-430.

Wang, Y., Chiba., L.I., Huang, C., Torres, I.M., Wang, L., and Welles, E.G. 2018. Effect of diet complexity, multi-enzyme complexes, essential oils, and benzoic acid on weanling pigs. Livest. Sci. 209:32-38.

Wardynski, F.A., Rust, S.R., and Yokoyama, M.T. 1993. Effect of microbial inoculation of high-moisture corn on fermentation characteristics, aerobic stability, and cattle performance. J. Anim. Sci. 71(8):2246-2252.

Weinberg, Z.G., Yan, Y., Chen, Y., Finkelman, S., Ashbell, G., and Navarro, S. 2008. The effect of moisture level on high-moisture maize (Zea mays L.) under hermetic storage conditions—in vitro studies. J. Stored Prod. Res. 44(2):136-144.

Wijtten, P.J.A, Verstijnen, J.J., van Kempen, T.A.T.G., Perdok, H.B., Gort, G., and Verstegen, M.W.A. 2033. Lactulose as a marker of intestinal barrier function in pigs after weaning. J. Anim. Sci. 89:1347-1357.

Wondra, K.J., Hancock, J.D., Kennedy, G.A, Behnke, K.C., and Wondra, K.R. 1995a. Reducing particle size of corn in lactation diets on energy and nitrogen metabolism in second-parity sows. J. Anim. Sci. 73(12):427-432.

Wondra, K., Hancock, J., Behnke, K., Hines, R., and Stark, C. 1995b. Effects of particle size and pelleting on growth performance, nutrient digestibility, and stomach morphology in finishing pigs. J. Anim. Sci. 73:757-763.

Wondra, K.J., Hancock, J.D., Kennedy, G.A, Hines, R.H., and Behnke, K.C. 1995c. Reducing particle size of corn in lactation diets from 1,200 to 400 micrometers improves sow and litter performance. J. Anim. Sci. 73(12):421-426.

Wondra, K.J., Hancock, J.D., Behnke, K.C., and Stark, C.R. 1995b. Effects of mill type and particle size uniformity on growth performance, nutrient digestibility, and stomach morphology in finishing pigs. J. Anim. Sci. 73(9):2564-2573.

Wong, J.M., de Souza, R., Kendall, C.W., Emam, A., and Jenkins, D.J. 2006. Colonic health: fermentation and short chain fatty acids. J. Clin. Gastroenterol. 40(3):235-243.

Woyengo, T.A., Beltranena, E., and Zijlstra, R.T. 2014. Non-ruminant nutrition symposium: Controlling feed cost by including alternative ingredients into pig diets: A review. J. Anim. Sci. 92(4):1293-1305.

Wurth, C.E., Fahrenholz, A.C., McKinney, L.J., and Behnke, K.C. 2010. Defining sieving methods used to determine and express fineness of feed materials. [Online]. Available: https://www.bookstore.ksre.ksu.edu/pubs/MF2952.pdf [13 Jun. 2018]

Yajima, T., and Sakata, T. 1986. Influences of short-chain fatty acids on the digestive organs. Bifidobacteria Microflora 6(1):7-14.

Yan, H., and Barbosa-Cánovas, G.V. 1997. Compression characteristics of agglomerated food powders: Effect of agglomerate size and water activity. Food Sci. Technol. Int. 3(5):351-359.

Young, L.G. 1970. Moisture content and processing of corn for pigs. Can. J. Anim. Sci. 50(3):705-709.

Zhang, Y., Lee, B., Thompson, M, Glass, R., Lee, R.C., Figueroa, D., Gilman, R., Taylor, D., and Stephenson, C. 2000. Lactulose-mannitol intestinal permeability test in children with diarrhea caused by *Rotavirus* and *Cryptosporydium*. J. Pediatric Gastroenterol. Nutr. 31:16-21.

Zijlstra, R.T., Jha, R., Woodward, A.D., Fouhse, J., and van Kempen, T.A.T.G. 2012. Starch and fiber properties affect their kinetics of digestion and thereby digestive physiology in pigs. J. Anim. Sci. 90(Suppl 4):49-58.

APPENDIX

A.1. Cost comparison of using dry grain and high-moisture wheat on pig diets from 6 to 130 kg.

	Drying	Acidification
I. Feed and grain requirement		
Initial BW	6.00	6.00
Final BW	130.00	130.00
Weight gain	124.00	124.00
Feed conversion ratio (FCR)	3.00	3.00
Total feed consumed (FCR × weight gain)	372.00	372.00
Amount of dry grain required, kg (assuming 50% inclusion, 86% DM)	186.00	
High-moisture grain equivalent, kg (80 % DM)	199.95	199.95
II. Preservation cost		
Variable costs related to drying and acidification		
Operating costs		
Repair costs	$ 0.21	
Labour ($20 h^{-1})	$ 0.74	
Power costs ($ 0.116 kWh^{-1})[a]	$ 0.06	
Propane costs ($0.65 L^{-1})[b]	$ 1.56	
Total operating costs[c]	$ 2.56	
Fixed costs		
Depreciation (10% on dryer, 5% on surge bins)	$ 1.07	
Investment cost (dryer $30,000; surge bins $12,000; auxiliary equipment $7,500; 20% salvage value)	$ 0.36	
Total fixed costs	$ 1.43	
Total drying costs	$ 3.99	
Cost of grain acidification by pig		
Amount high-moisture grain required, kg		199.95
Amount of acid required, kg (7 kg/ tonne inclusion rate)		1.40
Additional cost per pig due to acid (acid at $ 1.0 L^{-1})		$ 1.40
Estimated fixed costs		$ 0.82
Total acidification cost (Estimated based on reports by Palva, 2008)		**$ 2.22**
III. Cost of diet acidification		
Amount of weanling pig diet required (kg pig^{-1})	4.90	4.90
Cost of acidification ($1.0 per kg acid at 3.5 L t^{-1})	$ 0.02	
IV. Grinding cost[d]	**$ 0.081**	**$ 0.16**
Cost to grind wheat used for weanling pig diet	$ 0.002	$ 0.002
Cost to grind wheat used for growing-finishing pig diets	$ 0.066	$ 0.120
V. Overall cost	**$ 4.08**	**$ 2.34**

Difference vs dry grain	-$ 1.73

[a]Based on Jan 2018 power cost at CFRC, North Battleford, Saskatchewan.
[b]Propane cost is based on July 2018 prices (http://www.agric.gov.ab.ca/app21/farminputprices, Accessed Aug 2018))
[c]Fuel consumptions based on PAMI test reports and CSAE paper 84-211 "Heated Air Grain Dryer Performance."
[d]For weanling pig diets, dry wheat was ground using 2.0 mm screen and acidified wheat was ground using 4.8 mm screen. Wheat required for growing finishing diets were both ground using 4.8 mm screen. Actual grinding cost of dry wheat using 2.0 mm screen is $ 0.82 tonne^{-1} and using 4.8 mm screen is $0.36 tonne^{-1}. Actual grinding cost of acidified wheat using 4.8 mm screen is $ 0.61 tonne^{-1}.

A.2. Cost comparison of using dry and high-moisture barley on pig diets from 6 to 130 kg.

	Drying	Acidification
I. Feed and grain requirement		
Initial BW	6.00	6.00
Final BW	130.00	130.00
Weight gain	124.00	124.00
Feed conversion ratio (FCR)	3.00	3.00
Total feed consumed (FCR × weight gain)	372.00	372.00
Amount of grain required, kg (assuming 50% inclusion)	186.00	
High moisture grain equivalent, kg (80% DM)	204.60	204.60
II. Preservation cost		
Drying costs per pig		
Operating costs		
Repair costs	$ 0.26	
Labour ($20 h^{-1})	$ 0.94	
Power costs ($ 0.116 kWh^{-1})[a]	$ 0.08	
Propane costs ($0.65 L^{-1})[b]	$ 1.60	
Total operating costs[c]	**$ 2.88**	
Fixed costs		
Depreciation (10% on dryer, 5% on surge bins)	$ 1.36	
Investment cost (dryer $30,000; surge bins $12,000; auxiliary equipment $7500; 20% salvage value)	$ 0.46	
Total fixed costs	**$ 1.83**	
Total drying costs	**$ 4.71**	
Acidification cost per pig		
Amount of treated grain, kg		204.60
Amount of acid required, kg (7 kg/ tonne inclusion rate)		1.43
Additional cost per pig due to acid, acid at $ 1 L^{-1})		$ 1.43
Fixed cost (34% of total cost)		$ 0.84
Total preservation cost (Estimated based on reports by Palva, 2008)		**$ 2.27**
III. Cost of diet acidification		
Amount of weanling pig diet required, kg pig^{-1})	4.90	4.90
Cost of acidification ($1.0 kg^{-1} acid at 3.5 L t^{-1})	$ 0.02	
IV. Grinding cost[d]	**$ 0.13**	**$ 0.20**
Cost to grind barley used for weanling pig diet	$ 0.002	$ 0.003
Cost to grind barley used for growing-finishing pig diets	$ 0.125	$ 0.194
V. Overall cost	**$ 4.85**	**$ 2.47**
Difference vs dry grain		- $ 2.38

[a]Based on Jan 2018 power cost at CFRC, North Battleford, Saskatchewan.

[b]Propane cost is based on July 2018 prices (http://www.agric.gov.ab.ca/app21/farminputprices, Accessed Aug 2018))
[c]Fuel consumptions based on PAMI test reports and CSAE paper 84-211 "Heated Air Grain Dryer Performance."
[d]For weanling pig diets, dry barley was ground using 2.0 mm screen and acidified barley was ground using 4.0 mm screen. Wheat required for growing finishing diets were both ground using 4.8 mm screen. Actual grinding cost of dry wheat using 2.0 mm screen is $ 1.02 tonne^{-1} and using 4.8 mm screen is $0.68 tonne^{-1}. Actual grinding cost of acidified wheat using 4.0 mm screen is $ 0.96 tonne^{-1}.