Strategies to improve the microbial quality of liquid feed and optimise growth of liquid-fed grow-finisher pigs

By

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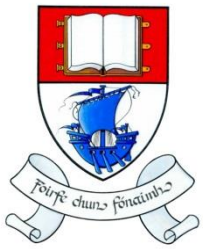

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Declaration

No element of the work described in this thesis has been previously submitted for a degree at this or any other institution. The work in this thesis has been performed entirely by the author.

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List of abbreviations

Abbreviation	Explanation
AA	Amino acid
ADG	Average daily gain
ADFI	Average daily feed intake
AIA	Acid insoluble ash
AOAC	Association of Official Analytical Chemists
AshD	Ash digestibility
ATTD	Apparent total tract digestibility
BA	Benzoic acid
BS EN	British Standard European Norm
CATTD	Coefficient of apparent total tract digestibility
CFU	Colony forming unit
CO_2	Carbon dioxide
CP	Crude protein
CV	Coefficient of variation
d	Day
DE	Digestible energy
DL	Detection limit
DM	Dry matter
DMD	Dry matter digestibility
EC	European Commission
EE	Ether extract
ESF	Electronic sow feeder
EU	European Union
FCE	Feed conversion efficiency
FCR	Feed conversion ratio
FIRE	Feed intake recording equipment

Abbreviation	Explanation
FLF	Fermented liquid feed
FM	Fresh matter
GC	Gas chromatography
GE	Gross energy
GeD	Gross energy digestibility
GIT	Gastro-intestinal tract
HCl	Hydrochloric acid
HPA	Health Protection Agency
HPLC	High-performance liquid chromatography
ISO	International Organization for Standardization
LA	Lactic acid
LAB	Lactic acid bacteria
LAF	Liquid acidified feed
Liq	Liquid
LW	Live-weight
Min	minute
N	Nitrogen
NaOH	Sodium hydroxide
ND	Not detected
NDF	Neutral detergent fibre
NitD	Nitrogen digestibility
NRC	Nutrient Requirements of Swine
NS	Not specified
NT	Not tested
OM	Organic matter
OMD	Organic matter digestibility
PVC	Polyvinyl chloride
pw	Post weaning
SD	Standard deviation
SI	Statutory Instrument
SID	Standardised ileal digestible
s.e.	Standard error
SEM	Standard error of the mean
TAEC	Teagasc Animal Ethics Committee
TOYO	Toyocerin
Trt	Treatment
TVC	Total viable count
VFA	Volatile fatty acid
WBC	White blood cell

Strategies to improve the microbial quality of liquid feed and optimise growth of liquid-fed grow-finisher pigs

Fiona Maria O' Meara

Abstract

The objectives of this thesis were to (1) characterise the microbiological quality of liquid feed on Irish pig units; (2) control spontaneous fermentation during liquid feeding using controlled fermentation and dietary acidification; (3) compare feed form and delivery in grow-finisher pigs; (4) determine the optimum water-to-feed ratio for grow-finisher pigs. A survey of eight commercial pig production units was conducted, in which spontaneous fermentation and amino acid degradation were found in liquid feed. Controlled whole diet fermentation and cereal-only fermentation were then compared to fresh liquid and wet/dry feeding of the same diet. Feeding a fermented whole diet reduced pig growth and caused feed conversion efficiency (**FCE**) to deteriorate. As no benefits were found for fermentation, feed form (meal, pellets) and delivery methods (dry, wet/dry, liquid) were compared. The conclusion was that wet/dry feeding of a pelleted diet to grow-finisher pigs optimises growth and FCE. In an attempt to improve FCE in liquid-fed pigs, four commercially used water-to-feed ratios (2.4:1, 3:1, 3.5:1 and 4.1:1, dry matter basis) were compared. The 3.5:1 ratio optimised FCE without reducing kill-out percentage. Lastly, diets supplemented with benzoic acid (**BA**) at 0, 2.5, 5 and 10kg/t were liquid-fed to grow-finisher pigs. While BA inclusion stabilised liquid feed pH and controlled lactic acid bacteria **(LAB)** growth in troughs, no improvements in growth or FCE were observed; however, FCE was excellent for the control and hence a response to BA was unlikely. In conclusion, wet/dry feeding of a pelleted diet optimised growth and FCE in grow-finisher pigs. Spontaneous fermentation occurs during fresh liquid feeding and even controlled whole diet fermentation leads to amino acid degradation. To optimise FCE, a 3.5:1 water-to-feed ratio is optimal for short-trough liquid feeding of grow-finisher pigs. Benzoic acid stabilised liquid feed pH and controlled LAB growth but did not improve pig growth.

1. Literature Review

1.1 Introduction to liquid feeding

For the purpose of this thesis, liquid feed is considered a combination .of dry ingredients, comprising of cereals, balancers, pre-mixes and/or co-products from industry mixed at a pre-determined water-to-feed ratio prior to feeding by an automated liquid feeding system. Wet/dry feeding, where pigs mix dry feed with water from a nipple in the trough at their desired water-to-feed ratio is described separately. Liquid feeding systems generally comprise of a central mixing tank where the dry feed ingredients or compound feed are mixed with water and/or co-products and the resulting mix is pumped through a series of pipes to feed troughs, from which the pigs eat (de Lange and Zhu, 2012). Figure 1.1 shows an example of the layout of a liquid feeding system.

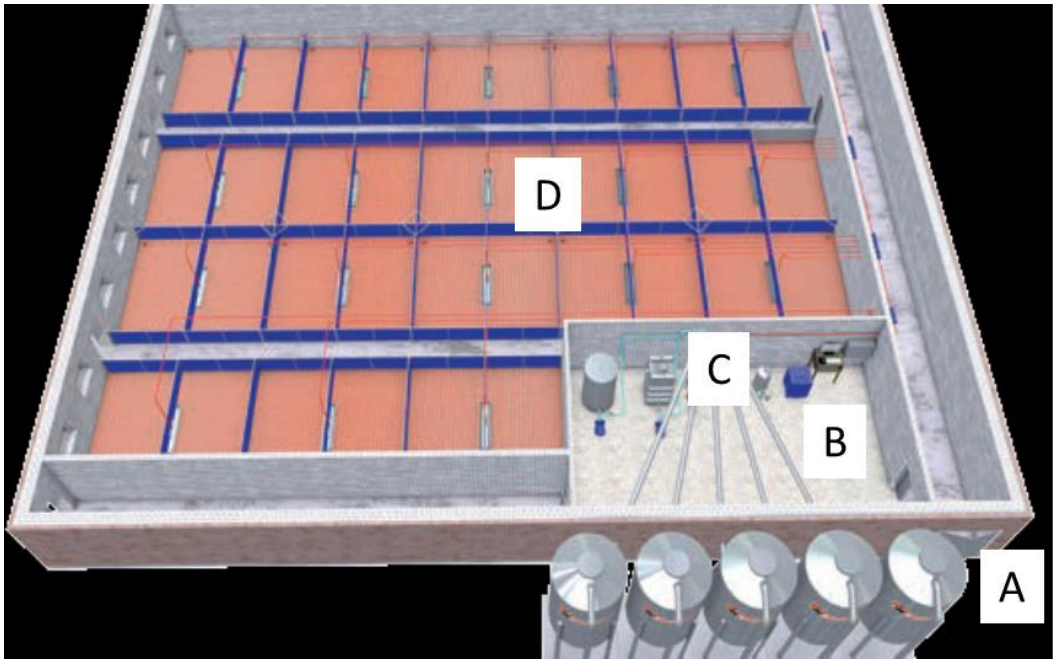


Figure 1.1 Schematic of a liquid feeding system with silos from which to draw feed into the feed preparation area and a series of pipelines to feed the pens of pigs (BigDutchman)
A:Feed storage silos for dry ingredients; B: Mixing area where dry ingredients are fed into the mixing tank and the computer that controls all liquid feeding practices is kept; C: Mixing tank area where the dry ingredients are agitated with water prior to feed-out; D: Pen area where pigs are kept and fed in troughs which have liquid feed delivered to them via a series of pipelines

1.1.1 Liquid feeding use worldwide

Approximately 70 % of pigs in Ireland are liquid-fed (McCutcheon, 2019). Data collected by Rodrigues da Costa (2018) from 56 Irish farrow-to-finish farms showed that 37.5 %, 48.2 % and 14.3 % of Irish pigs were wet-fed, dry-fed and fed using both delivery systems, respectively from weaning to slaughter; however, it should be noted that 'wet' feeding included both automated liquid feeding and wet/dry feeding so the figure of 37.5 % is not an accurate reflection of the number of pigs that are liquid-fed.

Liquid feeding is primarily used in Europe. Data from 91 farms across Spain and France revealed that 22 % of pigs were liquid-fed 2 to 4 times daily, 38 % were fed *ad-libitum* using dry hoppers, 37 % *ad-libitum* using wet feed hoppers and 3 % by turbomat (a round feeder that generally has a number of water drinkers above the trough) once or twice daily; however, liquid feeding was only observed in France and not in Spain (Temple et al., 2012). Figures from 2009 demonstrate the popularity of liquid feeding in Denmark and Sweden, with ~ 60 % of finisher pigs and the majority of sows liquid-fed (Best, 2009). Another study published in 2008 claimed that almost 70 % of fattening pigs in Sweden were liquid-fed (Persson et al., 2008). The Netherlands and France liquid-fed ~ 33 % and Germany ~ 40 % of grow-finisher pigs in 2009 (Best, 2009). These figures from Best (2009) are for automated liquid feeding only as they refer to 'pump and pipeline' feeding. Although liquid feeding is not as common in America, it was reported that ~ 20 % of pigs in Ontario, Canada were liquid-fed corn-based diets (de Lange and Zhu, 2012, Braun and de Lange, 2004b). These figures show that liquid feeding is popular worldwide and, therefore, optimising liquid feeding to maximise production should be financially beneficial for pig producers.

1.1.2 Liquid feeding systems

Modern liquid feeding systems have a central computer that controls all aspects of liquid feeding, such as feed timing and number of feeds per day, feed quantities delivered to valves on feed circuits, feeding curves, feed preparation (e.g. water-to-feed ratio) and diet composition. A revolution occurred in the liquid feeding sector in the early 1980s with the availability of inexpensive computing capability (Brooks, 2008). Liquid feeding is based around feeding curves, which allocate specific amounts of energy intake per day based on pig weight.

Older liquid feeding systems may not always have provided an equal distribution of feed ingredients, with differences in mineral content observed in feed distributed at the start compared to that at the end of the feed line (Braun and de Lange, 2004b, de Lange et al., 2006). Similarly, O' Reilly and Lynch (1992) concluded that inadequate agitation of liquid feed caused sedimentation of dense cereal particles, coarsely ground meat and bone-meal, and insoluble minerals which resulted in pens fed at the start of the feed line receiving more of these than pens near the end. This inadequate mixing of feed is less of a concern with modern liquid feeding systems due to the technologically sophisticated control equipment (de Lange and Zhu, 2012, Shurson, 2009).

The two basic feeding systems used for liquid feeding are long and short trough systems: long trough systems are a restricted feeding system that allow all pigs in the pen to eat simultaneously where troughs should be emptied between feeds. An accepted rule of thumb is that the troughs should be emptied 30 minutes to 1 hour after feed-out. Short trough systems work on an *ad-libitum* feeding basis and are equipped with sensors or probes (de Lange and Zhu, 2012). Sensor feeding

involves feeding once per pen at distinct mixing times based on one sensor reading; whereas, probe feeding involves mixing a large volume of feed after which probes are scanned regularly for multiple feed-outs from the same mix. It seems that feeding curves are more important in long trough compared to short trough systems because of their restrictive nature. Both long and short trough feeding systems work well once they are managed correctly.

Many farmers in Ireland have on-farm feed mills, so they buy in raw materials, home mill their ingredients and make up diets at the point of mixing liquid feed. This can be more economical than buying in compound feed once the initial capital investment for the mill has been paid off. Generally, the ingredients are fed into the mixing tank on separate augers from individual silos and are mixed as a diet for the first time in the mixing tank, when combined with water.

1.2 Advantages and disadvantages of liquid feeding

1.2.1 Advantages of liquid feeding

1.2.1.1 Improved growth and feed efficiency

There are many advantages associated with liquid feeding, some are based on improvements in productivity and efficiency, others offering opportunities that cannot be availed of using dry feeding. Firstly, improved growth rates have been reported in liquid-fed weaner (Russell et al., 1996, l'Anson et al., 2012, Kim et al., 2001, Partridge et al., 1992, Han et al., 2006) and grow-finisher (Hurst et al., 2008, Stotfold Research Centre, 2005) pigs compared to their dry-fed counterparts. However, results for feed conversion ratio (FCR) are contradictory, with some studies reporting improved FCR of liquid-fed vs dry-fed finisher (Hurst et al., 2008, Brooks et al., 2001, Forbes and Walker, 1968) and weaner pigs (l'Anson et al., 2013)

and others reporting the contrary, at least in weaned pigs (Lawlor et al., 2002, Han et al., 2006, Russell et al., 1996). Hurst et al. (2008) noted a close correlation between a higher eating rate and live weight gain, supporting the concept that liquid feed is more efficiently used for growth than dry feed. The faster intestinal flow of digesta associated with liquid feeding also requires less energy than dry feeding, so more energy is available for growth (Hurst et al., 2008). Dry matter (**DM)** intake in problem groups of pigs such as weaners or sows has also been shown to improve by liquid feeding (Scott et al., 2007, Brooks et al., 2001).

Other advantages associated with liquid feeding include reduced gastric ulceration and increased lean tissue growth rates compared to dry-fed pigs (Scott et al., 2007, Hurst et al., 2008). It has been suggested that the latter may be because extra nutrients are being partitioned towards protein deposition (Hurst et al., 2008). This ability to overcome appetite limitations and consequently reach the genetic potential of pigs to lay down muscle appears to be one of the main production advantages of liquid feeding (Hurst et al., 2008). Improved nutrient digestibility has been reported for liquid over dry feeding (UK Meat and Livestock Commission, 2003). Han et al. (2006) found improved nutrient digestibility at day 30 post-weaning (**pw**) in piglets fed liquid feed for 10 days pw compared to piglets that were liquid-fed for 20 days pw, a result for which there is no obvious biological explanation.

Although the focus of this thesis is grow-finisher pigs, the advantages of liquid feeding at weaning are important. Liquid feeding helps newly weaned pigs to avoid the dramatic growth check at weaning due to the fact that its consistency is similar to that of sow's milk (Missotten et al., 2015). It allows the young piglets'

stomach and small intestine (villous height and crypt depth) to adapt to dry feed and has been shown to stimulate feed intake post-weaning resulting in improved growth rates; moreover, it also means that piglets do not have to learn the difference between eating and drinking at weaning (Brooks et al., 1996, Jensen and Mikkelsen, 1998, Thacker, 1999).

1.2.1.2 Impacting the microbial profile of feed and the gastrointestinal tract

Liquid feeding also offers the opportunity to beneficially impact the microbial profile of feed using enzymes, inoculants and controlled fermentation, for example, thereby, potentially beneficially impacting the microbial profile of the gastrointestinal tract (GIT), a feat which is not as achievable using dry or wet/dry feeding (de Lange et al., 2006). This is important for efficient and economic production, as pigs do not produce fibre-digesting enzymes, for example, relying instead on microbiota in the GIT, particularly the hindgut, to ferment the fibre fraction of the diet, producing volatile fatty acids (VFAs) which can then be used as an energy source for the pig (Verschuren et al., 2018). Furthermore, in liquid feed that undergoes fermentation (either spontaneous or controlled), lactic acid bacteria (**LAB**) which naturally inhabit the raw materials used for liquid feed, increase in numbers, fermenting carbohydrates, with the resultant production of VFAs and lactic acid and a reduction in pH (Beal et al., 2005). Hence, liquid feeding can potentially seed the gut with LAB, resulting in a more desirable intestinal microbial profile, specifically LAB:coliform ratio (Scott et al., 2007). *Enterobacteriaceae* counts can also be reduced in liquid feed due to the high lactic acid concentrations and low pH (van Winsen et al., 2001). Interestingly, a lower prevalence of *Salmonella* on farms using liquid compared to dry feeding has also been reported (Van der Wolf et al., 2001, Beal et al., 2002, Farzan et al., 2006, Braun and De Lange, 2004a).

1.2.1.3 Other advantages of liquid feeding

Reduced labour costs were once considered an advantage of liquid feeding; however, there are now dry feed systems that are equally as labour-saving, so this argument is no longer valid. Other practical advantages associated with liquid feeding include reduced feed lost as dust, the ability to home mill diets, which is often cheaper than buying in compound pelleted diets, the ability to quickly adapt feeding curves and feed formulations based on ingredient availability and the possibility of 'step' and 'phase' feeding. 'Step-feeding' involves feeding a series of diets which closely match pigs' requirements at each stage of growth (Brooks et al., 2001). 'Phase feeding' requires a two pipeline system so that pigs can be phase fed on either an *ad-libitum* or a rationed basis (Brooks et al., 2001). These can in turn help to reduce environmental loading and nutrient content of effluent (Brooks et al., 2001). Liquid feeding can also reduce nitrogen and phosphorous output in effluent via the activation of endogenous phytase in the cereal grains (Brooks et al., 2001).

Liquid feeding also influences pig health and welfare. Restricted, long trough feeding reduces competition at the feeder face compared to short-trough *ad-libitum* feeding as it allows a feeding space for every pig (Stokes, 2015). Long trough feeding also allows for easy visual inspection of pigs at feeding time (Stokes, 2015). On the other hand, liquid feeding has been associated with reduced levels of dust as outlined above (Forbes and Walker, 1968), which may help to reduce pulmonary disease (Jericho and Harries, 1975); however, more recent data suggests that veterinary treatments for respiratory conditions and lung damage at slaughter were similar between dry- and liquid-fed pigs (Scott et al., 2007).

1.2.1.4 Inclusion of co-products

Liquid feeding provides the opportunity for dietary inclusion of liquid residues (co-products) from the food and drink industry (Scholten et al., 1999) which cannot be used with conventional dry or wet/dry feeding which is one of the main advantages of liquid feeding (UK Meat and Livestock Commission, 2003, de Lange et al., 2006, DeRouchey and Richert, 2010). For this reason, liquid feeding systems are more likely to have been introduced in areas where low-cost, high-quality, liquid food processing residues such as whey or skim milk were easily accessed. However, these co-products are no longer available to the same extent as products like whey are now used as a source of valuable human sports supplements as processing and extraction technologies have improved.

Nonetheless, co-products are still used by some pig producers; some of these and their associated DM content are outlined in Table 1.1. Recent research has shown that mayonnaise, almond meal, liquid bakery co-products and high-moisture corn are suitable for dietary inclusion for finishing pigs but that the high fibre content of brewery co-products, hominy feed, cocoa meal and kiwi make them unsuitable (Sol et al., 2016). Despite this, pot-ale is often formulated successfully into finishing pig diets in Ireland. Other co-products must be used with caution, for example, whey from cheese-making can contain up to 10 % salt on a DM basis, so this must be factored into formulations and pigs must have constant *ad-libitum* access to water (Shurson, 2009). However, once diets are reformulated regularly to account for the variability of composition and low DM of residues, there should be no loss of productivity and profitability can also be increased (Brooks et al., 2001). The use of co-products in liquid feed is also environmentally friendly and means that disposal costs are saved (Brooks et al., 2001). The transport of liquid co-products to

pig farms can, however, be expensive due to the volumes involved relative to the DM content of the feed (Shurson, 2009), but the use of 'back loads', where a truck would otherwise travel empty, is one way of minimising costs (Brooks et al., 2001).

Table 1.1 Co-products used in liquid feed for pigs (Scholten et al., 2001, Pedersen et al., 2004, Niven et al., 2007, de Lange et al., 2007, Lassén, 1995)

Co-product	Dry matter (g/kg)
Liquid wheat starch	225
Potato steam peelings	139
Cheese whey	70
Wet wheat-distillers grains	95
High moisture corn (maize)	750
Corn distillers solubles	300

1.2.2 Disadvantages of liquid feeding

1.2.2.1 Microbial aspects of liquid feed

Liquid feeding can be harder to manage on-farm than dry feeding, due to the possibility of spoilage and the careful manipulation and management of feeding curves required. Dry feeding has been associated with supplying the pig with a more consistent feed which requires less oversight by staff (DeRouchey and Richert, 2010). The potential for spontaneous fermentation by undesirable bacteria or yeast is one of the main disadvantages associated with liquid feed (Lawlor et al., 2002). Off-flavours can result when yeasts dominate the fermentation, and they have been shown to affect feed palatability and reduce average daily feed intake (**ADFI**) due to the production of acetic acid, ethanol and amylic alcohols (Plumed-Ferrer and Von Wright, 2009, Brooks et al., 2001, Scholten et al., 1999). Yeasts convert starch to alcohol and CO_2 which also signifies a loss of energy in the feed (Brooks et al.,

2001). The likelihood of mould growth and feed spoilage is also higher in liquid feed compared to dry feeding (DeRouchey and Richert, 2010).

1.2.2.2 Effects of liquid feed on productivity

Feed wastage from troughs is also more common with liquid feed compared to dry feeding, as found by (Russell et al., 1996). l'Anson et al. (2012) also concluded that the poorer feed conversion efficiency (**FCE**) observed in liquid- over dry-fed pigs was largely attributable to increased feed wastage. This wastage of liquid feed is difficult to monitor and measure on-farm. There have been some reports of decreased growth rates in liquid- compared to dry-fed weaner pigs (Lawlor et al., 2002, l'Anson et al., 2013). A poorer (i.e. increased) FCR in liquid-compared to dry-fed weaner pigs has been noted (Russell et al., 1996, l'Anson et al., 2012). However, a study of grow-finisher pigs by Zoric et al. (2015) found similar FCRs between dry- and liquid-fed pigs. A disadvantage associated with liquid feeding is a lower kill-out percentage compared to dry-fed pigs and although it is not consistently found, differences may be attributed to variations in the duration of pre-slaughter fasting or different feeding regimes which produce differences in gut fill (Hurst et al., 2008).

1.2.2.3 Other disadvantages with liquid feeding

Other disadvantages associated with liquid feeding include the fact that feed is used in meal form, so the digestibility improvements associated with pelleted diets are not attainable with liquid feeding. The increased cost of transporting liquid co-products due to their low DM has been cited as a disadvantage of liquid feeding (Brooks et al., 2001); however, if the transport is over a short distance and the co-products are good value then this argument may be invalid.

The manure volumes produced by liquid-fed pigs are consistently higher than those produced by dry-fed pigs, which results in increased environmental loading compared to volumes produced by dry-fed pigs (Brooks et al., 2001, Russell et al., 1996, DeRouchey and Richert, 2010). However, the nature of liquid feeding means that effluent volume is increased compared to dry feeding but nutrient load per litre is reduced (Brooks et al., 2001). Therefore, environmental loading must be expressed in terms of nutrients voided per kg growth/meat produced to ensure a fair comparison (Brooks et al., 2001).

In terms of pig health, Temple et al. (2012) revealed a significantly increased risk of severe faecal soiling in liquid-fed pigs compared with pigs fed via a wet-feed hopper. These results agree with those of Scott et al. (2007) who found that liquid-fed pigs had a significantly lower proportion of clean skin than dry-fed pigs, based on a hygiene score. As faeces can be a source of infectious agents, increased faecal soiling could contribute to the spread of disease (Temple et al., 2012). Interestingly, a questionnaire carried out in the UK revealed a significant association between diarrhoea in grow-finisher pigs and the use of a liquid feeding system (Pearce, 1999). However, the cause of this is not known and could be due to the microbial quality of the feed.

1.2.3 Liquid-fed pig behaviour

Scott et al. (2007) reported that liquid-fed pigs spent significantly more time sleeping and less time standing than dry-fed pigs, liquid-fed pigs drank less and performed less 'investigatory' behaviours than dry-fed pigs and there were no differences observed in eating time between dry- or liquid-fed pigs. However, Zoric et al. (2015) found that dry-fed finisher pigs spent longer eating at every feeding

occasion, expressed fewer re-groupings throughout the first week of the experiment and displayed less unwanted behaviours like belly-nosing and ear and tail nibbling than liquid-fed pigs.

Self-fed pigs will randomly space eating and drinking throughout the day, consuming 10 -12 meals per day (Persson et al., 2008, Ruckebusch and Bueno, 1976). A study that compared feeding 3 meals/day with 9 meals/day to grow-finisher pigs (27 – 112kg) with a restricted liquid feeding system concluded that increasing meals per day to 9 resulted in a poorer average daily gain (**ADG**) and an increased gastric lesion score compared to pigs fed 3 times/day (Persson et al., 2008).

1.3 Liquid feed system hygiene and effect on feed microbiology

The cleanliness of liquid feeding systems is a key consideration; however, there are no set guidelines on cleaning or disinfection. Liquid feed systems have been referred to as microbiological fermenters (Brooks et al., 2001, Plumed-Ferrer et al., 2004, Russell et al., 1996). This is because mixing tanks are generally not emptied, cleaned or sterilised before each refill, which leads to natural/spontaneous fermentation occurring and microbe-containing biofilms that form on the surface of mixing tanks, feed pipes and troughs are common to all liquid feeding systems (de Lange and Zhu, 2012, Plumed-Ferrer et al., 2004, Beal et al., 2005). Under farm conditions, due to the contamination of feed equipment, even fresh liquid feed which has just been mixed prior to feeding may have undesirable properties by the time it reaches the feed trough, as feed residue can act as a natural starter culture for fermentation (Canibe and Jensen, 2003). Every time this occurs in a new environment and/or with different ingredients, the characteristics of the resulting

feed will change (Canibe et al., 2010b). It is vital with both long and short-trough liquid feeding systems that troughs are completely emptied at least once per day to ensure that there is no spoilage or spillage of feed (de Lange and Zhu, 2012). Pockets in corners and under lips of troughs that are never emptied or cleaned, respectively can become areas for undesirable microbial proliferation, leading to palatability issues over time (UK Meat and Livestock Commission, 2003).

However, very few studies have investigated the microbial content of liquid feed that is not deliberately fermented and to our knowledge, none have related it to hygiene of the feeding system. Plumed-Ferrer et al. (2004) characterised the microbial community within liquid pig feed on one commercial pig production unit in Finland at time points during the standard 3 month fattening period. The diet was based on barley, soybean meal and liquid whey and pigs were fed 4 times/day at the start of the experiment and 5 times/day thereafter (Plumed-Ferrer et al., 2004). Of the microbial groups measured, LAB predominated, and this together with the low pH (4.7) indicated that natural fermentation had occurred. However, it should be noted that a 10-15 % portion of feed from the previous mix was held back and fresh feed added to this, a practice known as 'backslopping' which is usually used for deliberate fermentation (see Section 1.4.1.2). Therefore, it is not a true representation of spontaneous fermentation in freshly prepared liquid feed as carried out in Ireland, as the feed retained in the mixing tank acts as an inoculant for the new feed mix.

A French study that used 'contact water' as an indicator revealed an increase in microbial contamination from the mixing tank to the drop pipes and suggested that sanitation was only useful if the whole system was cleaned and not just the mixing

tank (Royer et al., 2004). They did reveal that although a reduction in microbes was observed after cleaning and disinfection, 'quick recontamination' was evident and concluded that 'without evidence of clinical problems, disinfection does not appear to be justified' (Royer et al., 2004). Cleaning pipelines may in fact have a negative impact as it has been reported to result in an undesirable 'bloom' of coliforms (Hansen and Mortensen, 1989). Brooks et al. (2001) suggested that if the microbial population established within the liquid feeding system is stable and favourable, it may be best not to clean the feed lines, as LAB and butyric acid may have positive effects on pig gastrointestinal health and growth. However, over time, populations of yeasts and unfavourable bacteria can increase in biofilms, affecting the nature of the fermentation occurring and in turn promoting the formation of biogenic amines and other unfavourable compounds, compromising feed quality and palatability and leading to a loss of nutrients such as synthetic amino acids (Pedersen et al., 2002, Niven et al., 2006, Canibe et al., 2007a). A Danish study reported almost complete loss of synthetic lysine in liquid feed that remained in pipelines during an 8-hour period (Pedersen et al., 2002). It is thought that microbes in the feed use up these free amino acids and that coliforms are the problem group as a larger disappearance has been reported in feed containing coliforms than in that dominated by *Lactobacillus* (de Lange et al., 2006, Niven et al., 2006). Mould is common on the inside walls of mixing tanks and its presence may also be a cause for concern due to mycotoxin production (Lawlor et al., 2002). However, the presence or absence of mycotoxin-producing moulds should not be used as a direct indicator of mycotoxin presence in feed. Diet composition is also an important factor in the microbial quality of liquid feed. For example, a diet for newly-weaned piglets containing milk

powder may be more affected as milk powder can spoil quickly, particularly at high room temperatures (de Lange and Zhu, 2012).

If feed quality is affected by microbial growth, de Lange and Zhu (2012) suggest that acids and bases for controlling yeasts and moulds and other undesirable microbes can be used to clean feed lines between batches of pigs. Many feeding systems installed in Ireland will hold feed in the pipes between feeds for a number of hours and moist conditions, warm temperatures and feed residues provide an ideal opportunity for undesirable microbes to proliferate. Most pig producers in Ireland do not use acid feed additives, acid rinses or disinfectants in their liquid feed, feed tanks and/or feed pipes. However, new technologies, such as hydro air systems where feed does not remain in pipes between feeding and high pressure air is forced through the feed lines to clean them between feeds, aim to improve the microbiological quality of liquid feed.

The standard values for microbiology, pH and organic acids in 'residue-free' liquid feed were reported as: LAB counts of 10^6 – 10^8 CFU/g, yeast counts of 10^4 - 10^6 CFU/g, *Enterobacteriaceae* counts of 10^4 – 10^5 CFU/g, pH of 5.0 – 6.0, lactic and acetic acid concentrations of 0 – 10 mmol/kg and ethanol concentrations of 0.0 – 0.5 g/kg liquid feed (Vils et al., 2018). However, it is unknown if liquid feed on commercial Irish units is similar to these standard values.

Overall, research into what happens to liquid feed microbial quality during the period from mixing to ingestion is missing. The use of in-feed acidifiers and/or disinfectants should also be investigated based on the results of analysis of the feed itself. With constant evolutions in liquid feeding technology, liquid feed

microbiological quality and feeding system contamination require further investigation.

1.4 Fermented liquid feed

There is a distinct difference between fresh and fermented liquid feed (FLF) based on the amount of time the feed spends mixed and stored prior to feed-out. FLF can be described as a mixture of feed and water which is stored for a period of time in a tank at a certain temperature prior to feed-out (Canibe and Jensen, 2003). The length of time that feed spends in a liquid medium is crucial as it affects microbial populations and nutrient availability of the resulting feed mix (Brooks et al., 2001). When feed and water are mixed, naturally occurring LAB and yeasts proliferate and produce lactic and acetic acids which reduce feed pH (Missotten et al., 2015, UK Meat and Livestock Commission, 2003). This reduced pH then inhibits the growth of pathogenic organisms e.g. enteropathogens developing (Missotten et al., 2015). The advantages and disadvantages associated with fermented liquid feeding are shown in Table 1.2.

Table 1.2 Advantages and disadvantages of fermented feed for pigs (Scholten et al., 1999, van Winsen et al., 2000)

Advantages	Disadvantages
Fermented co-products and compound diets may improve ADFI[1], ADG[2] and FCR[3]	Stage of fermentation is difficult to quantify; leads to variability in chemical composition and pig response
Gastric pH is reduced	Palatability may be reduced
Potential to ↑[4] desirable microbial populations in GIT[5]	Microbial decarboxylation of free amino acids likely
May improve digestion and absorption of nutrients	Production of undesirable microbial end-products e.g. biogenic amines
Reduced *Salmonella* incidence in feed and pigs	

[1] ADFI: Average daily feed intake; [2] ADG: Average daily gain; [3] FCR: Feed conversion ratio; [4]↑: Increase; [5]GIT: Gastrointestinal tract

1.4.1 Fermentation process

Spontaneous fermentation can occur in liquid feed as soon as it has been mixed resulting in altered physico-chemical and microbiological properties of the diet. This type of fermentation is uncontrolled and therefore inconsistent and mal-fermentation can occur, which can negatively impact pig health and performance, as described previously (Brooks et al., 2001).

Deliberate fermentation can be performed by soaking a compound feed or a source of starchy feed with water for a certain time period before feeding (Scholten et al., 1999, Dung et al., 2005). Starter cultures can be used to ensure that a population of desirable microbes (usually LAB) dominate the fermentation, thereby ensuring feed safety and maximising palatability, while reducing populations of undesirable microbes such as *Enterobacteriaceae* and moulds (Koeleman, 2015). Inoculants or starter cultures will be discussed in Section 1.4.1.6.

1.4.1.1 Characteristics of fermented liquid feed

Table 1.3 shows the physico-chemical and some microbiological characteristics of FLF as determined in a number of studies, both *in vitro* and on-farm. Characteristics of FLF include low *Enterobacteriaceae* counts, high counts of yeasts, lactic acid and VFAs and a low pH (Dung et al., 2005, van Winsen et al., 2001). However, a fermentation totally dominated by yeasts can result in off-flavours from the production of ethanol, acetic acid and amylic alcohols and can also result in a loss of energy due to starch being converted to and lost as CO_2 (Missotten et al., 2015, Koeleman, 2015, Brooks et al., 2001, Canibe et al., 2007b, Missotten et al., 2009, Brooks et al., 2003). Nonetheless, beneficial results of yeast fermentations have been reported as yeasts can bind *Enterobacteriaceae* which in turn prevents the

Enterobacteriaceae from binding to the gut epithelium (Mul and Perry, 1994, Missotten et al., 2015).

Table 1.3 Characteristics of feed fermentations reported in the literature, including the fermenting organism (if used), fermentation temperature and time, feed pH and concentrations of lactic and acetic acid, and ethanol where reported

Fermenting organism	Temp.	Time (hrs)	pH	Lactic acid	Acetic acid	LAB[1]/yeast count (log_{10} CFU/g)	Other	Reference
Lactobacillus plantarum	20 °C (*IV*[2])	24	4.4	~ 150 mmol/L	~ 10 mmol/L	*L. plantarum*[3]*:* > 9		van Winsen et al., 2000
Lactobacillus plantarum	20 °C (*IV*)	120	3.8	~ 275 mmol/L	~ 25 mmol/L	*L. plantarum:* > 9		van Winsen et al., 2000
Lactobacillus plantarum	20 °C	48	<4.5	> 150 mmol/L	< 40 mmol/L	LAB: 9.4 ± 0.26	Butyric acid < 5mmol/L, Ethanol < 0.8mmol/L	van Winsen et al., 2001
Pediococcus pentosaceus	20 °C	30	4.5	75 mM	9 mM	*P. pentosaceus*[4]*: >9*		Beal et al., 2002
Pediococcus pentosaceus	30 °C	10 - 20	4.5	75 mM	9 mM	*P. pentosaceus: >9*		Beal et al., 2002
None	20 °C	96	4.36	168.6 mmol/kg	25.8 mmol/kg	LAB: 9.4 ± 0.32 Yeast: 6.9 ± 0.69		Canibe and Jensen, 2003
NS[5](Fermented cereal diet)[6]	15 °C	NS	5	40 mmol/kg	13 mmol/kg	LAB: 8.9 – 9.2 Yeast: 5.9 – 7.8	26 mmol/kg ethanol	Canibe et al., 2007a
NS (Fermented whole diet)	15 °C	NS	4.45	160 mmol/kg	24 mmol/kg	LAB: 9.3 – 9.6 Yeast: < 4.1 – 7.2	17 mmol/kg ethanol	Canibe et al., 2007a
Pediococcus acidilactici (Bactocell ®)	30 °C *(IV)*	72	4	141.7 mmol/L	48.6 mmol/L	LAB: 9.4 ± 0.4 Yeasts: 5.7 ± 0.5	24.2 mmol/L ethanol	Missotten et al., 2007
Streptococcus infantarius & *Lactobacillus plantarum*	30 °C *(IV)*	72	4	150.1 mmol/L	30.6 mmol/L	LAB: 8.9 ± 0.7 Yeasts: 7.2 ± 0.8	17.6 mmol/L ethanol	Missotten et al., 2007

Fermenting organism	Temp.	Time (hrs)	pH	Lactic acid	Acetic acid	LAB[1]/yeast count (log_{10} CFU/g)	Other	Reference
(Adjulact ®)								
Lactobacillus plantarum	30 °C *(IV)*	16 - 17	3.36 - 3.42	248 – 269 mmol/L	11 - 14 mmol/L	Lactobacilli: 9.4 ± 0.4[7]		Missotten et al., 2009
Lactobacillus brevis	30 °C *(IV)*	23 - 41	3.48 - 3.97	103 - 170 mmol/L	8 - 26 mmol/L	Lactobacilli: 9.2 ± 0.1[7]		Missotten et al., 2009
Lactobacillus mucosae	30 °C *(IV)*	49 - >90	3.72 - 4.45	101 - 140 mmol/L	22 - 41 mmol/L	Lactobacilli: 9.1 ± 0.1[7]		Missotten et al., 2009
Lactobacillus casei group	30 °C *(IV)*	17	3.77	94 mmol/L	ND[8] (< 0.05 mmol/L)			Missotten et al., 2009
Lactobacillus johnsonii	30 °C *(IV)*	>90	4.62	128 mmol/L	ND (< 0.05 mmol/L)	Lactobacilli:9.0 ± 0.5, 8.8 ± 0.4, 8.8 ± 0.2[7]		Missotten et al., 2009
Lactobacillus kitasatonis	30 °C (*IV*)	>90	5.85 - 6.07	85 - 105 mmol/L	ND – 2 mmol/L	Lactobacilli: 8.7 ± 0.4[7]		Missotten et al., 2009
Lactobacillus reuteri	30 °C *(IV)*	49	3.84 - 3.95	85 – 132 mmol/L	14 – 29 mmol/L	9.0 ± 0.2[7]		Missotten et al., 2009
Pediococcus pentosaceus	30 °C (*IV*)	24	3.87	104 mmol/L	ND			Missotten et al., 2009

[1]LAB: Lactic acid bacteria; [2]*IV*: *In-vitro*; [3]*L. plantarum: Lactobacillus plantarum;* [4]*P. pentosaceus: Pediococcus pentosaceus*; [5]NS: Not specified; [6]Fermented cereal diet is the fermented cereal grains added to the other dietary components; [7] After 72 hours; [8]ND: Not detected

Off-flavours from acetic acid have affected feed intake in young pigs at concentrations as low as 30 mmol/L (Brooks, 2008, Missotten et al., 2015), but other research shows that ADG in piglets was not affected with concentrations of up to 120 mmol/L, despite a tendency for reduced ADFI compared to diets containing 30 mM and 60 mM acetic acid (Canibe et al., 2010a). Rudbäck (2013) added 0, 75, 100, 150 and 200 mmol/kg lactic acid to liquid feed in one trial and 0, 10, 50, 100 and 150 mmol/kg acetic acid in another. It was concluded that ADFI was not affected by even the highest inclusion rates of 200 mmol/kg lactic acid and 150 mmol/kg acetic acid, and that FCR was in fact improved by feeding 150 mmol/kg acetic acid compared to feeding 0 mmol/kg and 50 mmol/kg. Based on this, it is possible that yeast proliferation is not as detrimental to feed palatability as might be thought; however, more research is required to confirm this.

LAB fermentation is desirable and these are generally the dominant microbiota that develop in FLF (Brooks et al., 2001). Homofermentative LAB produce lactic acid only, whereas heterofermentative LAB produce lactic acid along with other products like acetic acid, ethanol and CO_2 (Scholten et al., 1999). Lactic and acetic acid were confirmed by van Winsen et al. (2000) as the metabolites produced during feed fermentation that were bactericidal to *Salmonella* (in combination with the resultant low pH).

Brooks (2008) concluded that when lactic acid levels are >100 mM in liquid feed, enteropathogen counts and the incidence of *Salmonella* can be greatly reduced (Brooks, 2008). It has been suggested that a pH of < 4.5 combined with 150 mM lactic or 80 mM acetic acid are the conditions necessary to reduce/eliminate *Salmonella* in FLF (Brooks, 2008).

1.4.1.2 Fermentation methods

There are different methods of producing FLF, including natural fermentation, fermentation with inoculants or fermenting only a portion of the diet. The amount of feed removed from and replenished to the fermentation will also influence stability of the microbial populations within the feed. 'Backslopping' involves holding back a portion of a previous successful fermentation as an inoculum for the next batch and this can also often occur unintentionally in feed lines where feed residue remains between feeds (Brooks, 2008). Backslopping was investigated in fermented wheat by Moran et al. (2006) who found that coliform bacteria could be eliminated within 48 hours using the technique, but that the pH had to be <4.0 for 24 hours to achieve this. It has been shown that if the proportion of fermented feed held back is too small, then lactic acid production can drop, eliminating the feed's ability to prevent growth of or kill off coliforms (Niven et al., 2006). A study carried out by Olstorpe et al. (2008) showed that a stable microbial population may appear to exist within FLF when viable counts are used; however, the yeast and LAB species composition can vary significantly when using backslopping to produce fermented feed over a period of time. They also found that the raw materials used in the diets had a considerable effect on the dominant microbial species and on species diversity (Olstorpe et al., 2008).

The addition of fresh feed is an important factor as it adds fermentation substrates, such as easily fermentable carbohydrates. Canibe and Jensen (2003) found that the fermentation process used low molecular weight sugars as the first substrate and only used a small amount of starch and non-starch polysaccharides, similar to the findings of Jensen and Mikkelsen (1998). On the other hand, Scholten

et al. (2001) did not add fresh feed and therefore noticed a decrease in the starch content during fermentation.

The variable growth performance data obtained when FLF is fed to pigs (as outlined in Section 1.4.1.4), is most likely a result of two main factors; poor palatability and microbial degradation of free amino acids (Brooks, 2008, Pedersen, 2001). A third factor is the differing feed preparation practices evident from the studies summarised above, and the fact that simple factors like different batches of feed ingredients could influence microbial populations in the feed.

1.4.1.3 Phases of fermentation

Spontaneous or natural fermentation occurs in an uncontrolled manner, when naturally occurring LAB and other microorganisms such as yeast grow in liquid feed (van Winsen et al., 2000). Fermentation of cereal grains or compound feed generally progresses through three phases, during which the pH is reduced and microbial populations change, which are shown in Figure 1.2 (Brooks, 2008). Phase 1 generally has a pH of 6 and coliform bacteria are common, while in phase 2, the pH drops to 4 and LAB take over and finally, in stage 3, the pH is maintained at ~ 4 and as LAB stabilise, yeast populations have the opportunity to increase (Brooks, 2008, Canibe and Jensen, 2003, Jensen and Mikkelsen, 1998, Lawlor et al., 2002). High levels of *Enterobacteriaceae* can proliferate in liquid feed during the early phase of fermentation due to the relatively high pH and low acidity of the mixture at that stage (Canibe et al., 2007b, Jensen and Mikkelsen, 1998, Beal et al., 2002, Canibe et al., 2001).

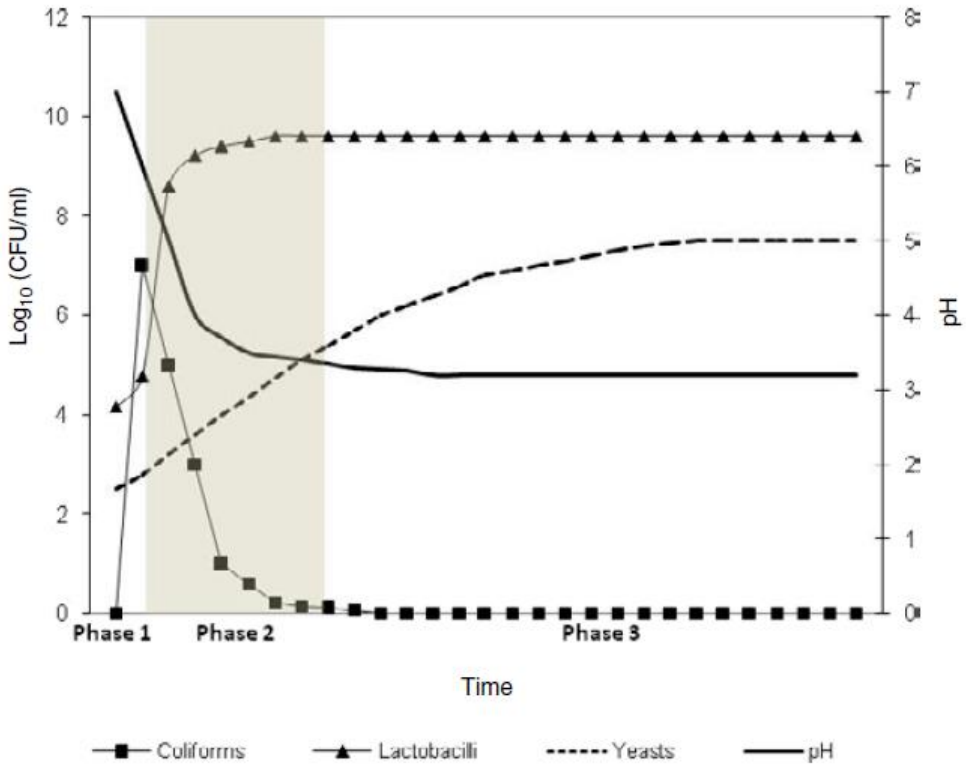


Figure 1.2. Phases of liquid feed fermentation (Brooks, 2008).

The length of each of these phases remains unclear and needs further research. In a controlled fermentation, Niven et al. (2006) found that after 7 hours, levels of LAB were high enough to exert antimicrobial effects; however, it took 21 hours for *E. coli* counts to fall below the detection limit. This suggests that during the initial stages of fermentation, firstly, LAB are not producing enough lactic acid to have an antimicrobial effect (see Section 1.4.1.1) and secondly, LAB concentrations and lactic acid production have increased but *Enterobacteriaceae* populations are not reduced. Interestingly, Beal et al. (2005) are of the opinion that spontaneous fermentation is not reliable enough to produce sufficient volumes of lactic acid or VFAs to reduce enteropathogen proliferation. Hence, inoculants and/or organic acids are just two examples of strategies used in an attempt to avoid the development of high levels of *Enterobacteriaceae* in liquid feed and these will be

discussed in sections 1.4.1.6 and 1.7, respectively (Niven et al., 2006, Geary et al., 1999, Canibe et al., 2001).

1.4.1.4 Effects of fermented liquid feed on pig growth, health and intestinal microbial populations

Properties specific to fermented diets include the microbial populations present, acidity and the concentration of organic acids (Scholten et al., 2001). The administration of beneficial bacteria, in particular LAB, via the feed can improve the intestinal microbial balance as it reduces the growth of undesirable bacteria (Brooks, 2008). For example, poultry by-products mixed with conventional ingredients that were fermented with *Lactobacillus plantarum* have been shown to reduce *Enterobacteriaceae* numbers numerically in the proximal small intestine and significantly in rectal content compared to a basic compound diet (Urlings et al., 1993). This study also showed improved FCR of pigs fed the fermented diet and reasons for this improvement, as well as the lower counts were suggested as a higher dietary energy content, an assumed improvement in nutrient digestibility and lower diarrhoea incidence in pigs fed the fermented treatment (Urlings et al., 1993). *Enterobacteriaceae* counts along the GIT were also reduced in grow-finisher pigs fed fermented liquid in comparison to dry or fresh liquid feed (Canibe and Jensen, 2003). Results showed that FLF increased the ratio of acetic acid to the sum of acetic, butyric and propionic acid in the stomach and that it resulted in higher lactic acid concentrations in the stomach contents, in comparison to pigs fed the dry and non-fermented diets. In terms of growth, pigs fed the FLF had a similar growth rate to pigs fed dry feed and a significantly worse growth rate than pigs fed fresh liquid feed. In contrast to this, Lawlor et al. (2002) compared the effect of dry pelleted, fresh, acidified and fermented liquid feed on pig performance from weaning at 26

days to harvest and concluded that from 28 days pw, any form of liquid feeding will not improve growth performance to harvest. However, Dung et al. (2005) found that grow-finisher pigs fed FLF had a similar FCR compared to those fed an acidified diet but a significantly better FCR than those fed dry and non-FLF diets (Dung et al., 2005). These results are somewhat in agreement with those of Geary et al. (1999) who showed that weaned pig performance is similar whether they are offered acidified liquid feed or FLF. This suggests that it is the acidic nature of FLF that is mainly responsible for the benefits and not necessarily the microbial content of the diet.

Feeding FLF has resulted in a reduced stomach pH compared with feeding dry and fresh liquid feeding (Canibe and Jensen, 2003). Naughton and Jensen (2001) showed that *Salmonella* inoculated into stomach content *in vitro* in bioreactors was killed off at pH 4, neither growth or killing took place at pH 5 in small intestine or stomach content and that it grew well in the small intestine at pH 7 (Naughton and Jensen, 2001). However, microbial growth/inhibition is not only pH-dependent. van Winsen et al. (2001), for example found a significant negative correlation between *Enterobacteriaceae* counts and the concentration of the undissociated form of lactic acid in the stomach contents of pigs fed a fermented diet. Digesta pH also impacts VFA production, with increased pH resulting in low production of VFAs, while a reduced pH will enhance VFA activity (van Winsen et al., 2002).

1.4.1.5 Fermenting the whole diet?

If a portion of the diet is retained to maintain continuous fermentation, a resident microbiota dominated by yeasts can develop which could compromise the

feed palatability, reduce its nutritional value and negatively impact pig health (Brooks, 2008). More consistent results can be achieved by batch fermentation of the cereal component of the diet using inoculants selected to generate high amounts of lactic acid (Brooks, 2008). However, Canibe et al. (2007a) compared the effect of dry feed, liquid feed containing fermented cereal grains and FLF on gastrointestinal microbial ecology and growth of piglets. They found that if only the cereal component of the diet is fermented, the growth of yeasts within the feed is promoted to a greater extent than when the whole diet is fermented. They also found a tendency for better ADG in piglets fed the cereal-fermented diet over those fed the fermented whole diet. This is probably because fermenting the cereal fraction alone avoids microbial decarboxylation of the free amino acids within the feed (Canibe et al., 2007a). The accumulation of free amino acids is caused by proteolysis by enzymes present in the ingredients (Canibe et al., 2007b). A decrease in the number of some individual amino acids in a fermented whole diet in a study conducted by Canibe et al. (2007b) suggested that they were degraded at a rate greater than that at which they were replaced by proteolytic activity. They suggested that the microbial breakdown of free amino acids was more significant than the breakdown of protein-bound amino acids like lysine, methionine and threonine.

Various studies have referred to a reduction of free amino acids during fermentation of liquid feed (Pedersen, 2001, Pedersen and Jensen, 2005, Niven et al., 2006). As previously mentioned, research performed in Denmark reported almost complete disappearance of synthetic lysine in liquid feed pipelines after 8 hours (Pedersen et al., 2002, Shurson, 2009). However, degradation can vary and Canibe et al. (2007b) points out that the way in which FLF is prepared and the microbial characteristics of the mixture can affect the disappearance of amino acids. They

suggest that reducing the amount of microorganisms in liquid feed could, in theory, be a strategy to eliminate microbial degradation of free amino acids; however, they did not test this theory. It has been shown that lysine loss from FLF is due to the metabolism of lysine by *E. coli* prior to their elimination from the diet by the reduced caused by LAB growth (Niven et al., 2006).

Amino acids can undergo undesirable microbial catabolism (transamination, deamination and decarboxylation) which can cause a loss of feeding value and the production of ammonia and toxic amines which negatively impact gastrointestinal mucosa and feed palatability (Canibe et al., 2007b, Smith and Macfarlane, 1997, Tavaria et al., 2002, Visek, 1978, Brooks et al., 2001). It has been suggested that synthetic amino acids should only be added once a successful fermentation has been established (75 mmol lactic acid or pH < 4.5) so that losses are minimised (Braun and de Lange, 2004b, Shurson, 2009).

Apart from resulting in amino acid loss from the diet, microbial decarboxylation of free amino acids, particularly lysine, during feed fermentation results in the production of biogenic amines, such as cadaverine (Canibe et al., 2007b, Brooks et al., 2001, Visek, 1978, Niven et al., 2006). Although their role is still unclear, the accumulation of biogenic amines is generally considered a defence mechanism by which bacteria counteract an acidic environment (Canibe et al., 2007b). The acidic environment of the FLF can then promote biogenic amine production if the pH is not low enough to kill the producing bacteria (Canibe et al., 2007b). Research has shown that LAB domination in fermented feed inhibits cadaverine production from lysine by *E. coli* (Niven et al., 2006).

There is a lack of studies comparing whole diet with cereal only fermentation in grow-finisher pigs, as the majority of the work on FLF has been performed in younger pigs around weaning and with whole diet fermentation.

1.4.1.6 Fermentation using inoculants

It is possible to increase the feeding value of ingredients by soaking with enzymes or via controlled fermentation with microbial inoculants or 'starter cultures' (de Lange and Zhu, 2012). The addition of selected LAB inoculants produces an acidic diet which rapidly and effectively excludes enteropathogens (Brooks et al., 2001) and leads to a product with better nutrient availability (Close, 2000). The final feed will be safe and of good microbial quality if the amount of inoculum, temperature and intervals between the addition of fresh feed and water are monitored and performed appropriately (Jensen and Mikkelsen, 1998, Scholten et al., 2001, Beal et al., 2002).

Many authors have recommended the use of microbial inoculants to control the fermentation and in turn, improve animal performance (Scholten et al., 1999, Brooks et al., 2001, Scholten et al., 2001, Canibe et al., 2007a, Russell et al., 1996, Jensen and Mikkelsen, 1998). Different LAB inoculants, usually *Lactobacillus*, *Pediococcus* or a combination of both as shown previously in Table 1.3, have been used to ferment pig feed (Beal et al., 2002, Missotten et al., 2007, Missotten et al., 2009, van Winsen et al., 2000, van Winsen et al., 2001). Some are probiotic, for example Bactocell®, which contains *Pediococcus acidilactici* and *Lactobacillus plantarum* and has been reported to have positive fermentation characteristics (Niven et al., 2006). A study by Missotten et al. (2007) compared two commercial probiotics (Bactocell® and Adjulact® Pro) to a control fermentation to which no

inoculum was added. They reported no significant differences in fermentation characteristics after 72 hours between treatments in terms of microbial counts, pH or concentrations of ethanol, lactic acid and acetic acid except for a higher yeast count in the feed inoculated with Adjulact®Pro (Missotten et al., 2007).

A study by (Olstorpe et al., 2010) prepared a fermented cereal grain with wet wheat distillers grains diet in 3 ways; fermenting ingredients with no additions, fermenting using a starter culture at the beginning of the fermentation and fermenting using a starter culture at the beginning and at every backslopping. They found that *Lactobacillus plantarum* from the starter culture dominated the latter two fermentations, but *Lactobacillus panis* dominated the diet with no starter culture. This highlights how a starter culture is responsible for the dominant populations in the resulting fermentation and that ingredients also have a natural flora associated with them. They also reported how *Enterobacteriaceae* populations were still present in all diets, indicating that lactic acid concentrations did not reach the 75 mmol/L required to eliminate *Enterobacteriaceae* reported by Beal et al. (2002). Levels of lactic acid in excess of 75 mM were reported by Beal et al. (2002) after 48, 72 and 96 hours of fermentation at both 20 and 30 °C in liquid feed fermented with *Pediococcus Pentosaceus.* Zhu et al. (2011) investigated the effect of enzymes and a *Pediococcus* inoculant on the lactic and acetic acid concentrations of FLF *in vitro*. They found an increase in lactic acid in liquid feed when enzymes and/or an inoculant were used (Figure 1.3). It appears that the use of inoculants is an effective way to produce FLF that is safe and dominated by desirable bacterial species.

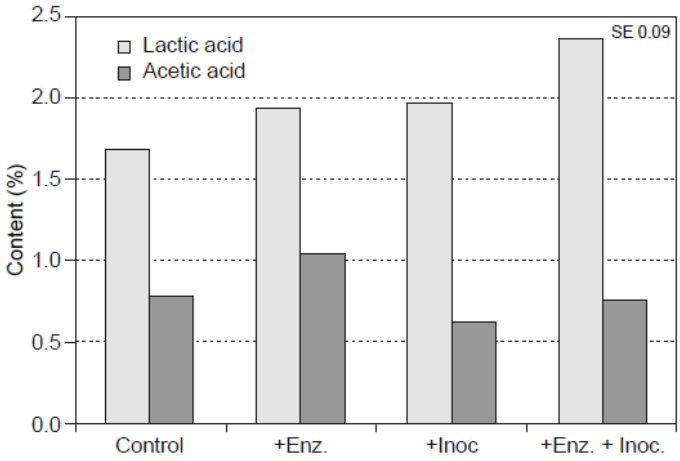


Figure 1.3. Lactic acid content (%) in the supernatant of corn distillers solubles mixed with water (17 % DM) in large scale fermentation tanks.

The four treatments were: Control (no added enzymes or inoculant), + Enz (added glucanase and xylanase), +Inoc (added *Pediococcus* inoculant) and +Enz +Inoc (added glucanase, xylanase and *Pediococcus*). The effect of adding enzymes and inoculants were significant ($P < 0.05$). but no interactive effect was observed ($P > 0.10$) (Zhu et al., 2011).

Some studies have investigated the development of specific inoculants for FLF, as currently none are available commercially. Missotten et al. (2009) screened 146 LAB strains isolated from pig gut digesta and successful feed fermentations as potential inocula for FLF. They selected three *Lactobacillus* strains (*Lactobacillus johnsonii*, *Lactobacillus salivarius* group and *Lactobacillus plantarum*) as promising; they reduced the pH of feed quickly, produced large volumes of lactic acid and displayed antimicrobial activity against *Salmonella*.

1.5 Water-to-feed ratio

When liquid feed is prepared, a volume of water is mixed with a volume of feed and expressed as a ratio; for example, if 3 kg water is mixed with 1 kg feed then the water-to-feed ratio is 3:1 on a fresh matter (**FM**) basis. The ratio can also be expressed on a DM basis. It is assumed that excessive amounts of water have a

detrimental effect on pig growth performance and environmental parameters; however, recent research on water-to-feed ratios is limited, so modern pig genotypes, management systems and accurate feeding technology have not been investigated in great detail (Hurst et al., 2008). Furthermore, the results that are available are quite variable, leading to varied suggestions of the optimum water-to-meal ratio in the literature.

It is expected that the ideal water-to-feed ratio is affected by diet and feed composition, the associated level of non-starch polysaccharides in the diet, the age of the pig, feed allowance (i.e. restricted or *ad-libitum* feeding), environmental conditions and water quality (Sol Llop, 2016, Choct et al., 2004, Hurst et al., 2008, de Lange and Zhu, 2012). It has been suggested by Chae (2000) that the optimal water-to-feed ratio likely changes with age of the pig and also with the way in which the feed mix is 'propelled' (pumped) around the system. High dietary inclusion rates of ingredients like maize require an increased water-to-feed ratio to help physically pump the diet around the system. Water-to-meal ratios are also affected by the distribution system. The agitation system, pump, pipe width and spreader type (to drop feed into the trough) must all be designed to transport a thicker, viscous liquid if a low water-to-meal ratio is used. At present, the amount of water included in liquid feed is primarily decided by how well the system can pump the mixture through the pipelines to troughs and not by the pigs' actual water requirements (de Lange et al., 2006).

In newly-weaned pigs, physical intake capacity normally limits nutrient intake so DM content should be at a maximum, (Russell et al., 1996). According to older research, pigs reliance on DM content is reduced as they get older, as finisher

pig growth performance and feed utilisation seem independent of feed DM content when between 20 % and 30 % (Gill, 1989, Russell et al., 1996). Manure volumes produced by finisher pigs can also be minimised by maximising the DM content of the diet; however, increased water may be required in cases of disease, high temperature, failure of the liquid feed system, or when co-products containing extreme mineral levels are included in the diet (de Lange and Zhu, 2012, de Lange et al., 2006).

The amount of water used to prepare liquid feed influences the positive growth performance effects associated with liquid feeding described in Section 1.2.1.1 (Hurst et al., 2008). Liquid feed delivered to troughs is generally 23 % DM; however, it is likely that this DM content varies hugely in practice, particularly where co-products are used and each batch could have a different DM content (de Lange and Zhu, 2012). It was noted by Sol Llop (2016) that, in practice, water-to-feed ratios of 3:1 FM (~ 3.5:1 DM) to 4:1 (~ 4.6:1 DM) are used.

A lot of the research on water-to-feed ratios is dated and was not performed on accurate, computer-controlled liquid feeding systems. Braude (1967) summarised a lot of this early work where 'very little difference' was observed in pig performance when 2, 2.5, 3 and 3.5 lb water to 1 lb feed were used (the equivalent of ~ 2.3:1, 2.9:1, 3.5:1 and 4.0:1 DM) (Barber et al., 1963). Older recommendations include 2.5:1 FM (~ 2.9:1 DM) (English et al., 1988) or 2:1 FM (~ 2.3:1 DM) (Pond and Maner, 1984) for pigs over 40 kg using commercial liquid feeding devices. It was suggested that these dilutions would even be too high for younger pigs due to reduced DM intake (English et al., 1988).

Hurst et al. (2008) has carried out one of the only modern investigations into water-to-feed ratio in which dry feeding, 1.5:1 FM (~ 1.7:1 DM) liquid feeding, 3:1 FM (~ 3.4:1 DM) liquid feeding and 3(4):1 FM (~ 3.4:1 DM) were compared (the latter is a diet in which the pH was reduced to 4 as discussed previously). Pigs fed the 3:1 FM and 3(4):1 FM diets had significantly improved FCRs compared to pigs fed the dry diet and the diet prepared at 1.5:1 FM as also mentioned previously. It should be noted that pigs were restricted-fed and not fed *ad-libitum.* It can be concluded from this study that a water-to-feed ratio of 3:1 FM is preferable over 1.5:1 FM in terms of FCR in pigs from 47.2 to 86 kg when restricted-fed. An interesting study would comprise the same treatments but fed in an *ad-libitum* fashion and with feed wastage compared between the 1.5:1 FM and 3:1 FM treatments. To our knowledge, no studies to date have accurately measured wastage in liquid feed prepared at different water-to-feed ratios.

At the other extreme, much higher water-to-feed ratios have been compared in older research. Kornegay and Vander Noot (1968) performed 4 experiments, the first of which compared a huge range of ratios from diets containing 10 (dry), 25 (~ 0.39:1 DM), 40 (~ 0.78:1 DM), 55 (~ 1.46:1 DM), 70 (~ 2.75:1 DM) and 85 % (~ 6.75:1 DM) water. At the higher 2 ratios, ADFI and ADG were supressed; however, during the overall trial period, FCR was no different to other treatments. The pigs were 16 kg at the start of this experiment so it is not directly comparable to results of a finisher trial. Their second experiment reported no differences in ADG or FCR between a dry diet and a diet containing 40 % water (~ 0.78:1 DM) with slightly heavier pigs of 22 kg. Their third study moved to pigs of 61 kg and compared diets containing 10 (dry), 40 (~ 0.78:1 DM) and 85 % (~ 6.75:1 DM) water and showed ADG and FCR were poorer and urine volume increased at the high ratio of 85 % (~

6.75:1 DM) water compared to the other treatments and, there were no differences observed in nutrient digestibility between treatments. Their final study confirmed that ADG and FCR were poorer in 48 kg pigs at a high ratio (85 % water, ~ 6.75:1 DM) and slurry volume increased and no differences were observed between treatments at slaughter. Energy digestibility and N-free extract digestibility were lower in pigs fed the diet prepared with 85 % water (~ 6.75:1 DM) (Kornegay and Vander Noot, 1968).

Older studies by Braude and Rowell (1967) and (Barber et al., 1991) showed that ratios of 2.5:1 FM (~ 2.9:1 DM) and 1.5:1 FM (~ 1.7:1 DM) failed to show a beneficial effect on pig performance. It was thought that by increasing the ratio to 3:1 FM (~ 3.5:1 DM) or 3.5:1 FM (~ 4:1 DM), ADG and FCR would be improved, as shown from research by Gill et al. (1987) and (Barber et al., 1991). The study by Geary et al. (1996) compared liquid diets of 149 (5.71:1 DM), 179 (4.59:1 DM), 224 (3.46:1 DM) and 255 g/kg DM (2.92:1 DM) water in weaner pigs that were 24 ± 2.6 days old for 28 days and showed no differences in ADG, ADFI or FCR.

Choct et al. (2004) compared dry feed with three water-to-feed ratios: 2:1 FM (~ 2.3:1 DM), 3:1 FM (3.5:1 DM) and 4:1 FM (4.6:1 DM) in weaner pigs (27 days of age, ratios expressed on a 100 % DM basis). They found that the water-to-feed ratio did not have a significant effect on any pig growth parameters during the trial period (27 days in the weaner stage). However, pigs fed the liquid diet had a significantly lower FCR than those fed dry diets. They also found that the DE value of the feed was significantly reduced when the diet was mixed at a ratio of 4:1 FM (4.6:1 DM); however, this difference was not considerable enough to have an effect on individual pig performance. They did not evaluate any additional water-to-meal

ratios or investigate a closer range as there was not a significant enough difference observed between the ratios used. They concluded that the lack of performance differences was not surprising because it is likely that above a certain threshold, water volume is unlikely to affect the release of endogenous enzymes or particle size changes (Choct et al., 2004).

To our knowledge, the most up-to-date research available on the topic of water-to-feed ratio was carried out by Sol Llop (2016) with pigs weighing 46.7 ± 1.98 kg for 52 days, divided into 2 x 26-day experiments. The first compared a dry diet with liquid diets containing 0.6:1 FM (~ 0.7:1 DM), 2.1:1 FM (~ 2.4:1 DM) and 2.7:1 FM (~ 3.0:1 DM) water:feed and it should be noted that these were mixed manually. The second compared a dry diet with ratios of 1.35:1 FM (~ 1.5:1 DM), 2.7:1 FM (~ 3.0:1 DM) and 3.5:1 FM (~ 3.9:1 DM). There were no differences in ADFI between treatments in experiment 1 or 2. In experiment 1, pigs fed the 0.6:1 FM (~ 0.7:1 DM), diet had a significantly higher weight gain than those fed the dry diet and weight gains on the 2.1:1 FM (~ 2.4:1 DM) and 2.7:1 FM (~ 3.0:1 DM) diets were intermediate. In experiment 2, pigs fed the 1.35:1 FM (~ 1.5:1 DM) and 2.7:1 FM (~ 3.0:1 DM) diets tended to have an improved weight gain over dry-fed pigs and those fed the 3.5:1 FM (~ 3.9:1 DM) ratio. They used quadratic regressions for both experiments and concluded that a water-to-feed ratio of 1.38:1 FM (~ 1.6:1 DM) in experiment 1 and 1.74:1 FM (~ 2.0:1 DM) in experiment 2 maximised ADG, while 1.30:1 FM (~ 1.5:1 DM) in experiment 1 and 1.58:1 FM (~ 1.8:1 DM) in experiment 2 produced the lowest, and therefore best, FCR.

It is a legal requirement in Europe, according to Council Directive 2008/120/EC (2008), that pigs have constant access to a clean, separate water

supply, regardless of the feed delivery system used. This is important, as Gill et al. (1987) showed that at water-to-meal ratios of 2:1, 2.5:1, 3:1 and 3.5:1 FM, (the equivalent of ~ 2.4:1, 2.9:1, 3.5:1 and 4.1:1 DM) all pigs still drank voluntary water from the additional source, despite the water inclusion level included in the liquid feed mix. They found that the greater the water content of the liquid feed mix, the less voluntary water required, but that the decrease was not directly proportional. They found that weight gain improved significantly and FCR improved numerically as the water content of the liquid feed increased (Gill et al., 1987). An interesting finding from this study is that when total water intake (water from liquid feed + voluntary water intake) was calculated for pigs fed the 2:1 FM ratio, their final ratio approached 3:1 FM. Furthermore, Barber et al. (1963) showed with their early research that growth rate was improved when an unrestricted water supply was given compared to pigs allowed just the water supplied in their liquid feed.

The water-to-feed ratio used can also influence carcass quality. However, Sol Llop (2016) found no differences in final carcass weight, carcass yield percentage or lean meat percentage between pigs fed any of the ratios investigated. The 3:1 FM (~ 3.4:1 DM) diet used by Hurst et al. (2008) improved lean tissue growth rate, but did not influence FCR.

Overall, there are obvious gaps in the literature as regards directly comparing different water-to-feed ratios to define an optimum for different stages of pig growth. In addition, a lot of the available data is old, and was therefore generated using less accurate feeding equipment compared to the modern systems currently available. Even the study carried out in 2016 used hand-mixed ratios so is not comparable to commercial production. Research is needed to determine the optimum water-to-feed

ratio at which liquid feed should be mixed for grow-finisher pigs and to establish if this optimum changes with pig age.

1.6 Feed form and delivery

Pig feed is generally available in two forms; meal and pellet. Three main feed delivery systems are used; dry, wet/dry and liquid feeding.

1.6.1 Feed delivery systems

Dry feeding of pigs was once carried out manually using feed bags and scoops. Nowadays, large scale intensive pig production units have automatic auger systems which are designed to deliver specific quantities of dry feed to pens (DeRouchey and Richert, 2010). Wet/dry feeders work similarly, where dry feed is delivered to a feeder and then the pigs can mix feed and water at their desired ratio using the water nipple in the trough. Figure 1.4 illustrates the layout of an automatic auger system that can be used to deliver feed to dry or wet/dry feeders. Systems vary in complexity and technological advancement and computer controlled systems to adapt feed curves and nutrient requirements are now available (DeRouchey and Richert, 2010). More complex feeders include electronic sow feeders (ESFs) and feed intake recording equipment (FIRE) which both require a transponder in each pig's ear to record individual pig feed intake data.

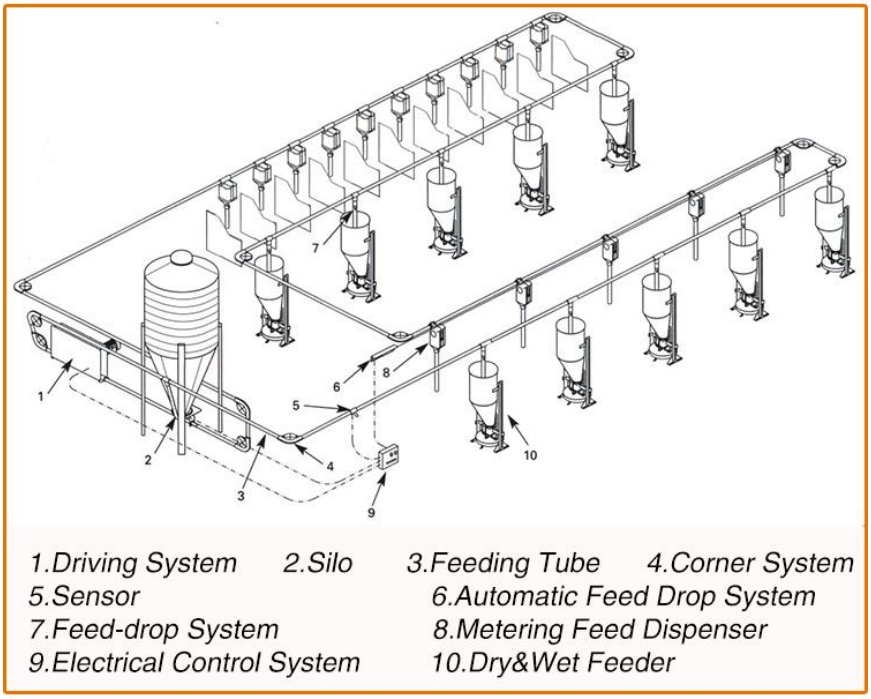
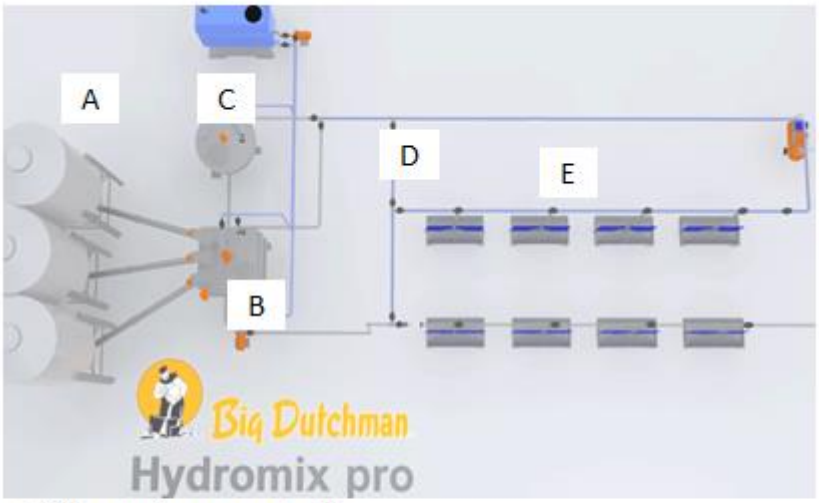


Figure 1.4 Illustration of dry auger feeding system (Available at http://m.goldenest1987.com/complete-farming-equipment/complete-pig-house-farming-equipment/pig-house-feeding-line-system.html)

1.6.2 Liquid and wet/dry feeding

Water and feed are kept separate in wet/dry feeders until the point of delivery to the pig and the key difference between wet/dry feeding and liquid feeding is the length of time that the DM fraction of the diet in is a liquid medium before consumption (Brooks et al. (2001). Another key difference is that the pig controls how much water to mix with the diet in a wet/dry feeder using the water nipple in the trough, whereas liquid-fed pigs are provided with a feed mixed at a pre-determined water-to-feed ratio. This should help to minimise spontaneous fermentation in the trough compared to liquid feeding, as the pig uses the water nipple just prior to eating. An advantage of wet/dry feeding is reduced water wastage (DeRouchey and

Richert, 2010). A study by Vermeer et al. (2009) compared wet/dry, long-trough liquid, sensor-liquid and Variomix (a hopper with a round feeder at the bottom from which you can feed liquid feed) grow-finisher pigs. They reported no significant differences in ADFI, ADG or FCR. Figure 1.5 shows the layout of a typical liquid feeding system.



A. Silos storing raw materials
B. Mixing tank where ingredients and water are mixed
C. Option of a satellite tank where liquid feed can agitate prior to feed-out, or a fermentation tank to ferment ingredients
D. Pipelines that delivery liquid feed to troughs
E. Drop pipes into each pen delivering liquid feed

Figure 1.5 Illustration of a liquid feeding system (Available at https://www.bigdutchmanusa.com/en/pig-production/products/sow-management/liquid-feeding/hydromix-pro/)

1.6.3 Liquid vs dry feeding systems

A summary of findings from studies that have compared feed delivery methods is shown in Table 1.4. It is evident that comparisons of liquid and dry feeding have produced variable results. Also from Table 1.4 it can be seen that a lot of work on liquid feeding has been performed around weaning. Lawlor et al. (2002)

compared liquid and dry feeding in newly weaned pigs. They found that DM intake was increased up to 27 days pw as a result of liquid feeding, but ADG decreased from day 0 to 27 in two of their experiments (Lawlor et al., 2002). Kornegay and Thomas (1981) showed little difference between the growth of liquid- and dry-fed weaner pigs when the results of their three experiments were reported. Similarly, Dung et al. (2005) noted that grow-finisher growth performance was not improved by fresh liquid feed compared to dry feed, in agreement with the findings of Lawlor et al. (2002) in weaner pigs.

However, these results are in contrast to those of Partridge et al. (1992) who noted an increase in ADG in weaner pigs fed liquid feed for 3 weeks compared to those fed a dry diet. Similarly, another study in which wet and dry feeding of weaner pigs were compared for 14 days from 11 days of age concluded that liquid-fed pigs were 21 % heavier and grew 44 % better than dry-fed pigs (Kim et al., 2001). After the 14-day trial period, all pigs were fed a dry diet but the liquid-fed pigs maintained the growth advantage to slaughter (Kim et al., 2001). However, it should be noted that the study took place in America where segregated early weaning is common; whereas, pigs in Europe would not be weaned onto dry feed from sows milk at such a young age (Kim et al., 2001).

Table 1.4 Findings from the literature comparing feed delivery systems for pigs

Delivery	Starting age/wt	Trial period	Effect of feed delivery	Reference
Liq[1] vs dry	Bacon pigs	Bacon pigs	ADG[2] similar liq & dry; FCR[3] ↓[4] in Exp.1 (tendency) & ↓ in Exp. 2 on liq	Forbes and Walker, 1968
Liq vs dry	22.6 ± 2.6 days	PW[5] for 28 days	ADG ↑[6] on liq; ADFI[7] ↑ on liq; FCR ↑ on liq	Russell et al., 1996
Liq vs dry	11.0 ± 0.1days old; 3.93 ± 0.05 kg	14 day trial & followed to slaughter	ADG ↑ on liq; ADFI ↑ on liq; Gain/feed ↑ on liq; LW ↑ at d14 on liq; Days to slaughter ↓ on liq	Kim et al., 2001
Liq vs dry	8.4 to 95.5 kg (Exp. 1)	Weaning to slaughter	ADG similar liq & dry; ADFI ↑ on liq; Gain: feed ↓ on liq	Lawlor et al., 2002
Liq vs dry	7.8 to 95.5 kg (Exp. 2)	Weaning to slaughter	ADG similar liq & dry; ADFI similar liq & dry; Gain:feed tended to ↓ on liq	Lawlor et al., 2002
Liq vs dry	34.2 - 102.95 kg	To target slaughter weight of 105 kg	ADG ↑ on liq; ADFI ↓ on liq; Feed:gain ↓ on liq	Stotfold Research Centre, 2005
Liq vs dry	47 kg	6 weeks	ADG ↑ on liq; ADFI similar liq & dry; FCR similar liq & dry; LW at slaughter ↑ on liq	Hurst et al., 2008
Liq vs dry	28 days, 7.4 ± 0.3 kg	26 day trial	ADG ↑ on liq; FCR ↑ on liq; LW at end of exp ↑ on liq	l'Anson et al., 2012

Delivery	Starting age/wt	Trial period	Effect of feed delivery	Reference
Liq vs dry	28 days; 7.3 ± 0.1 kg	26 day trial	ADG ↓ on liq; ADFI ↓ on liq; FCR ↓ on liq	l'Anson et al., 2013
Liq vs dry	20 kg	20 – 115 kg LW (100 days)	ADG similar liq & dry; FCR similar liq & dry	Zoric et al., 2015
W/Dry[8] vs dry	70.8 lb (32.1 kg)	69 days to finisher	ADG ↑ on wet/dry; ADFI ↑ on wet/dry; Final LW ↑ on wet/dry	Bergstrom et al., 2008
W/Dry vs dry	63.2 lb (28.7 kg)	104 days to finisher	ADG ↑ on wet/dry; ADFI ↑ on wet/dry; Final LW ↑ on wet/dry; FCR ↑ on wet/dry; Feed cost/pig ↑ on wet/dry; Carcass yield ↓ on wet/dry; Fatter carcases in wet/dry	Bergstrom et al., 2008
W/Dry vs dry	25 kg	25 kg - 106 kg	ADG ↑ on wet/dry; ADFI ↑ on wet/dry; Carcass lean ↓ on wet/dry	Gonyou and Lou, 2000
W/Dry vs dry	9.1 kg	Wean to finish	ADG ↑ by switching from dry MS[9] in grower to SS[10] W/Dry in finisher	Magowan et al., 2008
W/Dry vs dry	46.8 kg (Exp. 1) & 38.2 kg (Exp.2)	91 days (Exp. 1) & 104 days (Exp. 2)	ADG ↑ on wet/dry; ADFI ↑ on wet/dry	Myers et al., 2013

[1]Liq: Liquid; [2]ADG: Average daily gain; [3]FCR: Feed conversion ratio; [4] ↓: Decreased; [5]PW: Post-weaning; [6]↑: Increased; [7] ADFI: Average daily feed intake; [8] W/Dry: Wet/dry; [9]MS: Multi-space feeder; [10] SS: Single-space feeder

Hurst et al. (2008) compared dry feeding with liquid feed prepared at 1.5:1, 3:1 and 3:1 (4) as described previously, and found significantly lower ADG in dry-fed grow-finisher pigs compared to the other 3 treatments. They also showed similar ADFI between pigs fed the dry diet and the diet prepared at 1.5:1 which was higher than those fed 3:1 and the acidified diet (it should be noted that pigs were restricted fed at 5 - 10 % below their *ad-libitum* intake), resulting in a better FCR in pigs fed the 3:1 and acidified diet than in pigs fed dry and 1.5:1 (Hurst et al., 2008). l'Anson et al. (2013) showed that liquid-fed weaner pigs had a lower ADG and ADFI than dry-fed pigs which also resulted in a better FCR in liquid-fed than dry-fed pigs. However, a fair comparison is questionable because dry-fed pigs had *ad-libitum* access to feed while liquid-fed pigs were fed to appetite but only between the hours of 06.30 and 15.30 (l'Anson et al., 2013), making it harder to draw concrete conclusions based on the variation in feed supply. They did acknowledge in their abstract that poorer growth in liquid-fed pigs seemed to be as a result of some restriction of feed intake placed on the pigs (l'Anson et al., 2013). They also acknowledged that subsequent to the completion of their study, feed needed to be provided over a 12-hour period to achieve feed intakes as close to *ad-libitum* feeding as possible (l'Anson et al., 2013).

Interestingly, Han et al. (2006) noted that liquid feeding for 10 or 20 days pw and then switching to dry crumbled feed resulted in improved growth rates over dry-fed pigs for the 40-day experimental period. They noted that this improvement was not carried over into subsequent production periods and was only observed while the pigs were being liquid-fed. Brooks et al. (2001) concluded from work by Jensen and Mikkelsen (1998) and (Barber et al., 1991) that liquid-fed pigs can extract more

nutrients than pigs fed dry diets which may help to explain better growth rates in liquid-fed pigs.

1.6.3.1 Wastage in liquid feeding systems

A point that must not be overlooked when comparing feed delivery systems is the likelihood of wastage with liquid feed. Barber et al. (1991) described that water use = water intake + water wastage. A similar issue can be expected with liquid feed, especially with low troughs that pigs can climb into. Dry feed is much less likely to be wasted to the same extent as liquid feed and worsened FCR by liquid-fed pigs, often despite increased ADFI and ADG, has been attributed to feed wastage (Missotten et al., 2010, Russell et al., 1996, Han et al., 2006). As a result, in a follow-on experiment, Russell et al. (1996) improved trough design to minimise feed wastage and this resulted in an improvement in FCR compared to their initial results, although it was still worse than that found for dry-fed pigs. In a study by l'Anson et al. (2013) pigs were housed individually and it was concluded that wastage did not occur due to lack of competition at the feeder and also that when feed quantity was reduced and frequency of feeding increased, the wastage of liquid-fed pigs was negligible.

1.6.4 Dry vs wet/dry feeding

Bergstrom et al. (2008) concluded that pigs fed using wet/dry feeders grew faster and had increased feed intake, compared to pigs fed from dry feeders, but had poorer FCR. Wet/dry-fed pigs also produced fatter carcasses with a lower carcass yield, and were more costly to produce (Bergstrom et al., 2008).

With both dry and wet/dry feeders, single-space, double-space and multi-space feeders can be used. Gonyou and Lou (2000) observed the eating behaviours

of grow-finisher pigs using single and multi-space dry and wet/dry feeders. They found that the number of feed spaces did not affect pig productivity but that pigs eating from single-space feeders spent 15 % less time eating than those from double-space feeders. Pigs spent 17 % less time eating from wet/dry feeders than from dry feeders. They also concluded that single-space feeders can feed up to 12 pigs without affecting productivity. It should be noted that this research used a meal and not a pelleted diet.

Magowan et al. (2008) found that changing pigs from multi-space dry feeders in grower accommodation to single-space wet/dry feeders in finisher accommodation promoted highest growth rates for pigs from weaning to slaughter over pigs fed constantly from single-space wet/dry feeders, multi-space dry feeders or switching from single space wet/dry to multi-space dry feeders. Numerically, these pigs also had the best FCR, although the difference between treatments was not significant.

In conclusion, it is vital that new research is performed to compare pig growth and feed efficiency using up-to-date feed delivery systems, particularly in the case of liquid feeding.

1.6.5 Feed form: Pelleted vs meal feed

In general, pelleted feed is more popular for dry feeding, while meal is usually used for liquid feeding; however, there may be some cross-over between feed forms and delivery methods.

1.6.5.1 The pelleting process & impact on pig growth performance

Pelleting involves steam conditioning which involves the use of temperatures ranging from 55 to 80 °C. This heating process can improve diet digestibility by

breaking down the starch component of the diet. However, Svihus and Zimonja (2011) pointed out that only a small amount of dietary starch is gelatinised and that starch digestibility will not be affected to a great extent, but that some vitamins may be destroyed when pelleting diets. The pelleting process also converts feed to a more concentrated form, hence the increased nutrient density per unit volume, as shown by increased bulk density in pelleted diets compared to meal diets (Lundblad et al. (2011).

The temperature used is thought to affect diet quality and to determine the extent of the advantages associated with pelleting the diet. It is possible that protein degradation will occur due to the temperatures used in the pelleting process (Svihus and Zimonja, 2011); however, Ginste and De Schrijver (1998) found improved apparent total tract protein digestibility with grower and finisher diets pelleted at 80 °C compared to meal feeding. On the other hand, lower free lysine content was reported in a diet pelleted at 60 – 65 °C (1.59 ± 0.17 mg/g) compared to a meal diet (2.47 ± 0.06 mg/g) by Delgado-Andrade et al. (2010).

The influence of feed form on pig growth, health, diet quality and nutrient digestibility is shown in Table 1.5. Reasons for the improved growth performance of pigs fed pelleted diets and other advantages of pelleted feed can be summarised as follows; decreased segregation and dustiness; increased bulk density; improved palatability and handling properties; better thermal modification of starch and protein in the grain; homogenous feed intake; improved nutrient digestibility; increased nutrient density per unit volume (Delgado-Andrade et al., 2010, Jha et al., 2011, l'Anson et al., 2013, Lundblad et al., 2011, Lundblad et al., 2012, Svihus and Zimonja, 2011). The use of meal can therefore be seen as a potential disadvantage

associated with liquid feeding. However, some studies have not found any improvements in nutrient digestibility in pelleted compared to meal feed (l'Anson et al., 2013). It has also been hypothesised that the improvements in feed efficiency of pelleting compared to a meal diet is due to the fact that feed wastage is minimised with pelleted diets (Ball et al., 2015, Wondra et al., 1995, Kim et al., 2000).

Seerley et al. (1962) hypothesised that the pelleting process may cause chemical alterations that improve the nutritive value of the feed after pigs fed a pelleted diet grew faster than meal-fed pigs without an increase in feed intake. More recently, Flis et al. (2014) concluded from their review that finding a better gain-to-feed ratio from feeding finely ground feeds over coarser diets despite a lower feed intake is partly due to the higher nutritional value associated with them (i.e. higher ileal crude protein and amino acid digestibility along with higher energy concentrations). This is relevant as pelleted diets are generally more finely ground than meal diets. Other studies agree with this, with Ball et al. (2015) reporting significantly improved DM digestibility and digestible energy content of the diet and also a strong tendency of pelleting to improve both ash and energy digestibility. Lundblad et al. (2012) also reported increased ileal digestibility of starch from hydrothermal treatment (average of four heat treated pelleted diets) compared to a meal (control) diet. It is important to note that in their study, the meal diet was ground to avoid particle size becoming a confounding effect (Lundblad et al., 2012).

Table 1.5 Findings from the literature comparing the effects of feed form on pig growth performance, health, quality of the diet and nutrient digestibility

Feed form	Weight/age of pigs	Trial period	Effect of diet form	Reference
Pellet vs meal	81.6 kg - 90.7 kg	3 weeks	ADG[1] ↑[2] on pellets *ad-libitum* & equal intake ADFI[3] similar *ad-libitum* & equal intake FCR[4] ↓[5] when *ad-libitum* fed on pellets, similar when equal intake	Seerley et al., 1962
Pellet vs meal	81.6 kg - 90.7 kg	3 weeks	Energy digestibility ↑ on pellets *ad-libitum*	Seerley et al., 1962
Pellet vs meal	53.5 kg - finish	Finishing pigs	ADG ↑ by 3 % on pellets Feed:gain ↓ by 5 % on pellets	Stark et al., 1993
Pellet vs meal	55.2 kg – 114.8 kg	53 – 77 days (dependent on slaughter weight)	ADG ↑ 5 % on pellets Gain: feed ↑ 7 % on pellets Dry matter digestibility ↑ on pellets N[6] digestibility ↑ on pellets GE[7] digestibility ↑ on pellets	Wondra et al., 1995
Pellet vs meal (also particle size)	33 ± 7 kg	To slaughter	ADG similar on pellets & meal ADFI similar on pellets & meal FCR ↓ on pellets	Mikkelsen et al., 2004
Pellet vs meal (also acid)	27 kg	27 kg to 99 kg	ADG similar on pellets & meal ADFI similar on pellets & meal G:F similar on pellets & meal	Canibe et al., 2005
Pellet vs meal (also particle size)	33 ± 7 kg	4 weeks for content, 30-100 kg for performance	ADG similar on pellets & meal ADFI similar on pellets & meal FCR ↓ on pellets *Salmonella* protection lower on pellets	Hedemann et al., 2005

Feed form	Weight/age of pigs	Trial period	Effect of diet form	Reference
Pellet vs expanded vs meal	8.4 kg / 5 d PW	Weaning to slaughter	FCR ↓ by expanding & pelleting vs meal	Millet et al., 2012
Pellet vs meal	13.6 kg	13.6 kg to slaughter	Ulceration caused by pelleting & not by meal	Mößeler et al., 2012
Pellet vs meal	28 days, 7.4 ± 0.3 kg	26 day trial	ADG ↑ on pellets LW at end of trial ↑ on pellets FCR ↓ on pellets	l'Anson et al., 2012
Pellet vs meal	28 days; 7.3 ± 0.1 kg	26 day trial	ADG similar pellets & meal ADFI similar pellets & meal FCR ↓ on pellets	l'Anson et al., 2013
Pellets vs meal	2 Exps: 46.8 kg & 38.2 kgs	91 days & 104 days	Gain: feed improved by good quality pellets	Myers et al., 2013
Pellet (poor & good quality) vs meal	56.8 kg	69 days	Gain:feed ↑ on good quality pellets	Nemechek et al., 2015
Pellet vs meal	12 weeks	To target slaughter wt of 105 kg	ADFI ↓ on pellets FCR ↓ on pellets	Ball et al., 2015
Pellet vs meal	44.8 kg	14 day digestibility trial	Dry matter digestibility ↑ on pellets Energy digestibility tended to ↑ on pellets Ash digestibility tended to ↑ on pellets	Ball et al., 2015
Pellet vs meal	31.2 kg	118 day trial	ADG similar pellets & meal ADFI ↓ on pellets FCR ↓ on pellets Ulceration ↑ on pellets Pig removals ↑ on pellets	De Jong et al., 2016

[1]ADG: Average daily gain; [2]↑: Increased; [3]ADFI: Average daily feed intake; [4]FCR: Feed conversion ratio; [5]↓: Decreased; [6]N: Nitrogen; [7]GE: Gross energy

1.6.5.2 Feed particle size and pellet quality

Feed particle size differs between feed forms, with pelleted diets generally more fine, and meal diets often coarse. Canibe et al. (2005) found a higher starch concentration in the distal small intestine, caecum and mid-colon of pigs fed a meal diet versus a pelleted diet, indicating that the larger particle size in the meal diet impeded access of endogenous and microbial enzymes to nutrients in those feed particles. Despite the production advantages discussed above, pelleted feed is also associated with gastric ulceration (Table 1.5). This is due to the smaller particle size, as with finer particle size, passage rate is faster because the consistency of the stomach content is more liquid and the pH is reduced (Mößeler et al., 2012, Vukmirović et al., 2017, De Jong et al., 2016, van Winsen et al., 2000, Mößeler et al., 2010). Reese et al. (1966) for example, found more stomach lesions associated with fluid versus non-fluid digesta. The major concerns with gastric ulceration are animal welfare, mortality and the associated financial losses (Friendship, 2003). De Jong et al. (2016) reported increased ulceration in pigs fed a pelleted diet compared to a meal diet, despite the fact that both diets were from the same maize corn source and had identical particle sizes. They suggested that it is possible that the pelleting process itself further decreases particle size (De Jong et al., 2016).

Pellet quality is vitally important, and Myers et al. (2013) showed that high quality pellets can result in improved growth rates; however, they concluded that the advantages in feed efficiency associated with pelleting are lost if the pellets are of poor quality. This also supports the findings of Stark et al. (1993) who compared 5 treatments; a meal diet, a screened pelleted diet, and pelleted diets containing 20 %, 40 % and 60 % fines and found that pigs fed the meal diet or the diet with 60 % fines tended to have lower ADG compared to pigs fed the other diets (Stark et al., 1993).

They also found that pigs fed the screened pelleted diet had a 4.7 % improved gain:feed compared to pigs fed the meal diet (Stark et al., 1993). They concluded that pelleting diets improves the growth performance of finisher pigs but that a high percentage of fines in the diet result in a decrease in the advantage seen over meal feeding (Stark et al., 1993). The results of a study by Ball et al. (2015) suggest that feeding a finely ground pelleted diet is superior to a coarsely ground meal diet, based on FCR.

1.6.5.3 The microbiological impact of pelleting

The moisture and heat applied during the pelleting process lowers the microbial load of the feed (Attar et al., 2018) resulting in improved hygienic quality (Lundblad et al., 2012), as evidenced by lower *Enterobacteriaceae* counts in pelleted diets compared to meal compound pig feed (Burns et al., 2015).

Feeding of meal versus pelleted diets also impacts the pig intestinal microbiota. A coarsely-ground, non-pelleted diet, for example, leads to increased lactic acid concentrations and reduced pH in the stomach compared to a finely ground pelleted diet (Mikkelsen et al., 2004, Canibe et al., 2005, Flis et al., 2014). This may be due to the slower passage rate referred to in Section 1.6.5.2, which leads to increased microbial fermentation in the stomach, allowing for more proliferation of LAB. This then results in a healthier lower GIT, as undesirable bacteria are prevented from entering and proliferating by the low pH gastric barrier (Flis et al., 2014, Mikkelsen et al., 2004).

Another purpose of pelleting and heat treatment is to reduce *Salmonella* contamination in compound feed (Wong et al., 2004). However, Jørgensen et al. (1999) found that pigs fed pelleted feed had a 3.33 times higher risk of being

seropositive for *Salmonella* than pigs fed a meal diet and suggested that meal feed provides *Salmonella* with poor growth conditions compared to those provided in pelleted feed. A 60 % decrease in *Salmonella* adherence to ileal tissue in non-pelleted compared to pelleted diets has also been reported in a pig intestine organ culture model (Hedemann et al., 2005). Mikkelsen et al. (2004) also found an increased death rate of *Salmonella* Typhimurium in the stomach contents of pigs fed a coarse meal diet compared with coarse pelleted, fine meal and fine pelleted diets which was in agreement with the findings of Jørgensen et al., (1999). They concluded that feeding a coarse meal diet decreases *Salmonella* survival during gastric transit which then stops *Salmonella* getting to and proliferating in the lower GIT (Mikkelsen et al., 2004). However, a systematic review has shown a low level of confidence among qualified scientists that the relationship between reduced *Salmonella* prevalence and non-pelleted (i.e. meal) feed is scientifically valid (O'Connor et al., 2008).

In conclusion, with respect to feed form, there are growth and feed efficiency advantages associated with feeding a pelleted diet, but coarse meal diets result in a healthier GIT in the pig. However, there is a lack of recent studies, using up-to-date liquid feeding technology that compare the three feed delivery methods commonly used in this country. It also seems relevant that feed forms should be compared using these different feed delivery methods in an attempt to optimise grow-finisher pig growth and feed efficiency. It would be useful for pig producers investing in new units or re-furbishing existing units to know which feed form and delivery methods would maximise profitability for their system.

1.7 Acid inclusion in pig diets

1.7.1 The role of acid inclusion in pig diets

Acids have been added to pig diets for some time, particularly those of young pigs since the ban on routine use of antibiotic growth promoters came into effect in the EU in 2006 (Torrallardona et al., 2007). Interest in alternative feed additives for weaned pigs is also likely to increase with the EU ban on pharmacological levels of zinc oxide which comes into effect in 2022. There is also a desire to reduce the use of antibiotics in productive animals, because of the risks to human health of antibiotic resistance and drug residues in animal products (Papatsiros et al., 2011, Silbergeld et al., 2008, Partanen and Mroz, 1999).

Most of the research on acid inclusion in pig diets has been based around the stressful weaning period, during which disease susceptibility can become an issue (Papatsiros et al., 2011, Melin et al., 2004, Kim et al., 2005). Dietary acidification generally results in reduced gastric pH, leading to increased activity of proteolytic enzymes, an improvement in protein digestibility and inhibition of pathogenic bacteria in the GIT (Kim et al., 2005). The effect of low pH on growth and survival of Gram-negative bacteria, for example, is well-known with an *in-vitro* study by Knarreborg et al. (2002) showing that coliform populations were relatively constant at pH 5, at pH 4 growth was inhibited and at pH 3 a bactericidal effect was noted. Hence, a reduction in dietary pH is the main mechanism of antimicrobial action of organic acids, but their ability to change from undissociated to dissociated form based on environmental pH also contributes to their antimicrobial activity (Partanen and Mroz, 1999). To explain, an acid in its undissociated form can freely diffuse through the semi-permeable membrane of a bacterial cell (Partanen and Mroz, 1999, van Winsen et al., 2001, van Winsen et al., 2000). Once inside the cell, the acid can

then dissociate to suppress microbial enzymes, such as decarboxylases and catalases, and nutrient transport systems (Partanen and Mroz, 1999).

The supplementation of organic acids to pig diets reduces the dietary buffering capacity which is also thought to reduce the proliferation of undesirable microbes in the pig gut (Papatsiros et al., 2011). It is also thought that organic acid inclusion aids protein digestion by lowering gastric pH; however, this has not always been found to be the case (Partanen and Mroz, 1999, Halas et al., 2010). However, in growing pigs, improved apparent ileal digestibilities of protein and amino acids have been observed (Partanen and Mroz, 1999). Other contributing factors to improved digestion, absorption and retention of nutrients are the influence of organic acids on mucosal morphology and their possible stimulation of pancreatic secretions (Partanen and Mroz, 1999).

In terms of liquid feed hygiene, organic acids have been reported as the most suitable additive for control of bacteria, yeasts and moulds (Riemensperger, 2012). A study on an Austrian farm added a mixture of formic, propionic and lactic acids (Biotronic® SE Forte) to liquid feed at a rate of 3 L / 1000 L (i.e. 0.3 %) together with a 3 % inclusion rate in water remaining in the feed pipes overnight. Findings were that yeast were almost eliminated, particularly in residual feed in the troughs, and that feed pH was reduced from 5.57 to 4.89 (Riemensperger, 2012).

A range of organic acids have been supplemented to pig diets. These include formic, acetic, propionic, butyric, lactic, sorbic, fumaric, malic, tartaric, citric and benzoic, as well as blends and salt forms of these. Benzoic acid will be discussed here as it was used in one of the experimental chapters of this thesis.

1.7.2 Benzoic acid

Benzoic acid has been used as a food preservative for a long time due to its antimicrobial and antifungal properties (Mao et al., 2019). Benzoic acid increases digestive enzyme production and can activate digestive enzymes by decreasing the pH in the proximal GIT, both of which improve digestive ability, which is one of the reasons its inclusion in diets has lead to improved growth in pigs (Mao et al., 2019). Benzoic acid can also impact, immunity, gut microbiota and redox status (i.e. the balance between oxidants and antioxidants) (Mao et al., 2019). Inclusion levels of 0.5 – 2 % in feed was advised by Mao et al. (2019) based on their recent review, but it should be noted that benzoic acid is not authorised at inclusion levels > 1 % according to EU regulation No. 1138/2007/EC.

1.7.2.1 Influence of benzoic acid on the microbial quality and pH of feed and on the pig gastrointestinal tract

Benzoic acid can be added to both liquid and dry diets as it is available in powdered form. However, most of the research on benzoic acid has been performed in dry or wet/dry feed (Torrallardona et al., 2007, Guggenbuhl et al., 2007, Den Brok, 1999).

Benzoic acid supplementation has been shown to inhibit free amino acid degradation, yeast growth and lactic acid production in fermented liquid feed (Vils et al., 2018). In terms of effects on the gut microbiota, Guggenbuhl et al. (2007) found that LAB counts in the stomach of weaner pigs were reduced by 93 % and *E. coli* in the caecum by 92 % as a result of 0.5 % benzoic acid inclusion compared to a control diet with no benzoic acid added. A study by Øverland et al. (2008) also showed reduced concentrations of coliforms and *Enterococcus* in the jejunum,

reduced coliforms and LAB in the colon and coliforms and LAB in the rectum in grow-finisher pigs fed a 0.85 % benzoic acid-supplemented diet compared to a control diet that did not contain any acid.

An interesting finding by Halas et al. (2010) that agrees with the findings of a review conducted by Partanen and Mroz (1999) is that benzoic acid supplementation did not reduce gastric pH, which is thought to be a requirement for efficient protein digestion. This suggests that the effect of dietary organic acid inclusion may not be directly linked to lower gastric pH and agrees with the findings of Franco et al. (2005) where the addition of organic acid mixtures did not reduce gastric pH. However, Partanen and Mroz (1999) do mention that improved apparent ileal digestibilities of protein and amino acids have been observed in growing pigs administered organic acids or their salts, but not in weaners. Other beneficial effects of benzoic acid have been shown in the GIT of piglets, with enhanced nitrogen retention for example observed by Halas et al. (2010).

Considering the beneficial effects observed in the pig GIT with dietary supplementation of benzoic acid, it would be interesting to determine the effect of benzoic acid inclusion in liquid feed, specifically in terms of its antimicrobial effects and hence its possible role in controlling spontaneous fermentation in liquid feed.

1.7.2.2 The influence of benzoic acid on pig growth and feed efficiency

Table 1.6 summarises the results of a number of studies that have investigated the effects of benzoic acid-supplemented diets on the growth and feed efficiency of pigs. Growth has been improved by benzoic acid supplementation in some instances, depending on inclusion rate in weaner (Kluge et al., 2006, Torrallardona et al., 2007, Guggenbuhl et al., 2007, Halas et al., 2010, Papatsiros et

al., 2011, Diao et al., 2016) and grow-finisher pigs (Van der Peet-Schwering et al., 1999). On other occasions, dietary benzoic acid inclusion did not impact pig growth (Dierick et al., 2004, Øverland et al., 2008). Improvements in FCR have been reported by feeding benzoic acid, either as part of a blend or alone, again dependent on inclusion rate (Den Brok, 1999, Kluge et al., 2006, Torrallardona et al., 2007, Guggenbuhl et al., 2007, Van der Peet-Schwering et al., 1999); however, no improvements in FCR have also been shown (Papatsiros et al., 2011, Diao et al., 2016).

Table 1.6 Findings from the literature on the effects of benzoic acid on pig growth performance, nutrient digestibility and the environmental advantages

Dietary treatments	Trial duration	Weight/age of pigs	Feed form	Feed delivery	Finding	Reference
(1) Control (2) Benzoic acid blend	Grow-finisher period	26 -107.8 kg LW[1]	NS[2]	Wet/dry	FCR[3] ↓[4] in acidified diet vs control-fed pigs	Den Brok, 1999
(1) 0 % benzoic acid (2) 1 % benzoic acid (3) 2 % benzoic acid	Grow-finisher period	24.3 kg - 108.9 kg	NS	NS	ADG[5] ↑[6] on 1 % vs 0 % & 2 % benzoic acid ADFI[7] ↑ on 1 % vs 0 %, similar to 2 % FCR ↓ on 1 % vs 0 % & 2 % benzoic acid	Van der Peet-Schwering et al., 1999
(1) Control (2) 0.1 % lipase with 2.4 % MCT[8] (3) 1 % benzoic acid	11-12 days	8.67 ± 0.88 kg, 4 wks of age	NS	NS	ADG similar for all 3 diets	Dierick et al., 2004
(1) Control (2) 5 g/kg benzoic acid (3) 10 g/kg benzoic acid (4) 12 g/kg potassium diformate	35 days	28 days old; 7.5 kg	NS	NS	ADG ↑ on trt (3) & (4) vs (1), similar to (2) ADFI similar between all treatments FCR ↓ on (4) vs (1) & (2), similar to (3) LW on d35 heavier on (4) vs (1), similar to (2) & (3)	Kluge et al., 2006
(1) 0 % benzoic acid (2) 0.5 % benzoic acid	28 days	8.9 kg	Pelleted	Dry	ADG ↑ on benzoic acid ADFI ↑ on benzoic acid FCR ↓ on benzoic acid LW at day 28 ↑ on benzoic acid BA led to greater ileal microbiota diversity	Torrallardona et al., 2007

Dietary treatments	Trial duration	Weight/age of pigs	Feed form	Feed delivery	Finding	Reference
(1) 0 % benzoic acid (2) 0.5 % benzoic acid	32 days	28 days old; 7.40 ± 0.86 kg	Pelleted	Dry	ADG ↑ on benzoic acid FCR ↓ on benzoic acid	Guggenbuhl et al., 2007
(1) Control diet (2) 0.85 % benzoic acid (3)*	78 days	31.7 kg - 113.2 kg	NS	NS	ADG similar on benzoic acid & control ADFI similar on benzoic acid & control FCR improved on benzoic acid	Øverland et al., 2008
(1) Control diet (2) 10 g/kg benzoic acid (3) 20 g/kg benzoic acid	21 days	28 ± 1.7 kg - 39.1 ± 2.3 kg	Liquid (2.5:1 water-to-feed, individually housed pigs)	Mash	Ca, P & K utilisation ↑ on benzoic acid Mg utilisation unaffected on benzoic acid Na & Cl utilisation ↓ on benzoic acid	Sauer et al., 2009
(1) 0 % benzoic acid (2) 0.5 % benzoic acid (3) 750 IU/kg phytase (4) 750 IU/kg phytase + 0.5 % benzoic acid	Grow-finisher period	26 – 109 kg	NS	NS	Benzoic acid + phytase together in P-reduced diets can adversely affect nutrient & mineral digestibility; mechanisms of which are unclear	Bühler et al., 2010
2x3 factorial: 2 levels for benzoic acid: 0 & 5 g/kg 3 levels for Inulin: 0, 40 & 80 g/kg	21 days	21 ± 3 days old, 5.9 ± 0.08 kg	NS	NS	ADG ↑ on benzoic acid ADFI ↑ on benzoic acid LW at day 21 were similar	Halas et al., 2010
(1) 0 g/kg benzoic acid (2) 10 g/kg benzoic acid (3) 20 g/kg benzoic acid (4) 30 g/kg benzoic acid	10 days (individual metabolism crates)	64 ± 1.5 kg	Meal	Liquid (1:1 ratio)	Linear decrease in NH_3 as BA inclusion increased	Murphy et al., 2011

Dietary treatments	Trial duration	Weight/age of pigs	Feed form	Feed delivery	Finding	Reference
(1) Control (2) $1x10^9$ TOYO[9] (3) 5 g/kg BA (4) $1x10^9$ TOYO + 5 g/kg BA	35 days	7.93 ± 0.05 kg	Meal (home mixed)	NS	ADG ↑ on benzoic acid (& benzoic acid + toyocerin) vs control ADFI ↑ on benzoic acid vs control FCR similar benzoic acid + control Diarrhoea severity reduced by BA	Papatsiros et al., 2011
(1) Control (2) 5000 mg/kg (5 g/kg) benzoic acid	14 days	18.75 - 27.9 kg	NS	NS other than 'self-feeder'	ADG ↑ on benzoic acid ADFI ↑ on benzoic acid FCR similar on benzoic acid & control CP[10], DM[11], EE[12], GE[13] & Ash digestibility ↑ on benzoic acid	Diao et al., 2016

[1]LW: Liveweight; [2]NS: Not specified; [3]FCR: Feed conversion ratio; [4]↓: decreased; [5]ADG: Average daily gain; [6]↑: increased; [7]ADFI: Average daily feed intake; [8] MCT: Triacylglycerols; [9]TOYO: Toyocerin (probiotic containing *Bacillus toyonensis*); [10]CP: Crude protein; [11]DM: Dry matter; [12]EE: Ether extract; [13]GE: Gross energy

It is clear from Table 1.6 that a lack of studies exist on benzoic acid supplementation in grow-finisher diets and of those that do exist, some do not measure pig growth performance (Bühler et al., 2010, Murphy et al., 2011). To our knowledge, no studies to date have been carried out on feeding benzoic acid-supplemented diets to grow-finisher pigs using an automated liquid feeding system. It would be useful to do so in order to investigate the impact of benzoic acid on the microbial quality of liquid feed prior to ingestion as well as on pig performance.

To conclude on benzoic acid, there are many claims that it is anti-microbial and Knarreborg et al. (2002) concluded that it was the most efficient organic acid in terms of declining coliform viability in digesta compared to fumaric, lactic, butyric, formic and propionic. Hence, it was chosen as a suitable acid for one of the experimental chapters of this thesis in an attempt to improve liquid feeding system hygiene and to prevent the growth of undesirable microbes in liquid feed.

1.8 Conclusions

Liquid feeding systems have a number of advantages as well as disadvantages. They provide the opportunity to feed co-products from the food industry which are low-cost ingredients, thereby helping to reduce overall feed costs; however, this only becomes an advantage if a constant supply of high quality products is readily available and if diets can be easily re-formulated to match their nutritional characteristics. Increased feed intakes, growth rates, lean tissue deposition, improved gut microbiota populations and a shorter time to slaughter when compared with dry feeding are just some of the production advantages associated with liquid feeding.

Liquid feeding system hygiene is a debated topic, but currently, there are no guidelines available on the best course of action. There seems to be an argument for never cleaning or disinfecting liquid feeding systems, yet some advise that tanks should be emptied and cleaned on a regular basis. Further research in this area could help to improve the quality and consistency of liquid feed by helping to reduce the likelihood of contamination and controlling spontaneous fermentation.

One method of controlling spontaneous fermentation is to perform controlled fermentation to ensure that desirable microbes dominate, a favourable gut microbiota population can be achieved and palatability issues affecting feed intakes are avoided. However, fermented feed has produced variable results and also requires a lot of management on-farm. Acids have been supplemented to pig diets for some time, primarily to the diets of weaners. However, acid inclusion has also been examined at the finisher stage, albeit to a lesser extent, and seems to be beneficial in terms of pig growth, health and feed efficiency. The inclusion of acids could play a vital role in controlling spontaneous fermentation in liquid feed, as the resultant low pH will promote the growth of LAB and suppress the growth of *Enterobacteriaceae* and other undesirable bacteria.

It would be important to compare feed delivery methods, particularly for producers looking to install a new feeding system or update their current feeding regime. Alongside this, comparing feed forms (i.e. meal and pellets) would be vital in order to select the most efficient feeding strategy. A lack of research exists on water-to-meal ratios using modern liquid feeding technology. It is likely that optimisation of liquid feeding systems will provide producers with valuable

information which could increase production efficiency and reduce the overall feed cost per kg meat.

The topics discussed here may help to inform investment decisions for farmers in the future. Overall, the literature reviewed highlighted gaps in some of the basic liquid feeding practices which, if optimised, could be hugely beneficial to pig producers. These gaps include informed knowledge of the microbial content of liquid feed in a commercial setting, a direct comparison of whole diet and cereal fermentation with fresh liquid and dry feeding, a direct comparison of all available feed form and delivery methods, an optimised water-to-feed ratio for the different stages of pig growth and a trial of organic acids in liquid feed for grow-finisher pigs.

1.9 Overall objectives of the research

- Characterise the microbiological quality of liquid feed for grow-finisher pigs on commercial pig production units
- Control spontaneous fermentation during liquid feeding through controlled fermentation and dietary acidification
- Compare the impact of feed form and delivery on grow-finisher pig growth and **FCE**)
- Compare water-to-feed ratios to optimise growth and FCE of liquid-fed grow-finisher pigs.

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2. Microbiological assessment of liquid feed for finisher pigs on eight commercial pig units

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2.1 Abstract

The aim of this study was to investigate the microbiological quality of liquid feed for finisher pigs on eight commercial pig production units and the factors which influence this, including feeding system design, feeding practices and sanitation protocols. Liquid feed samples were collected from three sampling locations on each unit including the mix tank (**Mix Tank**; n=1), freshly dispensed feed at the trough (**Fresh**; n=3) and residual feed that remained in the trough from the previous feed just prior to dispensing new feed (**Residual**; n=3). Feed samples were microbiologically assessed and proximate, amino acid, lactate, ethanol and volatile fatty acid analyses were conducted. Details of feeding practices, sanitation protocols and diet composition were surveyed on each unit. Lactic acid bacteria counts ($P<0.001$), yeast counts ($P<0.001$) and sample temperature ($P<0.001$) increased from the Mix Tank to Fresh and again to Residual. Counts of *E. coli* were similar in the Mix Tank and Fresh but increased in Residual ($P<0.001$). The pH of liquid feed was similar in the Mix Tank and Fresh but was significantly lower in Residual ($P<0.001$). *Enterobacteriaceae* and mould counts did not differ across sampling locations. Lysine ($P<0.001$), methionine ($P<0.001$) and threonine ($P<0.001$) concentrations in the Mix tank and Fresh were similar, but significantly reduced in the Residual. The gross energy content was significantly reduced from the Mix tank to Fresh and again in Residual ($P<0.001$). Total lactic acid ($P<0.001$), ethanol ($P<0.001$) and acetate ($P<0.001$) concentrations in liquid feed were similar in the Mix tank and Fresh but significantly increased in Residual. Liquid co-products were included in the diet on three of the eight units surveyed and their use reduced *E. coli* in the Residual ($P<0.05$), pH in the Mix Tank ($P<0.01$) and Fresh ($P=0.01$) and mould counts at all three sampling locations ($P<0.01$). Sanitation practices implemented on five of the

eight units did not impact microbial counts; however, practices varied from unit to unit. The results of this study show that a considerable degree of spontaneous fermentation occurs in liquid feed on commercial units, with resultant negative effects on nutritional quality of the feed. The findings also show that nutritional approaches such as liquid co-product inclusion may have a greater influence on the microbial load of liquid feed than sanitation practices used on-farm.

2.2 Introduction

Liquid feeding systems must be carefully managed to optimise feed microbial quality. This will help to ensure optimal feed intake and growth performance, as well as nutrient utilisation and gut health (Roth, 2013). By mixing feed and water, naturally occurring lactic acid bacteria (**LAB**) and yeasts proliferate, resulting in a pH reduction, mainly due to lactic acid and acetic acid production (Canibe and Jensen, 2012; Missotten et al., 2015). Therefore, even in liquid feed that is not deliberately fermented, a certain amount of spontaneous fermentation will occur (Canibe et al., 2010) and microbiological examination has shown that LAB are very often the cause (Brooks et al., 2001b; Geary et al., 1999; Mikkelsen and Jensen, 1998; Plumed-Ferrer et al., 2004; Russell et al., 1996). However, the microbial species that dominate this fermentation can vary based on the dietary ingredients and/or the environment (Canibe et al., 2010) and this can in turn affect feed quality.

In addition, there are currently no generally accepted guidelines on hygiene practices for liquid feeding systems. Cleaning seems to be a requirement, to avoid the multiplication of undesirable microbes in pipelines and tanks and suppliers of liquid feed systems generally advise cleaning of mixing tanks and pipes; however, there is limited information on how best this should be done (Best, 2009). To date, no microbiological survey of liquid feed and/or associated hygiene practices has been conducted on Irish pig units. Feeding system and trough design are also important factors when considering the microbiological quality of liquid feed. Restricted feeding is practiced using long feed troughs which have sufficient space for each pig to eat simultaneously. Short trough feeding systems do not allow sufficient space for every pig to eat simultaneously, so a feed reservoir remains in the trough between feed-outs.

The hypothesis of the current study was that liquid feed quality would deteriorate in troughs due to spontaneous fermentation. The objective was to investigate, for the first time, the microbiological quality of liquid feed from the finisher section of Irish pig production units and the factors which influence this, including feeding system design, feeding practices and sanitation protocols. To achieve this, liquid feed from the mixing tanks and feed troughs on eight pig production units was subjected to a range of microbiological and physico-chemical analyses. A questionnaire on the feeding practices and cleaning protocols employed on each unit was also completed at the time of sampling.

2.3 Materials and Methods

2.3.1 Sample collection

Liquid feeding systems on eight Irish pig production units were investigated. Units were selected based on their proximity to the research centre, type of feeding system used, sanitation practices employed and the availability of accurate production data. Liquid feed samples were collected from the mix tanks and feed troughs of the grow-finisher stage from each unit. Each pig production unit had unique characteristics, including pen size, feed space allowance per pig, agitation time, feed system design and sanitation practices (Table 2.1). All units investigated were home milling i.e. buying in raw ingredients and manufacturing the diet on-farm at the point of mixing for liquid feeding. Feed remained in the pipelines between feeds on all units except for units D and H. The pipelines on unit D were filled with a formic acid solution between feeds (described later) and unit H had a hydro-air system which forced air through the pipelines at feeding and hence the pipelines remained empty between feeds. On all units except D and H, the feed remaining in the pipelines re-circulated into the mixing tank prior to the start of the next feed. The mixing tanks on all units except D and H were likely to have contained a very small amount of residual feed between feeds as is common practise on commercial units to prevent damage to feed pumps. On unit D, the pipelines were flushed with the formic acid solution from the mixing tank. This formic acid solution then returned to the mixing tank to make up some of the water fraction for the following feed. On unit H, water rinses were used between feed-outs to ensure there was no carry-over of liquid feed in the mixing tank. The rinse water on unit H was dumped prior to making up a new feed. Seven units (designated A to G) were investigated on one occasion and one unit (H) was investigated on seven different occasions; hence,

the data from this unit are means of data from samples collected on these seven occasions when all units are being compared. The repeated samplings from unit H were also analysed separately (see statistical analysis section).

A total of seven samples were collected from three sampling locations in the finisher section of each unit; one from the mixing tank after the agitation process; (**Mix Tank**; n=1), three samples of feed freshly delivered to each of three different troughs (**Fresh**; n=3) and three samples of liquid feed remaining in each of three different troughs just prior to feeding the next meal (**Residual**; n=3). Microbiological analysis was performed on each individual sample. Of the three Fresh and Residual samples collected, the start, middle and end of the feed circuit were represented by one of the three samples. This is referred to as 'trough location'. On units that had more than one feed circuit for finisher pigs, all troughs sampled were from the same feed circuit. Due to differences in liquid feeding system design and practices on each unit, the length of time that Residual feed remained in the trough between feeds varied from one unit to another. No Residual feed remained in troughs on unit D; therefore, a 30cm x 30cm area of each liquid feed trough was swabbed instead using a 10cm x 10cm sterile sampling sponge pre-soaked in maximum recovery diluent (Technical Service Consultants Ltd., Lancashire, United Kingdom).

To collect the Mix Tank sample, a specially designed autoclaved stainless steel sampler was lowered into the mixing tank using a new rope for each unit. Agitation in the mix tank should ensure homogeneity of the feed mix; however, to ensure a representative sample was taken, a minimum of three separate sub-samples were collected from each mixing tank and pooled. The Fresh samples were collected

as liquid feed was dispensed into the trough and the Residual feed was collected from troughs just prior to the next feed-out. At each sampling location, ~ 500 g liquid feed sample was collected into a disinfected (Virkon, DuPont, Sudbury, United Kingdom), thoroughly rinsed and dried 500 ml plastic container and transported on ice to the laboratory for same day microbiological analysis. The pH and temperature of all samples were recorded on-farm using a Mettler Toledo pH meter (Greisensee, Switzerland). A sub-sample (~ 20 g) was transported back to the research facility on ice and frozen at -20 °C for subsequent lactate, ethanol and volatile fatty acid (**VFA**) analysis. Another sub-sample (~ 250 g) was transported back to the research facility on ice and frozen at -20 °C for subsequent amino acid and proximate analysis. Prior to proximate and amino acid analysis, samples were oven dried at 55 °C for 72 hours.

A water sample was collected for microbiological analysis on all units, except unit A. The sample was collected into a sterile 250 ml bottle coated with a sodium thiosulphate preservative (Corning Life Sciences, Acton, MA). Water samples were transported to a commercial water testing laboratory for same-day analysis as outlined below.

Samples were collected between 24th January and 24th March 2017 from units A-G and between 13th June and 19th December 2017 from unit H. The mean monthly external temperatures recorded at the research centre during this period were as follows: January: 6.3 °C; February: 6.6 °C; March: 8.3 °C; June: 14.8 °C; July: 15.7 °C; August: 14.6 °C; September: 12.9 °C; October: 11.5 °C; November: 7.2 °C; December: 6.3 °C.

2.3.2 Microbiological analysis of feed and water samples

Approximately 10 g of each liquid feed sample was homogenized as a 10-fold dilution in maximum recovery diluent (MRD; Oxoid, Basingstoke, UK) and a 10-fold dilution series was performed in MRD. Relevant dilutions were plated in duplicate as follows; (1) pour-plated on de Man Rogosa & Sharpe, (MRS; Oxoid) agar, containing 50 U / mL nystatin (Sigma-Aldrich, Arklow, Co. Wicklow, Ireland), overlaid and incubated at 30 °C for 72 hours for LAB; (2) pour-plated on violet red bile glucose (VRBG; Oxoid), agar overlaid and incubated at 37 °C for 24 hours for *Enterobacteriaceae;* (3) pour-plated on ChromoCult tryptone bile X-glucuronide (CTBX; Merck, Damstadt, Germany) agar incubated at 44 °C for 24 hours for *E. coli*; and (4) spread-plated on yeast glucose chloramphenicol (YGC; Merck) agar incubated at 25 °C for 5 days for yeasts and moulds. Colonies were counted and the counts averaged and presented as $\log_{10}$ CFU/g of the original sample. The count from the Residual swab taken on unit D is presented as $\log_{10}$ CFU/cm^2.

The water sample was analysed as follows; (1) Coliform and *E. coli* were enumerated using a most probable number method (Colilert 18 Quanti-Tray; IDEXX, Westbrook, ME) according to the Health Protection Agency (HPA) standard method W18, ISO 9308-2 Part 2 and Microbiology of Drinking Water 2002:Part 4; (2) Enterococci were enumerated by membrane filtration according to the ISO 7899-2:2000 and the microbiology of drinking water (2010) part 5 methods; and (3) and a total viable count (TVC) was performed using an aerobic colony count technique with incubation at 22 °C for 72 hours as outlined in the BS EN ISO 6222:1999 method and HPA standard method W4.

2.3.3 Proximate, amino acid, lactate, ethanol and volatile fatty acid analysis of liquid feed samples

Proximate analysis included gross energy (GE), nitrogen (N) ash and neutral detergent fibre (NDF). Samples were ground through a 2mm screen in a Christy Norris mill (Christy and Norris Ltd, Chelmsford, UK). Ash (AOAC.942.05) and N content (AOAC.990.0) were determined according to methods of the Association of Official Analytical Chemists (AOAC, 2005). N content was determined using the LECO FP 528 instrument (Leco Instruments UK Ltd., Cheshire, UK). Crude protein (CP) was determined as N x 6.25. The NDF content was determined according to the method of Van Soest et al. (1991) using an Ankom 220 Fibre Analyser (Ankom Technology, Macedon, New York, USA). Gross energy was determined using an adiabatic bomb calorimeter (Parr Instruments, Moline, IL USA). Amino acid determination was carried out using cation exchange HPLC as previously described by McDermott et al. (2016) (AOAC 994.12).

Preparation of liquid feed samples for ethanol and lactate analysis was performed as described by van Winsen et al. (2000). Briefly, feed aliquots were defrosted prior to centrifugation at 2,000 g for 10 minutes at 4 °C. The supernatant was then centrifuged at 18,500 g for 10 minutes. The resulting supernatant was filtered through a 0.2 µm filter and stored at -20 °C until ethanol analysis by gas chromatography and lactate analysis by HPLC.

Samples were thawed slowly at room temperature prior to ethanol analysis by gas chromatography (Agilent 6890) using a flame ionization detector. A 1 µL volume of each sample was injected by split injection 5:1 onto the column (AT100 15 m x 0.53 mm i.d. x 1.2 micron) with a column flow rate of 3.4 ml/min helium.

The temperature programme was 40 °C for 3 minutes, ramped at 10 °C/min to 180 °C and held at 180 °C for 3 minutes.

For lactate analysis, samples were thawed slowly at room temperature, diluted with water as required and re-filtered through a 0.2µm filter prior to analysis by HPLC (Waters, Milford, USA). A 10µL volume of each diluted sample was injected onto a Phenomenex Chirex [5µm Chiral IV (ligand exchange) 3126 ®-PA 150 x 4.6mm] column under isocratic conditions. The column temperature was 22 °C, detector wavelength 254 nm and flow rate 1 ml/min with a run-time of 40 minutes.

For VFA analysis, extractions were carried out as described by McCormack et al. (2017) with some modifications. Briefly, 3.5g sample was weighed and the pH was recorded. Samples were diluted with 5 % trichloracetic acid (at 2.5 x weight of sample) and centrifuged at 1800 x *g* for 10 minutes at 4 °C. A 1.5 ml aliquot of the resultant supernatant was mixed with 1.5 ml internal standard and filtered through a 0.45 µm filter and stored at -20 °C until analysis by GC. An injection volume of 1 µl was injected into a Scion 456 gas chromatographer (SCION Instruments, Goes, The Netherlands) equipped with a EC^{TM} 1000 Grace column (15 m × 0.53 mm I.D) with 1.20 µm film thickness. The temperature programme set was: 75 °C – 95 °C increasing by 3 °C/minute, 95-200 increasing by 20 °C per minute, which was held for 30 seconds. The detector and injector temperature was 280 °C and 240 °C respectively while the total analysis time was 12.42 minutes.

2.3.4 Unit questionnaire

A questionnaire was completed with each producer or a member of unit staff before sample collection. This included questions on;

A) Feeding practices, such as the number of feed splits, percentages of total daily feed offering per feed, volume of feed mixed, number of pigs per pen and per trough, long/short trough feeding system, agitation time, amount of time required to feed out the mix, water to meal ratio, dietary ingredients and diet composition.
B) Technical aspects of the liquid feeding system including make, age and feed pumping method.
C) Sanitation practices (if any) including washing and disinfection of troughs, mixing tanks, feed pipes etc.

Data from questionnaires were used to categorise units into 'yes' or 'no' for sanitation practices. 'Yes' included all units that carried out some form of washing/disinfecting/power washing and 'No' included all units that did none of the above. The same grouping was used for dietary co-product inclusion with 'yes' for inclusion and 'no' for units that did not include co-products in the diet.

2.3.5 Pig growth data

Annual and quarterly unit technical animal performance data were gathered where possible for the period during which samples were collected. No data were available for unit B. Spearman correlations were performed using this data (see statistical analysis section).

2.3.6 Statistical analysis

Each of the seven samples from the individual units were analysed separately. Subsequently; results were averaged for sampling location (Mix tank, Fresh and Residual) as required for analysis of sampling location using the PROC MEANS statement of SAS® software version 9.4 (SAS Institute, Inc., Cary, NC,

US). Sampling location, the use of co-products, and sanitation practices were analysed for LAB, *Enterobacteriaceae*, *E.* coli, yeast, mould, sample pH and sample temperature using the MIXED procedure of SAS 9.4. Residual counts from unit D were removed from all analysis as they were measured from a swab in Log_{10} CFU/cm^2 while all others counts are expressed as Log_{10} CFU/g.

For the effect of sampling location, unit was included as a random effect. For the effect of co-product inclusion and sanitary protocols, units were grouped into 'Yes' and 'No'. To analyse the effect of dietary co-product inclusion, co-products and sampling location and their relevant interaction were included in the model with unit as a random effect and sampling location included as a repeated measure. The effect of sanitation was analysed similarly. Unit H was investigated on seven occasions. Data were analysed using PROC MIXED with sampling location in the model and sampling occasion as a random effect. The interaction of trough location on the feed circuit (start, middle and end) by sampling location (Fresh and Residual) was investigated for microbial counts, pH and temperature of liquid feed using the MIXED procedure of SAS 9.4. The equivalent mix tank measure (i.e. microbial count, pH or temperature) was included as a co-variate and pig unit was included as a random effect. For the results of proximate, amino acid, lactic acid, ethanol and VFA concentration analyses, sampling location was included in the model with unit as a random effect using PROC MIXED. Isobutyrate data were normal; however, all other VFA data were logged to normalise them prior to analysis.

Spearman correlations were carried out to investigate the relationship between liquid feed pH and temperature in the mix tank and microbial counts at each of the three sampling locations. Spearman correlations were also carried out to

investigate the relationship between microbial counts at each of the three sampling locations and carcass ADG and carcass FCR. To calculate carcass ADG, a kill-out percentage of 65 % was applied to the live-weight of pigs at transfer to the finisher section. Spearman correlations were also used to investigate the relationship between the room temperature where the feed was mixed and the microbial counts at each of the three sampling locations. Similar correlations were also performed to investigate the relationship between temperature in the pig house and microbial counts in the trough samples (Fresh and Residual).

2.4 Results

2.4.1 Microbial counts, pH and temperature of liquid feed at each of the sampling locations (mixing tank, fresh and residual feed)

The mean microbial counts and pH from all pig units at each sampling location are displayed in Figure 2.1. Lactic acid bacteria counts (P<0.001), yeast counts (P <0.001) and sample temperature (P<0.001) increased from the Mix tank to Fresh and again to Residual, while pH declined from Fresh to Residual (P<0.001). Counts of *E. coli* (P <0.001) increased from Fresh to Residual. *Enterobacteriaceae* and mould counts did not differ across sampling locations.

2.4.2 Microbial counts, pH and temperature of liquid feed sampled on multiple occasions from unit H

Results for liquid feed from 7 sampling occasions on unit H are shown in Figure 2.2. Lactic acid bacteria (P<0.001) and yeast counts (P<0.001) increased from the Mix tank to the Fresh and again to the Residual. *Enterobacteriaceae* counts (P<0.01) and *E. coli* counts (P<0.001) were similar in the Mix tank and Fresh but were significantly higher in the Residual. Mould counts were similar across all sampling locations. The pH and temperature of liquid feed in the Mix tank and the Fresh were similar, but the pH was lower in the Residual (P<0.001) and the feed temperature higher (P<0.001).

2.4.3 Effect of trough location on the feeding circuit and sampling location (fresh vs residual) on microbial counts, pH and temperature of liquid feed on the eight pig production units surveyed

There were no significant trough location (start, middle, end) by sampling location (Fresh and Residual only included here) interactions for microbial counts,

pH and temperature of liquid feed. As no interactions were found, the main effect of trough location was considered. Yeast counts were 5.79 $\log_{10}$ CFU/g, 5.94 $\log_{10}$ CFU/g and 5.59 $\log_{10}$ CFU/g (± 0.219 SEM, $P<0.01$) and mould counts were 4.17 $\log_{10}$ CFU/g, 3.91 $\log_{10}$ CFU/g and 3.85 $\log_{10}$ CFU/g (± 0.219 SEM, $P=0.05$) for the start, middle and end of the feed circuit, respectively. Yeast counts were significantly higher in troughs in the middle of the feed circuit than they were in troughs at the end of the feed circuit. Mould counts tended to be lower in troughs at the end of the feed circuit compared with troughs at the start of the feed circuit.

2.4.4 Microbiological analysis of water samples collected on pig units

Results for the microbiological analysis of water samples are shown in Table S 2.1. Coliform were detectable in the water samples from three of the four units on which this test was conducted, with 2×10^0, 4.1×10^1 and 4.3×10^1 CFU detected per 100 ml sample on units D, F and H, respectively. Of the seven units on which water was investigated for *E. coli*, only units E and G had detectable levels (1.09×10^2 and 1.6×10^1 CFU/100 ml, respectively). Water samples from six units were investigated for enterococci and the sample from unit F gave the only positive result. There was variation in the TVC of water samples, with counts ranging from 3.4×10^1 CFU/ml on unit B to 4.56×10^2 CFU/ml on unit E.

2.4.5 Data from questionnaires

2.4.5.1 Feeding practices and systems on the eight pig units surveyed

Results from the questionnaires are shown in Tables 2.1, S 2.2 and S 2.3. All pig units produced their own feed and all carried out night time feeding which was classified as feeding between 18:00 and 06:00. Three of the seven units investigated had long trough feeding systems (units A, B and D) while the other five units had

short trough feeding systems (units C, E, G, F and H). There was some variation in the length of time these feeding systems had been in place, referred to as the age of the feed system in Table 2.1. Feed systems on units A, C, E and H were less than 10 years old, while the feeding systems on units B, D, F and G were 10 or more years old.

The number of feed splits fed per day varied from between 4 and 5 on units A, B, C, D, G and H to more regular feeding (10 and 12 times per day, respectively) on units E and F. All units fed equal percentages of the daily feed allowance at each feeding time. Three units agitated the feed and water mixture for 20 min or longer (units A, B and G). Units C, D, E and F agitated the mixture for less than 20 min, while the agitation time on unit H varied from 5-20min depending on the occasion of sampling. The amount of time required to actually feed the pigs from the first to the last feed valve on the circuit varied hugely. This variation combined with the number of feed splits per day influenced the length of time between taking Fresh and Residual feed samples. On units B, C, D and F, it took 20 min or less to feed out the mixture while on units A, E, G and H it took over 30 min. Unit E took the longest at 120 min.

The number of pigs fed per trough was almost doubled on some units compared to others. However, it should be noted that on some units, one trough was shared between two pens of pigs. The lowest number of pigs fed per trough was on unit H, where only 6 pigs were fed per trough. The troughs on units A, B, C and D fed 25, 24, 28 and 26 pigs, respectively while units E, F and G fed larger groups of 56, 45 and 64 pigs, respectively. The number of pens fed on the sampled feed circuit

also varied hugely. The feed circuit on unit C fed 300 troughs and the number of troughs on the circuits of the other units was much smaller, ranging from 14 to 56.

2.4.5.2 Dietary ingredients used for grow-finisher pigs on the eight units surveyed

All units examined in the survey fed barley- and soybean meal-based diets. Six of eight units used wheat as one of the main ingredients. Four units also included maize as one of the main cereal ingredients, with units A and D combining it with wheat and barley while units F and G replaced wheat in the diet with maize. All units used oil and a premix containing minerals, vitamins and artificial amino acids, as expected. Four units used soya hulls (units A, C, F and G). Three units used liquid co-products; units A, F and G incorporated pot-ale syrup at dietary inclusion rates of 14 %, 5 % and 10 %, respectively, while unit A also used liquid whey at an inclusion rate of 21 %. The effect of dietary co-product inclusion on microbial counts and feed pH are shown in Table 2.2. Dietary co-product inclusion tended to reduce *Enterobacteriaceae* counts in Fresh ($P=0.086$; data not shown) and reduced *E. coli* counts in the Residual ($P<0.05$). Co-products also reduced mould counts at all sampling locations (Mix tank, Fresh and Residual; $P<0.01$). The pH of liquid feed was reduced by co-product inclusion in the Mix tank ($P<0.01$) and Fresh ($P<0.05$).

2.4.5.3 Sanitation practices implemented on the liquid feeding systems and pen troughs on the eight units surveyed

Three units, B, C and F, never cleaned the mixing tank. Unit D cleaned it after each feed and was the only unit to use an additive (formic acid) in the cleaning water. All of the other units used water only to clean the mixing tanks, with unit G

cleaning weekly, unit A every 2 weeks and unit H after each batch of pigs. The cleaning practices used for the feed pipes on each unit were similar to those used for the mix tanks, with units B, C, F and G never cleaning their pipes and unit D doing so after each feed with the formic acid solution allowed to sit in the pipes between feeds. Unit A cleaned the feed pipes every two weeks with water while unit E did likewise on a monthly basis. Unit H had a Hydro Air feeding system, which forces air at pressure through the feed pipes after each feed to clean them. Four units (units A, B, C and F) never cleaned their feed troughs. Unit E cleaned the troughs 2 to 3 times a year and spread lime in the pens and troughs afterwards; however, they did not power wash during the winter months. Unit G used a similar regime, where the troughs were cleaned 3 times per year but not during the winter months and they were the only unit to use a detergent. Units D and H both washed the feed troughs after each batch of pigs, unit D with water only while unit H was the only unit to use a disinfectant after washing. Results from statistical analysis of the impact of sanitation practices on the counts, pH and temperature of liquid feed are presented in Table S 2.4. There were no significant differences observed between sampling locations in response to sanitation practices. 'Yes' and 'no' units had similar microbial counts, pH and temperature of samples at each sampling location.

2.4.6 Correlations between liquid feed microbial counts, pH and temperature at each of the three sampling locations and carcass average daily gain and carcass feed conversion ratio

Results from the Spearman correlations are shown in Table S 2.5. The lactic acid bacteria count in Residual was positively correlated with carcass FCR ($P<0.05$, $r=0.505$).

2.4.7 Correlations between pH and temperature in the mix tank and counts at the three sampling locations on the eight units surveyed

Results of the correlations are shown in Table 2.3. A positive relationship between feed pH and *Enterobacteriaceae* counts (P=0.02, r=0.478) and feed pH with mould counts (P<0.001, r=0.706) was found in the Mix tank. Feed temperature in the Mix tank also had a positive relationship with LAB (P<0.01, r=0.595) and yeast (P=0.01, r=0.50) counts in the mixing tank, while a negative relationship was observed for the temperature of the feed in the mixing tank with mould (P<0.01, r= -0.524) counts at the same location.

When the pH of feed in the mixing tank was correlated with Fresh counts, positive relationships were noted for *E. coli* (P<0.01; r=0.577) and mould (P<0.001, r=0.762) counts while a negative relationship with yeast counts (P<0.001, r= -0.690) was noted. Temperature of liquid feed in the Mix tank was positively correlated with yeast counts in Fresh (P=0.05, r=0.402). The temperature of liquid feed in the mixing tank was also positively correlated with counts of LAB (P<0.01, r=0.607), *E. coli* (P=0.03, r=0.441), yeast (P<0.01, r=0.545) and mould (P=0.03, r=0.456) in Residual. Results of the correlations for unit H are shown in Table 2.4. The pH of liquid feed in the Mix tank was positively correlated with *Enterobacteriaceae* counts in Fresh (P<0.01; r=0.796) and negatively correlated with mould counts in Residual (P=0.02, r= -0.670). The temperature of liquid feed in the Mix tank was also positively correlated with *Enterobacteriaceae* (P<0.01, r=0.779), *E. coli* (P<0.01; r=0.804), and mould (P<0.001, r=0.981) counts while negatively correlated with LAB (P=0.03, r= -0.664) counts in the mixing tank. Negative correlations were also observed between liquid feed temperature in the Mix tank and *Enterobacteriaceae*

counts in Fresh (P=0.02, r= -0.689) and Residual (P<0.001, r=0.963) and *E. coli* (P=0.02, r= -0.675) counts in Residual.

2.4.8 Correlations between mix tank microbial counts and trough microbial counts (fresh and residual) on the eight units surveyed

The results of the correlations between counts in the Mix tank with Fresh and Residual counts are shown in Table S 2.6. Positive correlations were observed between counts of LAB in the Mix tank and counts of LAB (P<0.001, r=0.838), *E. coli* (P=0.03, r=0.451) and yeast (P<0.001, r=0.715) in Fresh and LAB (P<0.001, r=0.764), *E. coli* (P=0.04, r=0.426), yeast (P<0.001, r=0.855) and mould (P=0.02, r=0.470) in Residual feed. *Enterobacteriaceae* counts in the Mix tank had a positive relationship with *Enterobacteriaceae* (P<0.01, r=0.541) and yeast (P<0.01, r=0.541) counts in Fresh and with yeast (P=0.02, r=0.491) counts in Residual. *E. coli* counts in the mixing tank were positively correlated with *Enterobacteriaceae* (P<0.01, r=0.537) and *E. coli* (P=0.02, r=0.464) counts in Residual. Yeast counts in the Mix tank were positively correlated with yeast counts (P<0.001, r=0.759) in Fresh and negatively correlated with mould (P<0.001, r= -0.689) counts in Fresh. There was also a positive correlation between yeast counts in the Mix tank and yeast (P<0.001, r=0.642) counts in the Residual feed. Mould counts in the Mix tank were positively correlated with *E. coli* (P<0.01, r=0.562) and mould (P<0.01, r=0.600) counts in Fresh.

Correlations between microbial counts in the Mix tank and microbial counts in Fresh and Residual on unit H are shown in Table S2.7. When the counts from unit H on seven occasions were compared, *Enterobacteriaceae* counts in the Mix tank were negatively correlated with *E.coli* (P<0.01, r= -0.660) counts in the Residual

feed. *E. coli* counts in the Mix tank were negatively correlated with *E. coli* (=0.04, r= -0.504) counts in Residual and positively correlated with yeast (P<0.01, r=0.661) counts in Residual. Yeast counts in the Mix tank were positively correlated with yeast (P<0.01, r=0.616) counts in Residual. Mould counts in the Mix tank were negatively correlated with *Enterobacteriaceae* counts in Fresh (P<0.01, r= -0.675) and *Enterobacteriaceae* counts in Residual (P=0.02, r= -0.547).

2.4.9 Correlations between mix room temperature and pig room temperature and microbial counts on unit H

Correlations between the temperature in the mix room and the pig room with microbial counts on unit H are presented in Table S 2.8. The Mix tanks were located in the 'mix room' and Fresh and Residual trough counts are from troughs in the 'pig room'. For this reason, correlations between mixing tank counts and pig room temperature were not conducted. The temperature in the mix room was positively correlated with *E. coli* (P<0.01, r=0.675) and yeast (P=0.032, r=0.518) counts in the mixing tank. The temperature in the mix room was also positively correlated with yeast (P=0.03, r=0.535) and mould (P=0.02, r=0.575) counts in Residual and negatively correlated with *Enterobacteriaceae* (P<0.01, r= -0.678) counts in Residual. The temperature in the pig room was positively correlated with *Enterobacteriaceae* (P<0.01, r=0.631) and yeast (P=0.03, r=0.523) counts in Fresh and with LAB (P<0.01, r=0.649) and yeast (P=0.03, r=0.535) counts in Residual.

2.4.10 Gross energy, crude protein, ash, neutral detergent fibre and amino acid analysis of liquid feed from the eight units surveyed

The results of the proximate and amino acid analysis of the liquid feed samples are shown in Table 2.5. The GE (P<0.001) content and NDF (P<0.001)

percentage in liquid feed reduced from the Mix tank to Fresh and again to Residual. The CP ($P<0.001$), and ash ($P<0.001$) content were similar in the Mix tank and Fresh but were lower in Residual. The lysine ($P<0.001$), methionine ($P<0.001$) and threonine ($P<0.001$) content in the Mix tank and Fresh were similar, but lower in Residual.

2.4.11 Ethanol, lactate and volatile fatty acid content of liquid feed from the eight units surveyed

Results of the ethanol, lactate and VFA analysis of liquid feed samples are shown in Table 2.6. L-lactate ($P<0.001$), D-lactate ($P<0.001$), total lactic acid ($P<0.001$) and ethanol ($P<0.001$) concentrations were similar in the Mix tank and Fresh but increased in Residual. Ethanol was not detected in the mix tanks of 6 of the 8 units surveyed. Acetate ($P<0.001$), propionate ($P<0.001$) and total VFA ($P<0.001$) concentrations were similar in the Mix tank and Fresh but increased in Residual. Isobutyrate concentrations increased in the Fresh but were similar in the Mix tank and Residual ($P<0.001$). Butyrate concentrations were higher in the Residual than the Mix tank ($P<0.05$). Protein-derived VFAs were similar in the Mix tank and Fresh but lower in the Residual ($P<0.001$). There were no observed differences in isovalerate, valerate or the acetate:propionate ratio between sampling locations ($P>0.05$).

2.5 Discussion

This study examined, for the first time, the microbial and physio-chemical profile of liquid feed for grow-finisher pigs on commercial Irish pig units. As limited guidelines on liquid feeding system hygiene exist, this study is fundamental to identify existing problems and to aid in the design of sanitation protocols for liquid feeding systems when required. Spontaneous fermentation (Beal et al., 2005; Brooks et al., 2001a; Canibe and Jensen, 2003; Geary et al., 1999; Geary et al., 1996; Plumed-Ferrer et al., 2004; Russell et al., 1996) leading to amino acid degradation (Brooks, 2008; Canibe et al., 2007; Canibe and Jensen, 2003; Missotten et al., 2010; Pedersen et al., 2002; Shurson, 2009) have been well documented in liquid feed, but much of this work was performed *in-vitro* or using deliberately fermented liquid feed. The current study demonstrates that spontaneous fermentation and its associated problems are commonplace when fresh liquid feeding is practiced on commercial pig production units.

The characteristics of spontaneous fermentation as described by (Geary et al., 1996; Russell et al., 1996) were obvious in the current study. These included the incremental increase in LAB and yeast counts from the Mix tank to Fresh and again to Residual, the decrease in pH from Fresh to Residual and the increase in lactic acid concentrations from Fresh to Residual. The results of the current study agree with the findings of Plumed-Ferrer et al. (2004) who concluded that LAB growth caused spontaneous fermentation in liquid feed, thereby reducing feed pH. In that study this was not surprising, as even though the feed was referred to as 'fresh' liquid feed, 10 – 15 % of the liquid feed from the previous feed mix remained in the mixing tank at all times and would have acted as an inoculum, whereas in the current study feed was freshly mixed with water just before each feeding.

Yeast fermentation was also evident in the current study as demonstrated by the increase in yeast counts from the Mix tank to Fresh and again to Residual and an associated increase in ethanol concentrations in Residual. Yeast fermentation is considered undesirable, as the alcohol produced from starch in the feed negatively impacts feed palatability for pigs (Brooks et al., 2001b) and the energy value of the feed is reduced (Brooks, 2008). Amino acid degradation (particularly lysine, methionine and threonine) from the Mix tank and Fresh to Residual in the current study further supports the fact that spontaneous fermentation was occurring in the liquid feed. Lysine, methionine and threonine concentrations in liquid feed were reduced by 35.6 %, 15.4 % and 19.1 %, respectively, when the Mix tank and Residual feed are compared in the present study. Microbes use the free amino acids in liquid feed for their own growth, resulting in reduced amino acid concentrations being available for pig growth (de Lange et al., 2006; Niven et al., 2006). Amino acid concentrations were greatly reduced during the liquid feeding process.

Increased lactic acid, acetate, propionate and total VFA concentrations were observed in Residual compared to the Mix tank and Fresh in the current study. Lactic acid concentrations increased by 94.7 %, ethanol concentrations by 88.0 % and acetic acid concentrations by 66.7 % when concentrations in the Mix tank and Residual are compared. When the Fresh and Residual are compared, lactic acid concentrations increased by 71.6 %, ethanol by 68.4 % and acetic acid by 55.8 %. Concentrations of lactic acid were higher than the standard value for 'residue free' liquid feed of 0 – 10 mmol/kg liquid feed at all three sampling locations, while acetic acid concentrations were below the standard value of 0 – 10 mmol/kg liquid feed in the Mix tank and Fresh, but slightly higher in Residual (Vils et al., 2018). The lactic acid concentrations in the current study in Residual feed were higher than those

reported in the control diet by Vils et al. (2018); however, the acetic acid concentrations were lower in Residual feed in the current study. Nonetheless, natural or spontaneous fermentation cannot be relied upon to produce sufficient levels of short-chain fatty-acids to prevent pathogen proliferation (Beal et al., 2005). This is likely to have been the case in the current study as no differences in *Enterobacteriaceae* counts between sampling locations were observed and the pH in the feed troughs (even in the Residual feed) did not fall below 5 [pH 4.0 is required to inhibit enteric bacteria in feed; (Geary et al., 1999)]. It has also been suggested that a pH of <4.5 combined with 150mM lactic acid or 80mM acetic acid concentrations are the conditions necessary to reduce or eliminate *Salmonella* concentrations using FLF (Brooks, 2008). Concentrations of lactic acid exceeded these levels in Residual feed, but the pH and acetic acid concentrations did not, and it appears that the pH reduction was not sufficient enough to inhibit enteric bacteria in spontaneously fermented liquid feed. The conversion of complex carbohydrates into easily-digestible short-chain fatty acids that increase nutrient absorption and pig growth is considered an advantage of fermentation (Plumed-Ferrer et al., 2004). However, increased concentrations of acetic acid have also been associated with reduced feed palatability which can reduce the feed intake of pigs (Brooks et al., 2001a; Plumed-Ferrer and Von Wright, 2009; Scholten et al., 1999).

It is interesting to compare the results of the repeated sampling on unit H with the results from all commercial units which were sampled on one occasion only (it must be noted that the mean of the data from unit H was included as one unit's data for the eight unit analysis). The increase of LAB counts from the Mix tank to Fresh and again to Residual was evident in both sets of results, as were the changes

in sample pH and *E. coli*, yeast and mould counts. One difference observed was that on unit H, an increase in *Enterobacteriaceae* counts was observed in the Residual over the other two sampling locations while no differences in *Enterobacteriaceae* counts were observed for the eight units. Unit H had floor level troughs which may have facilitated greater contamination with faecal material; whereas step-up troughs were installed on the majority of the other units investigated. Nonetheless, faecal contamination of feed in troughs the most likely cause of increased *E. coli* counts in Residual over Fresh feed observed in the current study both on the eight units and particularly so on unit 8. Sample temperature increased from the Mix tank to Fresh and again to the Residual on the eight units, as expected due to the effect of heat within the pig houses, but on unit H, the Mix tank and Fresh were similar in temperature. A pneumatic feed delivery system and extremely short length feed delivery distance existed on unit H most likely explaining this difference. Nonetheless, results from repeated sampling of unit H were very consistent with those from the 8 units sampled only once.

Sanitation practices, did not impact the microbiological quality of liquid feed in the current study. On unit D, formic acid was used to rinse the mixing tank and pipes between feeds and this may explain why *E. coli* was not detected in the fresh trough-sampled feed on this unit. Likewise, unit F had undetectable *E. coli* in Fresh trough samples, however, the mix tank and pipelines were never washed on this unit. The liquid by-product pot-ale syrup was used as a feed ingredient on and it is highly likely that its low pH had an inhibitory effect on *E. coli* in the liquid feed. Presser et al. (1997) found that some *E. coli* strains grow slower below pH 5.5 at different concentrations of lactic acid. Cleaning and disinfection of liquid feeding systems can reduce bacterial counts by 2-3 log units on commercial units; however, microbial

count reduction is only temporary, returning to original levels as soon as one week after cleaning and disinfecting (Brooks, 2008; Royer et al., 2004). Additionally Royer et al. (2004) reported that cleaning the mixing tank is of little benefit if the feed delivery pipes/lines are not also cleaned and that sanitation of liquid feeding systems is not justifiable in the absence of clinical problems in pigs.

The use of co-products on units A and G may also explain the lower *E. coli* counts in the liquid feed from these units. The use of liquid co-products such as whey (unit A) and pot-ale syrup (units A, F and G) reduced liquid feed pH in the Mix tank and Fresh. These co-products have a low pH; 3.54 to 3.81 for pot-ale syrup (Graham et al., 2012) and pH ~ 4.0 for liquid whey (ranging from 3.7 to 5.7) (Plumed-Ferrer et al. (2004). Another strategy to help minimise spontaneous fermentation and pathogen growth in liquid feed may be to minimise the temperature of the water used for liquid feed preparation. Numerous positive correlations between the liquid feed temperature in the mixing tank and counts of bacteria, yeasts and moulds in the troughs were found in the present study.

As there is no legislation in place for animal drinking water, Ireland follows the standards for potable water for humans set out by the European Union (Drinking Water) Regulations 2014 S.I. No. 122 of 2014 which states that the tolerated level of *E. coli*, enterococci and total coliform bacteria is 0/100 ml. It was evident that the water used for preparing liquid feed on pig production units in the current study did not always comply with these standards. Despite this, *E. coli* or *Enterobacteriaceae* counts in liquid feed on units where the water standards were not met did not appear to be hugely impacted. This suggests that the bacterial load present in the feed

component of the liquid mix and resultant spontaneous fermentation likely has a greater impact on the microbial load of liquid feed than the water used for mixing.

Overall, this study shows clear evidence of uncontrolled spontaneous fermentation of liquid feed in feed troughs when fresh liquid feeding is practiced on Irish pig production units. This was evidenced by increased LAB and yeast counts, a decrease in pH and increased ethanol, lactic acid and acetic acid concentrations when Residual liquid feed from troughs was compared with feed from the Mix tank and Fresh troughs. Clear evidence of amino acid degradation and a reduction in gross energy was also evident in liquid feed troughs. Feed system sanitation as practiced in the current study had little impact on the microbiological quality of liquid feed. However, the inclusion of low pH liquid co-products in the liquid feed mixture reduced the pH and undesirable microbial load (i.e. *E. coli* and mould) and increased the counts of microbes considered beneficial (i.e. LAB) in liquid feed. Future work should focus on interventions such as dietary acidification and controlling water temperature used to prepare liquid feed to prevent spontaneous uncontrolled fermentation and the associated deterioration of nutritional quality of feed occurring when fresh liquid feeding is practiced.

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2.7 Tables

Table 2.1 Summary of survey results on liquid feeding practices in the finisher section on eight commercial pig production units

	Unit[1]							
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H[2]**
Model of feed system	Funki	Funki	Funki	Funki	Datamix	BD[3]	BD	BD
Trough type	Long	Long	Long[4]	Long	Short	Short	Short	Short
Age of feed system (yrs[5])	4	10	0.167	10	3	20	10	4
Feed splits (feeds/day)	5	4	4	4	10	12	4	4 or 5
Water to meal ratio (FM[6])	2.1:1	2.4:1	3.5:1	3:1	2.6:1	3.4:1	3.1:1	2.5:1
Volume in mix tank (kg)	2,632	1529	11,000	1,500	3,000	3,000	11,000	~ 150
Agitation time (min)	23	20	10	2[7]	8 to 10	6[7]	30	5 to 20
Time for feed-out (min)	45	10	20	15	120	5	30	60
Pigs fed per trough	50	24	28	26	56	45	64	6
Pens on feed circuit	56	36	300	14	24	17	23	36
Pump method	Hydraulic	Hydraulic	Hydraulic	Hydraulic	Hydraulic	Hydraulic	Hydraulic	Pneumatic
Co-product inclusion	LW[9], PAS[10]					PAS	PAS	

[1] All units were home milling, feeding at night-time (i.e. feeding between 18:00 and 06:00) and feeding equal percentages of the daily feed allowance at each feed time
[2] Unit H: Research unit where feeds/day, volume in mix tank, agitation time and time for feed-out varied depending on experimental criteria. This unit was investigated on seven occasions; whereas all other units were investigated on one occasion
[3] BigDutchman
[4] Although this unit had a long trough feeding system, a large volume of feed was mixed and fed continuously over a period of time

[5] Years
[6]FM: Fresh matter
[7] Unit D and F both had a satellite tank. Feed goes to the satellite tank after the mix tank prior to being fed out. On unit D feed resides there for a very short period prior to feed out so the 2 min agitation is in the Mix tank. On unit F, agitation is 3 min in the Mix tank and 3 min in the satellite tank
[8]Estimated percentage of the daily total feed mix volume prepared on the unit
[9]Liquid whey
[10]Pot-ale syrup

Table 2.2 The influence of dietary by-product inclusion on counts of *Enterobacteriaceae, E. coli*, mould and sample pH at the three sampling locations on the eight pig production units surveyed[1,2]

	Co-product inclusion[3]			
	No	**Yes**	**SEM**	**P-value**
E. coli **[2], log$_{10}$ CFU/g**				
Mix tank[4]	2.02	2.00	0.021	0.47
Fresh[5]	2.67	2.06	0.370	0.22
Residual[6]	3.90	3.26	0.210	0.04
Overall			0.070	0.001
Mould[2], log$_{10}$ CFU/g				
Mix tank	4.13	3.17	0.249	0.01
Fresh	4.29	3.31	0.249	0.01
Residual	4.56	3.24	0.249	<0.01
Overall			0.158	<0.001
pH				
Mix tank	6.29	5.32	0.231	<0.01
Fresh	5.94	5.11	0.231	0.02
Residual	5.02	5.04	0.231	0.95
Overall			0.201	0.04

[1]Least square means and pooled standard errors of the mean

[2]Dietary co-product inclusion did not influence lactic acid bacteria (LAB) or yeast counts or sample temperature at each of the sampling locations. LAB Mix tank = 6.81, log$_{10}$CFU/g and 6.28 log$_{10}$CFU/g (SEM=0.559; P=0.47); LAB Fresh 8.21 and 7.63 log$_{10}$CFU/g (SEM=0.559; P=0.42); LAB Residual = 9.02 and 8.58 log$_{10}$CFU/g (SEM=0.559; P=0.55); *Enterobacteriaceae* Mix tank = 5.06 and 4.79 log$_{10}$CFU/g (SEM=0.167; P=0.23); *Enterobacteriaceae* Fresh = 5.09 and 4.66 log$_{10}$CFU/g (SEM=0.182; P=0.09); *Enterobacteriaceae* Residual = 4.96 and 4.69 log$_{10}$CFU/g (SEM=0.338; P=0.54); Yeast Mix tank 4.44 and 5.12 log$_{10}$CFU/g (SEM=0.470; P=0.28); Yeast Fresh = 5.35 and 5.42 log$_{10}$CFU/g (SEM=0.470; P=0.90); Yeast Residual = 6.21 and 6.01 log$_{10}$CFU/g (SEM=0.470; P=0.74); Temperature Mix tank = 11.1 and 11.6 °C (SEM=0.74; P=0.59); Temperature Fresh = 13.1 and 13.9 °C (SEM=1.54; P=0.72); Temperature Residual = 15.7 and 14.7 °C (SEM=1.58; P=0.60) for 'no' and 'yes' co=product inclusion as above, respectively.

[3]Data from 5 pig units included for 'No' co-product inclusion; data from 3 pig units included for 'Yes'

Table 2.3 Spearman correlations of the pH and temperature of liquid feed in the mix tank with microbial counts at the three sampling locations on the eight pig production units surveyed

	Mix tank pH		Mix tank temperature	
	P[1]	r[2]	P	r
Mix tank				
Lactic acid bacteria	0.70	-0.084	<0.01	0.595
Enterobacteriaceae	0.02	0.478	0.67	0.095
E. coli	0.20	0.270	0.24	0.247
Yeast	0.19	0.279	0.01	0.050
Mould	<0.001	0.706	<0.01	-0.524
Fresh				
Lactic acid bacteria	0.83	-0.048	0.03	0.449
Enterobacteriaceae	0.83	0.048	0.33	0.208
E. coli	<0.01	0.577	0.85	0.041
Yeast	<0.001	-0.690	0.05	0.402
Mould	<0.001	0.762	0.15	-0.304
Residual				
Lactic acid bacteria	0.15	-0.302	<0.01	0.607
Enterobacteriaceae	0.59	0.116	0.15	0.302
E. coli	0.34	0.205	0.03	0.441
Yeast	0.19	-0.277	<0.01	0.545
Mould	0.46	0.158	0.03	0.456

[1]P=P-value
[2]r= correlation coefficient

Table 2.4 Spearman correlations of the pH and temperature of liquid feed in the mix tank with microbial counts at the three sampling locations from the seven sampling occasions (unit H)

	Mix tank pH		Mix tank Temperature	
	P[1]	r[2]	P	r
Mix tank				
Lactic acid bacteria	0.59	0.185	0.03	-0.664
Enterobacteriaceae	0.09	0.537	<0.01	0.779
E. coli	0.29	0.351	<0.01	0.804
Yeast	0.72	0.120	0.22	0.404
Mould	0.84	0.070	<0.001	0.981
Fresh				
Lactic acid bacteria	0.31	0.336	0.58	0.189
Enterobacteriaceae	<0.01	0.796	0.02	-0.689
E. coli	0.10	0.523	0.69	-0.136
Yeast	0.62	-0.167	0.91	0.038
Mould	0.82	-0.079	0.79	0.091
Residual				
Lactic acid bacteria	0.43	0.264	0.93	-0.029
Enterobacteriaceae	0.10	-0.528	<0.001	0.963
E. coli	0.23	-0.395	0.02	-0.675
Yeast	0.52	-0.217	0.12	0.500
Mould	0.02	-0.670	0.29	0.354

[1]P=P-value

[2]r= correlation coefficient

Table 2.5 Proximate and amino acid analysis of liquid feed for grow-finisher pigs sampled from three locations on the eight pig production units surveyed (presented on a DM basis)[1]

	Sampling location[2]				
	Mix tank	**Fresh**	**Residual**	**SEM**	**P-value**
Gross energy, MJ/kg	19.56[a]	18.86[b]	18.35[c]	0.198	<0.001
Crude protein, %	23.14[a]	23.20[a]	19.45[b]	1.081	<0.001
Ash, %	3.59[a]	3.52[a]	5.97[b]	0.425	<0.001
NDF, %	19.06[a]	16.19[b]	14.59[c]	0.785	<0.001
Amino acids, g/kg					
Lysine	10.20[a]	9.63[a]	6.57[b]	0.530	<0.001
Methionine	4.55[a]	4.72[a]	3.85[b]	0.214	<0.001
Threonine	8.59[a]	8.83[a]	6.95[b]	0.464	<0.001
Valine	11.36[a]	10.94[a]	9.05[b]	0.598	<0.001
Isoleucine	8.98[a]	9.10[a]	7.22[b]	0.528	<0.001
Leucine	16.36[a]	16.74[a]	13.83[b]	0.790	<0.001
Serine	10.65[a]	10.86[a]	8.76[b]	0.603	<0.001
Glutamic acid	43.44[a,b]	48.13[a]	39.68[b]	2.762	<0.001
Glycine	10.05[a]	9.58[a]	7.74[b]	0.472	<0.001
Alanine	9.53[a]	9.40[a]	7.85[b]	0.420	<0.001
Cysteine	1.59	1.27	1.46	0.136	0.26
Cysteic acid	5.64[a]	5.77[a]	4.77[b]	0.270	<0.001
Taurine	2.81[a]	2.05[b]	1.71[b]	0.155	<0.001

	Sampling location[2]				
	Mix tank	**Fresh**	**Residual**	**SEM**	**P-value**
Aspartic acid	20.37[a]	20.66[a]	15.62[b]	10.223	<0.001
Tyrosine	5.37[A]	4.64[A,B]	4.26[B]	0.526	0.09
Phenylalanine	10.67	8.75	9.22	1.218	0.20
Histidine	7.00[A]	6.17[A,B]	5.92[B]	0.442	0.06
Arginine	13.84[a]	13.51[a]	9.89[b]	0.847	<0.001
Proline	13.78[a,b]	15.14[a]	13.51[b]	0.714	0.01

[1]Least square means and pooled standard errors of the mean

[2]Values for the mix tank are the mean of data from eight samples (one per pig unit), Fresh represents mean of 24 samples (3 per unit) and Residual represents mean of 21 samples (3 per unit except for unit D which did not have any residual feed in troughs)

[a,b,c] Within each row, values that do not share a common superscript are significantly different (P<0.05)

[A,B,C] Within each row, values that do not share a common superscript tend to be different (0.05<P<0.10)

Table 2.6 D-lactate, L-lactate, total lactic acid, ethanol and volatile fatty acid concentrations in liquid feed from three locations the eight pig production units surveyed

	Sampling location[2]				
	Mix tank	**Fresh**	**Residual**	**SEM**	**P-value**
L-lactate, mmol/kg	5.3[a]	26.4[a]	105.1[b]	15.25	<0.001
D-Lactate, mmol/kg	6.0[a]	33.5[a]	107.0[b]	15.04	<0.001
Total lactic acid, mmol/kg	11.3[a]	59.9[a]	212.1[b]	30.00	<0.001
Ethanol, mmol	1.8[a]	5.0[a]	15.8[b]	2.13	<0.001
Volatile fatty acids mmol/kg					
Acetate	3.51[a]	4.66[a]	10.55[b]	0.225	<0.001
Propionate	0.03[a]	0.04[a]	0.12[b]	0.183	<0.001
Isobutyrate	0.19[b]	0.33[a]	0.12[b]	0.039	<0.001
Butyrate	0.017[b]	0.021[a,b]	0.032[a]	0.1671	0.024
Isovalerate	0.02	0.02	0.02	0.125	0.19
Valerate	0.07	0.06	0.04	0.286	0.19
Total VFA	3.91[a]	5.23[a]	11.00[b]	0.215	<0.001
Acetate:Propionate	102.06	108.79	89.44	4.603	0.40
Protein-derived VFA	0.27[a]	0.42[a]	0.14[b]	0.192	<0.001

[1]Least square means and pooled standard errors of the mean

[2]Values for the mix tank are the mean of data from eight samples (one per pig unit), Fresh represents mean of 24 samples (3 per unit) and Residual represents mean of 21 samples (3 per unit except for unit D which did not have any residual feed in troughs)

[a,b,c] Within each row, values that do not share a common supers

2.8 Figures

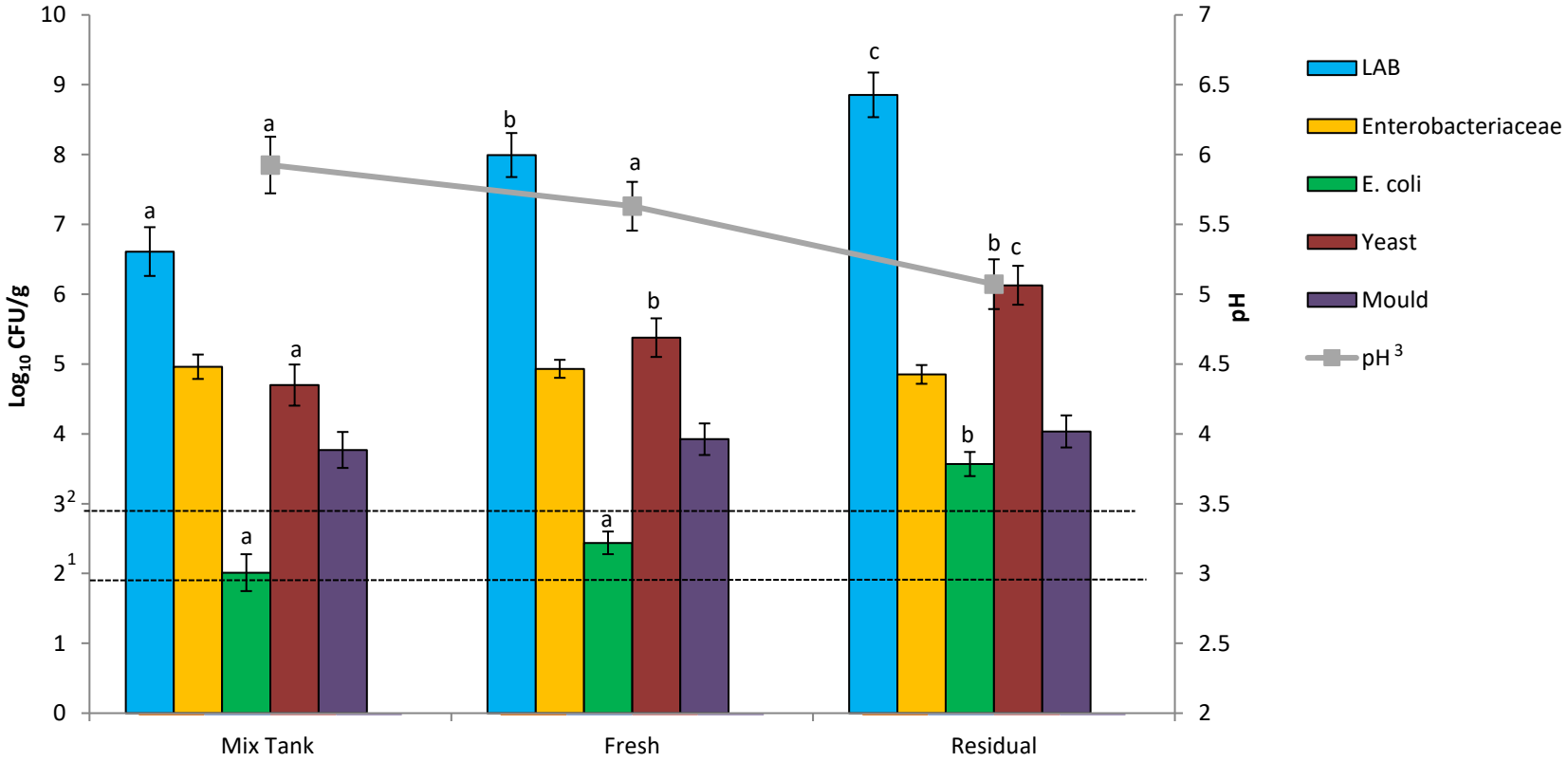
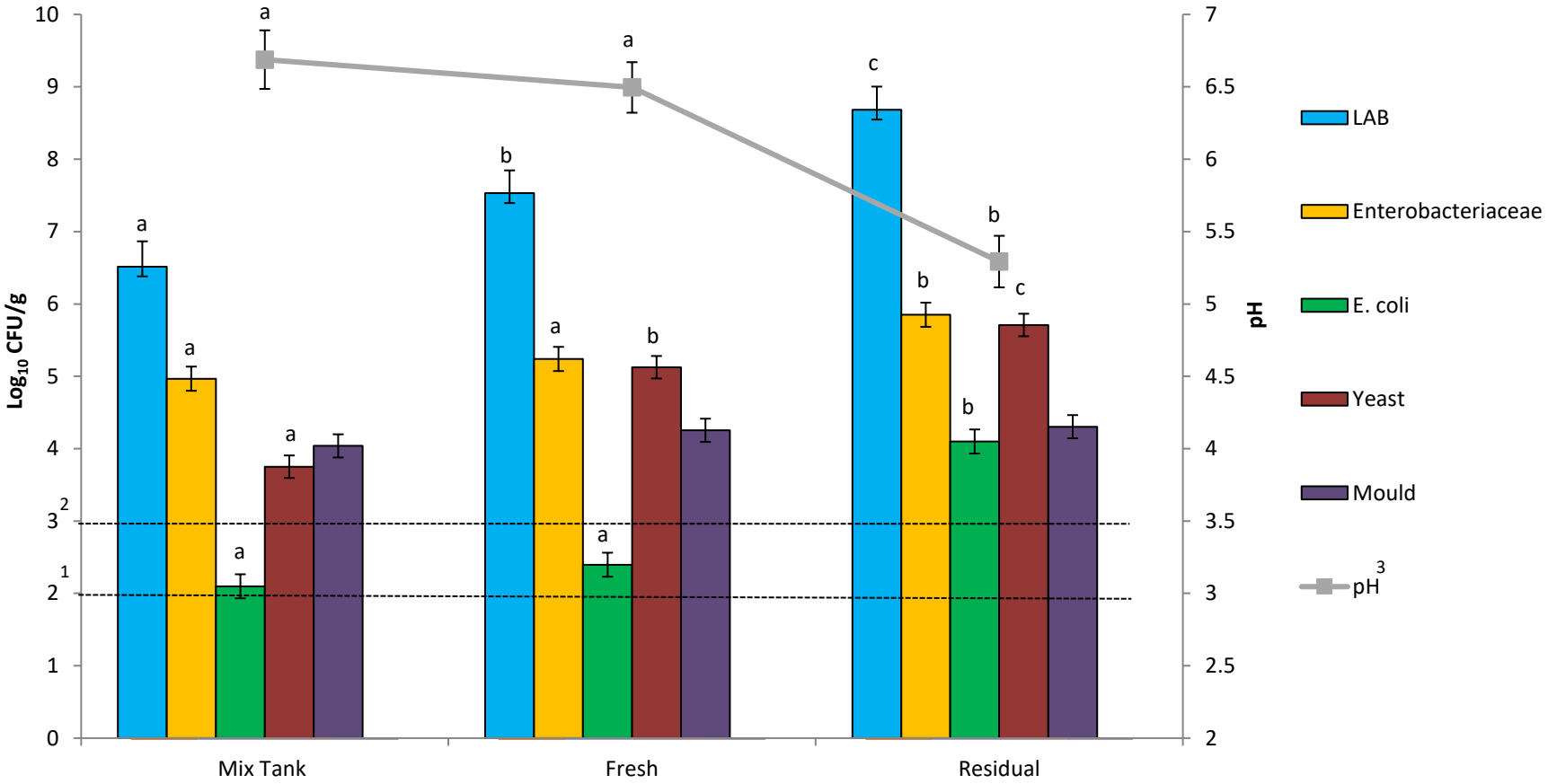

Log10 CFU/g
pH
Mix Tank
Fresh
Residual
LAB
Enterobacteriaceae
E. coli
Yeast
Mould
pH 3

Figure 2.1 Mean counts ± SEM of lactic acid bacteria, *Enterobacteriaceae, E. coli,* yeast and mould in liquid feed samples from the mixing tank, fresh feed from troughs and residual feed from troughs on eight commercial pig production units surveyed

[1] Detection limit for lactic acid bacteria, *Enterobacteriaceae,* and *E. coli* (2 $\log_{10}$ CFU/g)
[2] Detection limit for yeast and mould (3 $\log_{10}$CFU/g)
[3]Sample pH should be read from the secondary vertical axis. The mean sample temperature at each of the three sampling locations was: 11.3 °C, 13.4 °C and 15.4 °C ($P<0.001$; SEM 0.94) in the Mix tank, Fresh and Residual feed, respectively. Temperature in the mix tank was significantly lower than in Fresh, which was also significantly lower than in Residual.
[a,b,c] Within each bar colour and the line representing pH, bars and data points, respectively, that do not share a common superscript are significantly different ($P<0.05$)


Log10 CFU/g
pH
Mix Tank
Fresh
Residual
LAB
Enterobacteriaceae
E. coli
Yeast
Mould
pH
a
a
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b
a
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b
a
b
c
b
b
c

Figure 2.2 Mean counts ± SEM of lactic acid bacteria, *Enterobacteriaceae, E. coli,* yeast and mould in liquid feed samples from the mixing tank, fresh feed from troughs and residual feed from troughs (unit H)

[1] Detection limit for lactic acid bacteria, *Enterobacteriaceae,* and *E. coli* (2 $\log_{10}$ CFU/g)
[2] Detection limit for yeast and mould (3 $\log_{10}$CFU/g)
[3]Sample pH should be read from the secondary vertical axis. The mean sample temperature at each of the three sampling locations was: 13.8 °C, 14.8 °C and 16.6 °C ($P<0.001$, SEM 1.22) in the Mix tank, Fresh and Residual feed, respectively. Temperature in the mix tank and Fresh were similar, but significantly lower than in Residual.
[a,b,c] Within each bar colour and the line representing pH, bars and data points, respectively, that do not share a common superscript are significantly different ($P<0.05$)

2.9 Supplementary information

Table S 2.1 Microbiological analysis of water used to prepare liquid feed for finisher pigs on the eight pig production units surveyed

	Pig Unit							
	A[1]	B	C	D	E	F	G	H
Coliforms, CFU[2]/100 ml	NT[3]	NT	ND[4]	2.0×10^0	NT	4.1×10^1	NT	4.3×10^1
E. coli, CFU/100 ml	NT	ND	ND	ND	1.09×10^2	1.6×10^1	ND	ND
Enterococci, CFU/100 ml	NT	ND	NT	ND	ND	Positive[5]	ND	ND
Total viable count, CFU/ml	NT	3.4×10^1	5.4×10^1	1.40×10^2	4.56×10^2	3.64×10^2	UC[6]	4.52×10^2

[1]N/A: Not applicable
[2]CFU: Colony forming units
[3]NT: Not tested
[4]ND: Non-detectable
[5]Positive: Colonies above the detection limit but could not be enumerated
[6]UC: Uncountable

Table S 2.2 Dietary ingredients used in finisher diets on the eight pig production units surveyed

	Unit							
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**
Barley	✓	✓	✓	✓	✓	✓	✓	✓
Wheat	✓	✓	✓	✓	✓			✓
Soya bean meal	✓	✓	✓	✓	✓	✓	✓	✓
Maize	✓			✓		✓	✓	
Oil (soya or not specified)	✓	✓	✓	✓	✓	✓	✓	✓
Soya hulls	✓		✓			✓	✓	
Minerals & vitamins	✓	✓	✓	✓	✓	✓	✓	✓
Pot ale syrup	✓[1]					✓[2]	✓[3]	
Liquid whey	✓[4]							

[1] Inclusion rate 14 %
[2] Inclusion rate 5 %
[3] Inclusion rate 10 %
[4] Inclusion rate 21 %

Table S 2.3 Cleaning and sanitation practices on liquid feed systems for finisher pigs on the eight pig production units surveyed

	Unit A	Unit B	Unit C	Unit D	Unit E	Unit F	Unit G	Unit H
Cleaning MT[1]	2x/month[2]	Never	Never	4x/day	1x/month	Never	1x/week	1x/10weeks
Cleaning agent MT	Water	N/A[3]	N/A	Formic acid[4]	Water	N/A	Water	Water
Cleaning pipes	2x/month	Never	Never	4x/day[5]	1x/month	Never	Never	4 to 5x/day
Cleaning agent pipes	Water	N/A	N/A	Formic acid[6]	Water	N/A	N/A	Air[7]
Cleaning troughs	Never	Never	Never	1x/ ~ 12weeks	2 to 3x/year[8]	Never	3x/year[8]	1x/10weeks
Cleaning agent troughs	N/A	N/A	N/A	Water	Water & lime[9]	N/A	Water & det.[10]	Water & dis.[11]

[1]MT: Mix tank
[2] Twice per month
[3]N/A: Not applicable
[4] 85 % Formic acid (Water Technology Limited, Cork, Ireland) at a 1 % inclusion rate with water
[5]4x/day represents once after each feed daily
[6] 85 % Formic acid (Water Technology Limited, Cork, Ireland) at a 1 % inclusion rate with water sits in the feed pipes between feeds
[7]Hydro Air liquid feeding system
[8]Except during the winter months
[9] Water followed by lime
[10] Water followed by detergent (Top Foam™, MS Schippers, Bladel, The Netherlands)
[11] Water followed by disinfectant (Hyperox, Du Pont, Sudbury, United Kingdom)

Table S 2.4 The influence of sanitation practices at the three sampling locations on the eight pig production units surveyed

	Sanitation carried out			
	No	Yes	SEM	P-value
Lactic acid bacteria[1]				
Mix tank	6.41	6.73	0.573	0.66
Fresh	8.15	7.90	0.573	0.74
Residual	9.11	8.68	0.573	0.57
Overall			0.541	0.87
Enterobacteriaceae[1]				
Mix tank	5.08	4.89	0.179	0.41
Fresh	5.09	4.83	0.205	0.34
Residual	4.79	4.97	0.348	0.71
Overall			0.210	0.73
E. coli[1]				
Mix tank	2.00	2.02	0.021	0.47
Fresh	2.34	2.50	0.432	0.78
Residual	3.70	3.47	0.253	0.51
Overall			0.175	0.94
Yeast[1]				
Mix tank	4.82	4.63	0.487	0.76
Fresh	5.21	5.48	0.492	0.67
Residual	6.02	6.14	0.526	0.87
Overall			0.470	0.92
Mould[1]				
Mix tank	3.50	3.93	0.408	0.42

	Sanitation carried out			
	No	Yes	SEM	P-value
Fresh	4.03	3.86	0.408	0.76
Residual	4.23	3.91	0.408	0.55
Overall			0.368	0.97
pH				
Mix tank	5.79	6.00	0.333	0.63
Fresh	5.67	5.60	0.333	0.87
Residual	4.23	3.91	0.333	0.67
Overall			0.281	0.96
Temperature, °C				
Mix tank	11.8	11.0	0.71	0.37
Fresh	13.5	13.3	1.18	0.94
Residual	15.3	15.1	1.80	0.96
Overall			1.29	0.83

[1]Counts in Log_{10} CFU/g

Table S 2.5 Carcass average daily gain and carcass feed conversion ratio correlations with microbial counts, pH and temperature from liquid feed at the three sampling locations on the eight pig production units surveyed

	Carcass ADG		Carcass FCR	
	P[1]	r[2]	P	r
Mix tank				
Lactic acid bacteria	0.64	0.107	0.11	0.357
Enterobacteriaceae	<0.001	0.786	<0.01	-0.643
E. coli	0.07	0.408	1.00	0.000
Yeast	0.76	0.071	0.54	0.143
Mould	0.21	0.286	0.35	-0.214
pH	0.05	0.429	0.08	-0.393
Temperature	0.35	-0.214	0.01	0.536
Fresh				
Lactic acid bacteria	0.92	-0.024	0.23	0.271
Enterobacteriaceae	0.02	0.523	<0.01	-0.613
E. coli	0.44	0.178	0.91	-0.026
Yeast	0.02	0.523	0.56	-0.134
Mould	0.35	-0.217	0.91	-0.028
pH	0.77	0.069	0.28	-0.246
Temperature	0.91	-0.028	0.13	0.344
Residual				
Lactic acid bacteria	0.06	-0.455	0.03	0.505
Enterobacteriaceae	0.51	0.166	0.33	-0.241
E. coli	0.72	-0.091	0.21	0.310

	Carcass ADG		Carcass FCR	
	P[1]	r[2]	P	r
Yeast	0.10	0.405	1.00	0.000
Mould	0.61	-0.130	0.87	0.041
pH	0.42	-0.201	0.31	-0.251
Temperature	0.66	-0.113	0.10	0.399

[1]P=P-value

[2]r= correlation coefficient

Table S 2.6 Spearman correlations of mix tank microbial counts with trough microbial counts (fresh and residual) on the eight pig production units surveyed

	Lactic acid bacteria		*Enterobacteriaceae*		*E.coli*		Yeast		Mould	
	P[1]	r[2]	P	r	P	r	P	r	P	r
Fresh										
Lactic acid bacteria	<0.001	0.838	0.19	0.276	0.70	-0.082	0.29	0.226	0.23	0.252
Enterobacteriaceae	0.54	0.131	<0.01	0.541	0.07	0.373	0.96	0.011	0.76	0.066
E.coli	0.03	0.451	0.26	0.237	0.59	0.117	0.30	-0.222	<0.01	0.562
Yeast	<0.001	0.715	<0.01	0.541	0.52	-0.137	<0.001	0.759	0.40	-0.181
Mould	0.54	-0.130	0.59	-0.117	0.37	0.191	<0.001	-0.689	<0.01	0.600
Residual										
Lactic acid bacteria	<0.001	0.764	0.76	0.066	0.52	-0.137	0.25	0.244	0.85	0.042
Enterobacteriaceae	0.88	0.032	0.46	0.158	<0.01	0.537	0.24	-0.247	0.63	0.102
E.coli	0.04	0.426	0.93	0.018	0.02	0.464	0.46	-0.158	0.29	0.226
Yeast	<0.001	0.855	0.02	0.491	0.77	-0.064	<0.001	0.643	0.78	-0.060
Mould	0.02	0.470	0.31	0.216	0.18	0.283	0.27	-0.232	0.10	0.340

[1]P=P-value

[2]r= correlation coefficient

Table S 2.7 Spearman correlations of mix tank microbial counts with trough (fresh and residual) microbial counts for grow-finisher pigs (unit H)

	Lactic acid bacteria		*Enterobacteriaceae*		*E. Coli*		Yeast		Mould	
	P[1]	r[2]	P	r	P	r	P	r	P	r
Fresh										
Lactic acid bacteria	0.72	-0.093	0.85	-0.048	0.13	0.378	0.94	0.021	0.23	0.306
Enterobacteriaceae	0.32	-0.259	0.84	0.053	1.00	0.000	0.19	0.335	<0.01	-0.675
E.coli	0.93	-0.021	0.60	-0.138	0.64	0.123	0.69	-0.106	0.62	0.128
Yeast	0.26	-0.292	0.40	0.220	0.10	0.409	0.19	0.337	0.97	-0.010
Mould	0.50	0.175	0.16	-0.358	0.86	0.047	0.14	-0.374	0.15	0.363
Residual										
Lactic acid bacteria	0.64	-0.122	0.61	-0.132	0.05	0.473	0.05	0.479	0.31	-0.263
Enterobacteriaceae	0.53	-0.163	0.89	-0.037	0.27	-0.283	0.88	-0.040	0.02	-0.547
E.coli	0.06	0.464	<0.01	-0.660	0.04	-0.504	0.27	-0.286	0.09	-0.420
Yeast	0.46	-0.190	0.49	0.181	<0.01	0.661	<0.01	0.616	0.85	0.050
Mould	0.08	0.436	0.08	-0.433	0.63	0.126	0.48	-0.186	0.06	0.468

[1]P=P-value

[2]r= correlation coefficient

Table S 2.8 Spearman correlations of the temperature in the mix room and temperature in pig house with microbial counts in liquid feed for grow-finisher pigs at the three sampling locations (unit H)

	Mix Room Temp		Pig Room Temp	
	P[1]	r[2]	P	r
Mix Tank				
Lactic acid bacteria	0.74	0.086	.	.
Enterobacteriaceae	0.92	-0.028	.	.
E.coli	<0.01	0.675	.	.
Yeast	0.03	0.518	.	.
Mould	0.23	0.306	.	.
Fresh				
Lactic acid bacteria	0.25	0.294	0.40	0.219
Enterobacteriaceae	0.22	-0.314	<0.01	0.631
E.coli	0.95	0.018	0.55	0.155
Yeast	0.84	0.053	0.03	0.523
Mould	0.41	0.212	0.49	-0.180
Residual				
Lactic acid bacteria	0.09	0.419	<0.01	0.649
Enterobacteriaceae	<0.01	-0.678	0.12	0.403
E.coli	0.60	-0.139	0.41	-0.214
Yeast	0.03	0.535	0.03	0.535
Mould	0.02	0.575	0.12	-0.395

[1]P=P-value

[2]r= correlation coefficient

3. Effect of wet/dry feeding and fresh, fermented whole diet and fermented cereal liquid feeding on feed microbiology and growth in grow-finisher pigs

F.M. O' Meara, G.E. Gardiner, J.V. O' Doherty, D. Clarke, W. Cummins, P.G. Lawlor. 2020. Effect of wet/dry, fresh liquid, fermented whole diet liquid, and fermented cereal liquid feeding on feed microbial quality and growth in grow-finisher pigs. *Journal of Animal Science*. 98 (6): skaa166 doi:10.1093/jas/skaa166

3.1 Abstract

Fermented liquid feeding has proved beneficial for weaner pigs; however, there are limited reports on its effect on the growth and feed conversion efficiency (**FCE**) of grow-finisher pigs. Microbial decarboxylation of amino acids is associated with whole diet fermentation, while wet/dry and liquid feeding reportedly improve growth compared to dry feeding. The objective of this study was to determine the effect of wet/dry feeding and fresh, fermented whole diet and fermented cereal liquid feeding on pig growth, feed efficiency and carcass quality in grow-finisher pigs. Pigs were allocated to one of four dietary treatments in two experiments: 1. Single-space wet/dry feeders (**WET/DRY**), 2. Fresh liquid feeding (**FRESH**), 3. Fermented cereal liquid feeding where the cereal fraction (38 % barley, 40 % wheat) of the diet was fermented prior to feeding (**FERM-CER**), 4. Fermented whole diet liquid feeding where the whole diet was fermented prior to feeding (**FERM-WH**). In experiment 1, pigs were fed the experimental diets for 68 days prior to slaughter (29.8 kg ± 0.92 s.e to 102.3 kg ± 0.76 s.e.). Overall, average daily gain (**ADG**) was 1094, 1088, 1110 and 955 g/day (s.e. = 13.0; $P<0.001$) and FCE was 2.26, 2.37, 2.40 and 2.88 g/g (s.e. = 0.031; $P<0.001$) for treatments 1 through 4, respectively. Pigs fed FERM-WH were lighter at slaughter than pigs fed the other three treatments ($P<0.001$). In experiment 2, pigs were on treatment for 26 days prior to slaughter (85.3 kg ± 1.69 s.e. to 117.5 kg ± 0.72 s.e.). Overall ADG in experiment 2 was 1103, 1217, 1284 and 1140 g/day (s.e. = 27.87; $P<0.01$) and FCE was 2.78, 2.99, 2.95 and 3.09 g/g (s.e. = 0.071; $P=0.05$), for treatments 1 through 4, respectively. There were no significant differences observed between treatment groups in experiment 2 for dry matter, organic matter, nitrogen, gross energy (**GE**) or ash

apparent total tract digestibilities. Higher lactic acid bacteria counts and lower *Enterobacteriaceae* counts and pH were observed in FERM-CER and FERM-WH compared with WET/DRY and FRESH, although not statistically compared. Ethanol concentrations were almost four-fold higher in FERM-CER troughs than FRESH troughs and five-fold higher in FERM-WH than FRESH troughs. Amino acid degradation and a loss of GE found in FERM-WH help to explain the poorer growth and FCE of pigs fed this treatment. To conclude, whole diet fermented liquid feeding resulted in poorer growth and FCE compared with wet/dry, fresh liquid and fermented cereal liquid feeding.

3.2 Introduction

Fresh liquid feeding involves mixing the diet with water just prior to feed-out, while fermented liquid feed (**FLF**) is prepared by soaking all or part of the diet with water for a period of time prior to feeding, with/without an inoculum (Dung et al., 2005; Scholten et al., 1999). Wet/dry feeding has resulted in improved growth compared to dry feeding (Bergstrom et al., 2008; Gonyou and Lou, 2000; Myers et al., 2013). Diet fermentation can be beneficial to pig gastrointestinal health due to reduced feed pH and the resultant lower gastric pH, proliferation of lactic acid bacteria (**LAB**) and decreased *Enterobacteriaceae* (Canibe and Jensen, 2003; Lawlor et al., 2002; Mikkelsen and Jensen, 2000). Fermenting the cereal fraction of the diet may be preferable to whole diet fermentation to avoid microbial decarboxylation of free amino acids (Brooks, 2008; Canibe et al., 2007a; Canibe and Jensen, 2003; Canibe et al., 2007b). Recent research has focused on FLF as an alternative to antibiotics for young pigs (Close, 2000; Stein, 2002) and as a mechanism to reduce *Salmonella* in grow-finisher pigs (van Winsen et al., 2002). Lower feed intake and growth rates have been reported in grow-finisher pigs fed FLF than in pigs fed fresh liquid feed (Canibe and Jensen, 2003). Previous work by our group found improved growth rates and apparent total tract digestibility (**ATTD**) of nutrients by fermenting the cereal fraction of the diet compared to fresh liquid feeding (Torres-Pitarch, 2019). The aim of this study was to compare, for the first time, the effect of wet/dry feeding and fresh, fermented whole diet and fermented cereal liquid feeding on diet microbial quality and the growth, FCE, health and carcass quality of grow-finisher pigs. It was hypothesised that whole diet fermentation would result in reduced

growth due to decarboxylation of amino acids and that nutrient digestibility would improve in the fermented cereal diet compared to fresh and wet/dry diets.

3.3 Materials and Methods

3.3.1 Animal care and ethics

Ethical approval for this study was granted by the Teagasc Animal Ethics Committee (approval no. 107/2015). The experiment was conducted in accordance with Irish legislation (SI no. 543/2012) and the EU Directive 2010/63/EU for animal experimentation.

3.3.2 Experimental design and animals

The effect of wet/dry feeding and fresh, fermented whole diet and fermented cereal liquid feeding on pig growth, feed efficiency, health and nutrient digestibility was examined in 2 experiments. Experiment 1 used 216 Danavil Duroc x (Landrace x Large White) female and entire male pigs with an initial live-weight (**LW**) of 29.7 kg ± 0.92 SEM and its duration was 68 days following which pigs were slaughtered at days 69 and 70. Experiment 2 used 160 pigs with an initial LW of 85.3 kg ± 1.69 SEM and its duration was 26 days after which pigs were slaughtered on days 27 and 28. In experiment 1, pigs were penned in groups of 6 pigs/pen with a total of 9 pen groups/treatment. In experiment 2, pigs were penned in groups of 5 pigs/pen with a total of 8 pen groups/treatment.

In both experiments, all treatments were applied in the same room. Pen groups were blocked by sex and weight and assigned to one of four treatments, as follows; (1) Single space wet/dry feeders where pigs mixed water and meal at the point of feeding (**WET/DRY**); (2) Fresh liquid feeding (**FRESH**) where the diet and water were mixed immediately prior to feeding; (3) Fermented cereal liquid feeding (**FERM-CER**) where the cereal fraction of the diet was fermented and then mixed with balancer and water prior to feeding; (4) Fermented whole diet liquid

feeding (**FERM-WH**) where the whole diet was fermented prior to feeding. Pen groups were given a one week adaptation period prior to the start of the experiment, during which pigs fed WET/DRY were fed meal via wet/dry feeders while the other three treatment groups received fresh liquid feed prepared at 2.5:1 water:feed on a fresh weight basis.

Pen groups were housed in pens (2.36m x 2.37m) with concrete slatted floors and solid PVC partitions. Each pen group had access to supplementary water from a water bowl (DRINK-O-MAT, Egebjerg International A/.S, Egebjerg, Denmark) to comply with Council Directive 2008/120/EC (2008). Air temperature was maintained at 20 to 22 °C. The room was mechanically ventilated using ridge mounted exhaust fans and side inlets controlled by a Steinan PCS 8100 controller (Steinan BV, Nederwert, The Netherlands). Pigs were observed twice daily and pigs showing signs of ill-health were treated appropriately. All veterinary treatments were recorded including pig identity, symptoms, medication and dosage administered.

Each pen was equipped with a solenoid valve over a short trough fitted with an electronic sensor. For the three liquid treatments (FRESH, FERM-CER, FERM-WH), the sensors were checked 4 times per day increasing to 6 times per day and when the residual feed in the trough was below the sensor, additional feed was dispensed into troughs. Feeding was according to a feed curve for these three treatments that ensured *ad-libitum* access to feed. The feeding curve allowed for 23MJ digestible energy (**DE**)/pig/day at the start of the experiment, increasing to a maximum of 42MJ DE/pig/day during the experiment. Liquid feed levels in the troughs were manually inspected daily prior to and after feeding and feeding curves

increased or decreased accordingly to ensure *ad-libitum* access to feed while minimising wastage. The three liquid feeding treatments were fed from short steel troughs (100 cm x 32.5 cm x 21 cm) located on top of a rubber mat (1.5 x 1 m) to help minimise feed wastage.

The WET/DRY treatment was fed from single-space wet/dry feeders [Irish Dairy Services (IDS), Portlaoise, Ireland; 104.1 cm x 36.8 cm x 30.5 cm)] that were fitted with a water nipple at the point of feeding. Wet/dry feeders were monitored twice daily with feed in the hopper replenished as required and adjustments to feed flow made to ensure *ad-libitum* access to feed while minimising wastage

3.3.3 Feed preparation

All diets were formulated to contain 9.8 MJ NE/kg and 9.97 g/kg standardised ileal digestible (**SID**) lysine. All other amino acids were formulated relative to lysine according to the ideal protein concept (NRC, 2012). The full ingredient specification and nutrient composition of the dietary components and the experimental diet is reported in Table 3.1. The dietary components and experimental diet were manufactured in meal form at the Teagasc feed mill facilities (Teagasc, Moorepark, Fermoy, Co. Cork, Ireland) as follows: (1) Complete meal diet for WET/DRY treatment; (2) Cereal fraction, milled through a 3mm screen, composed of 38 % wheat and 40 % barley; and (3) Balancer fraction composed of soya bean meal, soya oil, synthetic amino acids, phytase, minerals and vitamins. Components (2) and (3) were stored in steel bins adjacent to the liquid feed preparation area during the experimental period and the complete diet was stored in 25 kg bags. Celite (2 g/kg) was added to the feed during the manufacturing process in order to

measure the coefficient of apparent total tract digestibility (**CATTD**) of nutrients using the acid insoluble ash (**AIA**) technique (McCarthy et al., 1977).

The three liquid-fed treatments (FRESH, FERM-CER, FERM-WH) were prepared and fed using a liquid feed system (HydroMix, BigDutchman, Vechta Germany) which had two fermentation tanks (2000 L) which were connected to two mixing tanks (500 L) for feed preparation and feed-out. Liquid feed in all tanks was agitated using a 6 pale agitator installed in each tank. A high-pressure air system delivered liquid feed from the mixing tanks to the feed troughs. The mixing tank and pipelines used to prepare and distribute liquid feed, respectively were empty between feeds.

The FRESH liquid dietary treatment was prepared by mixing the cereal and balancer components (at 0.784:0.216, cereal:balancer) with water. The diet was agitated for 120 seconds prior to delivery to the troughs. FERM-CER and FERM-WH were prepared by adding a starter culture containing *Lactobacillus plantarum* DSMZ166257 and *Pedicoccus acidilactici* NCIMB3005 (Sweetsile, Agway, Cork, Ireland) to the cereal plus water or cereal plus balancer (mixed at a ratio of 0.784:0.216) plus water, respectively and allowing an initial fermentation for 48 hours, during which no cereal/feed was removed from the tank. The starter culture was included at 20 g / 2000 L feed mix in experiment 1 and 15 g / 1500 L feed mix in experiment 2. Thereafter, to replace feed consumed by the pigs, the fermentation tanks were replenished once daily to a volume of 2000 L in experiment 1 and 1500 L in experiment 2 with either cereal or whole diet, according to treatment, at a water:meal ratio of 2.5:1 on a fresh matter basis. A minimum of 50 % of the total volume remained in the fermentation tanks to act as an inoculant for the next mix

when re-filled daily. The contents of the fermentation tanks were agitated on a constant 30 minutes on / 30 minutes off cycle for the duration of both experiments. The water:feed ratio (on a fresh matter basis) was 2.5:1 for each liquid feeding treatment. The WET/DRY treatment was fed from single-space wet/dry feeders as outlined above.

3.3.4 Records and Sampling

Individual pig weights were recorded on days 0, 34, 48 and 68 of experiment 1, and on days 0 and 26 in experiment 2. Pen-group weights were also recorded on day 13 in experiment 1. Feed delivered to troughs was recorded daily for each pen and feed disappearance calculated for the periods between each pig weighing in each experiment. Average daily gain (**ADG**), average daily feed intake (**ADFI**) and FCE were calculated for each period and the entire experiment. To calculate carcass ADG and carcass FCE, a kill-out percentage of 65 % was applied to the pig start weight in experiment 1 and a kill-out percentage of 75 % to start weights in experiment 2 due to their heavier start weights. The kill-out percentage at slaughter was then applied to the final LW of pigs prior to slaughter and carcass ADG and carcass FCE calculated accordingly.

During the initial 48 hour fermentation, feed samples were collected during agitation from a release valve at the base of each of the fermentation tanks at 0, 4, 8, 12, 16, 20, 24, 30, 36 and 48 hours for experiment 1 and every 6 hours for experiment 2 (0, 6, 12, 18, 24, 30, 36, 42 and 48 hours). The pH and temperature of these samples were recorded at each time point (Mettler Toledo pH meter, Greisensee, Switzerland) and samples were analysed microbiologically as explained below. The experiment began immediately at the end of the 48-hour initial

fermentation. Samples were also collected from each of the fermentation tanks and analysed on days 2, 8, 26 and 62 of experiment 1 and days 2, 6, 8 and 25 of experiment 2.

On day 26 and 62 of experiment 1 and days 6 and 25 of experiment 2, feed samples were collected from the WET/DRY hopper, mixing tanks (FRESH, FERM-CER and FERM-WH), liquid feed troughs (FRESH, FERM-CER and FERM-WH) and WET/DRY troughs for microbiological analysis. Liquid feed trough samples were sampled ~ 30 minutes before a new feed mix was delivered to the trough and included two trough samples per treatment on each sampling occasion which were analysed separately. All feed samples for microbiological analysis were put on ice and transported to the laboratory for analysis on the same day.

On day 6 and 25 of experiment 2, feed samples (~ 20 g) were collected from the mixing tanks and troughs of FRESH, FERM-CER and FERM-WH as were samples from the fermentation tanks of FERM-CER and FERM-WH. These were stored at - 20 °C for subsequent volatile fatty acid (**VFA**), ethanol and lactate analysis. On day 26 of experiment 2, liquid feed samples (~ 250 g) from the mixing tanks of FRESH, FERM-CER and FERM-WH were collected as was a dry feed sample (~ 250 g) from the WET/DRY hopper for amino acid analysis. These were frozen at - 20 °C for subsequent drying (trough samples were freeze-dried and the mixing tank and fermentation tank samples were oven dried at 55 °C for 72 hours) and amino acid analysis. The dry dietary components from the mill (cereal and balancer) were also frozen at - 20 °C prior to amino acid analysis.

Feed samples from each batch produced in the mill were collected during experiment 1 and experiment 2. These were pooled into one feed sample per

component for each experiment (i.e. one full diet, one cereal and one balancer) for proximate analysis. The full diet sample from experiment 2 was used for the apparent total tract digestibility (**ATTD**) determination. In experiment 2, freshly voided faecal samples for ATTD determination were collected from 3 pigs/pen from 24 pens (6 pens/treatment) on day 27. These faecal samples were frozen at -20 °C in aluminium foil trays for subsequent freeze-drying prior to chemical analysis.

3.3.5 Slaughter, carcass records and blood sampling

On days 69 and 70 of experiment 1 and days 27 and 28 of experiment 2, pigs were transported to a commercial abattoir. They were stunned using CO_2 and killed by exsanguination, during which blood samples were collected for haematology analysis from 36 pigs (9 pigs/treatment) in experiment 1 and 32 pigs (8 pigs/treatment) in experiment 2 using Vacuette tubes (Labstock, Dublin, Ireland) containing EDTA to prevent clotting.

The following measurements were taken: hot carcass weight was recorded 45 minutes after stunning, and back-fat thickness and muscle depth at 6 cm from the edge of the split back at the level of the 3rd and 4th last rib were determined using a Hennessy Grading Probe (Hennessy and Chong, Auckland, New Zealand). Lean meat content was estimated according to the formula: Estimated lean meat content (%) = 60.3 – 0.847x + 0.147y where x = fat depth (mm); y = muscle depth (mm) (Department of Agriculture and Food and Rural Development, 2001). Cold carcass weight was calculated as hot carcass weight x 0.98. Kill-out percentage was calculated from cold carcass weight and final live weight.

3.3.6 Haematological analysis of blood samples

Haematological analysis as an indicator of pig health was performed on whole blood within 6 hours of collection using a Beckman Coulter Ac-T diff analyser (Beckman Coulter, High Wycombe, United Kingdom). The following parameters were measured; white blood cell (**WBC**), lymphocyte number and percentage, monocyte number and percentage, granulocyte number and percentage, red blood cells, red blood cell distribution width, haemoglobin, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin, platelets and mean platelet volume.

3.3.7 Microbiological analysis of feed sampled from fermentation tank, mixing tanks and troughs

Approximately 10 g of each liquid or dry feed sample was homogenized in a stomacher as a 10-fold dilution in maximum recovery diluent (MRD; Oxoid, Basingstoke, UK) and a 10-fold dilution series was performed in MRD. Relevant dilutions were plated in duplicate as follows; (1) pour-plated on de Man Rogosa & Sharpe (MRS; Merck, Damstadt, Germany) agar, containing 50 U/mL nystatin (Sigma-Aldrich, Arklow, Co. Wicklow, Ireland), overlaid and incubated at 30 °C for 72 hours for enumeration of LAB; (2) pour-plated on violet red bile glucose (VRBG; Oxoid) agar, overlaid and incubated at 37 °C for 24 hours for *Enterobacteriaceae;* (3) pour-plated on ChromoCult tryptone bile X-glucuronide (CTBX; Merck) agar and incubated at 44 °C for 24 hours for *E. coli*; and (4) spread-plated on yeast glucose chloramphenicol (YGC; Merck) agar and incubated at 25 °C for 5 days for yeasts and moulds. Colonies were counted and the counts averaged and presented as $\log_{10}$ CFU/g of the original sample.

3.3.8 Feed analysis and apparent total tract digestibility determination

Prior to analysis, feed and faecal samples were ground in a Christy Norris mill (Ipswich, Suffolk, United Kingdom) through a 2 mm screen. Dry matter (**DM**, AOAC.934.01) and ash (AOAC.942.05) concentration was determined according to methods of the Association of Official Analytical Chemists (AOAC, 2005). The nitrogen (**N**) content was determined using the LECO FP 528 instrument (Leco Instruments, UK LTD., Cheshire, UK) (AOAC.990.0). Crude protein (**CP**) was determined as N x 6.25. The neutral detergent fibre (**NDF**) content was determined according to the method of Van Soest et al. (1991) using an Ankom 220 Fibre Analyser (Ankom Technology, Macedon, New York, USA). Gross energy (**GE**) was determined using an adiabatic bomb calorimeter (Parr Instruments, Moline, IL USA). Amino acid (**AA**) determination was carried out using cation exchange HPLC as previously described by McDermott et al. (2016) (AOAC 994.12). The concentration of AIA in dry diets was determined according to the method of McCarthy et al. (1977) in order to measure the CATTD of nutrients using the AIA technique.

Preparation of liquid feed samples for ethanol and lactate analysis was carried out as described by van Winsen et al. (2000). Briefly, feed aliquots were defrosted prior to centrifugation at 2,000 g for 10 minutes at 4 °C. The supernatant was then centrifuged at 18,500 g for 10 minutes. The resulting supernatant was filtered through a 0.2 µm filter and stored at -20 °C until ethanol analysis by gas chromatography and lactate analysis by HPLC.

Samples were thawed slowly at room temperature prior to ethanol analysis by gas chromatography (**GC**) (Agilent 6890; Agilent Technologies, Waghaeusel-

Wiesental, Germany) using a flame ionization detector. A 1 µL volume of each sample was injected by split injection 5:1 onto the column (AT-100 15 m x 0.53 mm i.d. x 1.2 micron) with a column flow rate of 3.4 ml/min helium. The temperature programme was 40 °C for 3 minutes, ramped at 10 °C / min to 180°C and held at 180 °C for 3 minutes.

For lactate analysis, samples were thawed slowly at room temperature, diluted with water as required and re-filtered through a 0.2 µm filter prior to analysis by HPLC (Waters, Milford, USA). A 10µL volume of each diluted sample was injected onto a Phenomenex Chirex [5µm Chiral IV (ligand exchange) 3126 ®-PA 150 x 4.6 mm] column under isocratic conditions. The column temperature was 22 °C, detector wavelength 254 nm and flow rate 1 ml/min with a run-time of 40 minutes.

For VFA analysis, extractions were carried out as described by McCormack et al. (2017) with some modifications. Briefly, 3.5 g sample was weighed and the pH was recorded. Samples were diluted with 5 % trichloracetic acid (at 2.5 x weight of sample) and centrifuged at 1800 x *g* for 10 minutes at 4 °C. A 1.5 ml aliquot of the resultant supernatant was mixed with 1.5 ml internal standard (0.05 % 3-methyl-n-valeric acid in 0.15 M oxalic acid dehydrate) and filtered through a 0.45 µm filter and stored at -20 °C until analysis by GC. An injection volume of 1 µl was injected into a Scion 456 gas chromatographer (SCION Instruments, Goes, The Netherlands) equipped with a EC^{TM} 1000 Grace column (15 m × 0.53 mm I.D) with 1.20 µm film thickness. The temperature programme set was: 75 °C – 95 °C increasing by 3 °C/minute, 95-200 increasing by 20 °C per minute, which was held for 30 seconds.

The detector and injector temperature was 280 °C and 240 °C respectively while the total analysis time was 12.42 minutes.

3.3.9 Statistical analysis

Growth parameters [ADFI, ADG, FCE and live weight (LW)], carcass quality parameters and blood haematology data were analysed using the MIXED procedure of SAS® 9.4 (SAS Institute, Inc., Cary, NC, US). For growth parameters; dietary treatment, sex, day of the experiment and their associated interactions were included in the model as fixed effects. Initial LW was included as a covariate and day as a repeated variable in the model while pen was the experimental unit. For carcass ADG and FCE, dietary treatment and sex and their associated interaction were included in the model as fixed effects, with initial weight included as a co-variate and pen as the experimental unit. For carcass quality parameters; dietary treatment and sex and their associated interaction were included in the model as fixed effects with pen as the experimental unit. Carcass cold weight was included as a co-variate for the analysis of muscle and fat depth and lean meat percentage while initial LW was included as a co-variate for the analysis of cold weight. For haematological analyses, data from both experiments were analysed together with dietary treatment, sex and experiment and their associated interactions included in the model for fixed effects. For ATTD determination, treatment, sex and their associated interaction were included in the model as fixed effects with pen as the experimental unit.

The normality of scaled residuals was investigated using the Shapiro-Wilk and Kolmogonov-Smirnov tests within the UNIVARIATE procedure of SAS. Results are presented as LS means ± SEM. Significance was reported for $P \leq 0.05$

and tendencies towards significance were reported for $P > 0.05$ but $P < 0.10$. The PROC MEANS procedure was used to obtain means and standard deviations for plate counts, lactate, ethanol, VFA, proximate and amino acid analysis of feed.

3.4 Results

3.4.1 Pig removals

There were no pigs removed from treatment during experiment 1. One pig was removed from the FERM-CER treatment on day 4 of experiment 2 due to lameness.

3.4.2 Microbiological analysis of fermented cereal and fermented whole diet during the initial fermentation

The initial fermentation refers to the first 48 hours after inoculant addition, during which time no feed was added to or removed from the fermentation tanks. Figure 3.1 shows the changes in counts of key microbial groups, as well as pH, observed within each fermentation tank during experiment 1 (Figure 3.1A) and experiment 2 (Figure 3.1C) during the initial fermentation and throughout the experiment (Figure 3.1B and 3.1D).

A similar pattern was observed in each tank when Experiments 1 and 2 were compared. During both experiments, the numbers of LAB increased steadily in both the cereal and whole diet fermentation tanks during the 48 hour start-up period. However, counts at 48hours were marginally higher in FERM-WH compared to the fermented cereal component (9.26 - 9.86 $\log_{10}$ CFU/g vs 8.84 - 9.23 $\log_{10}$ CFU/g).

Enterobacteriaceae counts behaved differently across experiments during the initial 48-hour start-up period. In experiment 1, *Enterobacteriaceae* counts were stable in the fermented cereal component until they began to decline at 20 hours up to 48 hours, except for a small increase at 36 hours. On the other hand, in FERM-WH, counts were stable up to 30 hours into the fermentation, at which point they

began to decrease. Final *Enterobacteriaceae* counts were similar in both fermentation tanks in experiment 1, with 3.33 $\log_{10}$ CFU/g detected in the fermented cereal component and 3.03 $\log_{10}$ CFU/g in FERM-WH. In experiment 2, *Enterobacteriaceae* counts were stable in the fermented cereal component for the first 12 hours, before an increase to 7.18 $\log_{10}$ CFU/g at 30 hours was observed, but these were reducing by 36 hours and counts in the tank at the end of the 48-hour period were 2.81 $\log_{10}$ CFU/g. *Enterobacteriaceae* counts in FERM-WH also began to increase at 18 hours, reaching a peak of 7.54 $\log_{10}$ CFU/g at 30 hours, before decreasing to 3.74 $\log_{10}$ CFU/g at 48 hours.

E. coli counts during experiment 1 were below the detection limit at the start and at the end of the initial fermentation in the fermented cereal component, despite some being detected between 16 and 24 hrs. However, in FERM-WH, *E. coli* was detected at 4.79 $\log_{10}$ CFU/g at the start of the fermentation before a steady decline to below the detection limit was observed, except at 30 hours when the *E. coli* count temporarily increased. In experiment 2, a similar result was obtained in the fermented cereal component, except that the temporary increase was not as high. On the other hand, counts increased from just above the detection limit of 2.10 $\log_{10}$ CFU/g at the start of the fermentation to 2.78 $\log_{10}$ CFU/g at 48 hours in the FERM-WH.

Yeasts grew similarly in both fermentation tanks during experiment 1, with a steady increase from 5.76 - 5.78 $\log_{10}$ CFU/g to 7.24 - 7.58 $\log_{10}$ CFU/g observed during the 48 hour period. Both initial and final counts were lower in experiment 2 and differed between fermentation tanks, ranging from 4.72 to 5.01 $\log_{10}$ CFU/g in

the fermented cereal component and from 3.74 $\log_{10}$ CFU/g to 6.61 $\log_{10}$ CFU/g in the FERM-WH.

During experiment 1, mould counts declined from 4.22 and 5.18 $\log_{10}$ CFU/g in the fermented cereal component and FERM-WH tanks, respectively, to below the detection limit at the end of the initial fermentation in both tanks. A similar pattern was observed during experiment 2, although initial counts were lower (3.47 $\log_{10}$ CFU/g in the fermented cereal component and 3.53 $\log_{10}$ CFU/g in FERM-WH).

The pH (Figure 1A and 1C) and temperature of the feed were recorded at each sampling during the initial fermentation. In experiment 1, the starting pH of the fermented cereal component was 6.17, lower than that of FERM-WH which was 6.35. The pH declined steadily in both tanks, dropping to pH 4 24 hours into the initial fermentation in the fermented cereal component and to 4.03 in the FERM-WH but after 48 hours. In experiment 2, the starting pH was similar in each feed at 6.15 in the fermented cereal component and 6.20 in FERM-WH. As in experiment 1, the pH of the fermented cereal component decreased faster than that of FERM-WH with the pH of the fermented cereal component dropping below 4 30 36 hours into the initial fermentation, while it was 42 - 48 hours before the pH decreased below 4 in the FERM-WH.

3.4.3 Microbiological analysis of fermented cereal component and fermented whole diet in the fermentation tanks during the experiments

Day 0 began when the initial 48 hour fermentation ended. Figure 3.1 shows the changes in counts of key microbial groups, as well as pH, observed within each fermentation tank throughout experiment 1 (Figure 3.1B) and experiment 2 (Figure 3.1D). Throughout the experimental period, counts of LAB in both the fermented

cereal component and FERM-WH remained relatively constant in the fermentation tanks for the duration of Experiments 1 and 2 (days 2-62 and 2-26, respectively). However, *Enterobacteriaceae* counts were more inconsistent; during experiment 1 they increased to 4.80 and 4.22 $\log_{10}$ CFU/g in the fermented cereal component and FERM-WH, respectively on day 2 compared to the counts obtained at the end of the initial fermentation (i.e. the start of day 0). Thereafter, counts were similar in both fermentation tanks, decreasing initially and then increasing to reach final counts of 5.35 and 5.89 $\log_{10}$ CFU/g, respectively on day 62 of experiment 1. In experiment 2, although *Enterobacteriaceae* counts had been very similar during the initial fermentation for the fermented cereal component and FERM-WH, they behaved very differently during the feeding experiment. In the fermented cereal component, counts remained just above the detection limit throughout the 26-day experiment, while in the FERM-WH counts increased to reach 5.57 $\log_{10}$ CFU/g at day 25 after a slight decrease at day 8.

E. coli were non-detectable in the fermented cereal component during both experiments, while in the FERM-WH, counts increased to 4.7 and 3.3 $\log_{10}$ CFU/g in the early stage of experiments 1 and 2, respectively, but subsequently declined and were non-detectable at the end of both experiments.

The levels of yeast in the fermented cereal component varied only slightly during experiment 1, ranging from 6.3 to 7.5 $\log_{10}$ CFU/g, while counts in the FERM-WH fluctuated more, ranging from 5.0 $\log_{10}$ to 7.1 $\log_{10}$ CFU/g. Nonetheless, counts were almost identical in both tanks at the end of the experiment. In experiment 2, yeast counts in both tanks were similar and remained relatively stable at 7.0 - 7.7 $\log_{10}$ CFU/g throughout the experiment.

Mould counts fluctuated throughout both experiments, ranging from non-detectable to 5.2 $\log_{10}$ CFU/g in the fermented cereal component and from 3.3 $\log_{10}$ CFU/g to 5.1 $\log_{10}$ CFU/g in the FERM-WH during experiment 1 and from non-detectable to 4.4 $\log_{10}$ CFU/g in the fermented cereal component and from non-detectable to 4.0 $\log_{10}$ CFU/g in FERM-WH during experiment 2. Final mould counts were almost identical in both fermentation tanks at the end of experiment 1, but mould counts were below the detection limit in FERM-WH while 4.00 $\log_{10}$ CFU/g was detected in the fermented cereal component on day 25 of experiment 2. The pH of the fermented cereal component in experiment 1 remained relatively constant at 3.54 - 3.71 up to day 26 and had dropped to 3.26 on day 62 of experiment 1, while the pH of FERM-WH remained ~ 4 up to day 26 after which it declined to 3.58 at day 62 of the experiment. In experiment 2, the pH of the fermented cereal component ranged from 2.92 to 3.20 throughout the experiment, while FERM-WH ranged from 3.24 to 3.74 throughout the 25 day experimental period.

3.4.4 Microbiological analysis of dietary treatments in hoppers, mixing tanks and troughs

Mean microbial counts as well as pH and temperature (where recorded) in wet/dry hoppers, mixing tanks and troughs are presented in Figure 3.2A (experiment 1) and Figure 3.2B (experiment 2).

In WET/DRY, counts of all microbes enumerated were higher in the trough than in the dry feed sampled from the hopper in both experiments 1 and 2, except for *Enterobacteriaceae* counts in experiment 2 which were similar in both locations.

In FRESH, LAB, *E.coli* and yeast counts were higher in the trough than in the mixing tank in experiment 1. Mould counts were slightly higher in the trough

than the mixing tank and there was a slight decrease in *Enterobacteriaceae* counts in the trough compared to the mixing tank. In experiment 2, counts of all microbes enumerated in FRESH were higher in the trough than in the mixing tank. The pH of FRESH in experiment 2 decreased slightly in the trough compared to the mixing tank and the temperature increased.

In FERM-CER in experiment 1, LAB and *Enterobacteriaceae* counts were similar in the mixing tank and the trough, but counts of *E. coli*, yeast and mould increased in the trough when compared with the mixing tank. In FERM-CER in experiment 2, LAB counts and temperature of feed were similar in the mixing tank and trough while counts of *Enterobacteriaceae*, *E. coli* and feed pH increased slightly and counts of yeast and mould decreased slightly from the mixing tank to the troughs.

In FERM-WH in experiment 1, counts of LAB were similar in the mixing tank and trough while counts of all other microbes increased in the trough compared with the mixing tank (albeit only slightly in moulds). In experiment 2 in FERM-WH, yeast counts remained similar and only a slight decrease in LAB counts and feed pH was noted; however, counts of *Enterobacteriaceae*, *E. coli* and mould increased in feed troughs compared with counts found in the mixing tank.

3.4.5 Effect of dietary treatment on the lactic acid, ethanol and volatile fatty acid concentrations in liquid diets during experiment 2

Results of lactate, ethanol and VFA analysis of the liquid diets are shown in Table 3.2. The total lactic acid (**LA**) concentration in FRESH varied greatly between the two analysed samples; nonetheless, LA concentration increased while feed resided in troughs compared to that sampled from the mixing tank.

Concentrations of LA in FERM-CER and FERM-WH were less variable across samples, as indicated by the smaller standard deviations. LA concentrations were lowest for FERM-CER sampled at all locations compared to the other two liquid treatments. There was a decrease in LA concentration in FERM-CER from the fermentation tank to the mixing tank, most likely due to the addition of balancer and fresh water followed by an increase once again while this diet resided in troughs. Total LA concentration in FERM-WH was highest in the mixing tank but only marginally, decreasing slightly then in the trough. The highest concentration of LA in the tanks (fermentation tank and mixing tank) was in FERM-WH but in the troughs it was in FRESH.

Ethanol concentrations were notably lower in both the mixing tank and trough samples of FRESH compared to the same locations for FERM-CER and FERM-WH. Concentrations in FERM-CER were lowest in the feed trough. The highest concentrations recorded across treatments were in the FERM-WH at all three locations, with the lowest concentration again observed in the trough.

A noticeable increase in acetate, propionate, isobutyrate, butyrate, isovalerate and valerate concentrations from the mix tank to the trough was observed for the FRESH treatment. Acetate and propionate concentrations in FERM-CER decreased in the mix tank, most likely due to the addition of fresh balancer before increasing once again in the trough samples. Concentrations of butyrate decreased slightly in the trough of FERM-CER compared to the fermentation tank and mix tank, while isobutyrate, isovalerate and valerate concentrations were higher in the trough than at the other two sampling locations.

Concentrations of acetate in the mix tank of FERM-WH were slightly higher than in the fermentation tank and trough, but generally remained quite constant. Increases in isobutyrate and valerate were observed in the trough when compared to the fermentation tank and mix tank, while butyrate and isovalerate remained quite constant. The concentration of propionate was higher in the mixing tank and the fermentation tank compared to the trough. The highest levels of acetate across all treatments were observed in FERM-WH at all three sampling locations.

3.4.6 Effect of dietary treatment on the gross energy, crude protein, ash and amino acid content of the diets

The results of these analyses are presented in Table 3.3. There was no decrease in GE from the dry bagged diet to WET/DRY troughs or from the FRESH mixing tank to FRESH troughs; however, there does appear to be a loss of GE when the values in the respective mixing tank and troughs of FERM-CER and FERM-WH are compared. The reduction in lysine concentration in the mixing tank and trough in FERM-WH when compared with the lysine content of the dry diet and that in the troughs of the other three treatments is also noteworthy. Methionine and threonine concentrations also appear lower in the troughs of FERM-WH than in troughs of the other three treatments.

3.4.7 Effect of wet/dry feeding and fresh, fermented whole diet and fermented cereal liquid feeding on growth and carcass quality of grow-finisher pigs from experiment 1

The effect of treatment on LW, ADFI, ADG, FCE and carcass quality is presented in Table 3.4. Pigs on all treatments had similar live weight at the start of experiment 1. At the end of the experimental period, pigs fed FERM-WH were

significantly lighter than those fed the other three treatments (P<0.001). Overall, pigs fed WET/DRY had a similar ADFI to pigs fed FRESH, but significantly lower than those fed FERM-CER and FERM-WH (P<0.01). Overall, pigs fed FERM-WH had a lower growth rate than those fed the other three treatments (P<0.001). This resulted in a significantly poorer FCE in FERM-WH-fed than pigs fed the other three treatments, while pigs fed WET/DRY also had a significantly better FCE than those fed FRESH (P<0.001). Pigs fed FERM-WH had a significantly poorer carcass ADG (P<0.001) and carcass FCE (P<0.001) than pigs fed the other three treatments. The coefficient of variation (**CV**) of live-weights in pens was similar in all treatments on day 1, while pigs fed FERM-WH had a significantly higher CV than all other treatments on day 68 (P<0.001).

At slaughter, pigs fed FERM-WH had lighter carcasses than those fed the other three dietary treatments (P<0.001). Pigs fed FERM-WH also had a significantly lower kill-out percentage than those fed FERM-CER (P<0.01), tended to have less muscle depth than pigs fed WET/DRY (P=0.09), had a greater fat depth than those fed WET/DRY (P<0.05) and had a lower lean meat percentage than WET/DRY- and FRESH-fed pigs (P<0.05).

3.4.8 Effect of wet/dry feeding and fresh, fermented whole diet and fermented cereal liquid feeding on growth and carcass quality traits of grow-finisher pigs from experiment 2

The effect of dietary treatment on LW, ADFI, ADG, FCE and carcass quality is presented in Table 3.5. Following the adaptation week, pigs fed WET/DRY were significantly lighter at the start of the experiment than those fed the other three dietary treatments (P<0.001). At slaughter, pigs fed FERM-CER were significantly

heavier than those fed FERM-WH and WET/DRY but similar in weight to those fed FRESH ($P<0.01$).

Overall, pigs fed FERM-CER had a significantly higher ADFI than those fed FERM-WH and WET/DRY but similar to pigs fed FRESH ($P<0.001$). Pigs fed FERM-CER had the highest overall ADG which was similar to those fed FRESH and significantly higher than those fed WET/DRY and FERM-WH ($P<0.01$). The FCE of pigs fed WET/DRY was significantly better than those fed FERM-WH ($P=0.05$). Pigs fed FERM-CER had a significantly higher carcass ADG than all other treatments, while FRESH also had a higher growth rate than WET/DRY ($P<0.001$). Pigs fed WET/DRY had better carcass FCE than those fed FERM-WH but similar to those fed FRESH and FERM-CER ($P<0.05$).

At slaughter, pigs fed FERM-CER had heavier carcass weights than those fed the other three treatments ($P<0.01$). Pigs fed FERM-CER had a higher kill-out percentage than those fed WET/DRY and FRESH but similar to those fed FERM-WH ($P<0.01$). Pigs fed FRESH and FERM-CER tended to have greater muscle depth than pigs fed FERM-WH ($P=0.06$). Pigs fed WET/DRY had significantly less fat depth ($P<0.05$) and a lower lean meat percentage ($P<0.05$) than those fed the other three dietary treatments.

3.4.9 Effect of dietary treatment on apparent total tract nutrient and energy digestibility

The effect of dietary treatment on ATTD of nutrients is shown in Table 3.6. There were no treatment differences for DM, organic matter, nitrogen, GE or ash digestibilities ($P>0.05$).

3.4.10 Effect of dietary treatment on the haematological profile of pigs at slaughter

The impact of dietary treatment on the haematological profile of pigs at slaughter is shown in Table 3.7. Pigs fed FERM-WH tended to have a lower ($P=0.06$) percentage of lymphocytes than and had a higher ($P<0.05$) percentage of granulocytes than those fed FRESH.

3.5 Discussion

To our knowledge, this is the first study to compare wet/dry feeding and fresh, fermented whole diet and fermented cereal liquid feeding of grow-finisher pigs under the same environmental and management conditions. Such a study is essential to help inform the decision making process of pig producers when choosing the most efficient feeding system to install for grow-finisher pigs. The carcass growth rate and carcass FCE of pigs fed FERM-WH were significantly poorer than for pigs fed the other three treatments in experiment 1 of the current study. Although based on a limited number of samples, this is most likely due to the degradation of amino acids and a loss of energy in FERM-WH when compared to the other treatments. Reduced levels of lysine, methionine and threonine were observed in the troughs of FERM-WH compared with troughs of the other three treatments. Amino acid degradation in fermented liquid feed has previously been reported (Canibe et al., 2007a; de Lange et al., 2006; Niven et al., 2006; Pedersen et al., 2002; Shurson, 2009). It is generally accepted that microbes in the liquid feed use free amino acids and losses are greater when coliforms predominate compared to *Lactobacillus* (de Lange et al., 2006; Niven et al., 2006). In the present study greater microbial growth was observed for both fermented liquid diets (FERM-CER and FERM-WH); however, because the synthetic amino acids and soybean meal were added to the FERM-CER just prior to feeding, the level of microbial decarboxylation of amino acids in this treatment appears to have been lower. It is also interesting to note that *E. coli* counts in the early stages of both experiments were higher in FERM-WH than FERM-CER when fermentation tank samples were compared.

Pigs fed FERM-CER and FRESH had similar carcass ADG and carcass FCE which were better than FERM-WH. This further suggests that AA degradation was

responsible for the poorer growth and feed efficiency in FERM-WH, as synthetic amino acids and soybean meal were added just prior to feed-out in FRESH and FERM-CER, and they produced similar results. Similar to the results of the current study, Canibe and Jensen (2003) found improved growth rates in grow-finisher pigs fed fresh liquid feed compared to those fed a fermented whole diet. It seems that to maintain the dietary concentration of amino acids and prevent consequent growth reduction and deterioration in FCE, fermenting only the cereal fraction and adding synthetic amino acids just prior to feeding is preferable to whole diet fermentation (Brooks, 2008; Canibe et al., 2007a; Canibe and Jensen, 2003; Canibe et al., 2007b). However, the current study would suggest that there is little additional benefit for FERM-CER over FRESH in terms of grow-finisher pig growth or feed efficiency. Previous work has also reported no advantage of fermentation of the cereal fraction of the diet on growth rate in the finisher phase compared with fresh liquid feeding and, in fact, reported a significantly worse growth rate during the grower-phase, resulting in no significant difference between treatments for the overall grow-finisher period (MLC, 2005).

In the fermentation tank, the pH of the FERM-CER dropped faster (20-24 hours in experiment 1 and 24-30 hours in experiment 2 into the fermentation) and as a result its final pH was lower than that of the FERM-WH after the initial 48-hour fermentation. This is likely due to the fact that cereals have a lower buffering capacity than whole diets (Lawlor et al., 2005; Scholten et al., 2001). A similar result was reported by Canibe et al. (2007a) in that the pH of a fermented cereal component was lower than that of a fermented whole diet. The pH of the FERM-CER also remained lower than the FERM-WH in the fermentation tanks throughout experiments 1 and 2. Counts of *Enterobacteriaceae* were lower in the mixing tank

and troughs of FERM-CER and FERM-WH than FRESH in both experiments, most likely due to the lower pH in these treatments, which in turn was probably due to the higher LAB counts. It would appear that FERM-CER and FERM-WH had reached phase 2, the steady phase of fermentation, as indicated by the low pH, high LAB counts and low *Enterobacteriaceae* counts. However, feed in the troughs of FRESH and WET/DRY were still at fermentation phase 1, with spontaneous fermentation occurring, as described by Canibe and Jensen (2003); this was evidenced in the FRESH by high *Enterobacteriaceae* and pH (in FRESH) compared to FERM-CER and FERM-WH. The concentration of lactic acid in FRESH troughs varied greatly by sample as evidenced by the large standard deviation; however, the lactic acid concentrations in both fermented diets were much more uniform. This highlights the unpredictability of spontaneous fermentation in fresh liquid feed while it resides in the feeding trough. It would seem reasonable to suggest that a stable feed pH of ~ 4.0 should be sustained for some time before phase 2 of fermentation is reached, which consequently causes a reduction in *Enterobacteriaceae* populations.

It has been well documented that ethanol is produced along with acetic acid and amylic alcohol when yeasts dominate the fermentation of liquid feed (Brooks et al., 2001; Brooks et al., 2003; Canibe et al., 2007b; Missotten et al., 2009; Missotten et al., 2015). Yeasts convert starch to alcohol and CO_2 resulting in a loss of energy in the feed (Brooks et al., 2001). It is therefore not surprising that because yeast counts were higher in the mix tank and troughs of the FERM-WH and FERM-CER than the FRESH, ethanol concentrations were also higher in these treatments and acetate concentrations were highest in the FERM-WD. Ethanol concentrations in the mix tank were 82.9 % higher in FERM-WD than FRESH and 88.9 % higher in FERM-CER than FRESH, while concentrations in the trough were 80.4 % higher in

FERM-WH than FRESH and 73.0 % higher in FERM-CER than FRESH in the current study. The ethanol concentrations in FERM-CER and FERM-WH reported in the current study were higher than those reported by Canibe et al. (2007a) despite yeast counts in the current study falling with their reported range of yeasts at 20 °C and 37 °C. Standard values for 'residue-free' liquid feed have been reported as 0 – 10 mmol/kg liquid feed for both lactic and acetic acid (Vils et al., 2018) which suggests that lactic acid concentrations in FRESH were extremely high, whereas acetic acid concentrations were within range in the mixing tank and were just outside the range in the trough. However, the huge variation in lactic acid measured in FRESH must be taken into consideration as previously discussed. Lactic acid bacteria counts were also within the reported range of 10^6 – 10^8 log CFU/g in the mixing tank but increased to above the range in the trough in FRESH. In contrast to the current study, Canibe et al. (2007a) found lower yeast counts and ethanol concentrations in a whole diet fermentation than a fermented cereal diet; however, in agreement with the current study, they did find higher levels of acetic and lactic acid in a fermented whole diet than the fermented cereal diet.

Despite higher ethanol and acetate concentrations in the fermented diets, it is evident from the high feed intakes in the current study that diet palatability was not adversely affected. It is likely that younger pigs may be more affected by this, as the reduced palatability associated with liquid feeding fermented whole diets has been found to reduce feed intake in weaned pigs (Brooks et al., 2001; Canibe et al., 2007a; Pedersen, 2001). Furthermore, most work on fermented liquid feeding, to date, has been conducted with weaned pigs (Canibe et al., 2007a; Geary et al., 1999; Lawlor et al., 2002; Rudbäck, 2013) in which the immature digestive tract may benefit most from the physical, microbial and chemical properties of fermented liquid feed.

In the current study, no differences in the ATTD of nutrients and energy were found in response to treatment. In contrast, our group previously found that cereal fermentation improved DM, organic matter, gross energy and crude protein digestibility compared to fresh liquid feeding which resulted in an improvement in ADG from cereal fermentation compared to fresh liquid feeding (Torres-Pitarch, 2019). It should be noted that the energy value in their diet was lower than in the diet in the current study, therefore, pigs fed the fermented cereal treatment in their study likely benefitted more from metabolites produced in the large intestine more than in the current study. Increased protein and organic matter digestibility have also been reported with fermented liquid feeding compared with dry feeding of grower pigs by Dung et al. (2005).

The fact that carcass feed efficiency of pigs fed WET/DRY was numerically best compared to all other dietary treatments suggests that increased physical feed wastage associated with liquid feeding may have been an issue in the FRESH, FERM-CER and FERM-WH treatments. Wastage in liquid feeding resulting in poorer feed efficiency has previously been reported (Han et al., 2006; l'Anson et al., 2012; Missotten et al., 2010; Russell et al., 1996) and improved feeding management must be implemented to ensure it is minimised.

In terms of pig health, the tendency for a lower percentage of lymphocytes and the higher percentage of granulocytes in pigs fed FERM-WH compared to those fed FRESH could be as a result of immune challenge, as both white blood cell types increase in response to infection (Wilson and Waugh, 2000). This could be due to the higher microbial load of the FERM-WH treatment, although the *Enterobacteriaceae* family, which could contain potential pathogens, was lower in

the FERM-WH treatment. Hence, a more detailed analysis of the feed microbiome is required. In any case, although the lymphocyte percentage for pigs on the FERM-WH treatment (37 %) was outside the normal range (39-62 %; Merck Manual) it was only marginally so. Furthermore, while no normal ranges can be found for granulocyte percentages, the values found for pigs on all treatments were within the normal ranges for humans (45-75 %) (IWMF).

In conclusion, this study shows that fermented whole diet liquid feeding results in poorer carcass growth rate and feed efficiency in grow-finisher pigs compared to wet-dry feeding and fresh or fermented cereal liquid feeding. It would appear that fermentation of the whole diet reduces gross energy and results in amino acid losses from the diet and that this contributes to the decreased growth and feed utilisation on this treatment. Wet/dry feeding, fresh liquid feeding and fermented cereal liquid feeding resulted in similar carcass growth rates and carcass feed efficiencies in grow-finisher pigs. Based on the results of the current study, whole diet fermentation is not recommended for grow-finisher pigs.

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3.7 Tables

Table 3.1 Composition of the experimental diet and dietary components (on an as-fed basis, g/kg unless otherwise stated)[1]

		Dietary components	
	Experimental diet	**Cereal**	**Balancer**
Ingredient composition[2]			
Wheat	400.0	400.0	
Barley	382.7	382.7	
Soybean meal	183.0		183.0
Limestone flour	11.0		11.0
Soya oil	9.7		9.7
Lysine HCl	3.8		3.8
Salt	3.0		3.0
L-Threonine	1.7		1.7
Celite	2.0		2.0
Vitamin and mineral premix[3]	1.0		1.0
Mono diCalcium phosphate	1.0		1.0
DL-Methionine	0.9		0.9
L-Tryptophan	0.2		0.2
Phytase[4]	0.1		0.1
Chemical composition			
Dry matter	875.0	871.0	900.0
Crude protein	173.0	94.0	420.0
Ash	40.0	29.0	118.0
Neutral detergent fibre	123.0	178.0	79.0
Gross energy, MJ/kg	16.1	16.0	17.0
Lysine	9.8	8.4	33.2
Methionine	4.4	4.2	10.9
Threonine	7.2	6.5	20.0
Digestible energy, MJ/kg[2]	13.8		
Net energy, MJ/kg[2]	9.8		
Oil[2]	25.7		

		Dietary components	
	Experimental diet	**Cereal**	**Balancer**
SID lysine[2,5]	10.0		
Total calcium[2]	6.6		
Total phosphorus[2]	2.6		

[1]Values are the mean of diets from 1 and 2 analysed for dry matter, crude protein, ash, neutral detergent fibre, gross energy. Values for amino acids are from experiment 2 only
[2]Calculated values
[3]Vitamin and mineral premix provided per kilogram of complete diet: Cu from copper sulphate, 15 mg; Fe from ferrous sulphate monohydrate, 24 mg; Mn from manganese oxide, 31 mg; Zn from zinc oxide, 80 mg; I from potassium iodate, 0.3 mg; Se from sodium selenite, 0.2 mg; retinyl acetate, 0.7 mg; cholecalciferol, 12.7 μg; DL-alpha-tocopheryl acetate, 40 mg; Vitamin K, 4 mg; vitamin B12, 15 μg; riboflavin, 2 mg; nicotinic acid, 12 mg; pantothenic acid, 10 mg; vitamin B1, 2 mg; vitamin B6, 3 mg and celite 2000 mg/kg.
[4]The diet contained 500 phytase units (FYT) per kg feed from RONOZYME HiPhos (DSM, Belfast, UK)
[5]SID Lysine = Standardized ileal digestible lysine

Table 3.2 Effect of dietary treatment on the lactic acid (mmol/kg), ethanol (mmol) and volatile fatty acid (mmol/kg) concentrations in liquid diets from experiment 2[1]

	FRESH[2]		FERM-CER[2]			FERM-WH[2]		
	Mixing tank	**Trough**	**Ferm. tank[3]**	**Mixing tank**	**Trough**	**Ferm. tank**	**Mixing tank**	**Trough**
D-lactate	164 ± 187.9	478 ± 313.7	128 ± 11.0	87[4]	101 ± 8.8	166 ± 3.8	179 ± 42.9	163 ± 18.7
L-lactate	166 ± 173.2	518 ± 305.1	127 ± 2.0	92[4]	96 ± 1.7	225 ± 14.0	232 ± 30.4	217 ± 19.1
Total LA[5]	330 ± 361.2	996 ± 616.6	255 ± 9.1	179[4]	197 ± 9.8	392 ± 10.2	411 ± 73.3	381 ± 37.3
Ethanol	7 ± 5.9	10 ± 6.8	50 ± 8.7	41 ± 9.8	37 ± 11.3	63 ± 5.2	63 ± 1.9	51 ± 3.6
VFA[6]								
Acetate	8.14 ± 2.000	14.76 ± 3.336	12.47 ± 0.513	7.96 ± 0.488	11.98 ± 2.330	18.07 ± 2.135	20.29 ± 2.300	18.64 ± 2.773
Propionate	0.06 ± 0.008	0.16 ± 0.057	0.20 ± 0.221	0.14 ± 0.129	0.19 ± 0.119	0.14 ± 0.129	0.23 ± 0.221	0.22 ± 0.077
Isobutyrate	0.24 ± 0.008	0.5 ± 0.151	0.02 ± 0.001	0.17 ± 0.202	0.41 ± 0.037	0.28 ± 0.017	0.35 ± 0.028	0.41 ± 0.042
Butyrate	0.02 ± 0.008	0.05 ± 0.022	0.04 ± 0.011	0.03 ± 0.016	0.02 ± 0.003	0.02 ± 0.011	0.04 ± 0.011	0.02 ± 0.009
Isovalerate	0.02 ± 0.010	0.046 ± 0.015	0.03 ± 0.004	0.03 ± 0.009	0.05 ± 0.015	0.02 ± 0.008	0.03 ± 0.000	0.03 ± 0.007
Valerate	0.03 ± 0.020	0.08 ± 0.019	0.06 ± 0.025	0.06 ± 0.000	0.08 ± 0.006	0.07 ± 0.080	0.05 ± 0.044	0.10 ± 0.023
Total VFA	8.51 ± 2.020	15.24 ± 3.400	12.82 ± 0.703	8.37 ± 0.151	12.73 ± 2.25	18.60 ± 1.940	20.99 ± 2.493	19.41 ± 2.667
A:P[7]	134.7 ± 50.67	108.0 ± 52.40	169.6 ± 188.29	99.4 ± 94.19	97.8 ± 73.56	231.4 ± 226.39	147.8 ± 129.49	97.58 ± 47.70
Protein-der[8]	0.29 ± 0.021	0.28 ± 0.144	0.11 ± 0.020	0.25 ± 0.192	0.54 ± 0.046	0.37 ± 0.055	0.43 ± 0.016	0.54 ± 0.054

[1]Mean ± standard deviation of samples collected on day 6 and day 25 of experiment 2 are presented at each location. Tank samples (Mix tank and Ferm. tank) represent the mean of 2 samples (except for FERM-CER in the mix tank where insufficient supernatant prevented analysis of a second sample) while trough samples represent the mean of 4 samples
[2] FRESH = fresh liquid feeding; FERM-CER = Fermented liquid feeding where the cereal fraction (wheat and barley) only of the diet was fermented prior to feeding; FERM-WH = Fermented liquid feeding where the whole diet was fermented prior to feeding
[3]Ferm. tank = Fermentation tank
[4]Value for one analysed sample; therefore, no standard deviation available
[5]LA = Lactic acid (Sum of D-lactate and L-lactate)
[6]VFA = volatile fatty acids
[7]A:P = Acetate:propionate ratio
[8]Protein-der = Protein-derived volatile fatty acids

Table 3.3 Effect of dietary treatment on the gross energy, crude protein, ash and amino acid content of the diets (presented on a dry matter basis)

	Dry[1]			Mixing tank[2]			Trough[3]			
	Bagged	**Cereal**	**Balancer**	**FRESH[4]**	**FERM-CER[4]**	**FERM-WH[4]**	**WET/ DRY**	**FRESH**	**FERM-CER**	**FERM-WH**
Gross energy (MJ/kg)	18.3	18.5	18.8	18.9	18.9	18.9	18.2	18.9	18.3	18.0
Crude protein, %	20.4	20.8	44.9	23.1	20.8	20.7	21.0	23.6	23.2	20.3
Ash, %	4.6	4.6	14.4	3.4	3.7	3.2	4.8	3.4	4.5	4.4
Amino acids (g/kg)										
Lysine	11.1	9.6	36.7	12.1	10.8	9.7	12.4	13.5	13.3	9.4
Cysteic acid	5.1	5.0	8.8	5.4	5.3	5.7	5.6	6.3	5.8	5.5
Taurine	1.5	1.4	1.2	1.7	0.7	0.6	0.7	0.6	0.6	0.5
Methionine	5.0	4.9	12.0	5.4	4.7	4.9	5.8	5.4	5.0	4.5
Aspartic acid	16.3	15.8	48.5	20.8	16.3	18.0	17.0	20.7	21.6	15.9
Threonine	8.3	7.4	22.2	9.5	7.9	8.3	8.1	9.5	9.1	7.6
Serine	8.9	8.9	21.2	10.8	8.7	10.1	9.3	11.3	10.8	9.0
Glutamic acid	42.0	42.8	78.6	46.2	37.0	44.5	43.6	50.0	45.1	41.0
Glycine	7.9	7.7	17.9	9.6	8.1	8.9	8.1	9.6	9.2	7.8
Alanine	7.3	7.2	17.8	9.1	8.3	9.1	7.7	9.2	9.5	8.8

	Dry[1]			Mixing tank[2]			Trough[3]			
	Bagged	**Cereal**	**Balancer**	**FRESH[4]**	**FERM-CER[4]**	**FERM-WH[4]**	**WET/ DRY**	**FRESH**	**FERM-CER**	**FERM-WH**
Cysteine	0.9	1.1	1.0	1.2	1.2	1.5	0.3	0.7	0.9	1.0
Valine	8.8	8.9	20.5	10.8	9.5	10.6	9.1	11.3	10.9	9.0
Isoleucine	7.3	7.3	19.0	9.0	7.5	8.5	7.6	9.2	9.3	7.4
Leucine	13.2	13.2	32.2	16.3	13.2	14.9	13.8	16.6	16.2	13.2
Tyrosine	3.8	3.7	12.3	5.5	5.1	6.0	4.9	6.2	6.4	5.1
Phenylalanine	9.2	9.3	21.7	11.0	9.2	10.4	9.5	11.5	11.0	9.3
Histidine	6.7	6.6	13.4	6.6	5.1	5.7	6.0	6.8	6.1	5.3
Arginine	11.1	10.9	29.5	13.7	10.8	11.6	11.7	14.0	13.6	10.3
Proline	13.5	13.9	19.4	15.2	12.4	14.0	13.1	14.9	13.1	12.3

[1]Dry samples pooled from 3 feed batches manufactured in the feed mill during experiment 2 and pooled prior to analysis (n=1/treatment)
[2]Mixing tank samples pooled from 3 samples at collection on day 26 of experiment 2 prior to analysis (n=1/treatment)
[3]Trough samples collected from 2 pens/treatment on day 26 of experiment 2 and analysed separately (n=2/treatment)
[4]FRESH = fresh liquid feeding; FERM-CER = Fermented liquid feeding where the cereal fraction (wheat and barley) only of the diet was fermented prior to feeding; FERM-WH= Fermented liquid feeding where the whole diet was fermented prior to feeding

Table 3.4 Effect of wet/dry feeding and fresh, fermented whole diet and fermented cereal liquid feeding on the growth, feed intake, feed efficiency, live weight and carcass characteristics of grow-finisher pigs in experiment 1[1]

	Treatment					P-value		
	WET/DRY[2]	FRESH[2]	FERM-CER[2]	FERM-WH[2]	SEM	Treatment	Sex	Treatment x sex
No. pens/trt[3]	9	9	9	9				
LW[4], kg								
Day 1	30.0	30.4	29.0	29.7	0.92	0.15	<0.01	0.09
Day 13	41.5[a]	41.8[a]	41.1[a,b]	37.5[b]	0.76	<0.001	0.80	<0.01
Day 34	63.2[a]	64.2[a]	65.6[a]	56.4[b]	0.76	<0.001	0.40	<0.001
Day 48	81.9[a]	80.9[a]	83.1[a]	71.6[b]	0.76	<0.001	0.44	<0.001
Day 68	103.7[a]	103.9[a]	105.7[a]	95.8[b]	0.76	<0.001	<0.001	<0.001
ADFI[4], g/day								
Day 1 – 13	1801	1851	1841	1799	72.1	0.93	0.05	0.59
Day 14 - 34	2326[b]	2519[a,b]	2691[a]	2606[a,b]	72.3	<0.01	0.13	0.02
Day 35 – 48	2808[b]	2814[b]	3096[a,b]	3148[a]	71.6	<0.001	0.36	<0.01
Day 49 – 68	2878[b]	3108[b]	3087[b]	3518[a]	71.6	<0.001	0.72	<0.001
Overall	2453[b]	2573[a,b]	2679[a]	2768[a]	62.4	<0.01	0.24	0.77
ADG[4], g/day								
Day 1 – 13	941[a]	954[a]	893[a]	613[b]	28.2	<0.001	0.03	<0.001
Day 14 - 34	1027[b,c]	1063[a,b]	1168[a]	905[c]	28.2	<0.001	0.97	<0.001

	Treatment					P-value		
	WET/DRY[2]	**FRESH[2]**	**FERM-CER[2]**	**FERM-WH[2]**	**SEM**	**Treatment**	**Sex**	**Treatment x sex**
Day 35 – 48	1325[a]	1188[a,b]	1249[a]	1086[b]	28.2	<0.001	<0.001	<0.001
Day 49 – 68	1081[b]	1146[a,b]	1130[a,b]	1217[a]	28.2	0.01	<0.001	<0.001
Overall	1094[a]	1088[a]	1110[a]	955[b]	13.0	<0.001	<0.001	0.08
FCE[4], g/g								
Day 1 – 13	1.92[b]	1.95[b]	2.07[b]	2.92[a]	0.056	<0.001	0.50	<0.001
Day 14 - 34	2.27[b]	2.41[b]	2.30[b]	2.81[a]	0.057	<0.001	<0.01	<0.001
Day 35 – 48	2.13[c]	2.39[b,c]	2.49[b]	2.90[a]	0.055	<0.001	<0.001	<0.001
Day 49 – 68	2.71	2.73	2.75	2.89	0.055	0.09	<0.001	<0.001
Overall	2.26[c]	2.37[b,c]	2.40[b]	2.88[a]	0.031	<0.001	<0.001	0.09
CV[4] of LW, %								
Day 1	4.7	4.2	6.2	5.0	0.69	0.21	0.09	0.96
Day 34	5.8[b]	4.4[b]	6.1[b]	9.1[a]	0.67	<0.001	0.06	<0.001
Day 48	4.9[b]	4.6[b]	5.7[b]	9.6[a]	0.67	<0.001	0.09	<0.001
Day 68	4.9[b]	5.1[b]	5.8[b]	9.6[a]	0.67	<0.001	0.35	<0.001
Carcass								
ADG[5], g/day	890[a]	893[a]	924[a]	788[b]	12.0	<0.001	0.16	0.15
FCE[6], g/g	2.80[a]	2.99[a]	2.95[a]	3.59[b]	0.065	<0.001	0.04	0.10
Cold-weight, kg	79.5[a]	79.7[a]	81.8[a]	72.9[b]	0.86	<0.001	0.28	0.14
Kill-out, %	77.0[a,b]	77.0[a,b]	77.5[a]	75.8[b]	0.33	0.01	0.13	0.63

	Treatment					P-value		
	WET/DRY[2]	**FRESH[2]**	**FERM-CER[2]**	**FERM-WH[2]**	**SEM**	**Treatment**	**Sex**	**Treatment x sex**
Muscle, mm	49.6^{A}	49.3^{A,B}	49.1^{A,B}	46.8^{B}	0.67	0.09	0.74	0.31
Fat, mm	12.0^{b}	12.3^{a,b}	12.6^{a,b}	13.4^{a}	0.30	0.04	0.10	0.84
Lean meat, %	57.4^{a}	57.1^{a}	56.9^{a,b}	55.8^{b}	0.30	0.02	0.15	0.77

[1]Least square means and pooled standard errors of the mean are presented
[2]WET/DRY = wet/dry feeding from a single space feeder; FRESH = fresh liquid feeding; FERM-CER = Fermented liquid feeding where the cereal fraction (wheat and barley) only of the diet was fermented prior to feeding; FERM-WH = Fermented liquid feeding where the whole diet was fermented prior to feeding
[3]No. pens/trt: pen replicates per treatment; 6 pigs per pen replicate
[4]LW = Live-weight; ADFI = Average daily feed intake; ADG = Average daily gain; FCE = Feed conversion efficiency; CV = Coefficient of variation as a measure of within pen pig weight variation
[5]Carcass ADG: From weight at start of experiment to slaughter = ((carcass weight in kg – LW on day 1 x 0.65)x1000) / number of days on treatment (Lawlor and Lynch, 2005)
[6]Carcass FCE: From start of experiment to slaughter = total average daily feed intake / carcass ADG (g)
[a,b,c] Within each row, values that do not share a common superscript are significantly different ($P<0.5$)
[A,B,C] Within each row, values that do not share a common superscript tend to be different ($0.05<P<0.10$)

Table 3.5 Effect of wet/dry feeding and fresh, fermented whole diet and fermented cereal liquid feeding on growth and carcass quality traits of grow-finisher pigs from experiment 2[1]

	Treatment					P-value		
	WET/DRY[2]	**FRESH[2]**	**FERM-CER[2]**	**FERM-WH[2]**	**SEM**	**Treatment**	**Sex**	**Treatment x sex**
No. pens/trt[3]	8	8	8	8				
LW[4], kg								
Day 1	81.2^{a}	86.6^{b}	86.5^{b}	86.7^{b}	1.69	<0.001	0.001	0.62
Day 26	115.4^{c}	$118.3^{a,b}$	120.1^{a}	$116.3^{b,c}$	0.72	<0.01	<0.001	0.07
ADFI[4],g/day	3068^{c}	$3602^{a,b}$	3743^{a}	3510^{b}	57.3	<0.001	0.42	0.50
ADG[4], g/day	1103^{c}	$1217^{a,b}$	1284^{a}	$1140^{b,c}$	27.7	<0.01	<0.001	0.07
FCE[4],g/g	2.78^{b}	$2.99^{a,b}$	$2.95^{a,b}$	3.09^{a}	0.071	0.05	<0.001	0.25
CV[4] of LW, %								
Day 1	4.8	4.0	3.5	4.1	0.67	0.33	0.01	0.32
Day 26	5.3	6.1	5.1	4.2	0.69	0.29	0.77	0.56
Carcass								
ADG[5], g/day	917^{c}	1002^{b}	1086^{a}	$967^{b,c}$	20.9	<0.001	0.24	0.19
FCE[6], g/day	3.35^{b}	$3.61^{a,b}$	$3.46^{a,b}$	3.64^{a}	0.078	0.04	0.24	0.47
Cold-weight, kg	89.1^{b}	91.0^{b}	93.7^{a}	90.1^{b}	0.62	<0.01	0.22	0.42
Kill-out, %	76.8^{b}	76.9^{b}	77.6^{a}	$77.4^{a,b}$	0.19	<0.01	0.16	0.17
Muscle, mm	$52.1^{A,B}$	52.5^{A}	52.7^{A}	51.3^{B}	0.39	0.06	0.47	0.28

	Treatment					P-value		
	WET/DRY[2]	**FRESH[2]**	**FERM-CER[2]**	**FERM-WH[2]**	**SEM**	**Treatment**	**Sex**	**Treatment x sex**
Fat, mm	11.7[b]	13.3[a]	13.4[a]	13.0[a]	0.32	0.02	0.47	0.58
Lean meat, %	58.1[a]	56.8[b]	56.7[b]	56.8[b]	0.29	0.02	0.41	0.52

[1]Least square means and pooled standard errors of the mean
[2]WET/DRY= wet/dry feeding from a single space feeder; FRESH = fresh liquid feeding; FERM-CER = Fermented liquid feeding where the cereal fraction (wheat and barley) only of the diet was fermented prior to feeding; FERM-WH = Fermented liquid feeding where the whole diet was fermented prior to feeding
[3]No. pens/trt = pen replicates per treatment; 5 pigs per pen replicate
[4]LW = Live-weight; ADFI = Average daily feed intake; ADG = Average daily gain; FCE = Feed conversion efficiency; CV = Coefficient of variation as a measure of within pen pig weight variation
[5]Carcass ADG: From weight at start of experiment to slaughter = ((carcass weight in kg – LW on day 1 x 0.75)x1000) / number of days on treatment (Lawlor and Lynch, 2005). A higher kill-out percentage was used in experiment 2 due to the heavier LW on day 1
[6]Carcass FCE: From start of experiment to slaughter = total average daily feed intake / carcass ADG (g)
[a,b,c] Within each row, values that do not share a common superscript are significantly different ($P<0.5$)
[A,B,C] Within each row, values that do not share a common superscript tend to be different ($0.05<P<0.10$)

Table 3.6 Effect of dietary treatment on apparent total tract nutrient (%) and energy (%) digestibility in grow-finisher pigs in experiment 2[1]

	Treatment[2]					P-value		
	WET/DRY	FRESH	FERM-CER	FERM-WH	SEM	Treatment	Sex	Treatment x sex
DMD[3]	87.5	86.2	86.9	87.6	0.53	0.29	0.23	0.31
OMD[3]	89.8	88.5	89.0	89.7	0.49	0.29	0.21	0.35
NitD[3]	87.0	84.8	87.4	87.8	0.84	0.11	0.33	0.15
GeD[3]	86.7	85.4	86.0	86.7	0.61	0.42	0.15	0.42
AshD[3]	61.1	58.9	63.9	64.5	1.85	0.18	0.76	0.28

[1]Least square means and pooled standard errors of the mean. Apparent total tract digestibilities were calculated from analysis of the experimental diet and faeces collected from a minimum of 3 pigs/pen from 24 pens (6 pens/treatment) on day 27 of experiment 2
[2]WET/DRY = wet/dry feeding from a single space feeder; FRESH = fresh liquid feeding; FERM-CER = Fermented liquid feeding where the cereal fraction (wheat and barley) only of the diet was fermented prior to feeding; FERM-WH = Fermented liquid feeding where the whole diet was fermented prior to feeding
[3]DMD = Dry matter digestibility; OMD = Organic matter digestibility; NitD = Nitrogen digestibility; GeD = Gross energy digestibility; AshD = Ash digestibility

Table 3.7 Effect of dietary treatment on the haematological profile of pigs at slaughter (n=68)[1]

	Treatment[2]					P-value					
	WET/DRY	FRESH	FERM -CER	FERM -WH	SEM	Trt[3]	Sex	Batch	Trt x sex	Trt x batch	Sex x batch
White blood cells, x 10^3 cells/µL	25.2	25.1	24.5	25.5	1.10	0.94	0.36	0.03	0.98	0.85	0.56
Lymphocytes											
%	$39.7^{A,B}$	43.0^{A}	$40.4^{A,B}$	37.0^{B}	1.51	0.06	0.58	0.09	0.86	0.03	0.83
no. x 10^3 cells/µL	9.88	10.74	9.91	9.22	0.459	0.15	0.20	0.37	0.92	0.12	0.40
Monocytes											
%	3.6	3.9	3.6	3.4	0.39	0.84	0.95	0.77	0.25	0.60	0.34
no. x 10^3 cells/µL	0.89	0.92	0.88	0.83	0.091	0.91	0.96	0.28	0.28	0.58	0.64
Granulocytes											
%	$56.7^{a,b}$	52.5^{b}	$56.1^{a,b}$	59.5^{a}	1.47	0.02	0.71	0.07	0.96	<0.01	0.89
no. x 10^3 cells/µL	14.41	13.24	13.69	15.38	0.890	0.37	0.60	0.03	0.98	0.38	0.78
Red blood cells, x 10^6 cells/µL	7.52	7.47	7.63	7.33	0.107	0.30	0.12	<0.01	0.84	0.20	0.75
Red cell distribution width (fL)	22.5	22.3	22.3	22.4	0.93	1.00	0.97	<0.01	0.83	1.00	0.90
Haemoglobin, g/dL	13.9	13.9	14.2	13.7	0.18	0.22	<0.0	0.18	0.79	<0.01	0.61
Haematocrit, %	41	42	40	41	1.0	0.33	0.11	0.77	1.00	0.05	0.29
Mean corpuscular volume, fL	54.1	55.1	54.0	55.0	0.52	0.22	0.37	<0.00	0.94	0.26	0.24
Mean corpuscular haemoglobin											
%	18.1	18.3	18.3	18.3	0.26	0.97	0.48	<0.00	0.41	0.44	0.12

	Treatment[2]					P-value					
	WET/DRY	**FRESH**	**FERM -CER**	**FERM -WH**	**SEM**	**Trt[3]**	**Sex**	**Batch**	**Trt x sex**	**Trt x batch**	**Sex x batch**
Pg	33.39	33.24	33.68	33.46	0.280	0.70	0.85	0.28	0.46	0.68	0.97
Platelets, x 10^3 cells/µL	216	186	195	217	22.5	0.61	0.30	0.84	0.99	0.17	0.98
Mean platelet volume (fL)	9.53	10.26	10.01	9.70	0.330	0.22	0.95	0.18	0.13	0.84	0.16

[1]Least squares means and pooled standard errors of the mean are presented. Values are the mean of data from experiments 1 and 2

[2] WET/DRY = meal diet fed from a wet/dry feeder; FRESH = fresh liquid feeding; FERM-CER = Fermented liquid feeding where the cereal fraction (wheat and barley) only of the diet was fermented prior to feeding; FERM-WH = Fermented liquid feeding where the whole diet was fermented prior to feeding

[3]Trt = Treatment

[a,b,c] Within each row, values that do not share a common superscript are significantly different (P<0.5)

[A,B,C] Within each row, values that do not share a common superscript tend to be different (0.05<P<0.10)

3.8 Figures

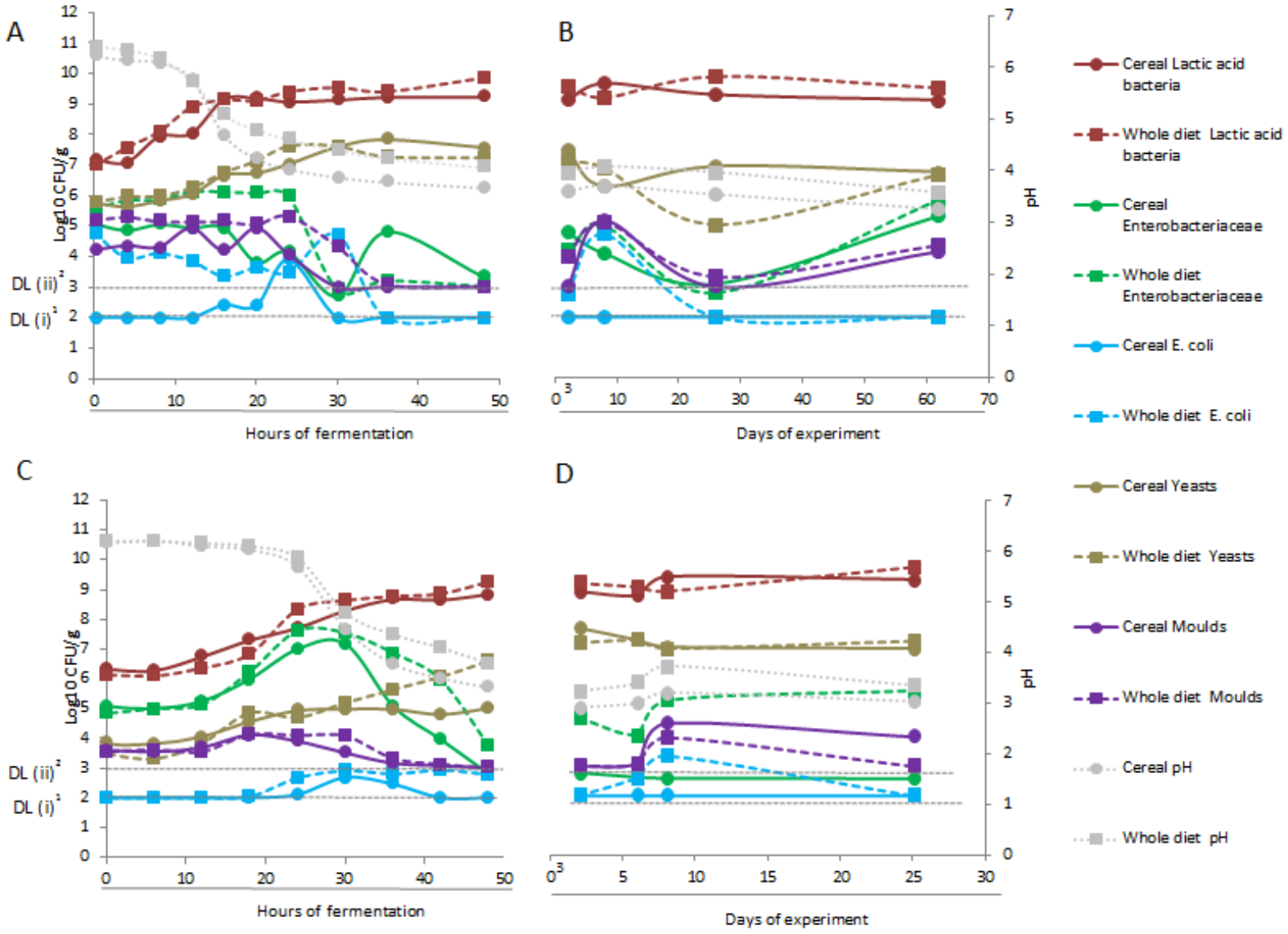
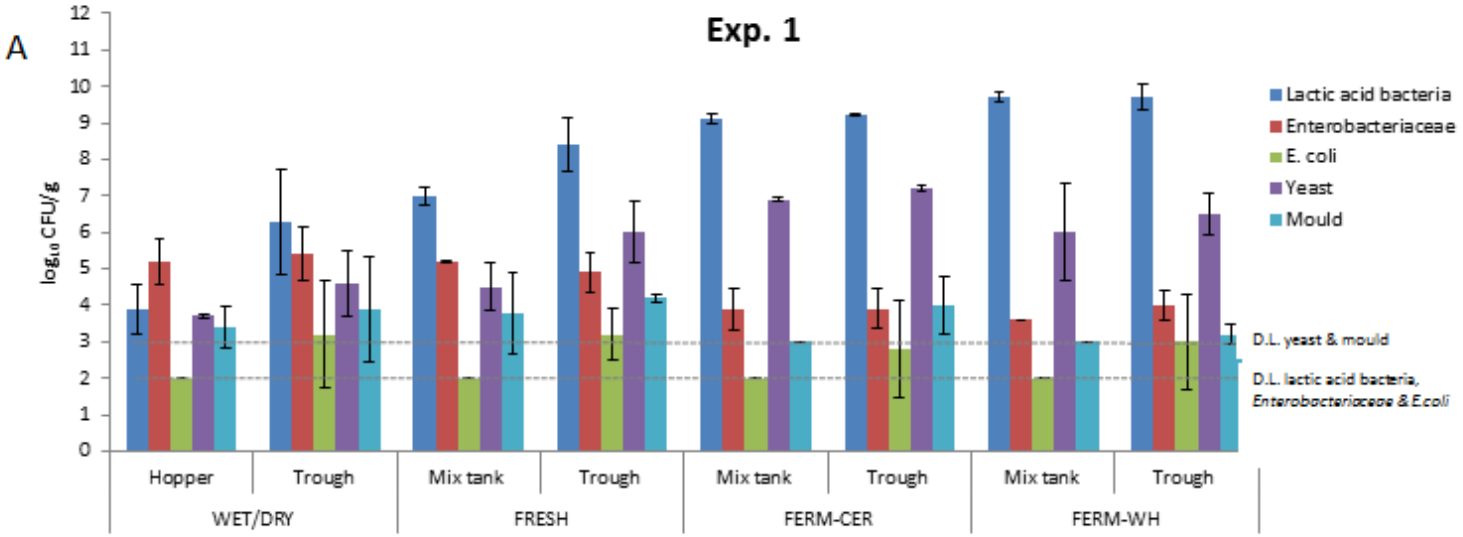

A
B
C
D
Log10 CFU/g
pH
DL (ii)²
DL (i)¹
Hours of fermentation
Days of experiment
Cereal Lactic acid bacteria
Whole diet Lactic acid bacteria
Cereal Enterobacteriaceae
Whole diet Enterobacteriaceae
Cereal E. coli
Whole diet E. coli
Cereal Yeasts
Whole diet Yeasts
Cereal Moulds
Whole diet Moulds
Cereal pH
Whole diet pH

Figure 3.1. Lactic acid bacteria, *Enterobacteriaceae, E. coli*, yeast and mould counts (log_{10} CFU/g) and pH of the fermented cereal diet and the fermented whole diet during the initial 48-hour fermentation (A, C) and for the duration of the experiments [Experiment 1 (B) and Experiment 2 (D)].

[1]DL (i) = Detection limit 1: 2 log_{10} CFU/g applies to lactic acid bacteria, *Enterobacteriaceae* and *E. coli*
[2]DL (ii)=Detection limit 2: 3 log_{10}CFU/g applies to yeast and mould
[3]Day 0 was when the initial 48 hour fermentation ended


A
Exp. 1
log10 CFU/g
Lactic acid bacteria
Enterobacteriaceae
E. coli
Yeast
Mould
D.L. yeast & mould
D.L. lactic acid bacteria, Enterobacteriaceae & E.coli
Hopper
Trough
Mix tank
Trough
Mix tank
Trough
Mix tank
Trough
WET/DRY
FRESH
FERM-CER
FERM-WH

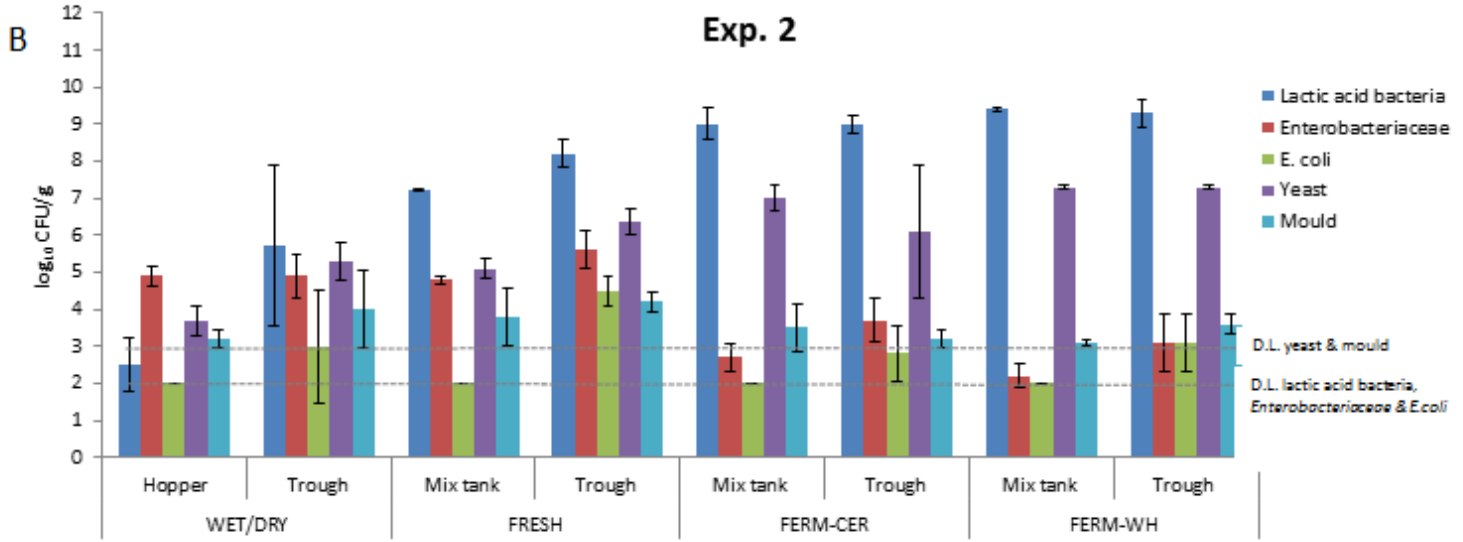

B
Exp. 2
log10 CFU/g
Lactic acid bacteria
Enterobacteriaceae
E. coli
Yeast
Mould
D.L. yeast & mould
D.L. lactic acid bacteria, Enterobacteriaceae & E.coli
Hopper
Trough
Mix tank
Trough
Mix tank
Trough
Mix tank
Trough
WET/DRY
FRESH
FERM-CER
FERM-WH

Figure 3.2 Lactic acid bacteria, *Enterobacteriaceae, E. coli*, yeast and mould counts (log_{10} CFU/g) of the four experimental diets sampled from the hopper/mixing tank and feed troughs during Experiment 1 (A)[1] and Experiment 2 (B)[2,3]

[1]Mean of data from counts performed on day 26 and day 62 of experiment 1
[2]Mean of data from counts performed on day 6 and day 25 of experiment 2
[3]The error bars represent the standard deviation

4. The effect of feed form and delivery method on feed microbiology and growth performance in grow-finisher pigs

F.M. O' Meara, G.E. Gardiner, J.V. O' Doherty, P.G. Lawlor, 2020. The effect of feed form and delivery method on feed microbiology and growth performance in grow-finisher pigs. *Journal of Animal Science*. 98 (3): skaa021. doi:10.1093/jas/skaa021

4.1 Abstract

Information is sparse regarding the optimum feed form and delivery methods for finisher pigs. The objective of this study was to compare the effect of feed form (meal and pellet) and delivery method (liquid, dry and wet/dry) on feed microbiology and growth, feed conversion efficiency (**FCE**) and carcass quality of finisher pigs. The experiment was conducted in two batches. In each batch 216 pigs (32.7 kg; ± 0.48 s.e.) housed in same sex (entire male or female) pens of 6 pigs/pen were on treatment for ~ 62 days prior to slaughter. The experiment was a 2x3 factorial arrangement with 2 factors for diet form (meal, pellet) and 3 factors for feed delivery (dry, wet/dry, liquid). The treatments were: 1. Meal from dry feeder, 2. Meal from wet/dry feeder, 3. Meal from liquid system, 4. Pellet from dry feeder, 5. Pellet from wet/dry feeder, 6. Pellet from liquid system. In total, there were 12 pen replicates per treatment. Pig growth performance was determined and blood samples collected at slaughter for haematological analysis. Microbiological and proximate analysis of feed was performed. Overall average daily gain (**ADG**) was 1114 and 1156 g/day (s.e. = 16.9; P<0.01) for pigs fed diets in meal and pellet form, respectively and 1080, 1114 and 1210 g/day (s.e. = 18.4; P<0.001) for dry-, wet/dry- and liquid-fed pigs, respectively. A significant feed form x delivery interaction was found for FCE. During the overall period FCE was 2.27, 2.34, 2.40, 2.14, 2.22 and 2.44 g/g (s.e. = 0.041, P<0.01) for treatments 1 through 6, respectively. When feed was pelleted, FCE was improved when feed delivery was dry or wet/dry compared to meal; however, when delivery was liquid, pelleting did not affect FCE. Carcass weight was 76.6 and 79.0 kg (s.e. = 0.55; P<0.001) for pigs fed in meal and pellet form, respectively, while it was 74.7, 77.3 and 81.5 kg (s.e. = 0.60; P<0.001) for pigs delivered dry, wet/dry and liquid diets, respectively. Counts of lactic acid bacteria

($P<0.05$) and yeasts ($P<0.01$) in troughs were higher for the liquid than the dry diet in both meal and pelleted form. There was also evidence of lysine degradation in the liquid diet; however, this did not impact pig growth. Pelleting the diet resulted in lower haemoglobin ($P<0.05$) and higher total white blood cell and neutrophil counts ($P<0.05$) than feeding the diet in meal form. To conclude, wet/dry feeding of a pelleted diet is recommended to maximise growth rate while optimising FCE in grow-finisher pigs.

4.2 Introduction

Pelleting improves nutrient digestibility, reduces feed wastage and increases flow-ability, thereby increasing pig growth and improving feed conversion efficiency (**FCE**) (Ball et al., 2015; Dinusson and Bolin, 1958; Nemechek et al., 2015; Seerley et al., 1962). Pelleted diets may also be microbiologically safer than meal, as evidenced by lower *Enterobacteriaceae* counts (Burns et al., 2015). Feed delivery methods can largely be classified as; dry (delivered in meal or pelleted form without being mixed with water); wet/dry (dry feed delivered as meal or pellets with pigs able to add water to the feed via a nipple or button drinker in the trough); and liquid (feed delivered pre-mixed with water from computer-controlled valves). Increased growth has been reported with liquid feeding of grow-finisher (Hurst et al., 2008; Stotfold Research Centre, 2005) and weaned (l'Anson et al., 2012; Russell et al., 1996) pigs; however, work by our group found poorer FCE when weaned pigs are liquid-fed (Lawlor et al., 2002). Wet/dry feeding of finisher pigs reportedly increases average daily feed intake (**ADFI)** and average daily gain (**ADG)** over dry-feeding (Bergstrom et al., 2008; Gonyou and Lou, 2000). Contrary to this, Magowan et al. (2008) reported that dry feeding finisher pigs maximised ADG but concluded that the optimal feed delivery system may be dependent on the stage of pig growth. It has also been reported that wet/dry feeding results in fatter carcasses with lower carcass yield than dry feeding (Bergstrom et al., 2008) which may also be the case with liquid feeding. The aim of this study was to compare dry, wet/dry and liquid feeding of the same diet in meal and pelleted form to grow-finisher pigs. It was hypothesised that liquid-fed grow-finisher pigs would have improved growth rates over dry and wet/dry-fed pigs, but with a poorer feed efficiency and that the feed efficiency of pigs fed pelleted diets would be better than those fed meal diets.

4.3 Materials and Methods

4.3.1 Ethical approval

Ethical approval for this study was granted by the Teagasc Animal Ethics Committee (approval no. TAEC 107/2015). The experiment was conducted in accordance with Irish legislation (SI no. 543/2012) and the EU Directive 2010/63/EU for animal experimentation.

4.3.2 Experimental design

The study comprised two batches of pigs, each fed the experimental diets for 62 days. A total of 432 Danavil Duroc x (Large White x Landrace) pigs (216 pigs per batch) penned in same gender pens of 6 pigs/pen with a starting weight of ~ 32.7 kg were used in the experiment. Feed form and delivery methods were compared in a 2 x 3 factorial arrangement, with two factors for feed form (meal and pellets) and three factors for feed delivery (dry, wet/dry and liquid). Pig pens were blocked on weight and sex. Pens were randomly assigned to 1 of 6 dietary treatments in a completely randomised block design as follows: (1) Dry meal diet;(2) Wet/dry meal diet; (3) Liquid meal diet; (4) Dry pelleted diet; (5) Wet/dry pelleted diet; and (6) Liquid pelleted diet. All treatments were fed on an *ad-libitum* basis.

4.3.3 Animal management

All treatments were applied in the same room to avoid an environmental effect. The groups of 6 pigs were penned in slatted pens (2.37 m x 2.36 m) with solid PVC partitions. Each pen was provided with a drinking bowl (DRIK-O-MAT, Egebjerg International A/.S, Egebjerg, Denmark). Air temperature was maintained at 20 to 22 °C and was recorded daily. The room was mechanically ventilated with fans and inlets controlled by a Steinen PCS 8100 controller (Steinen BV, Nederwert,

The Netherlands). Pigs were observed closely twice daily. Any pig showing signs of ill-health was treated as appropriate and all veterinary treatments were recorded including identity of pig, symptom, medication used and dosage. Pigs were allowed a 14-day adaptation period in the experimental facility to adapt to the new environment and feed delivery methods. During this period all pigs were fed their diet in meal form.

Treatments 1 and 4 were fed from double spaced dry feeders [Irish Dairy Services (IDS), Portlaoise, Ireland; 104.1 cm (H) x 35.6 cm (D) x 58.4 cm (W)], with one feeder per pen (6 pigs/feeder). Treatments 2 and 5 were fed from single-space wet/dry feeders [IDS; 104.1 cm (H) x 36.8 cm (D) x 30.5 cm (W)] that were fitted with a water nipple in the trough so that pigs could mix the dry feed with water at their preferred water to feed ratio. There was one single-space wet/dry feeder per pen (6 pigs/feeder). The liquid feed treatments (were fed from short steel troughs (100 cm x 32.5 cm x 21 cm, BigDutchman, Auf der Lage 2, Vechta Germany) located on top of a rubber mat (1.5 x 1 m) to help minimise feed wastage. The mixing tank and pipes in which the liquid feed was prepared and transported to pens remained empty between feeds. Each pen was equipped with a solenoid valve and a short trough fitted with an electronic sensor. The electronic sensors were checked 4 to 6 times per day and troughs with feed below the level of the sensor were refilled with their respective dietary treatments. The short-trough sensor liquid feed system ensured *ad-libitum* access to feed.

4.3.4 Diet preparation and feeding

Diets were manufactured in meal and pellet form at the Teagasc feed mill (Moorepark, Fermoy, Co. Cork, Ireland). Cereals were ground through a 3 mm

screen before mixing. The ingredient composition and nutrient content of the experimental diet is shown in Table 4.1 and the same diet specification was used in all treatments. Pelleted diets were manufactured to a diameter of 3 mm after steam heating to 50^{o}C. With all treatments, pigs were provided with *ad-libitum* access to feed and care was taken to minimise feed wastage. Where the delivery method was dry or wet/dry, feed flow to troughs was restricted so that half of the feed pan was covered with feed. Where liquid feeding was concerned, troughs were monitored closely before, during and after feeding and the feeding curve adapted daily for each pen as required to ensure that adequate feed amounts were delivered to troughs for *ad-libitum* access to feed but also to minimise liquid feed wastage. This was monitored by recording the feed level in troughs before and after feeding daily and increasing or decreasing the amount of feed delivered per pen as appropriate if a similar observation was recorded over 3 consecutive days.

Treatments 3 and 6 were fed using an automatic sensor liquid feeding system (HydroMix, BigDutchman). The diet was mixed with water (2.5:1 water:feed on a fresh matter basis) in mixing tanks with a 6 pale agitator. Diet agitation time was 10 minutes for batch 1 and 20 minutes for batch 2. Following agitation, liquid feed was delivered using air at high pressure from the mixing tanks to troughs which were fitted with electronic feed sensors. Separate mixing tanks were used for meal and pelleted diets. The mixing tank and pipelines used to prepare and distribute liquid feed, respectively were empty between feeds.

4.3.5 Records and feed sampling

Pigs were weighed on days 1, 14, 37, 55 and 62 during both batches of the experiment. Average daily gain was calculated on an individual pig basis, then

totalled for the pen and an average for the pen used. Average daily feed intake and FCE were calculated on a pen basis. Feed disappearance for liquid diets (treatments 3 and 6) were exported from the liquid feeding computer on a fresh matter basis and used to calculate ADFI. Feed disappearance for the dry (treatments 1 and 4) and wet/dry (treatments 2 and 5) delivery methods were calculated manually by subtracting the remaining feed at the end of each period from the total feed delivered to the trough during the period of interest. Feed intake and growth rates were monitored until Day 62 of each batch. Pigs removed from the trial for health reasons were weighed and their weight gain and feed intake accounted for when calculating growth and feed efficiency.

Feed samples from each of the 6 treatments were collected on days 1, 27 and 57 of each batch of the experiment for microbiological analysis. On each of the sampling days, one sample was collected from the feed storage bins for liquid feeding (one storage bin for meal, one storage bin for pellets), feed bags (from which the dry and wet/dry feeders were filled), hoppers and mixing tank, while samples from two troughs per liquid-fed treatment were sampled. All feed samples were put on ice and transported to the laboratory for analysis on the same day.

At the same time points, representative samples of each diet were also taken before feeding in each batch of the experiment for proximate and amino acid analysis. In addition, for proximate and amino acid analysis, feed samples (~ 250 g) were also taken from each of the storage bins used for the liquid diets, from the bagged diet used for wet/dry and dry feeding, from each of the mixing tanks used for the liquid diets and from the liquid diet troughs (2 per treatment) on day 57 of batch

1 and days 27 and 57 of batch 2. These samples were frozen at -20 °C prior to oven drying at 55 °C for 72 hours prior to analysis.

4.3.6 Slaughter, carcass records and blood sampling

Pigs were slaughtered at 101.0 kg ± 1.03 SEM live-weight (**LW**) by CO_2 stunning followed by exsanguination. Blood samples for haematological analysis were collected during exsanguination from 36 pigs (9 pigs/treatment) from each batch of the experiment (n=72) using Vacuette tubes (Labstock, Dublin, Ireland) containing EDTA to prevent clotting. Carcass weight was estimated by multiplying the weight of the hot eviscerated carcass 45 minutes after slaughter by 0.98. Kill out percentage was calculated as carcass weight/LW at slaughter. Back-fat thickness and muscle depth measured at 6 cm from the edge of the split back at the level of the 3rd and 4th last rib were determined using a Hennessy Grading Probe (Hennessy and Chong, Auckland, New Zealand). Lean meat content was estimated according to the following formula: Estimated lean meat content (%) = 60.3 – 0.847x + 0.147y where x = fat depth (mm); y = muscle depth (mm) (Department of Agriculture and Food and Rural Development, 2001).

4.3.7 Haematological analysis of blood samples

Haematological analysis was performed on whole blood within 6 hours of collection using an Abbot Cell-Dyn 3700 analyser (GMI-Inc, Minnesota, USA). Haematological analysis was carried out as the treatments were likely to result in differences in the microbial load of feed. Therefore, haematological analysis was used as a health indicator and to see if there was a treatment effect on white and red blood cells. The following parameters were measured; white blood cells (**WBC**), lymphocyte number and percentage, monocyte number and percentage, granulocyte

number and percentage, eosinophils number and percentage, basophil number and percentage, red blood cells, haemoglobin, mean corpuscular volume, mean corpuscular haemoglobin, platelets and packed cell volume.

4.3.8 Microbiological analysis of feed

Approximately 10 g of each feed sample was homogenized as a 10-fold dilution in Maximum Recovery Diluent (MRD; Oxoid, Basingstoke, UK) in a stomacher and a 10-fold dilution series was performed in MRD. Relevant dilutions were plated in duplicate as follows; (1) pour-plated on De Man Rogosa & Sharpe (MRS; Merck, Damstadt, Germany) agar, containing 50 U/mL nystatin (Sigma-Aldrich, Arklow, Co. Wicklow, Ireland), overlaid and incubated at 30 °C for 72 hours for lactic acid bacteria (**LAB**); (2) pour-plated on Violet Red Bile Glucose (VRBG; Oxoid) agar overlaid and incubated at 37 °C for 24 hours for *Enterobacteriaceae;* (3) pour-plated on ChromoCult Tryptone Bile X-glucuronide (CTBX; Merck) agar and incubated at 44 °C for 24 hours for *E. coli*; and (4) spread-plated on Yeast Glucose Chloramphenicol (YGC; Merck) agar and incubated at 25 °C for 5 days for yeasts and moulds. Colonies were counted and the counts from duplicate plates averaged and presented as $\log_{10}$ CFU/g of the original sample. Counts below the detection limit (30 CFU) were reported at the detection limit. Prior to statistical analysis, counts from dry feed samples from the storage bins and bagged diets were combined to create 'dry diet' data.

4.3.9 Proximate and amino acid analysis of feed

Prior to analysis, samples were ground in a Christy Norris mill through a 2 mm screen. Dry matter (DM, AOAC.934.01) and ash (AOAC.942.05) concentration was determined according to the method of the Association of Official Analytical

Chemists (AOAC, 2005). The nitrogen (N) content was determined using the LECO FP 528 instrument (Leco Instruments, UK LTD., Cheshire, UK) (AOAC.990.0). Crude protein (CP) was determined as N x 6.25. The neutral detergent fibre (NDF) content was determined according to the method of Van Soest et al. (1991) using an Ankom 220 Fibre Analyser (Ankom Technology, Macedon, New York, USA). Gross energy was determined using an adiabatic bomb calorimeter (Parr Instruments, Moline, IL USA). Amino acid determination was carried out using cation exchange HPLC as previously described by as McDermott et al. (2016) (AOAC 994.12).

4.3.10 Statistical analysis

Growth parameters (ADFI, ADG, FCE and LW), carcass quality parameters and haematology data were analysed using the MIXED procedure of SAS® 9.4 (SAS Institute, Inc., Cary, NC, US). Data from batch 1 and batch 2 were analysed together as all measurements were recorded at the same time points. For growth parameters; feed form, feed delivery and day of the experiment and their associated interactions were included in the model as fixed effects. Initial LW was included as a covariate and day as a repeated variable in the model and pen was the experimental unit. For carcass growth parameters (carcass ADG and carcass FCE), feed form and feed delivery and their interaction were included in the model as fixed effects with pen as the experimental unit. A random effect of pen within block was included in the model for all growth parameters. For carcass quality and haematology parameters; feed form and feed delivery and their interaction were included in the model as fixed effects with pen as the experimental unit. Carcass weight was included as a covariate for muscle, fat and lean meat percentage, while initial LW was used as a covariate for carcass weight.

Counts of LAB, *Enterobacteriaceae*, yeast and mould were analysed using the MIXED procedure of SAS ® 9.4. Counts from samples collected on days 1, 27 and 57 of each batch were included in the analysis. Sampling location (dry feed, mixing tank or liquid feed from the trough), feed form and their associated interaction were included as main effects in the model, with batch as a random effect and day included as a repeated measure. *E. coli* counts could not be analysed in the same way due to normality issues with the data so the LOGISTIC procedure was used on censored data with location and feed form in the model.

The normality of scaled residuals was investigated using the Shapiro-Wilk and Kolmogonov-Smirnov tests within the UNIVARIATE procedure of SAS. Results are presented as LS means ±SEM. Differences were considered significant at $P<0.05$ and as tendencies $0.05<P<0.10$.

4.4 Results

4.4.1 Pig removals

Three pigs were removed during batch 1; two due to lameness and one due to a rupture. Two pigs were removed from the wet/dry pellets treatment and one from the liquid pellets treatment. Six pigs were removed during batch 2; three due to lameness, one due to a broken back, one due to a rupture and one due to unusual discharge. Two pigs were removed from the dry meal treatment, one removed from the dry pellet treatment, two removed from the liquid meal treatment and one removed from liquid pellet treatment.

4.4.2 Effect of feed form x feed delivery on the growth and carcass quality of grow-finisher pigs

The effects of feed form by feed delivery interactions on pig growth parameters and carcass quality are reported in Table 4.2. The interaction for ADFI for the overall period was not significant. During the overall period, a tendency for a feed form x delivery interaction was observed for ADG. Pigs fed a meal diet using dry or wet/dry feed delivery grew similarly but slower than those fed a liquid meal diet; however, when fed a pelleted diet, both wet/dry and liquid feeding tended to increase ADG compared with dry feeding (P=0.07). During the overall period, a feed form x delivery interaction was observed for FCE. Feed efficiency was improved for pigs fed a pelleted diet compared to a meal diet when feed delivery was dry or wet/dry, but not when the pelleted diet was liquid fed (P<0.01).

There was a feed form x delivery interaction for pig LW at day 62 (P<0.001). When fed a meal diet, liquid fed pigs were heavier than dry and wet/dry fed pigs, while pigs fed a wet/dry pelleted diet were similar in weight to those fed a liquid

pelleted diet (P<0.001). There was a tendency for an interaction for carcass ADG (P=0.09). When fed a meal diet, wet/dry fed pigs grew faster than dry-fed, while pigs fed a pelleted dry and wet/dry diet grew similarly. There was an interaction for carcass FCE (P<0.01). Pigs fed a meal diet via dry, wet/dry and liquid delivery had similar carcass FCEs, while pigs fed a liquid pelleted diet had a worse carcass FCE than those fed dry or wet/dry. There were no feed form x feed delivery interactions for carcass quality in the current study.

4.4.3 Effect of feed form on the growth and carcass quality traits of grow-finisher pigs

Providing the diet in meal or pelleted form did not influence ADFI during the overall period. During the overall period, ADG was 1114 and 1156 g/day (s.e. = 16.9; P< 0.01) and FCE was 2.34 and 2.27 g/g (s.e. = 0.034; P<0.01) for meal- and pellet-fed pigs, respectively. Pig LW at day 62 was 99.7 and 101.7 kg (s.e. = 0.51; P<0.001) for meal- and pellet-fed pigs, respectively. At slaughter, carcass weight was 76.6 and 79.0 kg (s.e. = 0.55; P<.0001), kill-out yield was 76.7 and 77.6 % (s.e. = 0.22; P<0.001), backfat depth was 12.7 and 12.2 mm (s.e. = 0.20; P<0.05) and lean meat yield was 57.2 and 57.6 % (s.e. = 0.18; P<0.05) for pigs fed meal and pelleted diets, respectively.

4.4.4 Effect of feed delivery method on the growth and carcass quality traits of grow-finisher pigs

During the overall period, ADFI was 2329, 2483 and 2869 g/day (s.e. = 55.2; P<0.001), ADG was 1080, 1114 and 1210 g/day (s.e. = 18.4; P<0.001) and FCE was 2.21, 2.28 and 2.42 g/g (s.e. = 0.036; P<0.001) for dry-, wet/dry- and liquid-fed pigs, respectively. Pig LW at day 62 was 97.3, 99.8 and 104.9 kg (s.e. = 0.59, P<0.001)

for pigs fed using dry, wet/dry and liquid delivery systems, respectively. At slaughter, carcass weight was 74.7, 77.3 and 81.5 kg (s.e. = 0.60; P<0.001) and kill-out percentage was 76.7, 77.3 and 77.5 % (s.e. = 0.24; P<0.01) for dry, wet/dry and liquid-fed pigs.

4.4.5 Microbiological quality of dry diets fed via dry and wet/dry feeders and liquid feed prepared for grow-finisher pigs

Microbial counts in the dry diets and liquid feed samples collected from the mixing tanks and feed troughs are shown in Table 4.3. Significant feed form x sampling location interactions were observed for LAB, *Enterobacteriaceae*, and yeast counts. Lower LAB, *Enterobacteriaceae* and yeast counts were seen when the diet was dry in pelleted form compared to in meal form; however, no differences were observed in the mixing tank or troughs between feed forms.

In terms of feed form, LAB counts were 6.17 and 5.84 $\log_{10}$ CFU/g (s.e. = 0.126; P=0.07), *Enterobacteriaceae* counts were 5.47 and 4.86 $\log_{10}$ CFU/g (s.e. = 0.193; P<0.01), yeast counts were 4.25 and 4.03 $\log_{10}$ CFU/g (s.e. = 0.135; P=0.07) and mould counts were 4.11 and 3.78 $\log_{10}$ CFU/g (s.e. = 0.158; P<0.05) for meal and pellets, respectively (data not shown).

For sampling location, LAB counts were 2.79, 6.84 and 8.38 $\log_{10}$ CFU/g (s.e. = 0.155; P<0.001), *Enterobacteriaceae* counts were 4.25, 5.12 and 6.13 $\log_{10}$ CFU/g (s.e. = 0.217; P<0.001), yeast counts were 3.52, 3.63 and 5.27 $\log_{10}$ CFU/g (s.e. = 0.147; P<0.001) and mould counts were 3.38, 4.09 and 4.37 $\log_{10}$ CFU/g (s.e. = 0.173, P<0.001) for dry feed, mixing tank and liquid feed trough samples, respectively (data not shown). Results for *E. coli* counts showed that it was 143

times more likely to find *E. coli* in the liquid feed troughs than in the mixing tanks or dry feed.

4.4.6 Effect of feed form and delivery on gross energy, crude protein, ash, neutral detergent fibre and amino acid content of feed

The means and standard deviations of GE, CP, Ash, NDF and amino acid content of meal and pelleted feed at the sampling locations described above are shown in Table 4.4. There was no obvious decrease in GE or CP content of the feed when levels in the dry diet, mixing tank and trough are compared. There was evidence of some loss of NDF and lysine in liquid-fed troughs compared to the dry diet.

4.4.7 Effect of feed form and delivery methods on the haematological profile of pigs at slaughter

The haematological profile of pigs at slaughter is shown in Table 4.5. No significant feed form x feed delivery interactions were found. A tendency for feed form x feed delivery was observed for packed cell volume; however, there were no pairwise differences between treatments ($P>0.01$). Feed delivery did not affect any of the parameters measured. Haemoglobin levels in pigs fed the diet in meal and pellet form were 14.25 and 13.74 g/dL (s.e. = 0.169; $P<0.05$), mean corpuscular haemoglobin was 17.27 and 16.60 pg (s.e. = 0.194; $P<0.05$), white blood cell counts were 2.07×10^{10} and 2.30×10^{10} cells/L (s.e. = 6.93×10^{8}; $P<0.05$) and neutrophil counts were 1.28×10^{9} and 1.48×10^{9} cells/L (s.e. = 6.07×10^{8}; $P<0.05$), respectively. However, haemoglobin levels were within the normal range for pigs (10 – 16 g/dL), but mean corpuscular haemoglobin levels were slightly below the normal range of 17 – 21 pg for pigs on wet/dry meal, dry pellets and wet/dry pellets,

WBC counts were above the normal range for grow-finisher pigs (11 – 22 $x10^9$ cells/L) on each of the pelleted diets and neutrophil counts were above the normal range of 3.08 – 10.45 $x10^9$ cells/L for all treatments. The normal ranges referred to are those specified for pigs by Jackson and Cockcroft (2008).

4.5 Discussion

4.5.1 Optimum feed form and delivery for grow-finisher pigs

To our knowledge, this study is the first to compare feed form (meal, pellet) and feed delivery method (dry, wet/dry, liquid) under the same environmental conditions. Such a study is fundamental to determine the optimum mix of feed form and delivery methods to optimise pig growth, feed efficiency and ultimately profitability in commercial pig production. The results here can be utilised by commercial pig producers to inform investment decisions when it comes to planning new or upgrading existing facilities. Our results suggest that to optimise feed efficiency, dry or wet/dry feeding of a pelleted diet would be the preferred feeding strategy. Furthermore, the method of feed delivery chosen will determine whether feed should be pelleted or not. Our results suggest an advantage of pelleting when the diet is fed via dry or wet/dry hoppers, but that there is no growth or feed efficiency advantage of liquid feeding a pelleted diet over a meal diet. The results of the current study suggest that feeding a pelleted diet from a dry or a wet/dry feeder will optimise FCE in grow-finisher pigs compared with all other feed form and delivery methods compared.

The current study shows an FCE advantage with dry or wet/dry feeding of a pelleted diet compared with dry or wet/dry feeding of the same diet in meal form. These results are in contrast to those of Myers et al. (2013) who reported similar FCE for wet/dry feeding of a meal and pelleted diet but showed a poorer FCE in pigs fed a pelleted diet from a dry feeder compared with a meal diet from the same type of feeder. They explained that a high proportion of fines in their pelleted diet most likely explained the poorer FCE found with pellets indicating the importance of

pellet quality (Myers et al., 2013). A 5.7 % improvement in FCE was reported here by feeding a dry pelleted diet compared with a dry meal diet which agrees with the 7 % improvement reported by Wondra et al. (1995b). Carcass ADG in the current study increased for wet/dry compared to dry feeding of meal but not when the diet was pelleted which supports the suggestion of Gonyou and Lou (2000) that increases in ADG for wet/dry feeding compared to dry feeding a diet would be greater for a meal diet than a pelleted diet, due to the higher eating speed with pelleted diets.

The advantages of pelleting in terms of optimising FCE found when feeding dry or wet/dry diets was not found with liquid feeding. In agreement, l'Anson et al. (2013) found no improvement in FCE due to pelleting when liquid feeding was practiced, despite finding an improvement when dry feeding was practiced. It is likely that liquid feeding reduced dust losses normally associated with feeding meal, thereby negating the response to pelleting.

4.5.2 Impact of feed delivery method on pig growth and feed efficiency

In the current experiment, the treatments with the highest feed intakes (wet/dry and liquid) had water available at the point of feeding. One reason for increased feed intake in wet/dry over dry feeding suggested by Averos et al. (2012), is that both hunger and thirst motivate pigs to visit the feeder, compared with hunger alone with dry feeding. Additionally, eating speed is increased when diets are mixed with water (Gonyou and Lou, 2000; Bergstrom et al., 2012) which may also help to explain the increased ADFI found with wet/dry and liquid feeding compared with dry feeding in the current experiment. Others also found ADFI to increase in response to wet/dry feeding compared with dry feeding (Bergstrom et al., 2012;

Bergstrom et al., 2008; Brumm et al., 2000; Gonyou and Lou, 2000; Myers et al., 2013).

Average daily gain was increased by liquid feeding compared with dry- and wet/dry-feeding in the current study. Others have also found this to be the case when liquid when liquid feeding was compared with dry feeding (Braude and Rowell, 1967; Hurst et al., 2008; Kim et al., 2001; l'Anson et al., 2012; Russell et al., 1996; Stotfold Research Centre, 2005). A higher eating rate has been found with liquid feeding which helps explain the associated increase in growth rate (Braude, 1967; Hurst et al., 2008). In studies where no differences in ADG between liquid- and dry-feeding were found, (Dung et al., 2005; Zoric et al., 2015), it is likely that the feeding curves or daily feed allowances were restrictive, particularly in the study by Dung et al. (2005), and as such prevented pigs from reaching their true intake and growth potential.

The poorer FCE observed in the current study in liquid fed pigs is most likely due to increased feed wastage. Troughs were located at ground level and despite the use of solid rubber mats under and around them to minimise feed wastage it was possible for pigs to remove feed with their feet and faces at feeding time. Feed wastage was easier to control in the dry and wet/dry feeders due to good control of the release rate of the feed from hopper to trough. Increased feed wastage in liquid feed resulting in poor feed efficiency has also been reported in other studies (Han et al., 2006; l'Anson et al., 2012; Missotten et al., 2010; Plumed-Ferrer and Von Wright, 2009; Russell et al., 1996). Liquid feeding as practiced in the current study allowed *ad-libitum* access of pigs to the liquid feed. A 7 – 10 % improvement in FCE is possible if access to liquid feed is restricted (Hurst et al., 2008). Therefore,

one could speculate that if a restricted liquid feeding regime was used in the current study, the FCE of liquid fed pigs could be improved to levels observed for dry and wet/dry feeding.

4.5.3 Influence of feed form on the growth and feed efficiency of grow-finisher pigs

Pelleting the diet in the current experiment resulted in an increased ADG but not ADFI. It is likely that this is due to reduced feed wastage (Patterson, 1989) and improved nutrient digestibility (Ball et al., 2015; O'Doherty et al., 2001; Seerley et al., 1962; Wondra et al., 1995a) in response to pelleting. Pelleting diets has long been associated with improved feed utilisation efficiency and nutrient digestibility (Hanrahan, 1984). In agreement with previous reports (Ball et al., 2015; De Jong et al., 2016; Hedemann et al., 2005; l'Anson et al., 2012; l'Anson et al., 2013; Mikkelsen et al., 2004; Millet et al., 2012; Myers et al., 2013; Nemechek et al., 2015; Seerley et al., 1962; Stark et al., 1993; Wondra et al., 1995a), the FCE of dry and wet/dry pellet-fed pigs was significantly improved in the current study.

4.5.4 The impact of feed pelleting and delivery method on the microbiological quality and proximate analysis of feed

There is evidence that spontaneous fermentation occurred in the liquid feed in the current study, as counts of LAB and yeasts increased significantly from dry feed to the mixing tank and again to the trough in both meal and pelleted form. Despite the fact that there were significantly lower LAB and yeast counts in the dry pelleted diet than the dry meal diet, LAB and yeast counts in liquid feed troughs were similar between meal and pellets, suggesting that LAB and yeast growth is not affected by feed form once the feed is mixed with water. It also seems that pelleted

feed is of better microbiological quality, as evidenced by the lower *Enterobacteriaceae* counts observed in dry pelleted feed compared to dry meal feed. This is in agreement with previous work by our group, where lower *Enterobacteriaceae* counts were also found in pelleted versus meal feed in a survey of feed from commercial feed mills, likely due to the high temperatures used in the pelleting process (Burns et al., 2015). This may in part explain the improved growth rate in pigs fed the pelleted diet in dry or wet/dry form compared to meal-fed pigs using the same delivery system, as lower *Enterobacteriaceae* counts may indicate a lower prevalence of *Salmonella*, for example, in the feed (Jones and Richardson, 2004; Veldman et al., 1995). It was somewhat surprising that *Enterobacteriaceae* counts were similar between dry meal and liquid feed prepared from the meal when the latter was sampled form the feed trough. These high *Enterobacteriaceae* counts may negatively impact pig health and could be another reason that the FCE of pigs fed dry and wet/dry pelleted diets was improved compared to those fed dry and wet/dry meal diets.

Although based on a small number of samples, lysine concentrations in liquid feed from the mixing tank and troughs were lower than those in the corresponding dry meal and pelleted feed samples. Amino acid degradation is a disadvantage that has long been associated with fermented liquid feeding (Brooks, 2008; Pedersen, 2001), but evidence of its existence in fresh liquid feed is lacking. However, in the present study it did not seem to impact the growth of pigs but is likely to have contributed to the poorer FCE observed for liquid feeding.

4.5.5 The impact of feed form and delivery on the haematological profile of pigs

An interesting observation was that pelleting the diet resulted in lower haemoglobin and mean corpuscular volume and higher white blood cell and neutrophil counts in the blood compared to meal-fed pigs in the current study. However, haemoglobin levels were within the normal range, but mean corpuscular haemoglobin levels were slightly below the normal range for pigs on dry and wet/dry pellets, total white blood cell counts were above the normal range on all of the pelleted diets and neutrophil counts were above the normal range (3.08 – 10.45 cells $x10^9$/L) for all treatments [normal ranges for pigs as specified by Jackson and Cockcroft (2008)]. Pelleting reduces the particle size of the diet and is also associated with gastric ulceration (Krauss et al., 2018; Vukmirović et al., 2017). One could speculate that lower haemoglobin levels in pellet-fed pigs may be early indicators of sub-clinical ulceration associated with reduced particle size of the diet. Ulceration may also explain the elevated WBC count in these animals. In fact, previous work has shown higher WBC counts in piglets fed finely ground compared to coarsely ground corn (Huang et al., 2015).

In conclusion, the current study suggests that to achieve the best carcass FCE in grow-finisher pigs, dry or wet/dry feeding of a pelleted diet is optimal. Liquid feeding a meal or pelleted diet maximised carcass daily gain, however, as pelleting the diet did not increase carcass growth rate compared with meal in liquid-fed pigs, liquid feeding a meal will in practice be used to maximise growth rate. Wet/dry feeding a pelleted diet achieved a similar growth rate to liquid feeding a meal diet and because it also optimises FCE, subject to economic assessment, it is the recommended method of feeding grow-finisher pigs based on this study.

Investigations to determine if restricted liquid feeding in comparison to *ad-libitum* liquid feeding can improve FCE is warranted to optimise feed efficiency where liquid feeding is practiced.

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4.7 Tables

Table 4.1 Composition of the experimental diet (on an as-fed basis, g/kg unless otherwise stated)[1]

	Experimental diet
Ingredient composition[2]	
Wheat	400.0
Barley	382.7
Soya bean meal	183.0
Limestone flour	11.0
Soya oil	9.7
Lysine HCl	3.8
Salt	3.0
L-Threonine	1.7
Celite	2.0
Vitamin and mineral premix[3]	1.0
Mono DiCalcium Phosphate	1.0
DL-Methionine	0.9
L-Tryptophan	0.2
Phytase[4]	0.1
Chemical composition	
Dry matter	877.0
Crude protein	174.0
Ash	39.2
Neutral detergent fibre	163.3
Gross energy, MJ/kg	16.0
Lysine	10.6

	Experimental diet
Methionine	4.3
Threonine	7.2
Digestible energy, MJ/kg[2]	13.8
Net energy, MJ/kg[2]	9.8
Oil[2]	25.7
SID Lysine[2,5]	10.0
Total Calcium[2]	6.6
Total Phosphorus[2]	2.6

[1]Values are the mean of experimental diets from experiment 1 and experiment 2
[2]Calculated values
[3]Vitamin and mineral premix provided per kilogram of complete diet: Cu from copper sulphate, 15 mg; Fe from ferrous sulphate monohydrate, 24 mg; Mn from manganese oxide, 31 mg; Zn from zinc oxide, 80 mg; I from potassium iodate, 0.3 mg; Se from sodium selenite, 0.2 mg; retinyl acetate, 0.7 mg; cholecalciferol, 12.7 µg; DL-alpha-tocopheryl acetate, 40 mg; Vitamin K, 4 mg; vitamin B12, 15 µg; riboflavin, 2 mg; nicotinic acid, 12 mg; pantothenic acid, 10 mg; vitamin B1, 2 mg; vitamin B6, 3 mg and celite 2000 mg/kg.
[4]The diet contained 500 phytase units (FYT) per kg feed from RONOZYME HiPhos (DSM, Belfast, UK)
[5]SID Lysine = Standardized ileal digestible lysine

Table 4.2 Effect of feed form (meal or pellets) by delivery (dry, wet/dry or liquid) interaction on the live-weight, average daily gain, average daily feed intake, feed conversion efficiency, and carcass characteristics of grow-finisher pigs (N=12pens/treatment)[1]

Form	Meal			Pellet				P-value		
Delivery	Dry	Wet/dry	Liquid	Dry	Wet/dry	Liquid	SEM	Form x delivery	Form	Delivery
LW, kg[2]										
Day 1	32.9	32.8	32.4	32.7	32.4	33.2	0.48	0.10	0.80	0.67
Day 14	46.0	46.9	47.0	46.4	46.8	48.1	0.78	0.41	0.48	0.15
Day 37	69.7^{c}	$71.0^{a,b,c}$	$74.0^{a,b}$	$70.8^{b,c}$	$72.3^{a,b,c}$	74.6^{a}	0.78	0.001	0.11	0.001
Day 55	88.3^{c}	$91.0^{b,c}$	96.6^{a}	$91.0^{b,c}$	$93.3^{a,b}$	96.2^{a}	0.78	0.001	0.01	0.001
Day 62	95.7^{c}	$98.1^{b,c}$	105.1^{a}	$98.8^{b,c}$	$101.5^{a,b}$	104.7^{a}	0.78	0.001	0.001	0.001
ADFI, g/day[2]										
Day 1 – 14	1838^{b}	$1942^{a,b}$	$1946^{a,b}$	1706^{b}	$1848^{a,b}$	2116^{a}	55.2	0.001	0.67	0.001
Day 15 – 37	2325^{b}	2394^{b}	2719^{a}	2177^{b}	2349^{b}	2786^{a}	55.2	0.001	0.35	0.001
Day 38 – 55	2549^{c}	$2749^{b,c}$	3225^{a}	$2600^{b,c}$	2845^{b}	3150^{a}	55.2	0.001	0.59	0.001
Day 56 – 62	2639^{c}	$2787^{b,c}$	3509^{a}	$2795^{b,c}$	2950^{b}	3501^{a}	55.2	0.001	0.02	0.001
Overall	2338	2468	2850	2320	2498	2888	38.9	0.72	0.59	0.001
ADG, g/day[2]										
Day 1 – 14	889^{B}	$943^{A,B}$	$955^{A,B}$	901^{B}	$939^{A,B}$	1033^{A}	39.2	0.09	0.34	0.03
Day 15 – 37	1027^{d}	$1047^{c,d}$	1172^{a}	$1065^{c,d}$	$1105^{b,c}$	$1151^{a,b}$	39.2	0.03	0.41	0.01

Form	Meal			Pellet				P-value		
Delivery	Dry	Wet/dry	Liquid	Dry	Wet/dry	Liquid	SEM	Form x delivery	Form	Delivery
Day 38 – 55	1026^{b}	1109^{a,b}	1249^{a}	1130^{a,b}	1170^{a,b}	1204^{a,b}	39.2	0.001	0.19	0.001
Day 56 – 62	1289^{a,b}	1189^{b}	1472^{a}	1312^{a,b}	1412^{a}	1448^{a}	39.2	0.001	0.01	0.001
Overall	1057^{C}	1072^{C}	1212^{A}	1102^{B,C}	1156^{A,B}	1209^{A}	22.6	0.07	0.01	0.001
FCE, g/g[2]										
Day 1 – 14	2.11	2.10	2.10	1.93	2.02	2.09	0.062	0.16	0.05	0.39
Day 15 – 37	2.30^{a,b}	2.32^{a,b}	2.37^{a}	2.08^{b}	2.18^{b}	2.47^{a}	0.062	0.001	0.04	0.001
Day 38 – 55	2.53^{a,b}	2.52^{a,b}	2.67^{a}	2.35^{b}	2.50^{a,b}	2.69^{a}	0.062	0.001	0.20	0.001
Day 56 – 62	2.14^{b}	2.40^{a,b}	2.48^{a}	2.18^{b}	2.18^{b}	2.50^{a}	0.062	0.001	0.21	0.001
Overall	2.27^{b,c}	2.34^{a,b}	2.40^{a}	2.14^{d}	2.22^{c,d}	2.44^{a}	0.041	0.01	0.01	0.001
Carcass										
ADG, g/day[3]	823^{E}	868^{D}	954^{A,B}	880^{C,D}	918^{B,C}	967^{A}	12.1	0.09	<0.001	<0.001
FCE, g/g[4]	2.82^{a}	2.83^{a}	2.93^{a}	2.58^{b}	2.67^{b}	2.93^{a}	0.039	<0.01	<0.001	<0.001
Cold-weight, kg	73.0	75.8	81.0	76.4	78.8	82.0	0.75	0.11	0.001	0.001
Kill-out, %	76.1	77.0	77.0	77.2	77.5	78.0	0.30	0.39	0.001	0.01
Muscle, mm	51.1	51.8	51.3	51.1	51.7	51.2	0.50	1.00	0.91	0.26
Fat, mm	12.4	12.4	13.2	12.2	12.2	12.1	0.30	0.10	0.03	0.50
Lean meat, %	57.4	57.5	56.7	57.5	57.6	57.6	0.27	0.15	0.50	0.36

[1]Least squares means and pooled standard errors of the mean are presented. Values are the mean of data from both batches of the experiment.
[2]LW=Live-weight; ADFI= Average daily feed intake; ADG= Average daily gain; FCE= Feed conversion efficiency.

[3]Carcass ADG: From weight at start of experiment to slaughter = ((carcass weight in kg – LW on day 1 x 0.65)x1000) / number of days on treatment (Lawlor and Lynch, 2005)
[4]Carcass FCE: From start of experiment to slaughter = total average daily feed intake / carcass ADG (g)
[a,b,c] Within each row, values that do not share a common superscript are significantly different ($P<0.5$)
[A,B,C] Within each row, values that do not share a common superscript tend to be different ($0.05<P<0.10$)

Table 4.3 Microbial counts (log_{10} CFU/g) in dry diets and in the dietary treatments collected from the mixing tanks and from the feed troughs in the pig pens[1,2]

Form	Meal			Pellet				P-value		
Delivery	Dry[3]	Mixing Tank[4]	Trough[5]	Dry[3]	Mixing Tank[4]	Trough[5]	SEM	Sampling location x feed form	Sampling location	Feed form
LAB[6]	3.30^{b}	6.77^{c}	8.45^{d}	2.29^{a}	6.92^{c}	8.31^{d}	0.219	0.03	0.001	0.07
Enterobacteriaceae	$5.24^{b,c}$	5.09^{b}	$6.09^{b,c}$	3.26^{a}	$5.15^{b,c}$	6.18^{c}	0.276	0.001	0.001	0.01
Yeast	3.92^{b}	$3.64^{a,b}$	5.20^{c}	3.12^{a}	$3.63^{a,b}$	5.35^{c}	0.178	0.01	0.001	0.07
Mould	3.75	4.17	4.42	3.00	4.01	4.32	0.213	0.14	0.001	0.03

[1]*E. coli* counts were omitted from this analysis due to normality issues with the data. Therefore, a logistic procedure was used to calculate the likelihood of presence or absence of *E. coli* in the mixing tank or dry feed compared to in the trough and these data are presented in the text.
[2]Values are the mean of data from samples taken on days 1, 27 and 57 of batch1 and on the same days during batch 2, i.e. from 6 time points
[3]Dry: Represents dry feed samples collected from the storage bins used for preparing liquid feed and bagged diets used for feeding dry and wet/dry feed
[4]Mixing tank: Represents samples of liquid feed collected at the end of the agitation process
[5]Trough: Represents samples taken from liquid-fed troughs only
[6]LAB=Lactic acid bacteria
[a,b,c] Within each row, values that do not share a common superscript are significantly different ($P<0.5$)
[A,B,C] Within each row, values that do not share a common superscript tend to be different ($0.05<P<0.10$)

Table 4.4 Proximate analysis of dry diets and liquid feed collected from the mixing tanks and troughs for both the meal and pelleted diets[1] (presented on a dry matter basis)

Form	Meal			Pellets			
Delivery	**Dry[3]**	**Mixing tank[4]**	**Trough[5]**	**Dry[3]**	**Mixing tank[4]**	**Trough[5]**	**SD[2]**
Gross energy, MJ/kg	18.3	18.6	18.0	18.2	18.9	18.2	0.26
Crude protein, %	19.9	20.4	21.0	19.7	22.0	20.7	1.36
Ash, %	4.3	3.7	5.2	4.6	3.8	5.0	0.64
NDF, %	18.7	16.3	14.7	18.5	18.7	15.0	2.79
Amino acids, g/kg							
Lysine	12.1	9.7	10.0	12.1	11.3	9.3	1.29
Cysteic acid	5.4	5.3	5.9	5.3	5.6	5.5	0.27
Taurine	0.5	2.5	1.3	0.9	1.9	1.2	0.71
Methionine	5.0	5.2	4.7	4.8	5.0	4.6	0.28
Aspartic acid	17.1	17.4	18.3	17.1	18.3	16.4	1.49
Threonine	8.0	8.4	8.2	8.3	8.8	7.8	0.46
Serine	9.3	9.4	10.1	9.3	10.0	9.3	0.69
Glutamic acid	43.5	43.5	47.2	42.8	45.7	44.8	2.85
Glycine	8.1	8.5	9.0	8.1	9.0	8.3	0.57
Alanine	7.8	8.1	8.7	7.7	8.9	8.0	0.68
Cysteine	0.5	1.4	0.8	0.6	1.2	1.0	0.43
Valine	9.4	9.5	10.2	9.4	10.5	9.3	0.68

Form	Meal			Pellets			
Delivery	**Dry[3]**	**Mixing tank[4]**	**Trough[5]**	**Dry[3]**	**Mixing tank[4]**	**Trough[5]**	**SD[2]**
Isoleucine	7.6	7.9	8.4	7.6	8.3	7.5	0.68
Leucine	13.7	14.2	15.1	13.7	15.0	13.7	1.11
Tyrosine	5.2	4.4	5.6	5.2	5.3	5.2	0.60
Phenylalanine	9.6	9.7	10.4	9.5	10.3	9.5	0.72
Histidine	5.7	6.0	5.8	5.7	6.0	5.8	0.40
Arginine	11.8	11.9	11.7	11.8	12.3	10.9	0.88
Proline	12.9	13.8	14.9	13.0	14.7	13.8	1.05

[1]Means and their associated standard deviation are presented for each sampling location
[2]SD: Standard deviation
[3]Dry: Represents dry feed samples collected from the storage bins used for preparing liquid feed and bagged diets used for feeding dry and wet/dry feed; mean of 4 samples presented
[4]Mixing tank: Represents samples of liquid feed collected at the end of the agitation process; mean of 2 samples presented
[5]Trough: Represents samples taken from liquid-fed troughs only; mean of 2 samples presented

Table 4.5 Effect of feed form (meal or pellets) by delivery (dry, wet/dry or liquid) interaction on the on the haematological profile of pigs at slaughter (N=18/treatment)[1]

Form	Meal			Pellet				P-Value		
Delivery	Dry	Wet/dry	Liquid	Dry	Wet/dry	Liquid	SEM	Form x Delivery	Form	Delivery
White blood cells, $x10^9$ cells/L	20.8	20.7	20.5	22.7	22.5	23.7	1.20	0.80	0.02	0.91
Lymphocytes										
%	25.3	30.7	27.6	26.4	25.4	26.9	1.99	0.26	0.33	0.54
$x10^9$ cells/L	5.1	6.3	5.5	6.0	5.6	6.1	3.69	0.10	0.40	0.56
Monocytes										
%	8.7	8.9	9.6	9.0	8.6	7.5	0.84	0.34	0.31	0.92
$x10^9$ cells/L	1.8	1.8	2.0	2.0	2.0	1.8	2.09	0.57	0.86	0.99
Granulocytes										
Neutrophils										
%	64.4	58.8	61.0	62.7	64.0	64.2	1.97	0.20	0.17	0.55
$x10^9$ cells/L	13.6	12.3	12.6	14.3	14.5	15.5	10.52	0.58	0.03	0.79
Eosinophils										
%	0.2	0.1	0.1	0.2	0.2	0.1	0.84	0.42	0.54	0.12
$x10^9$ cells/L	0.03	0.03	0.03	0.05	0.04	0.02	0.099	0.37	0.39	0.29
Basophils										
%	1.4	1.5	1.7	1.7	1.8	1.3	0.24	0.25	0.75	0.82

Form	Meal			Pellet				P-Value		
Delivery	Dry	Wet/dry	Liquid	Dry	Wet/dry	Liquid	SEM	Form x Delivery	Form	Delivery
$x10^9$ cells/L	0.29	0.33	0.35	0.38	0.40	0.33	0.053	0.53	0.30	0.90
Red blood cells (x 10^{12} cells/L)	8.4	8.3	8.1	8.2	8.4	8.3	0.17	0.43	0.79	0.75
Haemoglobin (g/dL)	14.6	14.0	14.1	13.4	13.7	14.1	0.29	0.10	0.03	0.69
Mean corpuscular volume (fL)	58.8	57.5	57.2	56.6	55.6	57.9	1.32	0.49	0.29	0.64
Mean corpuscular haemoglobin Concentration (g/dL)	29.6	29.5	30.9	29.0	29.7	29.4	0.63	0.39	0.20	0.40
Pg	17.4	16.9	17.5	16.4	16.4	17.0	0.34	0.72	0.02	0.28
Platelets (x 10^9 cells /L)	201.5	220.2	252.5	284.2	241.0	260.1	269.70	0.34	0.10	0.64
Packed cell volume (L/L)[2]	0.49	0.47	0.46	0.46	0.46	0.48	0.010	0.06	0.34	0.60

[1]Least squares means and pooled standard errors of the mean are presented. Values are the mean of data from both batches of the experiment.
[2]An overall tendency was observed for the feed form x delivery interaction on packed cell volume; however, there were no pairwise differences between treatments (P>0.1)

5. Effect of water-to-feed ratio on the feed intake, growth rate, feed efficiency and carcass quality of liquid-fed grow-finisher pigs

F.M. O' Meara, G.E. Gardiner, J.V. O' Doherty, P.G. Lawlor. 2020. Effect of water-to-feed ratio on feed disappearance, growth rate, feed efficiency and carcass traits in growing-finishing pigs. *Translational Animal Science*. 4 (2): 1-11. doi:10.1093/tas/txaa042

5.1 Abstract

The optimum proportion of water for preparing liquid feed to maximise growth and optimise feed efficiency in grow-finisher pigs is not known. The aim of the current study was, using an automatic short-trough sensor liquid feeding system, to identify the water-to-feed ratio at which growth was maximised and feed was most efficiently converted to live-weight. Two experiments were conducted in which four commercially used water-to-feed ratios; 2.4:1, 3.0:1, 3.5:1 and 4.1:1 on a dry matter (**DM**) basis [the equivalent of 2:1, 2.5:1, 3.0:1 and 3.5:1 on a fresh matter (**FM**) basis] were compared. Each experiment comprised 216 pigs, penned in groups of 6 same sex (entire male and female) pigs/pen with a total of 9 pen replicates per treatment. The first experiment lasted 62 days (from 40.6 kg ± 0.76 s.e. to 102.2 kg ± 1.21 s.e. at slaughter) and the second experiment was for 76 days (from 31.8 kg ± 0.64 s.e. to 119.6 kg ± 0.99 s.e. at slaughter). Overall, in experiment 2, average daily gain was 1233, 1206, 1211 and 1177 (s.e. 12.7 g/day; $P<0.05$) for pigs fed at 2.4:1, 3.0:1, 3.5:1 and 4.1:1, respectively. Overall, in experiment 1, feed conversion efficiency (**FCE**) was 2.40, 2.41, 2.23 and 2.25 (s.e. 0.042 g/g; $P<0.01$) for pigs fed at 2.4:1, 3.0:1, 3.5:1 and 4.1:1, respectively. In experiment 2, pigs fed the 3.5:1 diet had the best FCE numerically; however, there was no significant difference between treatment groups for FCE ($P>0.05$). At slaughter, in experiment 1, kill-out percentage was 76.7, 76.6, 76.7 and 75.8 (s.e. 0.17 %; $P<0.01$) for 2.4:1, 3.0:1, 3.5:1 and 4.1:1, respectively. There were no significant differences between treatment groups for DM, organic matter, Nitrogen, gross energy or ash digestibilities ($P>0.05$). These findings indicate that liquid feeding a diet prepared at a water-to-feed ratio of 3.5:1 maximises FCE of grow-finisher pigs without negatively impacting kill-out percentage. Therefore, preparing liquid feed for grow-finisher

pigs at a water-to-feed ratio of 3.5:1 DM is our recommendation for a short-trough liquid feeding system.

5.2 Introduction

The optimum proportion of water to feed used for liquid feeding of grow-finisher pigs is not well known. Limited research has been conducted on this topic and there are no clear guidelines. Pigs limit their voluntary water intake in order to maximise dry matter (**DM**) intake (Geary et al., 1996; Yang et al., 1981). Consequently, high water-to-feed ratios are likely to prevent pigs adjusting their water intake to maximise DM feed intake. Water-to-feed ratio in the context of increased feed wastage associated with liquid feeding compared to dry feeding (l'Anson et al., 2012; Russell et al., 1996) must also be considered. Liquid feeding from long troughs normally involves restricted feeding and according to Hurst et al. (2008), feed conversion efficiency (**FCE**) is improved when liquid feeding is restricted compared with *ad-libitum.* However, more modern liquid feeding involves *ad-libitum* feeding from short troughs. O' Meara et al. (2020) found water-to-feed ratios ranging from 2.4:1 to 4.0:1 DM are used, while 3.1:1 to 5.9:1 DM are used in Ontario (Braun and de Lange, 2004b). Recommendations of 2.9:1 DM (English et al., 1988) and 2.3:1 DM (Pond and Maner, 1984) have been made but research has shown optimal FCE in growing pigs fed at 4.1:1 DM (Gill et al., 1987) and 3.4:1 DM (Hurst et al., 2008). Conflicting reports exist regarding the impact of water-to-feed ratio on nutrient digestibility, with some showing differences (Barber et al., 1991) and others not (Sol Llop, 2016). Our objective was to examine the effect of four commercially used water-to-feed ratios in an *ad-libitum* short-trough liquid feeding system on the growth rate, feed efficiency and carcass quality of finisher pigs and the apparent total tract digestibility (**ATTD**) of nutrients. Voluntary water intake of pigs allowed *ad-libitum* access to feed has been reported to be ~ 3:1 DM

(Cumby, 1986). Therefore, the hypothesis was that the optimum water-to-feed ratio would be ~ 3:1 DM for grow-finisher pigs.

5.3 Materials and Methods

5.3.1 Animal care and ethics

Ethical approval for this study was granted by the Teagasc Animal Ethics Committee (approval no. TAEC 107/2015). The experiment was conducted in accordance with Irish legislation (SI no. 543/2012) and the EU Directive 2010/63/EU for animal experimentation.

5.3.2 Animals and experimental design

The effect of water-to-feed ratio on the growth and feed efficiency of grow finisher pigs was examined in 2 experiments.

Experiment 1 used 216 Danavil Duroc x (Landrace x Large White) female and entire male pigs with an initial body weight of 40.6 kg ± 4.56 SD and its duration was 62 days. Experiment 2 used 216 pigs with an initial body weight of 31.8 kg ± 3.84 SD and its duration was 76 days. In each experiment, pigs were penned in same gender pens of 6 pigs/pen with a total of 9 pen groups/treatment. Pen groups were given a one week adaptation period to liquid feeding prior to the start of both experiments during which they were all fed a liquid diet prepared at 2.5:1 (DM). Pen groups were blocked by sex and weight and assigned to one of four dietary treatments, as follows: (1) Water mixed with the feed at a ratio of 2.4 kg water per kg feed DM, (2.4:1; 29.4 % DM); (2) Water mixed with the feed at a ratio of 3 kg water per kg feed DM, (3.0:1; 25.0 % DM); (3) Water mixed with the feed at a ratio of 3.5 kg water per kg feed DM**,** (3.5:1; 22.2 % DM); and (4) Water mixed with the feed at a ratio of 4.1 kg water per kg feed DM (4.1:1; 19.6 % DM).

Pen groups were housed in pens (2.37m x 2.36m) with concrete slatted floors and solid PVC partitions. Each pen group had access to a water bowl (DRIK-O-MAT, Egebjerg International A/.S, Egebjerg, Denmark) as per regulation Council Directive 2008/120/EC (2008). Air temperature was maintained at 20 to 22 °C and was recorded daily. The room was mechanically ventilated with fans and inlets controlled by a Steinen PCS 8100 controller (Steinen BV, Nederwert, The Netherlands). Pigs were observed closely twice daily and any pig showing signs of ill-health were treated appropriately. All veterinary treatments were recorded, including identity of pig, symptom, medication and dosage administered.

Each pen was equipped with a solenoid valve and a short trough fitted with an electronic sensor. The electronic sensors were checked 3 times per day increasing to 6 times per day, after 4 weeks, and additional feed was dispensed into troughs where the residual feed in the trough was below the level of the sensor. Feeding was according to a feeding curve to provide *ad-libitum* access to feed. The feed curve provided 23MJ digestible energy (**DE**)/pig/day at the start of the experiment, increasing to 42MJ DE/pig/day during the experiment. Feed level in the trough was manually inspected daily before and after feeding and feed allocation per pen increased or decreased accordingly. The short steel troughs (100 cm x 32.5 cm x 21 cm) were located on top of a rubber mat (1.5m x 1 m) which helped to minimise liquid feed wastage.

5.3.3 Diet preparation, storage and feeding

A common diet based on wheat, barley and soybean meal was used for all treatments in each experiment. The diet was manufactured in meal form at the Teagasc feed mill (Moorepark, Fermoy, Co. Cork, Ireland). Ingredient and chemical

composition of the diet is shown in Table 5.1. Celite (2 g/kg) was added to the feed during the manufacturing process in order to measure the coefficient of ATTD) of nutrients using the acid insoluble ash technique (McCarthy et al., 1977). The diet was stored in a steel bin adjacent to the liquid feeding system prior to use.

The dietary treatments were prepared and fed using an automatic sensor liquid feeding system (HydroMix, BigDutchman, Vechta, Germany). The liquid diets were prepared in a mixing tank with a 6 pale agitator and agitated for ~ 5 minutes prior to feed-out. A high-pressure air system delivered liquid feed from the mixing tanks to troughs which were fitted with electronic feed sensors. If feed was above the sensor in a trough, feed was not dispensed to that particular trough; if the feed was below the level of the sensor, feed was dispensed to that trough and sensors were checked automatically before each scheduled feeding. The mixing tank and pipelines used to prepare and distribute liquid feed, respectively were empty between feeds.

5.3.4 Records and sampling and analysis

Individual pig weights were recorded on days 0, 32 and 62 of experiment 1, and on days 0, 40 and 76 of experiment 2 and pen-group weights were also recorded on days 19, and 57 in experiment 2. Feed disappearance for each pen was recorded daily and calculated for the periods between each pig weighing in each experiment. Average daily gain (**ADG**), Average daily feed intake (**ADFI**) and FCE were calculated for each period and for the entire experiment.

Liquid feed samples (~ 250 g) were collected at day 42 from all 36 pens and stored at -20 °C prior to chemical analysis. Samples of the whole diet in dry form (~ 250 g) were collected on day 42 of experiment 1 and stored at - 20 °C for ATTD

determination. Freshly voided faecal samples (~ 250 g/day) were collected from all 36 pens (9 pens/treatment) on days 43 and 44 of experiment 1, and stored at -20 °C for ATTD determination. The faeces collected represented a pooled sample from a minimum of 3 pigs/pen group on each day. Liquid feed samples for chemical analysis and faecal samples for ATTD determination were oven dried at 55 °C for 72 hours.

5.3.5 Slaughter

At slaughter, pigs were stunned using CO_2 and killed by exsanguination in a commercial slaughterhouse after 62 or 76 days of receiving the experimental diets in experiments 1 and 2, respectively. Pigs were fasted for ~ 12 hours prior to slaughter.

The following measurements were taken: hot carcass weight was recorded 45 minutes after stunning, and back-fat thickness and muscle depth, measured at 6 cm from the edge of the split back at the level of the 3rd and 4th last rib were determined using a Hennessy Grading Probe (Hennessy and Chong, Auckland, New Zealand). Lean meat content was estimated according to the following formula: Estimated lean meat content (%) = 60.3 – 0.847x + 0.147y where x = fat depth (mm); y = muscle depth (mm) (Department of Agriculture and Food and Rural Development, 2001). Cold carcass weight was calculated as hot carcass weight (45 minutes after stunning) x 0.98. Kill-out percentage was calculated from final live-weight (**LW**) and cold carcass weight.

5.3.6 Feed analysis and apparent total tract digestibility determination

Samples were analysed for nitrogen (**N**), DM, ash, gross energy (**GE**), neutral detergent fibre (**NDF**), ether extract (**EE**), amino acids (**AA**) and acid insoluble ash (**AIA**). Feed and faecal samples were ground in a Christy Norris mill through a 2

mm screen. Faecal samples from the two collection days (day 43 and day 44 of experiment 1) were pooled into one sample per pen prior to analysis (n=9/treatment). Liquid feed samples for proximate and amino acid analysis were pooled into one sample per treatment prior to analysis. Dry matter (AOAC.934.01), ash (AOAC.942.05) and EE concentration (AOAC.920.39) was determined according to methods of the Association of Official Analytical Chemists (AOAC, 2005). The N content was determined using the LECO FP 528 instrument (Leco Instruments UK Ltd., Cheshire, UK) (AOAC.990.0). Crude protein (**CP**) was determined as N x 6.25. The NDF content was determined according to the method of Van Soest et al. (1991) using an Ankom 220 Fibre Analyser (Ankom Technology, Macedon, New York, USA). The concentration of AIA in dry diets was determined according to the method of McCarthy et al. (1977) in order to measure the CATTD of nutrients using the AIA technique. Gross energy was determined using an adiabatic bomb calorimeter (Parr Instruments, Moline, IL USA). Amino acid determination was carried out using cation exchange HPLC as previously described by McDermott et al. (2016) (AOAC 994.12).

5.3.7 Verification of water-to-feed ratios and liquid feed quantity delivered

Verification of water-to-feed ratios was carried out on d34 (n= 4 pens/treatment) and D55 (n= 5 pens/treatment) of experiment 1 and D22 (n=4 pens/treatment) and D75 (n=3 pens/treatment) of experiment 2 to verify that the liquid feeding system was mixing water and feed in accurate ratios. The entire feed delivery for each pen during feed-out was collected by diverting liquid feed from the main feed line, below the trough solenoid, into a clean, dry collection box. The feed collected was continuously agitated using a mechanical agitator and a representative sample (~ 250 g) taken during agitation to avoid any settling out of the feed. The

sample was weighed before oven drying at 65 °C for 72 hours. Samples were removed from the oven, cooled in a desiccator for 1 hour and weighed. The moisture content of the liquid feed was calculated by difference (weight of liquid sample - weight of dried sample) and was used to determine the water-to-meal ratio of each sample on a DM basis.

The liquid feed system was also checked during experiment 1 to ensure that the total mixed feed volume of liquid feed delivered to troughs was as recorded by the feeding computer. This process took place on days 21 and 49 of experiment 1. For this, the entire feed delivery for a pen was collected by diverting the feed from the main feed line as above. Three pen feed volumes per treatment were collected and weighed and compared with the volume displayed for feed-out on the computer. The difference in the actual feed delivery volume was calculated as a deviation percentage from the correct feed volume displayed on the computer.

5.3.8 Statistical analysis

Data were analysed using the MIXED procedure of SAS® 9.4 (SAS Institute, Inc., Cary, NC, US). For growth parameters [ADFI, ADG, FCE, LW] and coefficient of variation (**CV**) of LW; dietary treatment, sex, day of the experiment and their associated interactions were included in the model as fixed effects. For carcass growth parameters, dietary treatment, sex and their associated interaction were included in the model as fixed effects. Initial LW was used as a covariate and day as a repeated variable in the model and pen was the experimental unit for growth and carcass growth parameters. The CV of initial LW was used as a co-variate for CV of weights throughout the experiment. For carcass quality parameters; dietary treatment and sex and their associated interaction were included in the model as

fixed effects with pen as the experimental unit. Carcass cold weight was included as a co-variate in the analysis of kill-out percentage, muscle and fat depth and lean meat percentage. Initial LW was included as a co-variate for the analysis of cold weight. For ATTD data, dietary treatment was included in the model as a fixed effect with pen as the experimental unit. The normality of scaled residuals was investigated using the UNIVARIATE procedure of SAS. Results are presented as LS means ± SEM. Differences were considered significant at $P<0.05$ and as tendencies at $0.05<P<0.10$.

5.4 Results

5.4.1 Pig deaths and removals

Six pigs were removed during experiment 1; two due to lameness, three due to hernias or ruptures and one was found dead following a suspected heart attack. Three pigs were removed from the 2.4:1 treatment, one pig removed from the 3.0:1 treatment, one pig removed from the 3.5:1 treatment and one pig removed from the 4.1:1 treatment. One pig from the 2.4:1 treatment in experiment 1was also fully condemned at slaughter. No pigs were removed from treatment during experiment 2 and all 216 pigs were slaughtered.

5.4.2 Effect of water-to-feed ratio on the growth and carcass quality of grow-finisher pigs in Experiment 1

Treatment x sex interactions are shown in Table S5.1. There was a treatment x sex interaction for ADG in the period d 33 – 62 in which males fed the 4.1:1 treatment grew faster than females fed 4.1:1. There were treatment x sex interactions for FCE in the periods from d 1-32 ($P<0.01$) and from d 33-62 ($P<0.05$,). During d 1-32, male pigs fed the 3.5:1 treatment were more efficient than females fed 2.4:1 (data not shown). From d33 to 62, male pigs fed the 3.5:1 treatment were more efficient than female pigs fed 2.4:1 or 3.0:1. There was also an interaction for kill-out percentage. The kill-out percentage of male pigs was not affected by water-to-feed ratio whereas the kill-out percentage of females was reduced when a water-to-feed ratio of 4.1:1 was fed.

The effect of treatment on feed intake, growth, FCE and carcass characteristics in experiment 1 is shown in Table 5.2. Overall, pigs fed at a water-to-feed ratio of 3.5:1 had a lower ADFI than those fed at 2.4:1 and 3.0:1 ($P<0.05$) while

those fed at 4.1:1 had a similar ADFI to all other treatments. This was also reflected in the period from d 33 to 62. In the period from d 1 to 32, pigs fed at 3.5:1 had a lower ADFI than those fed at 3.0:1 ($P<0.01$), while those fed at 2.4:1 and 4.1:1 had a similar ADFI to all other treatments. There were no treatment differences observed for ADG during any period of the experiment or carcass ADG. During the overall experimental period, FCE of pigs fed at 3.5:1 was better than for pigs fed at 2.4:1 and 3.0:1 ($P<0.05$) while pigs fed at 4.1:1 had an FCE that was similar to that of pigs on all other treatments and the same results were observed for carcass FCE. During the d 1- 32 period, the FCE of pigs fed at 3.5:1 was better than for pigs fed at 2.4:1 ($P<0.05$), while pigs fed at 3.0:1 and 4.1:1 had a similar FCE to all other treatments. In the period from d 33 to 62, the FCE of pigs fed at 3.5:1 was better than for those fed at 2.4:1 and 3.0:1, but similar to those fed at 4.1:1 ($P<0.05$).

At d 0, pigs on the 3.0:1 treatment were heavier than pigs on 4.1:1 ($P<0.05$), while all other treatments had a similar weight. There were no treatment differences for LW at d 32 ($P>0.05$) or d 62 ($P>0.05$) and no treatment differences for CV of pig weight within pen during the experiment. At slaughter, pigs fed 4.1:1 had a significantly lower kill-out percentage than those fed the other 3 treatments ($P<0.01$). There were no treatment differences for carcass cold weight, muscle depth, fat depth or lean meat percentage ($P>0.05$).

The effect of sex was also investigated and results are presented in Table S 5.2. Male pigs had a higher overall ADG ($P<0.01$) and carcass ADG ($P<0.05$) than female pigs but there were no differences in ADFI between the sexes. This resulted in a better FCE (2.24 compared to 2.41; $P<0.001$) and a tendency for better carcass FCE (2.75 compared to 2.87; $P=0.06$) in male compared to female pigs. Males were

heavier than females at the start of the experiment (42.8 and 38.5 kg, respectively; P<0.01). Males were also heavier than females at slaughter with weights of 103.9 and 100.1 kg, respectively (P<0.05). At slaughter, females had a higher kill-out percentage (P<0.001), muscle depth (P<0.05) and lean meat percentage (P<0.01) than males, while females had a lower fat depth than males (P<0.001).

5.4.3 Effect of water-to-feed ratio on the growth, feed intake, feed conversion efficiency and carcass quality of grow-finisher pigs in Experiment 2

The treatment x sex interactions from experiment 2 are shown in Table S 5.3. There was a treatment x sex interaction for LW at d 57. Female pig weight was not affected by water-to-feed ratio, whereas males fed at 4.1:1 were lighter than those fed at 2.4:1 (P<0.05). There were treatment x sex interactions for ADG from days 41-57 and days 58-76 and for kill-out percentage at slaughter (data not shown). In the period from d 41 to 57, the growth of females was not affected by water-to-feed ratio, whereas the growth of males was reduced when fed at 4.1:1 compared to being fed at 2.4:1 (P<0.001). In the d 58 – 76 period, males fed the 2.4:1 ratio had a faster growth than females fed 2.4:1 and 4.1:1 (P<0.001). There was also a tendency for an interaction for carcass ADG where female growth rate was not affected by water-to-feed ratio but male pigs fed 2.4:1 grew faster than male pigs fed 4.1:1 (P=0.09). There was an interaction for FCE in the d 1 – 19 period. The FCE of females was not affected by water-to-feed ratio, whereas male pigs fed at 3.5:1 had a better FCE than those fed at 3.0:1 (P<0.05). At slaughter, the carcass weight of females was not affected by water-to-feed ratio, whereas males fed 2.4:1 tended to have heavier carcases than males fed at 4.1:1 (P=0.09).

The effect of treatment on the ADFI, ADG, FCE and carcass characteristics of grow-finisher pigs in experiment 2 is shown in Table 5.3. Overall, there was a tendency for feed intake to be reduced when pigs were fed at 4.1:1 compared with 2.4:1 ($P<0.05$), while the ADFI of pigs fed at 3.0:1 and 3.5:1 were similar to both. During the periods from d 1 to 19 and 41 to 57, pigs fed at 4.1:1 had a lower ADFI than those fed at 2.4:1 ($P<0.05$), while those fed at 3.0:1 and 3.5:1 had a similar ADFI to all other treatments.

In the period d 41–57 and overall, ADG was reduced when pigs were fed at 4.1:1 compared with 2.4:1 ($P<0.05$), while pigs fed at 3.0:1 and 3.5:1 had similar ADG to both. The same result was also noted in carcass ADG ($P=0.01$). There were no overall treatment differences observed for FCE ($P>0.05$). In the period d 1-19, pigs fed at 3.5:1 had a better FCE than pigs fed at 3.0:1 ($P<0.05$), while those fed at 2.4:1 and 4.1:1 had a similar ADFI to all other treatments.

On d 19, pigs fed at 3.5:1 were heavier than pigs fed at 4.1:1 ($P<0.01$), while those fed at 2.4:1 and 3.0:1 had similar weights to pigs fed all other treatments. On d 57, pigs fed at 2.4:1 were heavier than pigs fed at 4.1:1 ($P=0.05$) while those fed at 3.0:1 and 3.5:1 had similar weights to pigs fed all other treatments. At slaughter (d 76), pigs fed at 2.4:1 were heavier than pigs fed at 4.1:1 ($P=0.05$), while those fed at 3.0:1 and 3.5:1 had similar weights to pigs fed all other treatments. There was no treatment effect observed for the CV of pig weights within pen ($P>0.05$). At slaughter, pigs fed at 2.4:1 had heavier carcasses than those fed at 4.1:1 ($P<0.01$), while those fed at 3.0:1 and 3.5:1 had similar carcass weights to all other treatments. There were no treatment differences observed for kill-out percentage, muscle depth, fat depth and lean meat percentage between treatments ($P>0.05$).

The effect of sex was also investigated and results are presented in Table S 5.4. Males had a higher overall ADG ($P<0.001$) and carcass ADG ($P<0.001$) than females but there was no treatment effect on ADFI. This tended to result in a better FCE (2.22 g/g in males compared to 2.32 g/g in females; $P=0.05$) and carcass FCE (2.94 g/g in males compared to 3.06 g/g in females; $P=0.06$). There were no differences between the weight of males and females at the beginning of the experiment, but males were heavier than females on d 76, prior to slaughter, at 122.2 kg versus 116.5 kg ($P<0.001$). Male pigs also had a heavier carcass weight than females at 88.6 kg versus 85.8 kg ($P<0.001$). Female pigs had a higher kill-out percentage ($P<0.01$), muscle depth ($P<0.001$) and lean meat percentage ($P<0.001$) than male pigs, while female pigs had a lower fat depth than male pigs ($P<0.001$).

5.4.4 Verification of water-to-feed ratios and quantity of liquid feed delivered to troughs

The water-to-feed ratio verification results are reported in Table 5.4. During experiment 1, mean verification results were 2.5:1, 3.1:1, 3.5:1 and 4.2:1 for the 2.4:1, 3.0:1, 3.5:1 and 4.1:1 treatments, respectively. During experiment 2, mean verification results were 2.5:1, 3.4:1, 3.8:1 and 4.3:1 for the 2.4:1, 3.0:1, 3.5:1 and 4.1:1 treatments, respectively. The mean values in experiment 2 were slightly higher than the target values for the 3.0:1 and 3.5:1 ratios than those achieved in experiment 1.

Results to verify the quantity of liquid feed delivered to the troughs during experiment 1 showed that all treatment delivery volumes were within - 3.41 % and + 4.46 % of the planned delivery volume as displayed by the feed computer (data not shown). The deviation percentages from the computer volume for each treatment

were as follows; from - 3.41 % to + 4.46 % of expected delivery volume for 2.4:1; from - 2.48 % to + 2.15 % of expected delivery volume for 3.0:1; from - 0.42 % to + 3.81 % for 3.5:1 and from - 1.82 % to + 1.9 % for 4.1:1.

5.4.5 Effect of water-to-feed ratio on apparent total tract digestibility

The results from the determination on the ATTD are shown in Table 5.5. There were no treatment effects observed for DM, organic matter, N, GE or ash digestibilities.

5.4.6 Effect of water-to-feed ratio on gross energy, crude protein, ash and amino acid content in the diet

Results of proximate and AA analysis of dry feed and feed from troughs during experiment 1 are shown in Table 5.6. There were no obvious differences in crude protein, GE or ash between treatments in troughs. The lysine content of the trough samples from the 4.1:1 treatment were lower than those from other treatments at 8.6 g/kg DM compared to 10.6, 10.0 and 10.7 g/kg DM in the 2.4:1, 3.0:1, 3.5:1 and 4.1:1 treatments, respectively.

5.5 Discussion

This study compares four commercially used water-to-feed ratios (O' Meara et al., 2020) using a state of the art short trough ad-libitum liquid feeding system. Such a study is fundamental to identify the appropriate water-to-feed ratio for optimal growth and feed efficiency of liquid-fed grow-finisher pigs. The results here can be easily implemented on-farm to improve feed efficiency and, in turn, improve farm profitability.

5.5.1 Verification of water-to-feed ratios

The liquid feed system employed in the current study was a BigDutchman hydro-air system, which forces air through the feed pipes at high pressure to dispense feed and ensure that minimal residue remains in pipes between feeds. This, combined with the accurate weighing by load cells in the mixing tanks ensured that accurate volumes of correctly proportioned liquid feed was delivered to feed troughs for accurate comparison of water-to-feed ratios. Earlier studies have shown that older liquid feeding systems have not always provided equal distribution of DM and minerals to all troughs on a feed line (Braun and De Lange, 2004a; O' Reilly and Lynch, 1992). This is less of a concern with new liquid feeding technology, as shown in the current experiment.

5.5.2 Effect of water-to-feed ratio on overall grow-finisher pig performance

This study shows that the ADFI of pigs fed at the lower water-to-feed ratios (2.4:1 and 3.0:1) was numerically higher than those fed at the higher ratios (3.5:1 and 4.1:1) during all periods of both experiments. It would appear that, as water-to-feed ratio was increased, or the feed presented was more diluted with water, the associated voluntary feed intake of pigs was limited by the pig's physical intake

capacity. This was previously found by Kornegay and Vander Noot (1968) where growth rate was reduced and FCE deteriorated as a result of reduced DM intake when a water-to-feed ratio of ~ 5.7:1, on a DM basis, was fed compared with either a dry diet or a liquid diet at a water-to-feed ratio of ~ 0.7:1. It is also important to note that there are legal obligations (Council Directive 2008/120/EC, 2008) to supply supplementary water to pigs. In the current study, water intake for each treatment may have been higher than indicated by the water-to-feed ratio but water usage from the supplementary drinking bowls was not measured.

Growth rate was not affected by water-to-feed ratio in experiment 1; however, the growth rate of pigs fed at the highest ratio (4.1:1) in experiment 2 was reduced and carcass weight was lighter than in pigs fed at the lowest ratio (2.4:1). This mirrors feed intake observations. When grow-finisher pigs are provided with a very dilute diet, such as the 4.1:1 diet, physical intake capacity appears to limit DM intake and consequently growth rate. An early liquid feeding study by Braude and Rowell (1967) showed that liquid feeding at water-to-feed ratios greater than 4.1:1 DM does not provide production advantages in grow-finisher pigs, where improved growth rates and FCE were reported on a 2.9:1 DM water-to-feed ratio compared with 4.6:1 DM. It should be noted, however, that no supplementary water was provided to liquid-fed pigs in the study by Braude and Rowell (1967) and that pigs were only fed twice daily.

Both experiments in the current study found that FCE deteriorated, albeit numerically in Experiment 2, when the water-to-feed ratio was reduced below 3.5:1. During both periods of experiment 1, pigs fed at 3.5:1 had a better FCE than pigs fed at 2.4:1, while the only significance in experiment 2 was from d 0 – 19 where pigs

fed at 3.5:1 were more feed efficient than those fed at 3.0:1. Although every effort was made to minimise feed wastage through trough design and use of a rubber mat under and around the troughs, it is likely that feed wastage was responsible for the poorer FCE, particularly in experiment 1, at the lower water-to-feed ratios (2.4:1 and 3.0:1). Pigs fed these treatments had a higher ADFI but similar growth rate to those fed at 3.5:1 and 4.1:1. At a lower water-to-feed ratio, where wastage of liquid feed occurred, a greater proportion of DM is lost per kg of liquid feed wasted. This means that wastage by pigs fed at a lower water-to-feed ratio will decrease their actual feed intake more than those that waste feed at a higher ratio. The feed troughs in the current study were at floor level and this may have also impacted feed wastage, making it easy for pigs to remove feed on their feet and faces at feeding. In Experiment 2, management of the feeding system was improved by closer monitoring of feed disappearance which helped to improve feed efficiency while still ensuring *ad-libitum* feeding. It is possible that different results may be achieved using a long-trough, restricted liquid feeding system. Hurst et al. (2008) reported improved feed efficiencies when liquid feed was restricted-fed compared to *ad-libitum* and suggests that the difference was mainly down to feed wastage.

It was previously shown that the optimal ratio of water-to-feed for liquid feeding increases with age (Sol Llop, 2016). The intervals between weighing of pigs during the grow-finisher period in the current experiments can be used to investigate this. In experiment 1, a water to feed ratio of 3.5:1 was optimum throughout the entire experiment based on FCE, because increasing the water to feed ratio to 4.1:1 reduced kill-out yield, most likely due to increased gut fill and weight. In experiment 2, increasing water to feed ratio above 3.5:1 caused a reduction in ADG and carcass weight compared to pigs fed at 2.4:1 Results from the periods of both

experiments in the current study do not suggest that the optimum water-to-feed ratio changes throughout the grow-finisher period.

Sol Llop (2016) used regression analysis to conclude that ADG is maximised at 1.6:1 DM and 2.0:1 DM water-to-feed from 46.7 - 64.0 kg and 64.0 - 85.4 kg LW, respectively. They also concluded that FCE is best at 1.5:1 DM and 1.8:1 DM water-to-feed from 46.7 kg - 64.0 kg and 64.0 - 85.4 kg, respectively. Contrary to our study, they found no treatment differences in ADFI which may be as a result of the semi-restricted feeding management implemented. They only compared ratios ranging from 0.7:1 to 3.0:1 DM for the first period (46.7 – 64.0 kg) and from 1.5:1 to 3.9:1 for the second period (64.0 – 85.4 kg); therefore, the recommended ratios do not directly compare with the commercially used treatments employed in the current study. A constant water supply was available in both studies. It should be noted that diets were hand-mixed and fed twice daily in the latter experiment. With the feeding equipment currently available, feeding a water-to-feed ratio as low as 0.7:1 DM, or in fact below 2.4:1 DM, is simply not practical. Furthermore, pigs in the current study had *ad-libitum* access to feed; however, it is likely that pigs in the study by Sol Llop (2016) were feed-restricted, at least to some extent, since pigs in their study were only fed twice daily. Overall, our results suggest that a water-to-feed ratio of 3.5:1 is optimum based on FCE and kill-out percentage throughout the grow-finisher phase.

5.5.3 Effect of water-to-feed ratio on carcass quality at slaughter

In experiment 1, pigs fed at 4.1:1 had a significantly lower kill-out percentage than pigs fed the other three treatments. Although this was not found in experiment 2, LW at slaughter and carcass weight were reduced on the 4.1:1

treatment. This shows that increasing the water-to-feed ratio to 4.1:1 has negative consequences on carcass characteristics at slaughter.

It is hypothesised that the reduced kill-out percentage was due to increased intestinal weight in response to the larger volumes of liquid feed ingested at each feeding, despite the suggestion by Geary et al. (1996) that adding water to diets does not influence gut size in the same way that fibrous components do. While feed efficiency was not negatively impacted at the highest ratio in the current experiment, carcass characteristics were clearly affected, suggesting that 3.5:1 DM water-to-feed is optimum. In the study by Sol Llop (2016), the lack of impact of water-to-feed ratio on kill-out percentage may be attributed to the fact that pigs did not receive the highest ratio of 3.9:1 until they were ~ 64 kg, and then were only fed this ratio twice a day for 26 days prior to slaughter. Hence, there may not have been sufficient time for treatment to impact carcass characteristics, compared to the 62 and 76 - d treatment periods used in the current study.

5.5.4 Impact of water-to-feed ratio on feed composition, apparent total tract digestibility, water intake and slurry production

Despite limited dietary amino acid analysis, it would appear that a certain amount of lysine was lost in the liquid feed (at all water-to-feed ratios) compared to the dry diet, with the greatest losses occurring with the 4.1:1 treatment. The increased feed volume delivered to troughs on this high water-to-feed ratio may have resulted in a bigger quantity of feed sitting in the trough for a longer period of time, providing more opportunity for spontaneous fermentation compared to the other ratios. However, it should be treated with caution, as the proximate and amino acid analysis in the current study was based on only one pooled trough sample.

There were no treatment effects on nutrient digestibility in the current study, which is in agreement with previous work (Pedersen and Stein, 2010; Sol Llop, 2016). The fact that there were no differences in ATTD further supports our hypothesis that wastage of more concentrated liquid feed was responsible for the poorer FCE values when water to feed ratios below 3.5:1 were fed, particularly in experiment 1. In contrast, Barber et al. (1991) found that a water-to-feed ratio increase from 1.9:1 to 3.7:1 DM resulted in a significant linear improvement in DM digestibility. It is important to note that unlike in our experiment, pigs in this study did not have access to a supplementary water source and were provided with experimental diets only twice daily and as a consequence pigs were likely feed-restricted. It is likely that the increased DM digestibility reported by Barber et al. (1991) was more in response to meeting the animals' requirements for water than the water-to-feed ratio *per se*.

As mentioned, a supplementary water source must by law be provided to liquid-fed pigs in Europe (Council Directive 2008/120/EC, 2008); however, it was not possible to measure supplementary water consumption in the current study. Therefore, it is difficult to compare the results of the current study to those in the literature, as many older studies did not supply supplementary water when investigating liquid feeding (Barber et al., 1991; Barber et al., 1963; Braude and Rowell, 1967). Results from weaner work shows that pigs will consume more supplementary water when liquid feed is fed at low water-to-feed ratios (Geary et al., 1996; Gill et al., 1987). It is likely that, had we been able to record voluntary water intake, pigs on the lower water-to-feed ratio would have had higher voluntary water intakes, but all pigs would have used supplementary water, regardless of water-to-feed ratio.

Although not measured in the current study, slurry storage and disposal costs increase using liquid feeding compared to dry feeding (Stotfold Research Centre, 2005). Previous work has shown that increased slurry volumes are produced by pigs on high compared to low water-to-feed ratios (Kornegay and Vander Noot, 1968). It is interesting that growth rate on the lowest water-to-feed ratio was similar to all other treatments in experiment 1 and better than the highest ratio in experiment 2. If management of liquid feeding at lower water-to-feed ratios could be improved to minimise wastage, an improvement in FCE could be achieved, reducing slurry volumes produced.

In conclusion, results from the current study, in which water-to-feed ratios were shown to have been accurately delivered to troughs at feeding, show that grow-finisher pigs, on a sensor-fed short-trough liquid feeding system, are most feed efficient, have high growth rates and good kill-out yield when liquid feed is provided at a water-to-feed ratio of 3.5:1. Increasing the ratio to 4.1:1, reduced growth rate and negatively affected carcass characteristics, while reducing it below 3.5:1 negatively impacted FCE. However, decreasing the water-to-feed ratio to 2.4:1 improved growth; therefore, if management at 2.4:1 can be improved to reduce feed wastage, FCE could be further improved and higher growth rates achieved.

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5.7 Tables

Table 5.1 Ingredient and chemical composition of the experimental diet (on an air dry basis, g/kg) [1]

	Experimental diet[1]
Ingredient composition	
Wheat	400.0
Barley	382.7
Soy bean meal	183.0
Limestone flour	11.0
Fat, soya oil	9.7
Lysine HCl	3.8
Salt	3.0
L-Threonine	1.7
Celite	2.0
Vitamin and mineral premix[2]	1.0
Mono DiCalcium Phosphate	1.0
DL-Methionine	0.9
L-Tryptophan	0.2
Phytase[3]	0.1
Chemical composition	
Dry matter	879.0
Crude protein	179.0
Ash	39.0
Oil	28.7
Neutral detergent fibre	190.0
Gross energy, MJ/kg	16.1
Lysine	10.6
Methionine	6.6
Threonine	7.3
Digestible energy[4], MJ/kg	13.8
Net energy[4], MJ/kg	9.8
SID Lysine[4,5]	10.0

	Experimental diet[1]
Total Calcium[4]	6.6
Digestible Phosphorus[4]	2.6

[1]Values are the mean of experimental diets from experiment 1 and experiment 2
[2]Vitamin and mineral premix provided per kilogram of complete diet: Cu from copper sulphate, 15 mg; Fe from ferrous sulphate monohydrate, 24 mg; Mn from manganese oxide, 31 mg; Zn from zinc oxide, 80 mg; I from potassium iodate, 0.3 mg; Se from sodium selenite, 0.2 mg; retinyl acetate, 0.7 mg; cholecalciferol, 12.7 µg; DL-alpha-tocopheryl acetate, 40 mg; Vitamin K, 4 mg; vitamin B12, 15 µg; riboflavin, 2 mg; nicotinic acid, 12 mg; pantothenic acid, 10 mg; vitamin B1, 2 mg; vitamin B6, 3 mg and celite 2000 mg/kg.
[3]The diet contained 500 phytase units (FYT) per kg feed from RONOZYME HiPhos (DSM, Belfast, UK)
[4]Calculated values
[5]SID Lysine = Standardized ileal digestible lysine

Table 5.2 The effect of four commercially used water-to-feed ratios on the growth parameters and carcass quality of grow-finisher pigs[1] (Experiment 1)

	Water-to-feed ratio (DM[2])					P-value		
	2.4:1	**3.0:1**	**3.5:1**	**4.1:1**	**SEM**	**Treatment**	**Sex**	**Treatment x sex**
No. pens/trt[3]	9	9	9	9				
LW[4], kg								
d 0	40.8	41.4	40.6	39.8				
d 32	66.6	67.0	66.2	66.4	0.73	0.88	0.17	0.75
d 62	102.6	100.7	103.1	101.7	1.21	0.52	0.01	0.46
ADFI[4], g/day								
d 1 – 32	2167^{a,b}	2182^{a}	2000^{b}	2032^{a,b}	44.5	0.01	0.57	0.02
d 33 – 62	3147^{a}	3159^{a}	2877^{b}	2959^{a,b}	78.8	0.03	0.99	0.06
Overall	2657^{a}	2670^{a}	2439^{b}	2495^{a,b}	57.0	0.01	0.81	0.14
ADG[4], g/day								
d 1 – 32	980	994	973	978	22.3	0.92	0.20	0.80
d 33- 62	1220	1223	1220	1229	22.3	0.99	0.001	0.01
Overall	1100	1108	1096	1103	18.1	0.97	0.01	0.90
FCE[4], g/g								
d 1 - 32	2.23^{a}	2.21^{a,b}	2.07^{b}	2.09^{a,b}	0.038	0.01	0.01	0.01
d 33 – 62	2.58^{a,b}	2.61^{a}	2.39^{c}	2.41^{b,c}	0.056	0.01	0.001	0.001

	Water-to-feed ratio (DM[2])					P-value		
	2.4:1	**3.0:1**	**3.5:1**	**4.1:1**	**SEM**	**Treatment**	**Sex**	**Treatment x sex**
Overall	2.40[a]	2.41[a]	2.23[b]	2.25[a,b]	0.042	0.01	0.001	0.12
CV[5] weights, %								
d 1	6.8	5.2	5.7	4.3	0.81	0.11	0.56	0.41
d 32	6.3	6.7	6.1	5.9	0.61	0.82	0.93	0.24
d 62	5.8	6.4	6.4	5.6	0.61	0.67	0.52	0.05
Overall	6.2	6.4	6.3	5.7	0.41	0.62	0.97	0.02
Carcass								
Carcass ADG[6], g/day	907	915	905	896	14.6	0.83	0.03	0.81
Carcass FCE[7], g/g	2.91[a]	2.89[a]	2.69[b]	2.74[a,b]	0.051	0.01	0.06	0.12
Cold-weight, kg	78.7	77.2	79.0	77.1	0.95	0.37	0.11	0.32
Kill-out, %	76.7[a]	76.6[a]	76.7[a]	75.8[b]	0.17	0.01	0.001	0.05
Muscle, mm	47.5	46.2	47.0	45.7	0.60	0.17	0.001	0.40
Fat, mm	12.4	12.7	12.3	12.1	0.38	0.68	0.02	0.79
Lean meat, %	56.8	56.3	56.8	56.8	0.35	0.71	0.01	0.73

[1]Least square means and pooled standard errors of the mean
[2]DM=Dry matter; Water-to-feed ratios presented on a dry matter basis
[3]Pens/trt=pen replicates per treatment; 6 pigs per pen replicate
[4]LW=live-weight; ADFI=Average daily feed intake; ADG=Average daily gain; FCE=Feed conversion efficiency
[5]CV=Coefficient of variation, as a measure of within pen pig weight variation
[6]Carcass ADG: From live-weight at start of experiment to slaughter = ((carcass weight in kg – LW on day 1 x 0.65)x1000) / number of days on treatment (Lawlor and Lynch, 2005)
[7]Carcass FCE: From start of experiment to slaughter = total average daily feed intake / carcass ADG (g)
[a,b,c] Within each row, values that do not share a common superscript are significantly different (P<0.5)

[A,B,C] Within each row, values that do not share a common superscript tend to be different (0.05<P<0.10)

Table 5.3 The effect of four commercially used water-to-feed ratios on the growth and carcass parameters of grow-finisher pigs[1] (Experiment 2)

	Water-to-feed ratio (DM[2])					P-value		
	2.4:1	**3.0:1**	**3.5:1**	**4.1:1**	**SEM**	**Treatment**	**Sex**	**Treatment x sex**
No. pens/trt[3]	9	9	9	9				
Live-weight[3], kg								
d 0	31.9	32.2	31.9	31.1				
d 19	45.1[a,b]	44.5[a,b]	45.3[a]	44.0[b]	0.32	0.01	0.44	0.02
d 40	69.2	68.0	68.7	67.1	0.71	0.15	0.37	0.39
d 57	92.6[a]	90.5[a,b]	91.4[a,b]	89.0[b]	0.94	0.05	0.03	0.03
d 76	121.6[a]	119.5[a,b]	119.9[a,b]	117.4[b]	0.99	0.05	0.001	0.32
ADFI[4], g/day								
d 1 -19	1923[a]	1866[a,b]	1818[a,b]	1747[b]	51.5	0.04	0.84	0.12
d 20 - 40	2557	2468	2439	2382	67.4	0.24	0.78	0.62
d 41 – 57	3176[a]	3077[a,b]	3015[a,b]	2943[b]	65.4	0.05	0.41	0.20
d 58 – 76	3794	3719	3603	3540	98.8	0.24	0.31	0.39
Overall	2863[A]	2782[A,B]	2719[A,B]	2653[B]	62.4	0.06	0.54	0.57
ADG[4], g/day								
d 1 -19	905	870	914	852	18.9	0.07	0.29	0.25
d 20 - 40	1148	1118	1111	1097	20.8	0.35	0.03	0.22

	Water-to-feed ratio (DM[2])					P-value		
	2.4:1	**3.0:1**	**3.5:1**	**4.1:1**	**SEM**	**Treatment**	**Sex**	**Treatment x sex**
d 41 – 57	1368[a]	1327[a,b]	1336[a,b]	1285[b]	18.2	0.02	0.001	0.001
d 58 – 76	1512	1510	1481	1473	33.2	0.78	0.001	0.001
Overall	1233[a]	1206[a,b]	1211[a,b]	1177[b]	12.7	0.02	0.001	0.21
FCE[4], g/g								
d 1 -19	2.14[a,b]	2.15[a]	2.01[b]	2.07[a,b]	0.045	0.03	0.78	0.02
d 20 - 40	2.24	2.21	2.20	2.18	0.043	0.76	0.09	0.30
d 41 – 57	2.33	2.33	2.27	2.30	0.059	0.83	0.12	0.21
d 58 – 76	2.52	2.48	2.44	2.41	0.066	0.63	0.001	0.06
Overall	2.31	2.30	2.23	2.24	0.046	0.45	0.05	0.19
CV[5] weights, %								
d 1	5.6	5.1	5.5	6.2	0.80	0.82	0.14	0.84
d 40	8.0	7.3	7.1	7.8	0.71	0.79	0.22	0.59
d 76	7.0	6.3	7.2	6.8	0.71	0.83	0.38	0.95
Overall	6.9	6.4	6.6	6.8	0.53	0.89	0.56	0.85
Carcass								
Carcass ADG[6], g/day	932[a]	905[a,b]	908[a,b]	882[b]	8.7	0.01	0.001	0.09
Carcass FCE[7], g/g	3.03	3.04	2.96	2.97	0.064	0.58	0.20	0.18
Cold-weight, kg	89.1[a]	87.0[a,b]	87.3[a,b]	85.4[b]	0.66	0.01	0.001	0.09

	Water-to-feed ratio (DM[2])					P-value		
	2.4:1	**3.0:1**	**3.5:1**	**4.1:1**	**SEM**	**Treatment**	**Sex**	**Treatment x sex**
Kill-out, %	73.2	72.8	72.8	72.7	0.25	0.46	0.01	0.34
Muscle, mm	47.5	47.0	46.5	47.4	0.47	0.46	0.001	0.69
Fat, mm	15.0	14.6	14.9	14.2	0.32	0.32	0.001	0.90
Lean meat, %	54.6	54.9	54.5	55.2	0.26	0.25	0.001	0.80

[1]Least square means and pooled standard errors of the mean
[2]DM=Dry matter; Water-to-feed ratios presented on a dry matter basis
[3]pens/trt=pen replicates per treatment; 6 pigs per pen replicate
[4]ADFI=Average daily feed intake; ADG=Average daily gain; FCE=Feed conversion efficiency
[5]CV=Coefficient of variation, as a measure of within pen pig weight variation
[6]Carcass ADG: From live-weight at start of experiment to slaughter = ((carcass weight in kg – LW on day 1 x 0.65)x1000) / number of days on treatment (Lawlor and Lynch, 2005)
[7]Carcass FCE: From start of experiment to slaughter = total average daily feed intake / carcass ADG (g)
[a,b,c] Within each row, values that do not share a common superscript are significantly different (P<0.5)
[A,B,C] Within each row, values that do not share a common superscript tend to be different (0.05<P<0.10)

Table 5.4 Verification of water-to-feed ratios as fed during two experiments comparing commercially used water-to-feed ratios for grow-finisher pigs

	Water-to-feed ratio			
	2.4:1	**3.0:1**	**3.5:1**	**4.1:1**
Exp. 1, d 34[1]	2.5:1	3.1:1	3.5:1	4.2:1
Exp. 1, d 55[2]	2.5:1	3.2:1	3.6:1	4.2:1
Exp. 1 mean	2.5:1	3.1:1	3.5:1	4.2:1
Exp. 2, d 22[1]	2.5:1	3.6:1	3.9:1	4.4:1
Exp. 2, d 75[3]	2.5:1	3.2:1	3.6:1	4.3:1
Exp. 2 mean	2.5:1	3.4:1	3.8:1	4.3:1

[1]Mean value of samples from 4 troughs/treatment
[2]Mean value of samples from 5 troughs/treatment
[3]Mean value of samples from 3 troughs/treatment

Table 5.5 Effect of water-to-feed ratio on the apparent total tract digestibility (ATTD %) of grow-finisher pigs

	Water-to-feed ratio (DM)					P-value
	2.4:1	3.0:1	3.5:1	4.1:1	SEM	Treatment
Pens/trt	9	9	9	9		
DMD[1]	80.4	80.3	81.3	79.7	0.58	0.22
OMD[2]	82.6	82.5	83.5	82.0	0.56	0.23
NitD[3]	75.5	74.5	76.3	74.3	0.90	0.68
GeD[4]	78.4	78.3	79.3	77.6	0.67	0.28
AshD[5]	53.1	52.5	54.1	52.1	1.17	0.44

[1]DMD=Dry matter digestibility
[2]OMD=Organic matter digestibility
[3]NitD=Nitrogen digestibility
[4]GeD=Gross energy digestibility
[5]AshD=Ash digestibility

Table 5.6 Proximate and amino acid analysis of dry diets and trough samples from Experiment 1 (on a dry matter basis)

		Trough[1]			
	Dry diet	**2.4:1**	**3.0:1**	**3.5:1**	**4.1:1**
Gross energy (MJ/kg)	18.4	18.3	18.5	18.4	18.4
Crude protein, %	19.7	18.8	19.3	18.6	19.6
Ash, %	4.4	4.5	4.8	4.7	4.5
Amino acids (g/kg)					
Lysine	12.4	10.6	10.0	10.7	8.6
Cysteic acid	6.0	5.2	5.3	5.6	5.5
Taurine	1.5	1.3	1.9	1.2	1.3
Methionine	5.1	4.7	4.7	4.9	4.1
Aspartic acid	19.3	15.8	15.5	17.3	17.8
Threonine	8.6	7.7	7.6	8.0	7.7
Serine	10.6	8.8	9.0	9.6	9.6
Glutamic acid	48.9	40.5	41.0	43.5	42.7
Glycine	9.0	7.6	7.5	8.2	8.4

		Trough[1]			
	Dry diet	**2.4:1**	**3.0:1**	**3.5:1**	**4.1:1**
Alanine	8.5	7.8	7.7	8.4	8.6
Cysteine	0.6	0.5	0.5	0.5	0.8
Valine	10.2	8.3	8.1	9.0	9.7
Isoleucine	8.5	7.2	7.0	7.9	8.0
Leucine	15.4	13.8	13.9	15.1	15.1
Tyrosine	6.0	4.9	5.3	5.8	5.6
Phenylalanine	10.5	9.0	8.9	9.8	9.9
Histidine	6.5	5.7	5.1	5.6	5.7
Arginine	13.2	10.9	10.5	11.7	11.7
Proline	14.8	12.9	13.2	13.8	14.0

[1]Trough samples collected from 9 pens/trt on d 42 of experiment 1 and pooled by treatment prior to analysis (n=1/treatment)

5.8 Supplementary tables

Table S 5.1 Effect of water-to-feed ratio by sex interaction on growth parameters and carcass quality of grow-finisher pigs[1] (Experiment 1)

Sex	**Male**				**Female**					**P-value**
W:F ratio (DM[2])	**2.4:1**	**3.0:1**	**3.5:1**	**4.1:1**	**2.4:1**	**3.0:1**	**3.5:1**	**4.1:1**	**SEM**	**Treatment x sex**
No. pens[3]	5	5	5	5	4	4	4	4		
Live-weight, kg										
d 1	42.1	43.9	43.3	41.9	39.5	39.0	37.9	37.7	1.05	0.05
d 32	67.8	67.1	66.7	67.0	65.3	66.9	65.8	65.8	1.03	0.75
d 62	104.2	102.2	104.1	105.2	101.1	99.2	102.1	98.1	1.71	0.46
ADFI[4], g/day										
d 1 – 32	2145	2121	1963	2090	2190	2243	2037	1973	55.8	0.02
d 33 – 62	3083[a,b]	3110[a,b]	2837[b]	3109[a,b]	3211[a]	3207[a]	2917[a,b]	2809[b]	111.2	0.06
Overall	2614	2616	2400	2600	2700	2725	2477	2391	80.4	0.14
ADG[4], g/day										
d 1 – 32	1017	993	984	991	942	995	962	964	31.5	0.80
d 33-62	1263[a,b]	1277[a,b]	1264[a,b]	1297[a]	1178[a,b]	1169[a,b]	1176[a,b]	1161[b]	31.5	0.01
Overall	1140	1135	1124	1144	1060	1082	1069	1063	25.5	0.90
FCE[4], g/g										

Sex	Male				Female					P-value
W:F ratio (DM^2)	2.4:1	3.0:1	3.5:1	4.1:1	2.4:1	3.0:1	3.5:1	4.1:1	SEM	Treatment x sex
d 1 – 32	2.11[a,b]	2.15[a,b]	2.00[b]	2.11[a,b]	2.34[a]	2.27[a,b]	2.14[a,b]	2.06[a,b]	0.053	0.01
d 33-62	2.42[a,b]	2.44[a,b]	2.27[b]	2.39[a,b]	2.74[a]	2.77[a]	2.50[a,b]	2.44[a,b]	0.079	0.01
Overall	2.27	2.29	2.14	2.25	2.54	2.52	2.32	2.25	0.060	0.12
Carcass										
Carcass ADG, g/day	927	921	921	922	887	908	889	869	20.6	0.81
Carcass FCE, g/g	2.77	2.82	2.63	2.77	3.05	2.97	2.75	2.71	0.072	0.12
Cold-weight, kg	79.1	77.4	79.2	79.5	78.3	76.9	78.7	74.6	1.35	0.32
Kill-out, %	76.0[c]	75.8[c]	76.2[b,c]	75.6[c]	77.5[a]	77.5[a]	77.1[a,b]	76.0[b,c]	0.23	0.05
Muscle, mm	47.0	44.3	45.3	44.2	48.1	48.1	48.7	47.3	0.85	0.40
Fat, mm	12.8	13.0	12.9	12.8	12.1	12.4	11.6	11.3	0.54	0.79
Lean meat, %	56.4	55.8	56.0	55.9	57.2	56.9	57.6	57.7	0.49	0.73

[1]Least square means and pooled standard errors of the mean
[2]DM=Dry matter; Water-to-feed ratios presented on a dry matter basis
[3]No. pens = pen replicates per treatment; 6 pigs per pen replicate
[4]ADFI=Average daily feed intake; ADG=Average daily gain; FCE=Feed conversion efficiency
[a,b,c] Within each row, values that do not share a common superscript are significantly different (P<0.5)
[A,B,C] Within each row, values that do not share a common superscript tend to be different (0.05<P<0.10)

Table S 5.2 Effect of sex on growth and carcass quality parameters of grow-finisher pigs fed at four commercially used water-to-feed ratios[1] (Experiment 1)

	Sex		SEM	P-value
	Male	**Female**		**Sex**
No. pens[2]	20	16		
Live-weight, kg				
d 1	42.8	38.5	0.96	0.01
d 32	67.1	66.0	0.59	0.17
d 62	103.9	100.1	0.93	0.01
ADFI[3], g/day				
d 1 – 32	2080	2111	38.0	0.57
d 33 – 62	3035	3036	59.6	0.99
Overall	2557	2573	45.5	0.81
ADG[3], g/day				
d 1 – 32	1017	942	31.5	0.20
d 33 – 62	1263	1178	31.5	0.001
Overall	1136	1068	14.0	0.01
FCE[3], g/g				
d 1 – 32	2.09	2.20	0.027	0.01
d 33 – 62	2.38	2.61	0.04	0.001

	Sex			P-value
	Male	**Female**	**SEM**	**Sex**
Overall	2.24	2.41	0.03	0.001
Carcass				
Carcass ADG, g/day	923	888	10.6	0.03
Carcass FCE, g/g	2.75	2.87	0.044	0.06
Cold-weight, kg	78.8	77.2	0.68	0.32
Kill-out, %	75.9	77.0	0.12	0.001
Muscle, mm	45.2	48.1	0.44	0.001
Fat, mm	12.9	11.9	0.28	0.02
Lean meat, %	56.0	57.3	0.25	0.01

[1]Least square means and pooled standard errors of the mean
[2]No. pens = pen replicates per treatment; 6 pigs per pen replicate
[3]ADFI=Average daily feed intake; ADG=Average daily gain; FCE=Feed conversion efficiency

Table S 5.3 Effect of water-to-feed ratio by sex interaction on growth parameters and carcass quality of grow-finisher pigs[1] (Experiment 2)

Sex	**Male**				**Female**					**P-value**
W:F ratio (DM[2])	**2.4:1**	**3.0:1**	**3.5:1**	**4.1:1**	**2.4:1**	**3.0:1**	**3.5:1**	**4.1:1**	**SEM**	**Treatment x sex**
No. pens[3]	5	5	5	5	4	4	4	4		
Live-weight, kg										
d 1	31.3	32.1	32.2	30.8	32.4	32.3	31.6	31.4	0.89	0.35
d 19	45.0	4.3	45.2	43.7	45.1	44.7	45.3	44.3	0.43	0.02
d 40	70.1	68.2	68.9	67.2	68.4	67.7	68.5	67.0	1.00	0.39
d 57	94.6^{a}	91.6^{a,b}	92.3^{a,b}	89.1^{b}	90.5^{a,b}	89.5^{b}	90.5^{a,b}	88.8^{b}	1.32	0.03
d 76	125.9	122.3	121.6	119.5	116.9	117.4	116.8	118.1	1.40	0.32
ADFI[4], g/day										
d 1 – 19	1909	1866	930	1798	1937	1866	1878	1697	70.8	0.12
d 20 - 40	2532	2491	2422	2444	2583	2445	2456	2320	93.7	0.62
d 41 – 57	3162	3108	3033	3031	3189	3046	2997	2856	90.9	0.20
d 58 – 76	3882	3738	3566	3683	3707	3699	3640	3398	138.5	0.39
Overall	2871	2801	2695	2739	2854	2764	2743	2568	86.6	0.57
ADG[4], g/day										
d 1 – 19	900	853	908	837	910	886	920	867	26.7	0.25

Sex	Male				Female					P-value
W:F ratio (DM[2])	2.4:1	3.0:1	3.5:1	4.1:1	2.4:1	3.0:1	3.5:1	4.1:1	SEM	Treatment x sex
d 20 - 40	1189	1142	1124	1115	1107	1094	1099	1079	29.3	0.22
d 41 – 57	1442[a]	1377[a,b]	1376[a,b]	1288[b]	1294[b]	1277[b]	1297[b]	1283[b]	25.6	0.001
d 58 – 76	1634[a]	1606[a,b]	1534[a,b]	1578[a,b]	1389[b]	1414[a,b]	1427[a,b]	1368[b]	46.9	0.001
Overall	1291	1244	1235	1204	1175	1168	1186	1149	17.9	0.21
FCE[4], g/g										
d 1 – 19	2.13[a,b]	2.18[a]	1.95[b]	2.16[a,b]	2.16[a,b]	2.15[a,b]	2.07[a,b]	1.98[a,b]	0.062	0.02
d 20 - 40	2.13	2.17	2.16	2.20	2.35	2.25	2.25	2.17	0.060	0.30
d 41 – 57	2.19	2.25	2.21	2.36	2.47	2.40	2.32	2.24	0.083	0.21
d 58 – 76	2.37	2.33	2.32	2.33	2.68	2.63	2.56	2.50	0.092	0.06
Overall	2.20	2.24	2.16	2.26	2.41	2.36	2.30	2.22	0.064	0.19
Carcass										
Carcass ADG, g/day	971[A]	919[A,B]	918[A,B]	894[B]	892[B]	891[B]	898[B]	871[B]	12.3	0.09
Carcass FCE, g/g	2.89	3.00	2.89	3.00	3.17	3.09	3.03	2.94	0.090	0.18
Cold-weight, kg	92.1[a]	88.1[a,b]	88.1[a,b]	86.2[b]	86.1[b]	85.9[b]	86.5[b]	84.5[b]	0.94	0.09
Kill-out, %	73.1	72.1	72.4	72.1	73.4	73.6	73.3	73.2	0.35	0.34
Muscle, mm	45.2	45.0	45.0	45.5	49.8	49.1	48.0	49.2	0.66	0.69
Fat, mm	15.9	15.4	15.6	14.8	14.1	13.7	14.2	13.6	0.45	0.90

Sex	Male				Female					P-value
W:F ratio (DM[2])	2.4:1	3.0:1	3.5:1	4.1:1	2.4:1	3.0:1	3.5:1	4.1:1	SEM	Treatment x sex
Lean meat, %	53.5	53.8	53.7	54.4	55.7	55.9	55.4	56.0	0.38	0.80

[1]Least square means and pooled standard errors of the mean
[2]DM=Dry matter; Water-to-feed ratios presented on a dry matter basis
[3]No. pens = pen replicates per treatment; 6 pigs per pen replicate
[4]ADFI=Average daily feed intake; ADG=Average daily gain; FCE=Feed conversion efficiency
[a,b,c] Within each row, values that do not share a common superscript are significantly different (P<0.5)
[A,B,C] Within each row, values that do not share a common superscript tend to be different (0.05<P<0.10)

Table S 5.4 Effect of sex on growth and carcass quality parameters of grow-finisher pigs fed at four commercially used water-to-feed ratios[1] (Experiment 2)

	Sex			P-value
	Male	**Female**	**SEM**	**Sex**
No. pens[2]	20	16		
Live-weight, kg				
d 1	31.6	31.9	0.80	0.77
d 19	44.5	44.9	0.30	0.44
d 40	68.6	67.9	0.54	0.37
d 57	91.9	89.8	0.69	0.03
d 76	122.2	116.5	0.90	0.001
ADFI[3], g/day				
d 1 – 19	1832	1845	46.6	0.84
d 20 – 40	2472	2451	55.8	0.78
d 41 – 57	3083	3022	54.6	0.41
d 58 – 76	3717	3611	75.6	0.31
Overall	2776	2732	52.9	0.54
ADG[3], g/day				
d 1 – 19	1244	1175	14.0	0.29
d 20 – 40	1291	1168	15.2	0.03

	Sex			P-value
	Male	**Female**	**SEM**	**Sex**
d 41 – 57	1244	1186	13.5	0.001
d 58 – 76	1235	1149	23.8	0.001
Overall	1244	1169	9.9	0.001
FCE[3], g/g				
d 1 – 19	2.10	2.09	0.039	0.78
d 20 – 40	2.17	2.26	0.038	0.09
d 41 – 57	2.25	2.36	0.048	0.12
d 58 – 76	2.34	2.59	0.051	0.001
Overall	2.22	2.32	0.040	0.05
Carcass				
Carcass ADG, g/day	925	888	6.1	0.001
Carcass FCE, g/g	2.94	3.06	0.063	0.020
Cold-weight,kg	88.6	85.8	0.47	0.001
Kill-out, %	72.4	73.4	0.17	0.01
Muscle, mm	45.2	49.0	0.33	0.001
Fat, mm	15.5	13.9	0.24	0.001
Lean meat, %	53.9	55.7	0.21	0.001

[1]Least square means and pooled standard errors of the mean
[2]No. pens = pen replicates per treatment; 6 pigs per pen replicate
[3]ADFI=Average daily feed intake; ADG=Average daily gain; FCE=Feed conversion efficiency

6. Effect of dietary inclusion of benzoic acid (VevoVitall®) on the microbial quality of liquid feed and the growth and carcass quality of grow-finisher pigs

F.M. O' Meara, G.E. Gardiner, J.V. O' Doherty, P.G. Lawlor. 2020. Effect of dietary inclusion of benzoic acid (VevoVitall®) on the microbial quality of liquid feed and the growth and carcass quality of grow-finisher pigs. *Livestock Science*. 237: 104043. doi.org/10.1016/j.livsci.2020.104043

6.1 Abstract

Benzoic acid has long been used as a food preservative due to its antibacterial and antifungal effects. Supplementation to pig diets has also been shown to inhibit microbial free amino acid degradation and to control yeast growth in fermented liquid feed. However, the effect of dietary inclusion of benzoic acid (**BA**) in fresh liquid feed for grow-finisher pigs on feed quality and the resultant effects on pig growth remain unclear. The objective of the current study was to compare four inclusion levels of BA (VevoVitall®) on feed microbial quality and the health, growth performance and carcass quality of grow-finisher pigs. Two-hundred and sixteen pigs with a starting weight of 30.0 kg (± 7.43 SD) were used in the experiment. The four dietary treatments were as follows: (1) Basal diet + 0 kg/t BA (**0 kg/t BA**), (2) Basal diet + 2.5 kg/t BA (**2.5 kg/t BA**), (3) Basal diet + 5 kg/t BA (**5 kg/t BA**), (4) Basal diet + 10 kg/t BA (**10 kg/t BA**). Lactic acid bacteria (**LAB**) counts in the mixing tank were similar across treatments ($P>0.05$) but were lower in the troughs for the feed supplemented with 10 kg/t BA than for all other treatments ($P<0.01$). The pH of the 10 kg/t BA treatment was also lower than that of the other three treatments. However, this only occurred in the mixing tank ($P<0.01$), as in the trough, the basal diet had the lowest pH (lower than the other three treatments; $P<0.01$). Dietary BA inclusion did not affect average daily gain, average daily feed intake, feed conversion efficiency, final live-weight, carcass weight or carcass quality during the experimental period ($P>0.05$). In conclusion, while BA may limit the growth of LAB in liquid feed and stabilise feed pH, its inclusion in the diet did not improve the growth performance or carcass quality of grow-finisher pigs.

6.2 Introduction

Benzoic acid (**BA**) has been authorised as a feed additive for grow-finisher pigs at inclusion levels of 0.5 % - 1 % in the diet and is included in the functional group of 'other zoo technical additives' (EU regulation No. 1138/2007/EC; (EFSA, 2007). The metabolic end product of BA is hippuric acid which can decrease urinary pH, so one of the main reasons for using BA is to reduce ammonia emissions from manure. Benzoic acid is a monocarboxylic acid which is used as an antibacterial and antifungal chemical preservative in the food industry (E-number: E210)(Mao et al., 2019). It has also been shown to reduce the loss of free amino acids in fermented liquid feed, which occurs via microbial degradation (Vils et al., 2018). This is presumably by inhibition of microbial growth, as the same study also showed an inhibition of yeast growth and a reduction in the amount of lactic acid produced in the benzoic-acid supplemented feed.

Improved feed conversion efficiency (**FCE**) has also been reported with dietary BA supplementation in grow-finisher pigs (Den Brok, 1999; Van der Peet-Schwering et al., 1999; Øverland et al., 2008) and improved growth rates have been found in weaner pigs (Partanen and Mroz, 1999; Kluge et al., 2006; Guggenbuhl et al., 2007; Torrallardona et al., 2007; Halas et al., 2010; Diao et al., 2016). This enhanced growth performance is most likely due to the antibacterial activity of BA in the pig gut, particularly against coliforms (Knarreborg et al., 2002; Kluge et al., 2006; Øverland et al., 2008; Papatsiros et al., 2011).

The objective of the current study was to compare the effect of four dietary inclusion levels of BA (0 kg/t, 2.5 kg/t, 5 kg/t and 10 kg/t) on the microbial quality of liquid feed and on the growth and carcass quality of grow-finisher pigs. It was

hypothesised that BA would have an antimicrobial effect in liquid feed, thereby limiting spontaneous fermentation and improving feed microbial quality. Furthermore, it was hypothesised that increasing dietary BA inclusion would improve growth and feed efficiency in liquid fed grow-finisher pigs.

6.3 Materials and methods

6.3.1 Ethical approval

Ethical approval for this study was granted by the Teagasc Animal Ethics Committee (approval no. TAEC 107/2015). The experiment was conducted in accordance with Irish legislation (SI no. 543/2012) and the EU Directive 2010/63/EU for animal experimentation.

6.3.2 Experimental design and animal management

The experiment used 216 Danavil Duroc x (Landrace x Large White) female and entire male pigs with an initial live-weight (**LW**) of 30.0 kg ± 7.43 SD. Pigs were penned in groups of 6 pigs with a total of 9 pen replicates/treatment. Pen groups were given a 7-day adaptation period to liquid feeding prior to the start of the experiment, during which all were fed a control diet (0 kg/t BA). Pen groups were blocked by sex and weight following which pens were randomly assigned to one of four dietary treatments, as follows; (1) Basal diet, 0 kg/tonne VevoVitall® (**0 kg/t BA**); (2) Basal diet + 2.5 kg/tonne VevoVitall® (**2.5 kg/t BA**); (3) Basal diet + 5 kg/tonne VevoVitall® (**5 kg/t BA**) ; and (4) Basal diet + 10 kg/tonne VevoVitall® (**10 kg/t BA**).

All pigs were assigned to dietary treatments on the same day of the experiment (day 0). The heaviest two blocks of pigs were on trial for 56 days and slaughtered on day 57 (108.1 kg ± 5.39 SD), while the lighter pigs were on trial for 76 days and slaughtered on days 77 and 78 (118.1 kg ± 8.95 SD). Pigs were slaughtered at a mean LW of 115 kg ± 9.2 SD.

Pen groups were housed in pens (2.37m x 2.36m) with concrete slatted floors and solid PVC partitions. Each pen group had access to a water bowl (DRIK-O-MAT, Egebjerg International A/.S, Egebjerg, Denmark) as per regulation Council Directive 2008/120/EC (2008). Air temperature was maintained at 20 to 22 °C and was recorded daily. The room was mechanically ventilated with exhaust fans and air inlets controlled by a Steinen PCS 8100 controller (Steinen BV, Nederwert, The Netherlands). Pigs were observed closely twice daily and any pig showing signs of ill-health were treated appropriately. All veterinary treatments were recorded, including identity of pig, symptom, medication and dosage administered.

Each pen was equipped with one solenoid valve above a short liquid feeding trough fitted with an electronic sensor. The electronic sensors were checked, 4 times per day, increasing to 6times per day, after 4 weeks, and additional feed was dispensed into troughs where the residual feed in the trough was below the level of the sensor. Feeding was according to a feeding curve to provide *ad-libitum* access to feed. Feed level in the trough was manually inspected daily before and after feeding and feed allocation per pen increased or decreased accordingly. The short stainless-steel troughs (100 cm x 32.5 cm x 21 cm) were located on top of a rubber mat (1.5 x 1 m) which helped to minimise liquid feed wastage.

6.3.3 Diet preparation and feeding

A common diet based on wheat, barley and soybean meal formulated to 9.8 MJ NE/kg and 9.97 g/kg standardised ileal digestible lysine was used. All other amino acids were supplied relative to lysine according to the ideal protein concept (NRC, 2012). A commercially available BA product (VevoVitall®, DSM Nutritional Products, Basel, Switzerland) was included in the diet at 0 kg/t, 2.5 kg/t,

5 kg/t and 10 kg/t, for treatments 1, 2, 3 and 4 respectively, and directly replaced wheat in the diet. The diets were manufactured in meal form at the Teagasc feed mill (Moorepark, Fermoy, Co. Cork). Ingredient and chemical composition of the diet is shown in Table 6.1.

The dietary treatments were prepared and liquid-fed using an automatic sensor feeding system (HydroMix, BigDutchman, Vechta, Germany). Diets were prepared in a mixing tank with a 6 pale agitator and agitated for ~ 5 minutes prior to feed-out. The high-pressure air system delivered liquid feed from the mixing tanks to troughs, each of which was fitted with a solenoid valve and an electronic feed sensor. If feed was above the sensor in a trough, feed was not dispensed to that particular trough. If feed was below the level of the sensor, feed was dispensed to the trough and sensors were checked automatically before each scheduled feeding. A 12.5 litre rinse of the mixing tanks was carried out after feeding each treatment to prevent contamination from one mix to the next. The mixing tank and pipelines used to prepare and distribute liquid feed, respectively were empty between feeds. The water-to-feed ratio used to prepare the liquid feed was 2:1 on a fresh matter basis (FM) or 2.4:1 on a dry matter basis (DM).

6.3.4 Titrations

Titrations were carried out in order to determine the quantity of BA required to reduce the pH of the diet to 4 as described by Lawlor et al. (2005). Four samples of the basal diet were titrated in duplicate prior to the start of the experiment to determine the amount of acid required to bring the diet to pH 4. Briefly, a 0.5 g sample of the diet was added to 50 ml deionised water and continuously stirred using a magnetic stirrer. Hydrochloric acid (HCl, 0.1N) was added in 0.2 ml increments every 3 minutes and the pH recorded (Mettler Toledo pH meter, Greisensee,

Switzerland) prior to the addition of each increment. Four replicates of the VevoVitall® product were also titrated against sodium hydroxide (NaOH, 0.1N) in 1 ml increments every 3 minutes to assess how much base would be required to raise the pH to 4. A Pearson square calculation was used to determine the proportions of feed and acid that would produce a diet of pH 4.

6.3.5 Records and sampling

All pigs were weighed on Day 0 and prior to slaughter at the end of the experiment (i.e. day 56 or day 76). Feed disappearance for each pen was recorded daily and average daily gain (**ADG**), average daily feed intake (**ADFI**) and FCE were calculated for the entire experiment.

The pH and temperature of liquid feed from each treatment from the mixing tank was recorded using a pH meter (Mettler Toledo) 3 times/week throughout the experiment. To do so, three ~ 100 ml aliquots were removed from the mixing tank during agitation prior to feed-out and the pH and temperature recorded immediately. The pH and temperature of liquid feed from all 36 troughs was recorded once/week during the experiment, provided feed was available.

Liquid feed samples (~ 50 g) were collected on days 1, 42 and 70 into sterile containers from the mixing tank and 2 troughs/treatment and transported to the laboratory on ice for same-day microbiological analysis. Liquid feed samples for ethanol analysis were collected on day 42 and day 70 from the mixing tank and from 2 troughs/treatment and stored in ~ 20 g aliquots at - 20 °C until analysis. Dry samples of each diet from each batch of feed produced in the feed mill were pooled into one diet sample per treatment for chemical analysis. Liquid feed samples (~ 250 g) were also collected from the mixing tank (1/treatment) and troughs (2/treatment)

on day 42 and day 70 and stored at -20 °C for proximate analysis and amino acid determination.

During exsanguination at the slaughter house, blood samples were collected from 36 pigs (9 pigs/treatment) using Vacuette tubes (Labstock, Dublin, Ireland) for haematological analysis.

6.3.6 Slaughter

Pigs were fasted for ~ 12 hours prior to slaughter by CO_2 stunning followed by exsanguination in a commercial slaughterhouse. The following measurements were taken: hot carcass weight was recorded 45 minutes after stunning, and back-fat thickness and muscle depth measured at 6cm from the edge of the split back at the level of the 3rd and 4th last rib were determined using a Hennessy Grading Probe (Hennessy and Chong, Auckland, New Zealand). Lean meat content was estimated according to the following formula: Estimated lean meat content (%) = 60.3 – 0.847x + 0.147y where x = fat depth (mm); y = muscle depth (mm) (Department of Agriculture Food and Rural Development, 2001). Cold carcass weight was calculated as hot carcass weight (45 minutes after stunning) x 0.98. Kill-out percentage was calculated from final LW prior to slaughter and cold carcass weight.

6.3.7 Microbiological analysis of liquid feed

Approximately 10 g of each liquid feed sample was homogenized in a stomacher as a 10-fold dilution in maximum recovery diluent (MRD; Oxoid, Basingstoke, UK) and a 10-fold dilution series was performed in MRD. Relevant dilutions were plated in duplicate as follows; (1) pour-plated on de Man Rogosa & Sharpe, (MRS; Merck, Damstadt, Germany) agar, containing 50 U/mL nystatin (Sigma-Aldrich, Arklow, Co. Wicklow, Ireland), overlaid and incubated at 30 °C for

72 hours for enumeration of lactic acid bacteria (**LAB**); (2) pour-plated on violet red bile glucose (VRBG; Oxoid) agar, overlaid and incubated at 37 °C for 24 hours for *Enterobacteriaceae;* (3) pour-plated on ChromoCult tryptone bile X-glucuronide (CTBX; Merck) agar and incubated at 44 °C for 24 hours for *E. coli*; and (4) spread-plated on yeast glucose chloramphenicol (YGC; Merck) agar and incubated at 25 °C for 5 days for yeasts and moulds. Colonies were counted and the counts averaged and presented as $\log_{10}$ CFU/g of the original sample.

6.3.8 Feed analysis

The four diets used in the experiment were ground through a 2mm Christy Norris mill and analysed for DM, ash, gross energy (**GE**) neutral detergent fibre (**NDF**) ether extract (**EE**), nitrogen (**N**) and amino acid (**AA**) concentration. The DM (AOAC.934.01), ash (AOAC.942.05), and EE concentration (AOAC.920.39) were determined according to methods of the Association of Official Analytical Chemists (AOAC, 2005). Gross energy was determined using an adiabatic bomb calorimeter (Parr Instruments, Moline, IL USA). The neutral detergent fibre (**NDF**) content was determined according to the method of Van Soest et al. (1991) using the Ankom 220 Fibre Analyser (Ankom Technology, Macedon, New York, USA). The N content was determined using the LECO FP 528 instrument (Leco Instruments, UK Ltd., Cheshire, UK) (AOAC.990.0). Crude protein (**CP**) was determined as N x 6.25. Amino acid determination was carried out using cation exchange HPLC as previously described by McDermott et al. (2016) (AOAC 994.12).

Liquid feed samples collected from the mixing tank and troughs on day 42 and day 70 were oven-dried at 55 °C for 72 hours and milled through a 2mm screen using a Christy Norris mill. These samples were pooled prior to analysis to give one

mixing tank and one trough sample per treatment which were analysed for GE, N, CP, ash and AA as above.

Preparation of liquid feed samples for ethanol analysis was carried out as described by van Winsen et al. (2000). Briefly, feed aliquots were defrosted prior to centrifugation at 2,000 g for 10 minutes at 4 °C. The supernatant was then centrifuged at 18,500 g for 10 minutes. The resulting supernatant was filtered through a 0.2 µm filter and stored at -20 °C until analysis. Samples were thawed slowly at room temperature prior to ethanol analysis by gas chromatography (Agilent 6890; Agilent Technologies, Waghaeusel-Wiesental, Germany) using a flame ionization detector. A 1 µL volume of each sample was injected by split injection 5:1 onto the column (AT-100 15 m x 0.53 mm i.d. x 1.2 micron) with a column flow rate of 3.4 ml/min helium. The temperature programme was 40 °C for 3 minutes, ramped at 10 °C/min to 180 °C and held at 180 °C for 3 minutes.

6.3.9 Haematological analysis of blood samples

Blood samples for haematology were analysed on the day of slaughter using an Abbot Cell-Dyn 3700 analyser (GMI-Inc., Minnesota, USA). The following parameters were measured; white blood cells, neutrophil number and percentage, lymphocyte number and percentage, eosinophil number and percentage, monocyte number and percentage, basophil number and percentage, red blood cells, haemoglobin, packed cell volume, mean corpuscular volume, mean corpuscular haemoglobin and platelets.

6.3.10 Statistical analysis

Growth parameters [ADFI, ADG, FCE and LW], carcass quality parameters and haematology data were analysed using the MIXED procedure of SAS®9.4 (Sas

Institute, Inc., Cary, NC, US). For growth parameters; treatment, sex and their associated interaction were included in the model as fixed effects. Initial LW and number of days on trial were included in the model as co-variates with pen as the experimental unit and pen nested within block as a random effect. A general linear model procedure was also used to check for linearity. For carcass quality parameters, carcass growth parameters and haematological analysis; treatment and sex and their associated interaction were included in the model. Carcass cold weight was used as a co-variate for analysis of muscle depth, fat depth and lean meat percentage and initial LW was used as a co-variate for cold weight. The microbial counts, pH and temperature on days 1, 42 and 70 were also analysed using the MIXED procedure of SAS with treatment, sampling location and their associated interaction included in the model as fixed effects and day of sampling included as a random effect. The normality of scaled residuals was investigated using the UNIVARIATE procedure of SAS. Results are presented as least square means ± SEM. Differences were considered significant at $P<0.05$ and as tendencies $0.05<P<0.10$. The MEANS procedure was also used to calculate means and standard deviations for the weekly pH trough recordings carried out.

6.4 Results

6.4.1 Pig Deaths and Removals

Seven pigs were removed from treatment throughout the experimental period and their weights were taken into account in feed intake and growth calculations. This included two pigs from the 0 kg/t inclusion rate of BA, one due to suspected stomach ulcers and one due to a prolapse; one pig from the 2.5 kg/t BA treatment due to a rupture; three pigs from the 5 kg/t BA treatment, one due to a suspected heart attack, one due to pneumonia and one due to a burst rupture; one pig from the 10 kg/t BA treatment due to a burst rupture.

6.4.2 Titrations of benzoic acid product against HCl and NaOH (data not shown)

Firstly, it was found that, on average, 0.55 ml 0.1N HCl was required to bring the pH of the basal diet to pH 4 which equated to 110 mEq/g feed. Titrations of the VevoVitall® benzoic acid additive against the NaOH determined that 8 ml NaOH was required to raise the pH of the VevoVitall® product to pH 4 which equated to 1600 mEq/g. The Pearson square calculation showed that a ratio of 93.57 % feed to 6.43 % VevoVitall® was optimal to achieve a pH of 4 with this diet.

6.4.3 Effect of benzoic acid on the microbiological quality, pH and temperature of liquid feed for grow-finisher pigs

The effect of sampling location by treatment interactions on the microbial counts, pH and temperature of liquid feed are shown in Table 6.2. A sampling location x treatment interaction was observed for LAB. Counts of LAB were lower in troughs of the 10 kg/t BA treatment than in troughs of all other treatments, but LAB counts in the mixing tank were not influenced by BA inclusion rate. A

tendency for a sampling location x treatment interaction was observed for *Enterobacteriaceae* counts. In liquid feed from the 2.5 kg/t BA treatment, counts of *Enterobacteriaceae* were higher in the trough than in the mixing tank; however counts in the mixing tank and trough were similar at all other BA inclusion rates. There was also a sampling location x treatment interaction for pH. In the mixing tank, the pH of the 10 kg/t BA treatment was lower than the 0 kg/t BA and 2.5 kg/t BA treatments, whereas in the trough, the pH of 0 kg/t BA was lower than for all other treatments. There were no sampling location x treatment interactions for *E. coli*, yeast, or mould counts or feed temperature.

The results of the microbiological, pH and temperature analysis of the four liquid feed treatments in the mixing tanks and troughs carried out on days 1, 42 and 70 of the experiment are shown in Table 6.3. In the mixing tank, counts of LAB, *E. coli*, yeast and mould and liquid feed temperature were similar for all treatments. Counts of *Enterobacteriaceae* in the 2.5 kg/t BA treatment tended to be lower than those in the 0 kg/t BA and 5 kg/t BA treatments but the same as the 10 kg/t BA treatment ($P=0.06$). The pH of the 10 kg/t BA treatment was lower than all other treatments in the mixing tank ($P<0.05$).

There were no differences in *E. coli*, yeast or mould counts in feed samples collected from the troughs; however, counts of LAB were lower in the 10 kg/t BA treatment than in the other three treatments ($P<0.01$). *Enterobacteriaceae* counts tended to be lower in the 10 kg/t BA treatment than the 2.5 kg/t BA treatment, but were similar to those in the 0 kg/t BA and 5 kg/t BA treatments ($P=0.06$). In the pen troughs the opposite occurred in terms of pH, with the pH of the 0 kg/t BA treatment lower than that of the other three treatments ($P<0.01$). A tendency for a treatment

effect on temperature was observed in troughs; however, there were no pairwise differences between treatments (P>0.01).

Figure 6.1 shows the pH of liquid feed from the mixing tank (28 recordings in total; 3 recordings weekly) and troughs (9 recordings in total; 1 recording weekly from 9 troughs/treatment where feed was available) of each treatment. The mean pH of liquid feed from the 28 recordings from the mixing tank of each treatment throughout the trial period was 6.14, 5.93, 5.72 and 5.41 for treatments 1 through 4, respectively. The mean pH of liquid feed from 9 recordings from the troughs of each treatment was 5.12, 5.26, 5.29 and 5.40 for treatments 1 through 4, respectively. The results of these mixing tank and trough recordings are similar to those reported in Table 6.3 on days 1, 42 and 70 when liquid feed was microbiologically analysed. The mean temperature of liquid feed from 28 recordings from the mixing tank of each treatment was 20.6 °C, 20.4 °C, 20.5 °C and 20.7 °C for treatments 1 through 4, respectively. The mean temperature of liquid feed from 9 recordings of liquid feed from troughs was 22.1 °C, 22.0 °C, 22.3 °C and 22.3 °C for treatments 1 through 4, respectively.

6.4.4 Proximate and amino acid analysis of feed

The results of chemical analyses of the dry diets and liquid feed from the mixing tank and troughs of each treatment are shown in Table 6.4. There were no obvious differences in GE content of the diet between dry and liquid (mixing tank/ trough) feed. There appears to be a loss of lysine in all four treatments when dry and liquid (mixing tank and trough) feed are compared; however, the loss of methionine from the liquid feed in the mixing tank appear greater in the 0 kg/t BA treatment than the other three treatments. Ethanol was not detected in liquid feed sampled from the

mixing tank in any treatment on either day 42 or day 70 of the experiment (data not shown). Ethanol concentrations in liquid feed troughs on day 70 were 20.1mM, 1.8mM and 0.5mM from the 0 kg/t, 5 kg/t and 10 kg/t BA treatments respectively.

6.4.5 Effect of dietary benzoic acid on the growth, feed efficiency and carcass quality of grow-finisher pigs

The effect of treatment on pig growth, feed efficiency and carcass quality is shown in Table 6.5. No treatment x sex interactions were observed for any of the growth performance parameters or carcass quality traits measured in the current study. There were also no treatment differences observed for ADFI, ADG, FCE, slaughter weight, carcass ADG or carcass FCE during the experiment ($P>0.05$). Similarly, no treatment differences were observed for kill-out percentage, muscle depth, fat depth or lean meat percentage at slaughter ($P>0.05$).

6.4.6 Effect of dietary benzoic acid on the haematological profile of pigs at slaughter

Results from the haematological analysis are shown in Table S 6.1. There were no significant treatment x sex interactions for any of the haematological parameters measured ($P>0.05$). The only treatment effect observed was that pigs fed the 0 kg/t BA treatment tended to have a lower number of platelets than pigs fed the 2.5 kg/t BA treatment ($P=0.09$). Platelet counts were slightly below the normal range reported in the Merck manual of 200 – 500 cells $x10^9$/L in pigs fed the 0 kg/t treatment.

6.5 Discussion

Benzoic acid has long been used as a food preservative due to its antibacterial and antifungal activity (Mao et al., 2019). It can also be used as a feed additive; however, most of the research to date on dietary inclusion of benzoic acid has been performed in dry or wet/dry feed (Den Brok, 1999; Guggenbuhl et al., 2007; Torrallardona et al., 2007). To our knowledge, this is the first study to investigate the impact of BA both on the microbial quality of fresh liquid feed and growth of grow-finisher pigs in the same study. Our objective was to improve the microbial quality of liquid feed by adding BA to the diet and, consequently to improve pig growth.

While dietary BA inclusion did not affect overall or carcass growth or feed efficiency in the current study, it is important to note that the growth rate was exceptionally high and feed efficiency was extremely good for pigs on all treatments. For this reason, it would have been difficult to obtain a biological improvement in ADG, FCE or both in response to dietary inclusion of BA. Management of the liquid feeding system was extremely good in the current experiment, in an attempt to minimise wastage which was previously found to be the most likely cause of poorer FCE when liquid feeding (Russell et al., 1996; l'Anson et al., 2012). It is evident from the growth rates and FCEs achieved that feed wastage was minimised while still ensuring *ad-libitum* access to feed by the pigs in the current study.

The benefits of dietary BA inclusion are not as pronounced in older pigs as in younger pigs (Bühler, 2009). It has been suggested that as pigs age, the BA supplementation-mediated improvement in digestive ability from the associated pH reduction and the increase in activity of digestive enzymes declines (Diao et al.,

2016). This, combined with the excellent feed efficiency found for the control group in the current experiment help explain why no treatment differences in pig growth and feed efficiency were found in the current experiment. While no studies, to date have investigated the growth performance of pigs fed fresh liquid feed supplemented with BA, Van der Peet-Schwering et al. (1999) reported a 0.1 unit improvement in FCE when dry-feeding 10 kg/t BA to finisher pigs. However, the FCE for the control diet in the Van der Peet-Schwering et al. (1999) was 2.78, whereas it was 2.27 in the present study. Hence, it would appear that, there was greater scope for FCE to improve due to dietary BA inclusion than in the current study.

The results of this study show that, although dietary BA inclusion did not influence pig growth, at 10 kg/t BA inclusion it did stabilise liquid feed pH from the mixing tank to the troughs and reduced the growth of LAB in residual feed in the troughs. This resulted in the highest pH recording in the troughs of the 10 kg/t BA treatment, despite the fact that the opposite was true in the mixing tank, where the lowest pH was recorded in the treatment with the highest inclusion level of BA. It is well known that the production of lactic and acetic acid by LAB and yeasts in liquid feed reduces the pH of the mixture (Missotten et al., 2015). The reduced growth of LAB in the 10 kg/t BA treatment while in the feed trough therefore seems to have reduced microbial acid production. The potential of BA to inhibit yeast growth and lactic acid production in liquid (fermented) feed has previously been reported (Vils et al., 2018). The pH reduction and increase in LAB counts and ethanol concentrations between the mixing tank and troughs for the control diet without BA suggest that spontaneous fermentation was occurring (Scholten et al., 1999) in the present study while feed resided in the troughs. Our results suggest that 1 % dietary BA inclusion prevented this spontaneous fermentation, as evidenced by the stabilised

pH and limited growth of LAB over time compared to the control diet (although there was no effect on yeast growth).

Despite the fact that enteric bacteria are reportedly reduced in the gut by BA supplementation (Kluge et al., 2006; Guggenbuhl et al., 2007), no BA-mediated reduction in *Enterobacteriaceae* was found in the liquid feed in the present study. This is most likely because the pH reduction achieved in the liquid feed was not sufficient. A pH of ~ 4.0 is required to reduce coliform counts in liquid feed (Geary et al., 1999; Plumed-Ferrer et al., 2004) and pH 4.72 was the lowest pH recorded in our study.

Amino acid analysis from the current study shows a loss of lysine in the mixing tanks and troughs of all four treatments when compared with the dry feed; however, the biggest loss appears to be in the control treatment without BA supplementation. This suggests that the BA might be preventing microbial degradation of free amino acids as a result of controlling spontaneous fermentation, in agreement with previous findings for fermented liquid feed (Vils et al., 2018). However, the results from the current study should be treated with caution, as the liquid feed data are from only one pooled sample at each location.

No treatment differences were observed in the haematological profile of pigs in the current study, except that the platelet count tended to be higher in pigs supplemented with 2.5 g/t BA. This is likely due to the fact that the count was slightly below the normal range in the non-BA supplemented pigs. Overall, the findings show that, although white blood cell counts were slightly higher than the normal range in the 2.5 kg/t BA and 5 kg/t BA treatments, and the mean corpuscular haemoglobin was slightly above the normal range in all BA-supplemented pigs

(Jackson and Cockcroft (2008), health status was not impacted by BA inclusion in the diet.

The upper dietary benzoic acid inclusion limit for pigs is 10 kg/t (EU regulation No. 1138/2007/EC). The titrations conducted as part of the current study suggested that if we wish to reduce the pH of liquid feed to 4, to help reduce *Enterobacteriaceae* counts, that 64.3 kg BA/t feed would be required. Previous work has shown that increasing dietary inclusion from 10 kg/t to 20 kg/t worsened pig growth rate and feed efficiency (Van der Peet-Schwering et al., 1999) and hence controlling feed hygiene using BA is unlikely to be feasible.

In conclusion, findings from the present study showed that dietary inclusion of BA at 10 kg/t reduced LAB growth and the associated reduction in feed pH while feed resided in the feed trough, indicating that spontaneous microbial fermentation was somewhat controlled. However, dietary BA supplementation did not improve growth or feed efficiency in liquid-fed grow-finisher pigs, most likely because the growth and feed efficiency of unsupplemented pigs was already very high.

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6.7 Tables

Table 6.1 Ingredient and chemical composition of the experimental diets (on an as-fed basis, g/kg unless otherwise stated)

	Inclusion rate of benzoic acid (kg/t)			
	0.0	**2.5**	**5.0**	**10.0**
Ingredient composition				
Wheat	400.0	397.5	395.0	390.0
Barley	382.7	382.7	382.7	382.7
Soya bean meal	183.0	183.0	183.0	183.0
Benzoic acid[1]	0.0	2.5	5.0	10.0
Limestone flour	11.0	11.0	11.0	11.0
Lysine HCl	3.8	3.8	3.8	3.8
Mono DiCalcium phosphate	1.0	1.0	1.0	1.0
Salt	3.0	3.0	3.0	3.0
L-Threonine	1.7	1.7	1.7	1.7
Soya oil	9.7	9.7	9.7	9.7
Vitamin and mineral pre-mix[2]	1.0	1.0	1.0	1.0
DL-Methionine	0.9	0.9	0.9	0.9
Celite	2.0	2.0	2.0	2.0
L-Tryptophan	0.2	0.2	0.2	0.2
Phytase[3]	0.1	0.1	0.1	0.1
Chemical composition				
Dry matter	880.0	880.0	881.0	880.0
Crude protein	182.0	175.0	179.0	175.0
Ash	37.7	35.4	40.4	42.3
Oil	40.6	35.3	36.1	41.7
Neutral detergent fibre[4]	138.0	138.0	138.0	138.0
Gross energy, MJ/kg	15.7	16.2	16.2	16.2
Lysine	10.8	10.8	10.8	10.8
Methionine	4.4	4.3	4.3	4.3
Threonine	7.2	8.1	7.3	7.0
Digestible energy, MJ/kg[4]	13.8	13.8	13.8	13.8

	Inclusion rate of benzoic acid (kg/t)			
	0.0	**2.5**	**5.0**	**10.0**
Net energy, MJ/kg[4]	9.8	9.8	9.8	9.8
SID[5] lysine[4]	9.97	9.97	9.97	9.97
Total calcium[4]	6.59	6.59	6.59	6.59
Digestible phosphorus[4]	2.6	2.6	2.6	2.6

[1] VevoVitall ® (DSM Nutritional Products, Basel, Switzerland)

[2] Vitamin and mineral premix provided per kilogram of complete diet: Cu from copper sulphate, 15 mg; Fe from ferrous sulphate monohydrate, 24 mg; Mn from manganese oxide, 31 mg; Zn from zinc oxide, 80 mg; I from potassium iodate, 0.3 mg; Se from sodium selenite, 0.2 mg; retinyl acetate, 0.7 mg; cholecalciferol, 12.7 µg; DL-alpha-tocopheryl acetate, 40 mg; Vitamin K, 4 mg; vitamin B12, 15 µg; riboflavin, 2 mg; nicotinic acid, 12 mg; pantothenic acid, 10 mg; vitamin B1, 2 mg; vitamin B6, 3 mg and celite 2000 mg/kg.

[3] The diet contained 500 phytase units (FYT) per kg feed from RONOZYME HiPhos (DSM, Belfast, UK)

[4]Calculated values

[5]SID: Standardised ileal digestible

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Table 6.2 Effect of sampling location (mixing tank or trough) and dietary benzoic acid inclusion rate on the microbial quality, pH and temperature of liquid feed[1,2]

Location	Mixing tank				Trough					P-value		
Benzoic acid, kg/t[3]	**0.0**	**2.5**	**5.0**	**10.0**	**0.0**	**2.5**	**5.0**	**10.0**	SEM	**Location x Treatment**	**Location**	**Treatment**
Lactic acid bacteria[4]	$6.76^{a,b}$	5.65^{a}	$6.47^{a,b}$	6.11^{a}	9.15^{c}	8.65^{c}	8.72^{c}	7.42^{b}	0.258	0.04	0.001	0.01
Enterobacteriaceae[4]	$5.32^{A,B}$	4.70^{A}	$5.28^{A,B}$	$4.96^{A,B}$	$5.36^{A,B}$	5.52^{B}	$5.24^{A,B}$	$4.84^{A,B}$	0.178	0.07	0.18	0.12
E. coli[4]	2.15	2.00	2.00	2.00	3.61	3.81	3.51	3.06	0.243	0.50	0.001	0.42
Yeast[4]	4.33	3.91	3.89	4.09	6.26	6.38	6.17	5.95	0.157	0.21	0.001	0.31
Mould[4]	3.15	3.09	3.08	3.08	3.66	3.64	3.51	3.27	0.182	0.70	0.01	0.56
pH	6.2^{d}	6.0^{d}	$5.9^{c,d}$	$5.5^{b,c}$	4.7^{a}	$5.4^{b,c}$	5.2^{b}	$5.5^{b,c}$	0.17	0.001	0.001	0.08
Temperature, °C	19.6	19.5	19.8	19.6	19.4	19.8	21.5	21.2	1.45	0.40	0.09	0.27

[1]Least square means and pooled standard errors of the mean
[2] Results are the mean of data obtained on day 1, day 42 and day 70 of the experiment
[3] VevoVitall ® (DSM Nutritional Products, Basel, Switzerland)
[4]Counts in $\log_{10}$ CFU/g
^{a,b,c} Within each row, values that do not share a common superscript are significantly different ($P<0.05$)
^{A,B,C} Within each row, values that do not share a common superscript tend to be different ($0.05<P<0.10$)

Table 6.3 Effect of four dietary inclusion rates of benzoic acid on the microbial quality, pH and temperature of liquid feed for grow-finisher pigs from the mixing tank and troughs[1,2]

	Inclusion rate of benzoic acid (kg/t)[3]					P-value
	0.0	2.5	5.0	10.0	SEM	Treatment
Feed from the mixing tank						
Lactic acid bacteria[4]	6.76	5.65	6.47	6.11	0.329	0.15
Enterobacteriaceae[4]	5.32^{A}	4.70^{B}	5.28^{A}	4.96^{A,B}	0.140	0.06
E. coli[4]	2.15	2.00	2.00	2.00	0.073	0.45
Yeast[4]	4.33	3.91	3.89	4.09	0.153	0.25
Mould[4]	3.15	3.09	3.08	3.08	0.077	0.77
pH	6.16^{a}	6.01^{a}	5.87^{a}	5.50^{b}	0.190	0.01
Temperature, °C	19.6	19.5	19.8	19.6	1.83	0.47
Feed from troughs						
Lactic acid bacteria[4]	9.15^{a}	8.65^{a}	8.72^{a}	7.42^{b}	0.159	0.01
Enterobacteriaceae[4]	5.36^{A,B}	5.52^{A}	5.24^{A,B}	4.84^{B}	0.210	0.06
E. coli[4]	3.61	3.81	3.51	3.06	0.336	0.47
Yeast[4]	6.26	6.36	6.17	5.95	0.160	0.37
Mould[4]	3.66	3.64	3.51	3.27	0.246	0.30
pH	4.72^{b}	5.40^{a}	5.24^{a}	5.48^{a}	0.151	0.01
Temperature[5], °C	19.4	19.8	21.5	21.2	0.92	0.07

[1]Least square means and pooled standard errors of the mean
[2] Results are the mean of data obtained on day 1, day 42 and day 70 of the experiment
[3] VevoVitall ® (DSM Nutritional Products, Basel, Switzerland)
[4]Counts in $\log_{10}$ CFU/g
[5] A tendency for a treatment effect on temperature was observed in troughs; however, there were no pairwise differences between treatments (P>0.01).
[a,b,c] Within each row, values that do not share a common superscript are significantly different (P<0.05)
[A,B,C] Within each row, values that do not share a common superscript tend to be different (0.05<P<0.10)

Table 6.4 Gross energy, crude protein, ash and amino acid analysis of dry diets and liquid feed from the mixing tank and troughs containing different inclusion rates of benzoic acid (presented on a DM basis)[1]

Sampling location		**Mixing tank**				**Trough**			
Benzoic acid (kg/t)[2]	**Dry[3]**	**0.0**	**2.5**	**5.0**	**10.0**	**0.0**	**2.5**	**5.0**	**10.0**
Gross energy, MJ/kg	18.3	18.7	18.8	18.7	18.4	18.4	18.7	18.1	18.4
Crude protein, %	20.2	22.1	22.2	23.2	21.3	19.2	21.9	21.1	21.0
Ash, %	4.42	3.83	4.20	4.05	4.07	5.04	5.43	6.49	5.33
Amino acids, g/kg									
Lysine	12.4	8.6	10.4	10.7	9.8	8.2	8.4	NT[4]	8.4
Methionine	4.9	4.5	5.1	5.4	5.1	4.5	4.7	NT	4.9
Threonine	8.4	7.7	8.9	9.4	9.0	7.7	8.3	NT	8.7
Cysteic acid	5.9	5.3	5.9	6.2	6.1	5.7	5.0	NT	5.3
Taurine	1.4	1.5	1.3	1.5	2.3	1.6	3.4	NT	3.6
Aspartic acid	19.1	16.5	19.5	20.7	20.1	16.5	17.8	NT	19.3
Serine	10.2	9.2	10.5	11.0	10.8	9.4	9.6	NT	10.1
Glutamic acid	46.7	43.2	47.5	49.1	48.5	45.2	44.7	NT	49.0
Glycine	9.0	8.2	9.3	9.7	9.5	8.5	8.7	NT	9.5
Alanine	8.5	7.7	8.9	9.1	8.9	8.2	7.7	NT	8.6
Cysteine	0.7	0.9	1.0	0.9	0.8	0.9	1.4	NT	2.7

Sampling location		**Mixing tank**				**Trough**			
Benzoic acid (kg/t)[2]	**Dry[3]**	**0.0**	**2.5**	**5.0**	**10.0**	**0.0**	**2.5**	**5.0**	**10.0**
Valine	10.2	9.2	10.4	11.0	10.5	9.6	9.2	NT	11.5
Isoleucine	7.9	7.6	8.6	9.1	8.8	7.7	8.0	NT	8.7
Leucine	15.1	13.6	15.3	16.3	15.9	13.9	14.4	NT	15.4
Tyrosine	5.6	5.3	6.1	6.5	6.2	5.2	3.9	NT	4.5
Phenylalanine	10.4	9.4	10.5	11.1	10.8	9.9	9.6	NT	10.2
Histidine	6.2	5.4	6.1	6.6	6.5	5.4	6.2	NT	6.8
Arginine	12.7	11.0	12.6	13.7	13.2	11.0	11.5	NT	12.2
Proline	14.7	14.0	14.5	15.3	15.2	15.3	15.5	NT	14.9

[1]Results are from pooled samples: Dry sample pooled from 3 feed batches from the mill for each diet (n=3/treatment prior to pooling); Mixing tank sample pooled from 1 sample/treatment on day 42 and 1 sample/treatment on day 70 for each treatment (n=2/treatment prior to pooling); Trough sample pooled from 2 samples/treatment on day 42 and 2 samples/treatment on day 70 (n=4/treatment prior to pooling).
[2]VevoVitall ® (DSM Nutritional Products, Basel, Switzerland)
[3]Mean results for 4 dry diets
[4]NT=Not tested

Table 6.5 Effect of four dietary inclusion rates of benzoic acid on the growth, feed efficiency and carcass quality of liquid-fed grow-finisher pigs[1]

	Inclusion rate of benzoic acid (kg/t)[2]					P-value		
	0.0	**2.5**	**5.0**	**10.0**	**SEM**	**Treatment**	**Sex**	**Treatment x sex**
No pens/trt[3]	9	9	9	9				
Start-weight, kg	34.4	34.2	35.8	35.6	2.30	0.61	0.08	0.26
ADFI[4], g/day	2785	2876	2766	2826	59.6	0.58	0.43	0.58
ADG[4], g/day	1224	1264	1239	1263	17.9	0.23	0.001	0.24
FCE[4], g/day	2.27	2.29	2.23	2.24	0.034	0.62	0.001	0.90
Slaughter weight, kg	114.6	116.5	114.0	117.0	2.76	0.84	0.001	0.90
Carcass								
ADG[5], g/day	903	931	903	920	9.7	0.14	<0.001	0.17
FCE[6], g/g	3.00	3.02	3.03	3.05	0.054	0.94	0.01	0.27
Cold-weight, kg	84.3	85.6	83.5	85.5	1.86	0.84	0.01	0.90
Kill-out, %	73.7	73.6	73.3	73.1	0.65	0.91	0.01	0.86
Muscle, mm	46.9	47.4	48.1	48.2	0.47	0.20	0.001	0.42
Fat, mm	13.8	14.3	13.7	13.3	0.36	0.28	0.001	0.64
Lean meat, %	55.5	55.2	55.8	56.1	0.31	0.182	0.001	0.75

[1]Least square means and pooled standard errors of the mean. There were 9 pen replicates per treatment with 6 pigs per pen replicate
[2] VevoVitall ® (DSM Nutritional Products, Basel, Switzerland)

[3]No. pens/trt= number of pens per treatment, each pen had 6 pigs per pen
[4]ADFI=Average daily feed intake; ADG=Average daily gain; FCE=Feed conversion efficiency
[5]Carcass ADG: From weight at start of experiment to slaughter = ((carcass weight in kg – LW on day 1 x 0.65) x1000) / no. days on treatment (Lawlor and Lynch, 2005)
[6]Carcass FCE: From start of experiment to slaughter = total average daily feed intake / carcass ADG (g)

Table S 6.1 Effect of different inclusion rates of a commercially available benzoic acid product in the grow-finisher diet on the haematological profile of pigs at slaughter[1]

	Inclusion rate of benzoic acid (kg/t)[2]					P-value		
	0.0	**2.5**	**5.0**	**10.0**	**SEM**	**Treatment**	**Sex**	**Treatment x sex**
No. pigs sampled /trt	9	9	9	9				
White blood cells, x10^9 cells/L	20.82	22.13	23.75	20.78	1.667	0.58	0.84	0.58
Neutrophils								
%	54.8	59.0	59.3	23.8	2.17	0.24	0.13	0.73
no. x 10^9 cells/L	11.65	13.13	14.21	11.22	1.240	0.36	0.41	0.48
Lymphocytes								
%	34.7	31.1	31.1	36.4	2.07	0.25	0.06	0.68
no. x 10^9 cells/L	7.15	6.59	7.28	7.52	0.489	0.61	0.10	0.81
Eosinophils								
%	0.2	0.1	0.1	0.1	0.02	0.21	0.26	0.77
no. x 10^9 cells/L	0.03	0.03	0.03	0.02	0.005	0.65	0.40	0.84
Monocytes								
%	8.1	7.8	7.0	7.6	0.78	0.81	0.27	0.67
no. x 10^9 cells/L	1.70	1.70	1.67	1.57	0.201	0.96	0.26	0.67
Basophils								

	Inclusion rate of benzoic acid (kg/t)[2]					P-value		
	0.0	**2.5**	**5.0**	**10.0**	**SEM**	**Treatment**	**Sex**	**Treatment x sex**
%	2.5	2.4	2.5	1.9	0.23	0.58	0.15	0.72
no. x 10^9 cells/L	0.48	0.51	0.59	0.42	0.067	0.39	0.43	0.83
Red blood cells, x10^{12} cells/L	7.83	7.60	7.67	7.86	1.611	0.62	0.01	0.12
Haemoglobin, g/dL	14.0	14.3	14.1	14.6	0.27	0.55	0.01	0.21
Packed cell volume, L/L	0.47	0.47	0.47	0.48	0.008	0.75	0.01	0.15
Mean corpuscular volume, fl	59.9	62.1	60.8	61.3	1.01	0.54	0.41	0.68
Mean corpuscular haemoglobin								
Pg	17.8	18.8	18.3	18.6	0.27	0.13	0.61	0.38
g/dL	29.7	30.3	30.2	30.3	0.20	0.20	0.43	0.42
Platelets, x10^9 cells/L	197.2^{A}	266.6^{B}	249.8^{A,B}	255.6^{A,B}	17.80	0.09	0.48	0.17

[1]Least square means and pooled standard errors of the mean
[2]Benzoic acid was VevoVitall ® (DSM Nutritional Products, Basel, Switzerland)
^{A,B,C} Within each row, values that do not share a common superscript tend to be different (0.05<P<0.10)

6.9 Figure

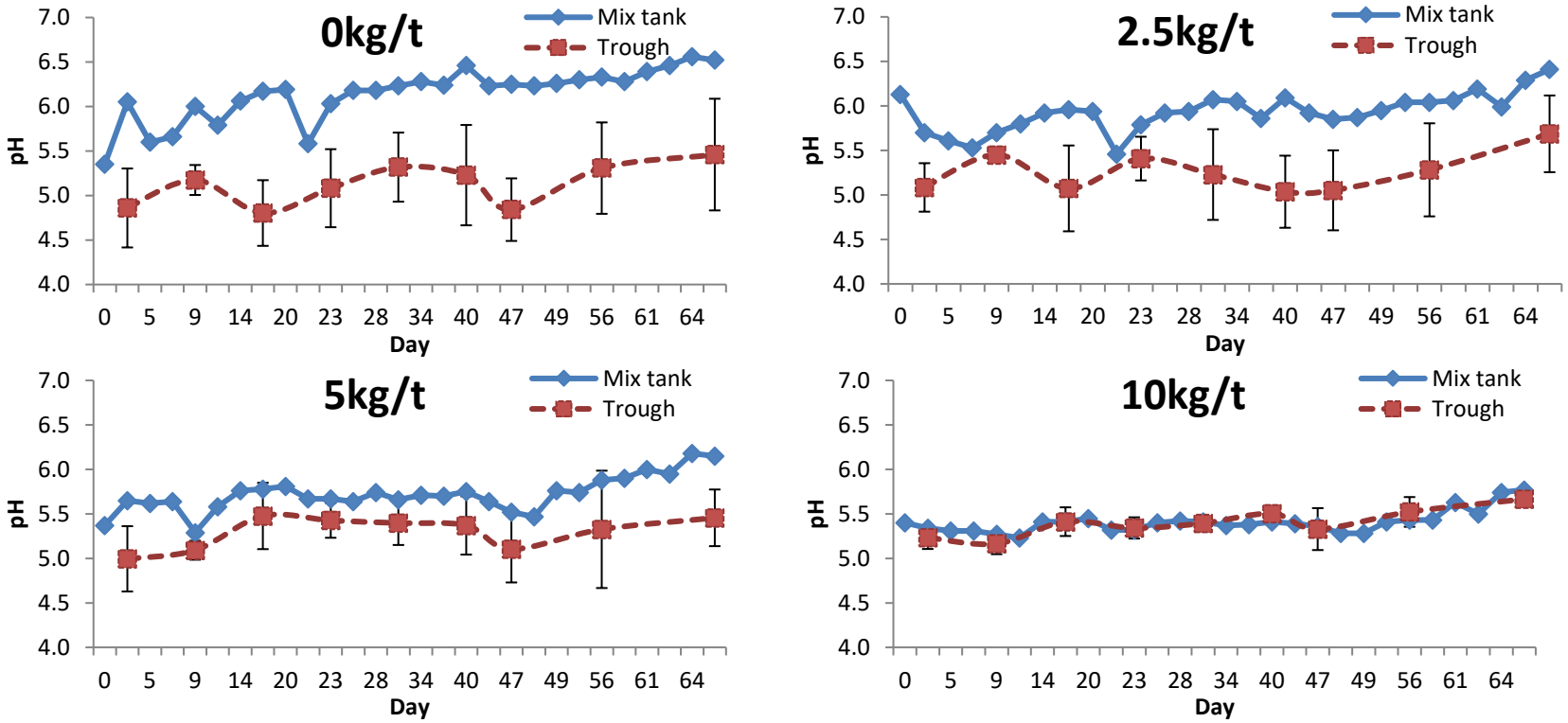

Figure 6.1 pH of liquid feed for grow-finisher pigs containing four dietary inclusion rates of benzoic acid (VevoVitall ®; 0 kg/t, 2.5 kg/t, 5 kg/t and 10 kg/t) sampled from both the mixing tank and troughs

7. Overall conclusions regarding liquid feed for grow-finisher pigs

- Spontaneous fermentation occurred in fresh liquid feed residing in troughs on eight commercial pig production units. This was evidenced by decreased liquid feed pH, increased lactic acid bacteria and yeast counts, increased lactic acid, ethanol and acetate concentrations and decreased amino acid and gross energy concentrations in residual feed collected from liquid feed troughs.

- Whole diet fermentation resulted in poorer pig growth and feed efficiency than fermenting the cereal fraction of the diet, fresh liquid and wet/dry feeding of the same diet, likely due to amino acid degradation and reduced gross energy concentrations.

- Fermenting the cereal fraction of the diet and adding a balancer containing soybean meal, soya oil, synthetic amino acids, phytase, minerals and vitamins just prior to feed-out negated this, resulting in growth rates and feed efficiencies similar to that of pigs fed fresh liquid feed.

- Advantages of pelleting over meal feeding in terms of feed efficiency were observed when the same diet was fed from dry and wet/dry feeders but when the diet was fed in liquid form, no advantage of pelleting was observed.

- Liquid feeding of a meal or pelleted diet maximised growth rate; however, liquid feeding of pelleted diets is not performed commercially. Liquid feeding of a meal diet and wet/dry feeding of a pelleted diet resulted in similar growth rates. Dry and wet/dry feeding of a pelleted diet optimised FCE. Therefore, to optimise growth and feed efficiency, wet/dry feeding of a pelleted diet is optimal.

- Liquid feed prepared at a water-to-feed ratio of 3.5:1 on a dry matter basis (3.0:1 on a fresh matter basis) resulted in the best feed efficiency without negatively affecting kill-out percentage. Increased growth rates were achieved when liquid feed was prepared at a lower water-to-feed ratio of 2.4:1 on a dry matter basis (2.0:1 on a fresh matter basis).

- Benzoic acid stabilised liquid feed pH and the growth of lactic acid bacteria and minimised ethanol production in liquid feed. However, it did not improve growth or feed efficiency of pigs.

8. Overall discussion

Reports from the literature are inconsistent regarding how best grow-finisher pigs should be fed in order to optimise growth, feed conversion efficiency (**FCE**) and carcass quality. Liquid feeding is very popular in countries like Ireland and the Netherlands, where liquid dietary co-products (e.g. whey and skim milk) from the food and drinks industry were historically available as cheap energy and nutrient sources for inclusion in pig diets. Such co-products are no longer as available, and where available, their nutritional value is considerably lower than before due to more advanced processing methods. In many cases, liquid feeding now simply involves mixing water with cereal-based diets. A review of the available literature indicates that liquid feeding can increase growth rate compared to dry feeding in grow-finisher pigs (Hurst et al., 2008; Stotfold Research Centre, 2005); however, poorer feed efficiency is associated with liquid feeding compared to dry feeding, likely due to feed wastage (Han et al., 2006; l'Anson et al., 2012; Missotten et al., 2010; Plumed-Ferrer and Von Wright, 2009; Russell et al., 1996). Spontaneous fermentation of liquid feed has been described as unpredictable, therefore, controlled fermentation using specially selected lactic acid bacteria (**LAB**) has been recommended (Brooks, 2009).

This thesis aimed to improve the microbiological quality of liquid feed and optimise the growth of liquid-fed grow-finisher pigs. The objectives of this work were to:

1. Characterise the microbiological quality of liquid feed for grow-finisher pigs on commercial pig production units
2. Control spontaneous fermentation during liquid feeding through controlled fermentation and dietary acidification

3. Compare the impact of feed form and delivery on grow-finisher pig growth and **FCE**)
4. Compare water-to-feed ratios to optimise growth and FCE of liquid-fed grow-finisher pigs.

Spontaneous fermentation is unreliable and if the fermentation is dominated by yeasts, off-flavours can result from acetic acid, ethanol and amylic alcohol production (Brooks et al., 2001; Plumed-Ferrer and Von Wright, 2009; Scholten et al., 1999). Moreover, energy loss can result from the conversion of starch to alcohol and CO_2 (Brooks et al., 2001). Amino acid degradation has been documented in deliberately fermented liquid feed (Canibe and Jensen, 2003; Canibe et al., 2007; Pedersen, 2001); however, evidence of this in spontaneously fermenting liquid feed on commercial units was lacking. To our knowledge, there are no studies available that have looked at the microbiological and chemical composition of liquid feed on commercial pig production units.

Therefore, the aim of chapter 2 was to characterise the microbial quality of liquid feed on Irish commercial units and investigate factors that influence this. The results clearly showed evidence of spontaneous fermentation occurring in liquid feed, as evidenced by increased LAB and yeast counts, increased ethanol, lactic acid and acetate production and decreased liquid feed pH in residual feed, collected from troughs. This residual liquid feed had not reached the 'steady' phase of fermentation as described by Canibe and Jensen (2003), as no reduction in *Enterobacteriaceae* counts was observed. Evidence of amino acid degradation and gross energy loss in liquid feed residing in the trough was also very clear with a 35.5 % loss of lysine and 6.2 % reduction in gross energy observed from the mix tank to residual feed in the

trough. It is therefore plausible to suggest that producers are unintentionally feeding a lower quality diet than what they have formulated for liquid-fed grow-finisher pigs.

As the results from chapter 2 show that spontaneous fermentation is unreliable and causes a deterioration in liquid feed quality, a comparison of controlled fermentations (whole diet and cereal only) was conducted in chapter 3. Whole diet and cereal fermentations produced very different results, where pigs fed the fermented whole diet had poorer growth and feed efficiency than those fed the same diet where only the cereal component was fermented. This supports the argument that amino acid degradation is responsible for the poorer performance of pigs fed a fermented whole diet. When synthetic amino acids and soybean meal were added to the fermented cereal just prior to feeding, pig performance was similar to that of fresh liquid- and wet/dry-fed pigs. A 22.3 % reduction in lysine concentration was observed between the fresh liquid mixing tank and the fermented whole diet in the trough. This is in between the 17 % loss of synthetic lysine reported by Shurson (2009) after 24 hours storage of fermented liquid feed and the 25 to 28 % degradation reported during fermentation by Pedersen (2001); however, it is less than the 35.5 % decrease found on commercial units in chapter 2. The results of chapter 3 proved that the amino acid degradation confirmed in liquid feed on commercial units in chapter 2 results in poorer pig growth and feed efficiency. Therefore, whether the whole diet has been deliberately or spontaneously fermented, if amino acid degradation occurs, a negative impact on pig performance is likely. There was little evidence of amino acid degradation in the fresh liquid feed in chapters 3 and 4 and only a small loss in lysine was found in chapter 6 when the mix tank and trough concentrations are compared. This highlights the unpredictability of

spontaneous fermentation. Amino acid analysis is expensive and it was not possible to carry out analysis on a large number of samples from chapter 3, 4 or 6.

As wet/dry feeding of a meal diet resulted in a numerical improvement in FCE compared to fresh liquid feeding in chapter 3 and no advantage of fermentation was observed, the natural progression for this work was to compare feed form and delivery methods. Liquid feeding is associated with increased growth compared to dry feeding in grow-finisher (Hurst et al., 2008; Stotfold Research Centre, 2005) and weaner pigs (Han et al., 2006; Kim et al., 2001; l'Anson et al., 2012; Partridge et al., 1992; Russell et al., 1996). The results of chapter 4 confirmed the FCE advantage of wet/dry feeding compared to liquid feeding found in chapter 3. Liquid feed wastage is difficult to eliminate completely, but feeding management practices should be improved to minimise it. It is hypothesised that feed wastage was the main cause of poorer FCE in liquid-fed pigs in chapters 3 and 4. This was previously reported in a number of studies comparing liquid and dry-feeding of pigs (Han et al., 2006; l'Anson et al., 2012; Missotten et al., 2010; Russell et al., 1996). Wet/dry feeding of a pelleted diet resulted in a similar growth rate to fresh liquid feeding but a superior feed efficiency. Therefore, wet/dry feeding of a pelleted diet is recommended for grow-finisher pigs.

As the FCE of liquid-fed pigs has been shown in previous chapters (3 and 4) to be poorer than dry and wet/dry feeding, an attempt was made to improve the FCE of liquid-fed pigs by altering the water-to-feed ratio used to prepare liquid feed. There were inconsistent recommendations on the best water-to-feed ratio found in the literature, from 2.9:1 DM (English et al., 1988) and 2.3:1 DM (Pond and Maner, 1984) to 4.1:1 DM (Gill et al., 1987) and 3.4:1 DM (Hurst et al., 2008). A lot of the

more recent on-farm liquid feeding installations in Ireland have been short-trough *ad-libitum* systems, and research using this feeding method was lacking. From the range of water-to-feed ratios tested in chapter 5 (2.4:1 DM to 4.1:1 DM), our results show that liquid feed prepared at a water-to-feed ratio of 3.5:1 DM is optimum for grow-finisher pigs using short-trough *ad-libitum* liquid feeding as it optimises FCE without negatively impacting kill-out percentage. The hypothesis is that wastage of liquid feed prepared at lower water-to-feed ratios results in poorer FCE because a larger volume of nutrients are lost with every unit of liquid feed wasted. Increased growth rates were achieved when liquid feed was prepared at a lower water-to-feed ratio of 2.4:1 on a dry matter basis (2.0:1 on a fresh matter basis). Therefore, if management at this lower ratio could be improved to minimise feed wastage, it is likely that improved feed efficiencies could also be achieved at water-to-feed ratios lower than 3.5:1. The voluntary water intake of pigs from supplementary drinkers in this thesis, particularly chapters 3, 4 and 5 would have been a very interesting measurement, had it been possible. Further to recording water intake, slurry production by pigs fed at different water-to-feed ratios would have been an interesting measurement.

Another method of controlling spontaneous fermentation is diet acidification. Benzoic acid was previously found to inhibit free amino acid degradation and yeast growth, and reduce lactic acid production during fermentation (Vils et al., 2018). In chapter 6, there was evidence to suggest that fermentation was somewhat controlled in liquid feed in response to BA inclusion. The initial pH of the liquid feed in the mix tank was reduced as dietary BA inclusion increased. The growth of LAB in troughs was controlled and pH stabilised in the trough compared to feed without BA supplementation. However, pigs fed the control diet with no BA supplementation

had excellent growth rate and FCE and consequently, no growth or FCE response was found in response to BA.

The best FCE achieved in pigs fed fresh liquid meal in this thesis was 2.23 g/g (chapter 5) and the worst was 2.41 g/g (chapter 5). Although, in a commercial setting these FCEs are excellent, the range highlights the possibility for improved feeding management to improve feed efficiency in liquid-fed grow-finisher pigs. Producers using long-trough liquid feeding should apply a restricted feeding approach, which was clearly not evident on commercial farms in chapter 2, as producers using long-trough systems allowed feed to remain in troughs between feed-outs. Producers using short-trough *ad-libitum* feeding systems can not restrict feed pigs to improve feed efficiency and feed trough design, system hygiene, and feeding management will all be important in improving feed efficiency.

Spontaneous fermentation is unpredictable as evidenced by the large variations in microbial counts between studies in this thesis. Despite using the same diet throughout chapters 3, 4, and 6, quite a lot of differences were observed in the microbial quality of the fresh liquid feed (i.e. fresh treatment in chapter 3, liquid meal and liquid pellet treatments in chapter 4 and 0 kg/t BA in chapter 6), particularly in residual feed that remained in the trough between feed-outs. LAB counts in liquid-fed troughs ranged from 7.39 to 9.61 $\log_{10}$ CFU/g, *Enterobacteriaceae* counts from 4.52 to 6.87 $\log_{10}$ CFU/g, *E.coli* counts from 2.43 to 5.39 $\log_{10}$ CFU/g, yeast counts from 4.45 to 6.61 $\log_{10}$ CFU/g and mould counts from 3.38 to 5.22 $\log_{10}$ CFU/g in chapters 3, 4 and 6. While the range of *E. coli* counts, and consequently *Enterobacteriaceae* counts, may be at least partially explained by faecal contamination of troughs, other counts such as LAB and yeast

resulted from spontaneous fermentation, which clearly has varying results. The amount of feed and length of dwell time in the trough may also have contributed to this variation. Despite using the same diet throughout this thesis, different batches of wheat, barley and soybean meal were used throughout the 22 month trial period, which likely had unique natural microbiota associated with them that contributed to variation in spontaneous fermentation. This highlights the unpredictable nature of uncontrolled/spontaneous fermentation.

It seems that to control spontaneous fermentation in fresh liquid feed, the mix should be prepared and fed-out to pigs quickly. Furthermore, if dwell time in the trough is minimised and a build-up of residual feed is avoided, liquid feed nutritional quality may not be as adversely affected as found in chapter 2. Benzoic acid may have more of an impact in pigs on a commercial unit with a lower disease status and lower growth rates than the research farm at which the work for chapters 3, 4, 5 and 6 was carried out, as improved gastrointestinal health has been reported in grow-finisher pigs fed a BA supplemented diet (Øverland et al., 2008). It is likely that each farm will have its own dominant microbiota in tanks and troughs, and will have their own feeding and management practices, sanitation protocols and dietary ingredients which influence the resultant fermentation. Dry and wet/dry feeding avoids the uncontrollable and unpredictable spontaneous fermentation that is evident with liquid feed and has less feed wastage, thereby resulting in improved FCE. Nonetheless, liquid feeding is likely to be economically beneficial for producers who can avail of dietary co-products as cheap nutrient sources.

Overall, the work in this thesis provides vital information to optimise liquid feeding for grow-finisher pigs. Amino acid degradation occurs in deliberate whole diet

fermentation and during spontaneous fermentation of fresh liquid feed in troughs. This was shown to result in poorer pig growth and feed efficiency with whole diet fermentation in chapter 3. For producers undertaking investment decisions, installation of wet/dry feeders and feeding a pelleted diet would be the best choice of feeding system to optimise growth and feed efficiency. For units with short-trough liquid feeding systems installed, this research suggests that liquid feeding at a water-to-feed ratio of 3.5:1 DM is optimum.

Future work would include:

(1) A comparison of more intense sanitisation protocols and a comparison of a range of co-products in an attempt to control spontaneous fermentation on a large number of commercial units.

(2) Strategies to minimise amino acid degradation in spontaneously fermenting fresh liquid feed in troughs, such increasing the frequency of feed splits per day. This would minimise the dwell time of residual liquid feed in the trough reducing the opportunity for fermentation to occur. This could also help to minimise wastage with *ad-libitum* liquid feeding.

(3) Comparing the impact of over-formulated fresh liquid diets in terms of amino acid and gross energy content (to compensate for the losses observed) with the diet fed throughout this thesis on grow-finisher pig growth and feed efficiency.

(4) A comparison of restricted and *ad-libitum* liquid feeding to improve feed efficiency of liquid-fed grow-finisher pigs, incorporating management strategies such as no intervention or daily adjustment of feeding curves.

(5) Benzoic acid supplementation to the diet of pigs on a commercial unit with poorer growth rates and feed efficiencies and a lower health status than the research unit used in chapter 6.

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Dedication

This thesis is dedicated to the loving memory of

- *Kate O' Meara, my wonderful Granny, I hope you're getting the same kick out of the submission of this thesis as you got when you heard I was going to start it.*

- *Jonathan Fitzpatrick, who's life sadly ended prematurely during this PhD - you loved to slag me about pigs, so it only seems right that I give you this one!*

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9. Appendix

Publications, conference attendance and presentations

Journal publications

- F.M. O' Meara, G.E. Gardiner, D. Clarke, W. Cummins, J.V. O' Doherty, P.G. Lawlor. 2020. **Microbiological assessment of liquid feed for finisher pigs on commercial pig units**. *Journal of Applied Microbiology* (in press) doi.org/10.1111/jam.14785
- F.M. O' Meara, G.E. Gardiner, J.V. O' Doherty, D. Clarke, W. Cummins, P.G. Lawlor. 2020. **Effect of wet/dry, fresh liquid, fermented whole diet liquid, and fermented cereal liquid feeding on feed microbial quality and growth in grow-finisher pigs**. *Journal of Animal Science*. 98 (6): skaa166 doi:10.1093/jas/skaa166
- F.M. O' Meara, G.E. Gardiner, J.V. O' Doherty, P.G. Lawlor, 2020. **The effect of feed form and delivery method on feed microbiology and growth performance in grow-finisher pigs**. *Journal of Animal Science*. 98 (3): skaa021. doi:10.1093/jas/skaa021
- F.M. O' Meara, G.E. Gardiner, J.V. O' Doherty, P.G. Lawlor. 2020. **Effect of water-to-feed ratio on feed disappearance, growth rate, feed efficiency and carcass traits in growing-finishing pigs.** *Translational Animal Science*. 4 (2): 1-11. doi:10.1093/tas/txaa042

- F.M. O' Meara, G.E. Gardiner, J.V. O' Doherty, P.G. Lawlor. 2020. **Effect of dietary inclusion of benzoic acid (VevoVitall®) on the microbial quality of liquid feed and the growth and carcass quality of grow-finisher pigs.** *Livestock Science*. 237: 104043. doi.org/10.1016/j.livsci.2020.104043

Conference oral presentations, abstracts and short papers

- O'Meara FM, Torres-Pitarch A, O'Doherty JV, Lawlor, PG (2017). **Comparing the effects of four liquid feeding practices on finisher pig growth.** In proceedings of the Teagasc Pig Research Dissemination Day, Co. Cork & Co. Cavan, 29th and 31st May, 2017 (page 19). *Supplementary abstract.*
- O'Meara FM, Gardiner GE, O'Doherty JV, Lawlor PG (2018). **The effect of four liquid feeding strategies on the growth, carcass quality and feed efficiency of grow-finisher pigs.** Presented at the British Society of Animal Science Annual Conference (BSAS), Dublin, 9th – 11th April 2018 (page 167). *Oral presentation.*
- O'Meara FM, Torres-Pitarch A, O'Doherty JV, Gardiner GE, Lawlor PG, (2018). **The effect of four liquid feeding strategies on the growth, carcass quality and feed efficiency of grow-finisher pigs.** In proceedings of the Teagasc Pig Research Dissemination Day, Co. Cork & Co. Cavan, 24th and 25th April, 2018 (page 17). *Supplementary abstract.*
- Lawlor PG, O'Meara FM, Torres-Pitarch A, Ryan T, Clarke D, O'Doherty JV, Gardiner GE (2018). **The effect of feed form and delivery on growth, feed efficiency and carcass quality of grow-finisher pigs.** Presented at the Teagasc Pig Research Dissemination Day, Co. Cork & Co. Cavan, 24th and 25th April, 2018 (page 14). *Oral presentation.*

- O'Meara FM, Gardiner GE, O'Doherty JV, Lawlor PG (2018). **Effects of feed form and delivery on growth, feed efficiency and carcass quality of grow-finisher pigs.** Presented at the 69th Annual Meeting of the European Federation of Animal Science (EAAP), Dubrovnik, Croatia, 27th – 31st August 2018 (page 95). *Oral presentation.*
- O'Meara FM, Gardiner GE, O'Doherty JV, Lawlor PG (2019). **Microbiological survey of liquid feed for finisher pigs on eight commercial pig farms.** Presented at the British Society of Animal Science Annual Conference (BSAS), Edinburgh, Scotland, 9 – 11th April 2019 (page 41). *Oral presentation.*
- O'Meara FM, Gardiner GE, O'Doherty JV, Lawlor PG (2019). **Strategies to improve the microbial quality of liquid feed and optimise growth of liquid-fed grow-finisher pigs.** Presented at Teagasc Pig Research Dissemination Day, Co. Cork & Co. Cavan, 30th April and 1st May 2019 (page 10). *Oral presentation.*
- O'Meara FM, Gardiner GE, O'Doherty JV, Lawlor PG (2019). **Strategies to improve the microbial quality of liquid feed and optimise growth of liquid-fed grow-finisher pigs.** Presented at the Teagasc Walsh Fellowship Annual Seminar, Grange, Co. Meath, 2nd October 2019. *Oral presentation.*

Popular Press

- O' Meara FM (2017). **Liquid feeding**. Teagasc Pig Development Department monthly newsletter, December issue.

- Lawlor PG and O'Meara FM (2018). **To pellet or not to pellet?** Irish Farmers Monthly, June 2018.

- Lawlor PG and O'Meara FM (2018). **Comparison of dry, wet/dry and wet feeding for finisher pigs.** Irish Farmers Monthly, June 2018.