

*Effect of liquid feeding at different water-to-feed ratios on the morphological adaptations in the gastrointestinal tract of growing pigs*

Article

Published Version

Hurst, D., Juniper, D., Clark, L., Litten-Brown, J., Corson, A. and Lean, I. (2020) Effect of liquid feeding at different water-to-feed ratios on the morphological adaptations in the gastrointestinal tract of growing pigs. *Journal of Food Nutrition and Agriculture*, 3. pp. 1-8. ISSN 2616-6550 doi: 10.21839/jfna.2020.v3.314 Available at <https://centaur.reading.ac.uk/91640/>

It is advisable to refer to the publisher's version if you intend to cite from the work. See [Guidance on citing](#).

Identification Number/DOI: 10.21839/jfna.2020.v3.314  
<<https://doi.org/10.21839/jfna.2020.v3.314>>

Publisher: Phoenix

All outputs in CentAUR are protected by Intellectual Property Rights law, including copyright law. Copyright and IPR is retained by the creators or other copyright holders. Terms and conditions for use of this material are defined in

the [End User Agreement](#).

[www.reading.ac.uk/centaur](http://www.reading.ac.uk/centaur)

## **CentAUR**

Central Archive at the University of Reading

Reading's research outputs online

## Research Article

# Effect of liquid feeding at different water-to-feed ratios on the morphological adaptations in the gastrointestinal tract of growing pigs

David Hurst<sup>1</sup>, Darren Thomas Juniper<sup>2\*</sup>, Lynne Clark<sup>3</sup>, Jennie Litten-Brown<sup>4</sup>, Anne Corson<sup>1</sup>, Ian Lean<sup>1</sup>

<sup>1</sup>Faculty of Life Sciences, Imperial College London (Wye Campus), United Kingdom, <sup>2</sup>Department of Animal Sciences, University of Reading, United Kingdom, <sup>3</sup>Faculty of Life Sciences, Imperial College London (Wye Campus), United Kingdom, <sup>4</sup>Animal Sciences, Canterbury College, Canterbury, United Kingdom

(Received: 15-01-2020; Accepted: 23-06-2020; Published: 03-07-2020)

\*Corresponding Author: Darren Thomas Juniper (d.t.juniper@reading.ac.uk)

## ABSTRACT

The study examined the morphology of the gastro-intestinal tract (GI) of growing pigs offered dry feed or liquid feed with differing water-to-feed ratios. Twenty male pigs were randomly allocated to one of four treatment groups; treatments included a standard dry pelleted diet (D) or the same diet soaked in water at a feed-to-water ratio of 1:1.5 (T1:1.5), 1:3 (T1:3) or 1:3 with the addition of lactic acid to adjust the feed to pH 4 (T1:3[4]). Animals were humanely slaughtered after 6 weeks to enable sampling and histological examination (light, scanning electron (LSEM) and binocular dissection microscope) of the GI tract. Samples were taken at the 2% position (duodenum), the 20% position and 50% position (jejunum) along the small intestines (SI). Liquid-fed pigs were heavier ( $P < 0.05$ ) and exhibited improved feed conversion ratios (FCR) when compared to D animals. These differences in live weight were attributed to alterations in the weight and volume of the stomach and SI, which were also heavier and larger in liquid-fed pigs ( $P < 0.05$ ). However, these differences were no longer apparent when intestinal weights and stomach volumes were adjusted for animal live weight. Differences in villus type between the treatments were noted throughout, particularly in the duodenum and proximal jejunum sections. Mean villus height was taller in all liquid-fed animals when compared to D pigs ( $P < 0.05$ ); this was particularly apparent in T1:1.5 and T1:3[4] pigs. There were no differences in crypt depths between treatment groups at the 2% and 20% positions, but at the 50% position the T1:1.5 group crypts were deeper ( $P < 0.05$ ) than those in the D and T1:3 pigs. It is concluded that liquid feeding alters the morphology of the GI tract, which may in part, explain the differences in growth performance observed between liquid and D fed pigs. The inclusion of organic acid in the diets of T1:3[4] pigs appeared to exert an effect on villus height and crypt depth when compared to those pigs receiving the T1:3 diet.

**Key words:** liquid feeding, gastrointestinal tract morphology, pigs

**Abbreviations:** ADG, average daily gain; DEFRA, Department for Environment, Food, and Rural Affairs; DM, dry matter; FCR, feed conversion ratio; GI, gastrointestinal; LSEM, light scanning electron microscope; SI, small intestine.

## INTRODUCTION

The majority of studies on pigs have demonstrated advantages in liquid feeding compared to dry feeding. Liquid feeding of pigs can reduce production costs, by allowing the use of low, dry matter co-products and may offer some degree of welfare benefit to animals under hot, summer conditions (Scott *et al.*, 2007). It can also result in improved animal performance, with regards to rates of growth, time to slaughter, and feed conversion ratio (FCR) (Jensen and Mikkelsen 1998; Lawlor *et al.*, 2002; Canibe and Jensen, 2003; Hurst *et al.*, 2008). These improvements in pig performance associated with wet feeding cannot be adequately explained by any differences in the physical or chemical composition of the feeds (Hurst *et al.*, 2008). The gastrointestinal tract and, in particular, the small intestinal mucosa, is at the interface between nutrient intake and its entry into the circulation, and is known to undergo a marked change in structure and function in response to a variety of

post-weaning stimuli (Diamond and Karasov 1983; Pluske *et al.*, 1997; Xui *et al.*, 2000).

Previous studies have observed changes in the small intestine (SI) of newly weaned pigs, including reductions in villus height, and increases in crypt depth (Hampson and Kidder 1986; Cera *et al.*, 1988; Deprez *et al.*, 1987). Changes to villus height and crypt depth have been correlated with activity of disaccharidase enzymes on the brush border (Hampson and Kidder, 1986; Pluske *et al.*, 1996a; Tsukahara *et al.*, 2016) and a reduction in the absorption capacity of the SI. Overall, these changes reduce the digestive and absorptive function of the gut, which is likely to be reflected in the growth and performance efficiency of the pig. The causes of villus atrophy and crypt hyperplasia have been predominantly linked to lower feed intake and reduced energy availability but the effect of the physical form of the diet has received little attention.

The effects of diet composition on the small intestinal epithelium of pigs has been reported previously (e.g. Deprez

*et al.*, 1987; Cera *et al.*, 1988; Jin *et al.*, 1994, Pluske *et al.*, 1996a; Yang *et al.*, 2001). Weight, volume and the capacity of the gastrointestinal tract have been shown to increase with higher levels of dietary fibre in otherwise similar diets (Hansen *et al.*, 1992), and also alter gastrointestinal morphology in pigs (Jin *et al.*, 1994). Despite much work on weaner pigs the availability of literature concerning changes in the small intestinal mucosa of pigs offered wet diets is much more limited. The aim of the current study was to determine if changes occur in the whole gastrointestinal tract, but more specifically the small intestinal mucosa, when growing pigs are fed diets of differing water-to-feed ratios. It is hypothesised that providing liquid diets of different feed to water ratios and with the inclusion of organic acid on iso-energetic rations fed at equal DM intakes will alter the structure of the small intestinal mucosa when compared to a dry pelleted diet.

## MATERIALS AND METHODS

### Animals and experimental design

All pigs used in the study were supplied by Cotswold Pig Development Company Ltd and maintained at their Pig Research and Development Unit at Wye, Kent, UK. At all stages of life animals were kept within the guidelines set out by the Department for Environment, Food, and Rural Affairs (DEFRA, 2003), and the study had been approved by the Local Ethical Committee. Pigs were housed in pens with a lying area measuring 2.54 x 2.75 m, with a dunging area of 2.75 x 1.8 m. Individual feeding crates were situated within the pens to enable feed intake to be restricted; pigs had free access to each of these feeding crates. All pens contained two self-drinkers and fresh water was provided *ad libitum*. The test house was mechanically ventilated and an ambient temperature of between 15 and 20°C maintained by adjusting the ventilation rate.

The study was a completely randomised design involving twenty male Meishan x Large White pigs, weighing  $47.2 \pm 1.61$  kg at the start of the six-week study period. Pigs were randomly assigned to one of four dietary treatments. The four treatments were: Control diet comprising air-dry commercial dry pellets (BOCM Paul Ltd, UK; 19 % Crude Protein, 1.15 % lysine and 14 MJ/kg digestible energy) (D); The same air-dry pellets soaked in water at a feed-to-water ratio of 1:1.5 (weight-for-weight) (T1:1.5); Dry pellets soaked in water at a feed to water ratio of 1:3 (T1:3); and dry pellets soaked in water at a ratio of 1:3 with the addition of lactic acid immediately prior to feeding (Lactic Acid Feed 80, Purac biochem) to adjust the feed to a pH of 4 (T1:3-4), as previously described by Hurst *et al.* (2008). The acid was substituted for an equal volume of water to maintain the same liquid to dry matter ratio of T1:3. The amount of lactic acid required to achieve the correct pH varied to some extent but was approximately 6ml of lactic acid per litre of wet feed. The feed to water ratios used in T1:1.5 and T1:3 have been used in previous studies (Barber *et al.*, 1963; Holme and Robinson, 1965; Gill *et al.*, 1987), and the pH used in T1:3(4) represents a pH value similar to that of

fermented feed (Brooks, 1999). The pH of the water added to the feed and supplied to pigs for drinking did not vary from 7.25 during the entire trial. The pH of each meal was tested and recorded immediately prior to feeding.

Rations were prepared fresh daily and pigs were individually fed three times daily (07.00, 12.00 and 17.00 hours); fresh potable water was available *ad libitum* at all times except during meals. Animals voluntarily entered the individual feeding crates at the specific feeding times and were allowed sufficient time to finish their allocated ration, following which they were released and any feed refusals collected and weighed. Feeding three times daily was adopted in order to ensure a high daily feed intake with minimum refusals of feed. The amount of dry pelleted feed offered to pigs was based on *ad libitum* intake rates previously recorded by this research group for a similar genotype and size of dry fed pigs; the total amount of feed offered per day was approximately 5-10% below these previously recorded *ad libitum* levels. Initially pigs were given seven days to adapt to their respective trial diets and the amount of food offered was adjusted to a level where all animals readily consumed their entire ration. The amount of feed offered was reviewed weekly and increased from 1.45 kg to 2.65 kg of air-dry feed per day from week 1 of the study to slaughter (week 6). Pigs were weighed at weekly intervals throughout the six-week experimental period to enable average daily gain (ADG) to be determined. Feed intakes were recorded to allow FCR to be calculated.

### Tissue sampling

Pigs were offered a final feed on the evening before they were slaughtered by means of electrical stunning and exsanguination. The abdomen was opened along the midline from sternum to pubis and the entire gastrointestinal tract removed. The gastrointestinal tract was carefully dissected out by dissecting away the visceral peritoneum and the associated mesenteric fat and tissues and subsequently divided into its component sections. Stomachs were incised along their greater curvature, and after any food residue removed, laid out flat and the radius measured. They were washed, carefully dried and weighed. The stomach volume was calculated assuming it approximated the shape of a sphere and using the radius dimension in the formula:  $\frac{4}{3}\pi r^3$ . The SI, caecum and colon were unravelled and the length of each was measured. Each component was carefully emptied of its contents, washed and dried before being weighed.

The SI was dissected from the mesentery, unravelled, measured and two ring-shaped intestinal segments, approximately 1cm wide, were carefully removed at distances of proportionately 2% (duodenum), 20 % (proximal jejunum), and 50 % (mid jejunum) along the intestine from the gastric pylorus. The excised segments were cut longitudinally, spread out with the serosal side facing down and pinned to dental wax. The tissue was immediately fixed in 2.5% glutaraldehyde in 0.1M phosphate buffer (pH 7.2) for subsequent treatment and examination using a light scanning electron (LSEM) and light microscopy.

## Histology

After fixation for several days, the SI samples were longitudinally divided for light and scanning electron microscopy. The samples for light microscopy were dehydrated through an ethanol series and then embedded in paraffin wax. From each of these, four transverse sections (6µm) were cut and stained with haemotoxylin and eosin and examined under a light microscope. Measurements of villus height and crypt depth were made where the plane of section ran vertically from the tip of the villus to the base of an adjacent crypt. From each section, a calibrated eyepiece graticule was used to measure 8-10 of the best-orientated villi and associated crypts.

Three further sections were also prepared and stained with PAS Schiff stain to highlight the presence of mucin and enable an estimation of the number of goblet cells present on the villi. The number of goblet cells observed within the first 150 µm from the apical tip of the villi were recorded. The remaining fixed intestinal material was trimmed and areas of the tissue excised and placed in 0.1m phosphate buffer (pH 7.4) and dehydrated through an acetone series, critical point dried, mounted on aluminum stubs and gold coated prior to examination under a LSEM (Hitachi S430). The samples for LSEM were also examined under a binocular dissection microscope and the villus pattern graded according to a scale based on Mouwen (1971; Table 1). One LSEM sample was prepared for each SI position in each animal. The predominant villus form was scored on each by binocular dissection microscopy and the results re-evaluated with the LSEM to ensure consistency.

## Statistical analysis

The results were analysed statistically using one-way analysis of variance in Minitab release 13 in a complete randomised design for treatment effects with missing values fitted for one of the samples due to sectioning problems. Significance between treatments was determined by Fisher's least significant difference procedure. One-way analysis of variance using Genstat 5.4 was used to analyse the gross organ data both before and after adjustment for differences in animal live weights. Pearson correlation coefficients were calculated for the relationships between villus height and FCR.

## RESULTS

### Intake and growth performance

The addition of water, or the addition of an organic acid to wet feed, had no appreciable effect on rates of feed intake (Table 2). Despite this lack of difference between dietary treatments in feed intake behaviour, pigs offered liquid feed had better ADG than D pigs ( $P < 0.05$ ). However, FCR in pigs receiving T1:1.5 and T1:3 diets were not different to those offered D or T1:3[4], although pigs receiving the T1:3[4] diet had better FCR than D pigs ( $P < 0.05$ ).

### Bodyweight and organ weight

On the day of slaughter, pigs offered wet diets were heavier ( $P < 0.05$ ) than D animals but carcass weights post-mortem were similar between treatment groups (Table 2). Consequently, despite ADG being improved in liquid fed animals ( $P < 0.05$ ), their killing out percentage were lower when compared to D pigs ( $P < 0.05$ ). These differences in live weight were attributed to alterations in the weight and volume of the stomach, which were heavier and larger in liquid-fed pigs ( $P < 0.05$ ), and the SI, which were also heavier and longer in these groups ( $P < 0.05$ ). However, these differences were no longer apparent when the values were adjusted for live weight (data not shown). Caecum length, colon length and weight, and total intestinal weight and length, were similar between groups (Table 2).

### Villus form and distribution

Differences in villus type between treatments, based on the scoring system of Mouwen (1971), were noted throughout the small intestine sections with differences being greatest at the duodenum and proximal jejunum (Figure 1). In all segments of the D group there were a mixture of short tongue, leaf, ridge and some convoluted villi, whereas tongue villi became more apparent in the duodenum and proximal jejunum in liquid fed animals, especially as the water to feed ratio increased ( $P < 0.05$ ). The addition of an organic acid in the T1:3[4] group appeared to result in mainly long tongue villi being observed when compared to other treatments ( $P < 0.05$ ). Differences in mucosal form became less distinct between treatment groups in the mid jejunum, but the T1:3[4] group mucosa had fewer complex villi forms, particularly when compared with the D and T1:1.5 groups ( $P < 0.05$ ).

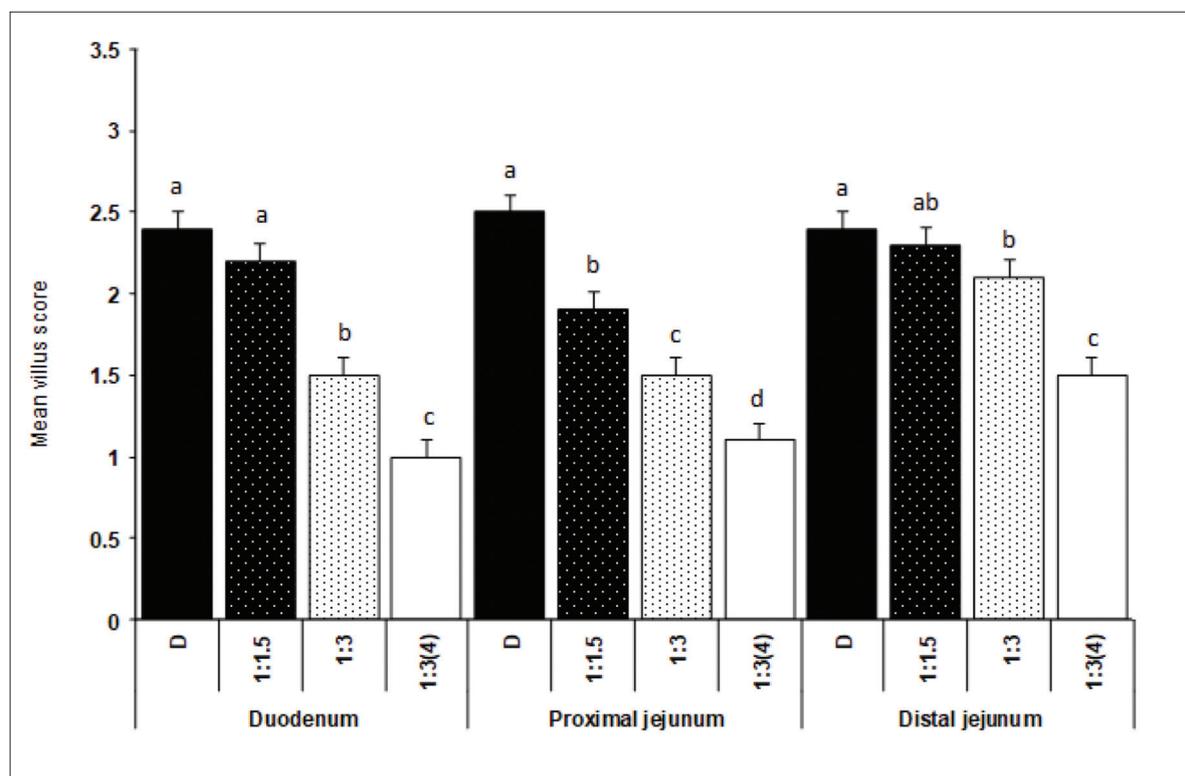
**Table 1:** Criteria for grading of small intestine mucosa (Taken from Mouwen, 1971)

Grade	Criteria
0	All finger-shaped villi or mostly finger-shaped villi with few tongue-shaped villi
0.5	Mixed finger-shaped and tongue-shaped villi
1	Predominantly long to short tongue-shaped villi with few finger-shaped and leaf-shaped villi
1.5	Predominantly short tongue-shaped and leaf-shaped villi with few long tongue-shaped and ridge-shaped villi
2	Mixtures of short tongue-shaped, leaf-shaped, ridge-shaped, and convoluted villi
2.5	Similar to Grade 2 with flat areas
3	Flat mucosa

**Table 2:** The effects of feeding either a standard dry pelleted diet (D) or varying the feed-to-water ratio and pH of the diet over a period of six weeks of restricted feeding (5 % to 10 % below ad libitum intake) on the size and weight of the gastrointestinal organs in slaughter weight pigs

	Feed:Water ratio (pH)				SED
	D (n = 4)	1:1.5 (n = 4)	1:3 (n = 4)	1:3(pH4) (n = 4)	
Live weight (kg)	75.2 <sup>a</sup>	82.0 <sup>b</sup>	84.5 <sup>b</sup>	81.2 <sup>ab</sup>	4.09
Average daily gain (g/d)	850 <sup>a</sup>	970 <sup>b</sup>	1000 <sup>b</sup>	960 <sup>b</sup>	33
Average daily feed intake (g DM/d)	1836	2051	2170	1872	135
FCR (g/g)	2.16 <sup>a</sup>	2.10 <sup>ab</sup>	2.27 <sup>a</sup>	1.95 <sup>b</sup>	0.067
Carcass weight (kg)	57.5	59.3	60.3	58.5	3.31
Killing out %	76.5 <sup>a</sup>	72.3 <sup>b</sup>	71 <sup>b</sup>	72 <sup>b</sup>	1.35
Stomach wt (kg)	0.53 <sup>a</sup>	0.59 <sup>ab</sup>	0.65 <sup>b</sup>	0.59 <sup>ab</sup>	0.073
Stomach Volume (l)	1.2 <sup>a</sup>	1.91 <sup>ab</sup>	2.39 <sup>b</sup>	1.96 <sup>ab</sup>	0.681
Small Intestine length (m)	17.3 <sup>a</sup>	19.3 <sup>b</sup>	19.3 <sup>b</sup>	19.2 <sup>b</sup>	0.108
Small Intestine weight (kg)	1.87 <sup>a</sup>	2.07	2.11	2.21 <sup>b</sup>	0.184
Caecum length (m)	0.279	0.28	0.30	0.28	0.038
Colon length (m)	5.42	5.35	4.97	5.70	0.929
Colon weight (kg)	1.09	1.14	1.13	1.18	0.131
Total Intestinal length (m)	23.0	24.9	24.5	25.2	1.139
Total Intestinal weight (kg)	3.68	3.99	4.07	4.16	0.243

Different superscripts within row differ significantly ( $P < 0.05$ ).



**Figure 1:** The effects of offering a standard dry pellet diet (D) or varying the water-to-feed ratio (1:1.5 or 1:3) and pH (1:3(4)) of the diet over a period of six weeks of restricted feeding (5 % to 10 % below ad libitum intake) on the predominant villus types (Mouwen, 1971) observed in the small intestine mucosa of pigs (Different letters within intestinal section differ significantly  $P < 0.05$ ).

LSEM examination revealed a consistent difference in villus surface characteristics between treatments; D animals exhibited surfaces as normally described with individual enterocytes displayed convex surfaces bulging into the luminal space; this was not confined to the villus tips but

epithelial surface down the villus sides (Figure 2, a & c). This differed markedly from liquid fed pigs where individual enterocytes displayed convex surfaces bulging into the luminal space; this was not confined to the villus tips but

extended down the sides to the villus/crypt junction (Figure 2, b & d). This effect was noted in the majority of villi in T1:3 animals, and to a lesser extent in T1:1.5 animals. Abrasion damage to the villi tips was frequently observed in D animals (Figure 2, a) whilst the incidence of similar damage in wet fed animals was considerably less (Figure 2, b). No attempt was made to quantify the comparative levels of villus tip damage or erosion in this study. The microvilli of the villi in liquid-fed pigs were observed to be dense and largely intact across all areas of the villus surface and although the microvilli of D pigs were generally similar to the liquid-fed pigs, in areas close to enterocyte erosion microvilli were seen to be less dense (Figure 3).

### Villus height and crypt depths

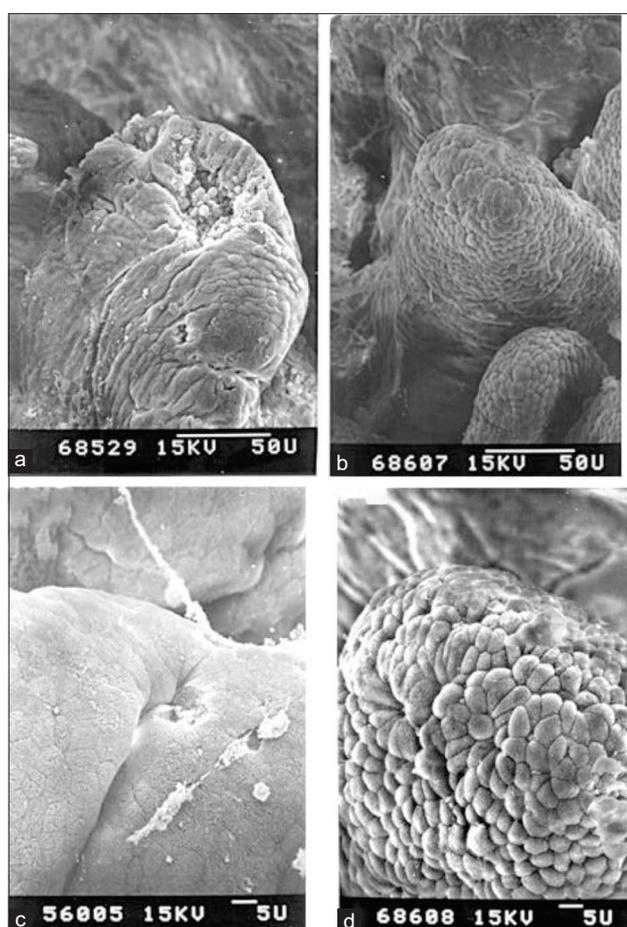
Diet form had an overall effect on villus height (Table 3); mean villus heights were between 68 and 95% taller in all

liquid-fed animals when compared to D pigs ( $P < 0.05$ ). When the three sections were considered individually, although liquid-fed pigs had taller villi differences between liquid-fed and D animals were not always statistically significant (Table 3). In the duodenum T1:3 and T1:1.5 groups were taller than D pigs ( $P < 0.05$ ) whilst in the proximal jejunum the T1:3[4] and T1:1.5 groups differed from the D group ( $P < 0.05$ ). In the mid jejunum villi height only differed between the T1:1.5 group and D animals ( $P < 0.05$ ). There were no differences in crypt depths between treatment groups in the duodenum and proximal jejunum, but in the mid jejunum the T1:1.5 group crypts were deeper ( $P < 0.05$ ) than those in the T1:3 and D group pigs. Overall crypt depths were deeper in T1:1.5 and T1:3(4) group pigs than those of D and T1:3 groups.

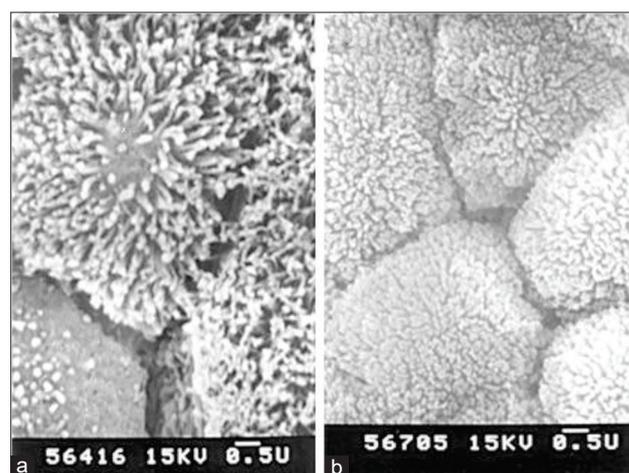
A Pearson correlation coefficient of  $-0.545$  ( $P = 0.036$ ) indicated that as villus height increased there were commensurate improvements to FCR; the poorest FCR was recorded for D fed animals, which corresponded with the shortest villi, whilst wet fed animals had greater mean villus heights and better FCR.

### DISCUSSION

The findings of the present study demonstrate that the growth performance is improved with liquid feeding, reflecting the work of others (Jensen and Mikkelsen 1998; Lawlor *et al.*, 2002; Canibe and Jensen, 2003; Hurst *et al.*, 2008). As carcass weights were similar between treatment groups, differences in live weight can be partly attributed to the weight and volume of the stomach and SI, which were also heavier and larger in liquid-fed pigs. However, when the weight of the whole gastrointestinal tract and individual organs are adjusted for live weight differences between treatment groups cease to be statistically different. The fact that the liquid-fed animals in the present study did not exhibit larger, heavier guts in relation to body weight may be partly explained by an increase in the rate of passage (Castle and Castle, 1957) and gastric emptying



**Figure 2:** Typical villus of: (a) a dry fed pig displaying a surface with slight delineation of the enterocyte borders and typical erosion of the enterocytes at the tip of the villus (x500); (b) liquid fed pig showing prominent enterocyte outlines with surfaces bulging into the small intestine lumen; this effect can be observed extending from the villus apex towards the villus base (x 500); (c) dry fed pig exhibiting the hexagonal outline of the enterocytes with folding of the villus (x1200); and (d) liquid fed pig showing the bulging enterocyte surface(x1200).



**Figure 3:** The microvilli of (a) dry fed pigs and (b) liquid fed pigs (x12000).

**Table 3:** The effects of offering a standard dry pelleted diet (D) or varying the water-to-feed ratio and pH of the diet over a period of six weeks of restricted feeding (5 % to 10 % below ad libitum intake) on mean villus height, crypt depth and goblet cell numbers at three positions in the small intestine of pigs

	Feed:Water ratio (pH)			
	D (n=4)	1:1.5 (n=4)	1:3 (n=4)	1:3(pH4) (n=4)
Villus height (µm)				
Duodenum	219 ± 20.4 <sup>a</sup>	410 ± 29.5 <sup>b</sup>	347 ± 49.7 <sup>b</sup>	316 ± 26.5 <sup>ab</sup>
Proximal jejunum	199 ± 57.8 <sup>a</sup>	333 ± 57.5 <sup>b</sup>	261 ± 27.4 <sup>ab</sup>	382 ± 30.0 <sup>b</sup>
Mid jejunum	143 ± 84.5 <sup>a</sup>	396 ± 88.6 <sup>b</sup>	341 ± 65.1 <sup>ab</sup>	366 ± 47.1 <sup>ab</sup>
Overall	188 ± 27.6 <sup>a</sup>	366 ± 60.5 <sup>b</sup>	317 ± 12.8 <sup>b</sup>	354 ± 23.5 <sup>b</sup>
Crypt depth (µm)				
Duodenum	172 ± 19.5	247 ± 24.4	209 ± 40.2	211 ± 21.5
Proximal jejunum	151 ± 67.3	212 ± 48.6	145 ± 12.7	236 ± 14.1
Mid jejunum	182 ± 93.0 <sup>a</sup>	350 ± 24.0 <sup>b</sup>	201 ± 4.1 <sup>a</sup>	275 ± 54.3 <sup>ab</sup>
Overall	175 ± 42.7 <sup>a</sup>	269 ± 23.2 <sup>b</sup>	185.2 ± 11.2 <sup>a</sup>	241 ± 16.5 <sup>b</sup>
Goblet cells				
Duodenum	7.4 ± 0.66 <sup>a</sup>	3.5±0.72 <sup>b</sup>	5.3±0.53 <sup>b</sup>	4.5±0.32 <sup>b</sup>

Values are presented as means ± sem. Within row means with different superscripts are significantly different,  $P < 0.05$ .

(Rayner and Millar, 1990). This may indicate that rate of passage is adjusted to maintain equal levels of gutfill or intragastric pressure between the diet forms. Although D animals in the present study had *ad libitum* access to water and their water intakes were not recorded, Barber *et al.* (1991) previously reported that dry fed animals drink water before and after a meal up to an intake comparable with wet fed animals, which may also provide the same overall level of gut fill.

During examination the stomachs sampled from D pigs exhibited signs of inflammation and mild ulceration. This combined with the higher number of goblet cells recorded in the intestinal mucosa, the larger amounts of mucus evident in the SEM samples, and the abrasion damage to villi tips is suggestive of a pathological irritant effect of the D diet. Furthermore, these observations are similar to those of Wondra *et al.* (1995) and Regina *et al.* (1999), who reported increased incidence of ulceration in the stomach of pigs fed pelleted diets. DeJong *et al.* (2016) and Mösseler *et al.* (2012) attributed this increased incidence of ulceration in pelleted diets to finer dietary particle size, resulting in a more liquid digesta and homogenous pH. However, Ayles *et al.* (1999) reported that particle size did not influence the incidence of gastric ulcers, but severity was modulated by gastric tissue melatonin concentrations. Mösseler *et al.* (2012) also reported that the addition of water to pelleted diets did not reduce the incidence of ulceration, concluding that diet structure was more important than water content. In contrast, the findings of the current study would suggest that the addition of water to pelleted diets may have protective effects with regards to the formation of gastric ulcers, but the reason for this remains unclear.

A natural transformation in the stereomorphological features of villi occurs with age (Mouwen, 1971) but other factors are responsible for changes in villus form, including diet viscosity, fibre, energy intake, hormones and disease. A

great deal of work has been carried out on the causes of villus shape and height changes in weaner pigs (e.g. Mouwen, 1971; Cera *et al.*, 1988; Pluske *et al.*, 1996bc; van Beers-Schreurs, 1998) but similar work in growing pigs is more limited, especially studies relating to the feeding of liquid diets. Deprez *et al.* (1987) reported villus height was decreased in growing pigs offered dry feed, suggesting that one potential difference could be due to levels of feed intake, a known cause of villus atrophy especially in young weaner pigs (Pluske *et al.*, 1996bc). However, Yang *et al.* (2001) also reported a trend towards taller villi in growing pigs fed wet diets but with identical intakes, mirroring the findings of the current study. It has been suggested that villi may be damaged/compromised in the early weaning stage as a consequence of lack of intestinal stimulation, a result of post-weaning anorexia (Lalles *et al.*, 2007). Liquid feeding may help ameliorate this effect by promoting gastric emptying, resulting in increased flow or availability of nutrients to the intestine which may stimulate an increase in villi height, crypt depth, and subsequent absorptive surface area (Vrabcova *et al.*, 2016).

In the current study although villi height was greater in liquid fed pigs crypt depths did not differ between treatments in the duodenum or proximal jejunum, although mean overall crypt depth was greater in 1:1.5 and 1:3(4) groups. Yasar and Forbes (1999) also observed increased villus height when feeding wet diets to poultry, and that crypt depths also remained unchanged between wet and dry diets. However, they also found that crypt cell proliferation rates were substantially reduced in wet diet animals and suggested that this could give considerable energy and nitrogen savings in gut maintenance. However, this does not explain why villus height was comparatively longer in wet fed animals. As mentioned previously in the current study abrasion damage to villi tips was frequently observed in D animals, whilst the incidence of similar damage in wet fed animals was considerably less. If enterocyte erosion from the

villus apex on dry diets is high, it is probable that villi height and absorptive function may be compromised as migrating enterocytes might not have differentiated and matured sufficiently to express full absorptive capacity.

A number of studies have reported on the beneficial effects that organic acids have on weaning pigs, these include lowering of gastric pH resulting in improvements to proteolysis (Mayer, 1994; Partanan and Mroz, 1999; Suiryranrayna and Ramana, 2015), providing a rapidly utilised energy source for intestinal and extra-intestinal tissues (Ferrara *et al.*, 2017), as well as providing antibacterial activity (Partanen and Mroz, 1999) and modulation of gut specific immune responses (Lee *et al.*, 2007).

In the current study the addition of an organic acid to a wet diet (1:3(4)) improved feed conversion ratio when compared to a non-acidified wet diet (1:3). This improvement has been noted in a number of trials and attributed to enhanced proteolysis in the stomach due to a reduced pH (Mayer, 1994; Partanen and Mroz, 1999). In contrast, Lee *et al.* (2007) reported no improvement in growth performance or feed efficiency of weaned pigs offered diets supplemented with organic acids. Furthermore, this lack of effect was also reflected in intestinal morphology as the addition of an organic acid did not affect villi height or crypt depth in the study of Lee *et al.* (2007).

## CONCLUSIONS

In conclusion, changes in villus form, surface morphology and villus height point to a substantial increase in the surface area of the SI for digestion and absorption in liquid-fed animals, which is further modified by the inclusion of an organic acid to the diet, and may explain differences in production performance.

## ACKNOWLEDGEMENTS

The authors would like to thank the staff at the Wye College pig unit for their help and support in this study.

## REFERENCES

- Ayles, H.L., Friendship, R.M., Bubenik, G.A. and Ball, R.O. (1999). Effect of particle size and dietary melatonin supplementation on gastric ulcers in swine. *Canadian Journal of Animal Science*, 79(2), 179-185.
- Barber, R.S., Braude, R. and Mitchell, K.G. (1963). Further studies on the water requirements of the growing pig. *Animal Production*, 5, 277-282.
- Barber, J., Brooks, P.H. and Carpenter, J.L. (1991). The effect of four levels of food on the water intake and water to food ratio of growing pigs. *Animal Production*, 52, 602(abstract).
- Brooks, P.H. (1999). The Potential of liquid feeding systems. In: Concepts in Pig Science. Eds, Lyons, T.P and Cole, D.J.A. Chapter 7. Nottingham University Press.
- Canibe, N. and Jensen, B.B. (2003). Fermented and non-fermented liquid feed to growing pigs: Effect on aspects of gastrointestinal ecology and growth performance. *Journal of Animal Science*, 81, 2019-2031.
- Castle, E.J. and Castle, M.E. (1957). Further studies of the rate of passage of food through the alimentary tract of pigs. *Journal of Agricultural Science*, 49, 106-112.
- Cera, K.R., Mahan, D.C., Cross, R.F., Reinhart, G.A. and Whitmoyer, R.E. (1988). Effect of age, weaning and postweaning diet on small intestinal growth and jejunal morphology in young swine. *Journal of Animal Science*, 66, 574-584.
- DEFRA. 2003. Code of Recommendations for the Welfare of Livestock. Available online:
- DeJong, J.A., DeRouchey, J.M., Tokach, M.D., Dritz, S.S., Goodband, R.D., Woodworth, J.C. and Allerson, M.W. (2016). Evaluating pellet and meal feeding regimes on finishing pig performance, stomach morphology, and carcass characteristics. *Journal of Animal Science*, 94, 4781-4788.
- Deprez, P., Deroose, P., van den Hende, C., Muyelle, E. and Oyaert, W. (1987). Liquid versus dry feeding in weaned pigs. *Journal of Veterinary Medicine*, 34, 254-259.
- Diamond, J.M. and Karasov, W.H. (1983). Trophic control of the intestinal mucosa. *Nature*, 304, 18.
- Ferrara, F., Tedin, L., Pieper, R., Meyer, W. and Zentek, J. (2017). Influence of medium-chain fatty acids and short-chain organic acids on jejunal morphology and intra-epithelial immune cells in weaned piglets. *Journal of Animal Physiology and Animal Nutrition*, 101, 531-540.
- Gill, B.P., Brooks, P.H. and Carpenter, J.L. (1987). Voluntary water use by growing pigs offered liquid foods of differing water-to-meal ratios. In: Eds. A.T. Smith and T.L.J. Lawrence. Pig Housing and the Environment. British society of Animal production, Edinburgh, U.K. Occasional publication No. 11, 1-2.
- Hampson, D.J. and Kidder, D.E. (1986). Influence of creep feeding and weaning on brush border enzyme activities in the piglet small intestine. *Research in Veterinary Science*, 40, 24-31.
- Hansen, I., Bach Knudsen, K.E. and Eggum, B.O. (1992). Gastrointestinal implications in the rat of wheat bran, oat bran and pea fibre. *British Journal of Nutrition*, 68, 451-462.
- Holme, D.W. and Robinson, K.L. (1965). A study of water allowances for the bacon pig. *Animal Production*, 7, 377-384.
- Hurst, D., Clarke, L. and Lean, I.J. (2008). Effect of liquid feeding at different water-to-feed ratios on the growth performance of growing-finishing pigs. *Animal*, 2:9, 1297-1302.
- Jensen, B.B. and Mikkelsen, L.L. (1998). Feeding liquid diets to pigs. In: Recent Advances in Animal Nutrition (eds. Garnsworthy PC and Wiseman J), Chapter 7, p 107-123. Nottingham University Press.
- Jin, L., Reynolds, L.P., Redmer, D.A., Caton, J.S. and Crenshaw, J.D. (1994). Effects of dietary fiber on intestinal growth, cell proliferation, and morphology in growing pigs. *Journal of Animal Science*, 72, 2270-2278.
- Lalles, J-P., Bosi, P., Smidt, H. and Stoles, C.R. (2007). Nutritional management of gut health in piglets around weaning. *Proceedings of the Nutrition Society*, 66, 260-268.
- Lawlor, P.G., Lynch, P.B., Gardiner, G.E., Caffrey, P.J. and O'Doherty, J.V. (2002). Effect of liquid feeding weaned pigs on growth performance to harvest. *Journal of Animal Science*, 80, 1725-1735.
- Lee, D.N., Liu, S.R., Chen, Y.T., Wang, R.C., Lin, S.Y. and Weng, C.F. (2007). Effects of diets supplemented with organic acids and nucleotides on growth, immune responses and digestive tract development in weaned pigs. *Journal of Animal Physiology and Animal Nutrition*, 91, 508-518.
- Mayer, E.A. (1994). The physiology of gastric storage and emptying. In: Physiology of the Gastrointestinal Tract (eds. Johnson LR,

- Alpers DH, Christensen J, Jacobsen ED and Walsh JH), Third edition. Vol 1, p.929-976. Raven Press, New York.
- Mösseler, A., Wintermann, M., Sander, S.J. and Kamphues, J. (2012). Effect of diet grinding and pelleting fed either dry or liquid feed on dry matter and pH in the stomach of pigs and the development of gastric ulcers. *Journal of Animal Science*, 90, 343-345.
- Mouwen, J.M.V.M., (1971). White Scours in piglets. *Veterinary Pathology*, 8, 364-380.
- Partanen, K.H. and Mroz, Z. (1999). Organic acids for performance enhancement in pig diets. *Nutrition Research Reviews*, 12, 117-145.
- Pluske, J.R., Thompson, M.J., Atwood, C.S., Bird, P.H., Williams, I.H. and Hartman, P.E. (1996a). Maintenance of villous height and crypt depth, and enhancement of disaccharide digestion and monosaccharide absorption, in piglets fed on cow's whole milk after weaning. *British Journal of Nutrition*, 76, 409-422.
- Pluske, J.R., Williams, I.H. and Aherne, F.X. (1996b). Maintenance of villous height and crypt depth in piglets by providing continuous nutrition after weaning. *Animal Science*, 62, 131-144.
- Pluske, J.R., Williams, I.H. and Aherne, F.X. (1996c). Villous height and crypt depth in piglets in response to increase in the intake of cows' milk after weaning. *Animal Science*, 62, 145-158.
- Pluske, J.R., Hampson, D.J. and Williams, I.H. (1997). Factors influencing the structure and function of the small intestine in the weaned pig: a review. *Livestock Production Science*, 51, 215-236.
- Rayner, D.V. and Miller, S. (1990). Cholecystokinin effects on wet and dry meals in pigs. *Proceedings of the Nutrition Society*, 49, 222A.
- Regina, D.C., Eisemann, J.H., Lang, J.A. and Argenzio, R.A. (1999). Changes in gastric contents in pigs fed a finely ground and pelleted or coarsely ground meal diet. *Journal of Animal Science*, 77, 2721-2729.
- Scott, K., Chennells, D.J., Armstrong, D., Taylor, L., Gill, B.P., Edwards, S.A. (2007). The welfare of finishing pigs under different housing and feeding systems: liquid versus dry feeding in fully-slatted and straw-based housing. *Animal Welfare*, 16(1), 53-62.
- Suiryanrayna, V.A.N. and Ramana, J.V. (2015). A review of the effects of dietary organic acids fed to swine. *Journal of Animal Science and Biotechnology*, 6:45.
- Tsukahara, T., Inoue, R., Nakatani, M., Fukuta, K., Kishino, E., Ito, T. and Ushida, K. (2016). Influence of weaning age on the villous height and disaccharidase activities in the porcine small intestine. *Animal Science Journal*, 87, 67-75.
- van Beers-Schreurs, H.M.G., Nabuurs, M.J.A., Vallenga, L., Kalsbeek-van der Valk, H.J., Wensing, T. and Breukink, H.J. (1998). Weaning and the Weanling diet influence the villous height and crypt depth in the SI of pigs and alter the concentrations of short-chain fatty acids in the large intestine and blood. *Journal of Nutrition*, 128, 947-953.
- Vrabcova, M., Mikuska, L., Vazan, R., Miko, M., Varga, I. and Mravec, B. (2016). Effect of chronic intake of liquid nutrition on stomach and duodenum morphology. *Acta Histochemica*, 118, 435-442.
- Wondra, K.J., Hancock, J.D., Behnke, K.C. and Stark, C.R. (1995). Effects of mill type and particle size uniformity on growth performance, nutrient digestibility, and stomach morphology in finishing pigs. *Journal of Animal Science*, 73, 2564-2573.
- Xui, R.J., Wang, F. and Zhang, S.H. (2000). Postnatal adaptation of the gastrointestinal tract in neonatal pigs: a possible role of milk-borne growth factors. *Livestock Production Science*, 66, 95-107.
- Yang, J.S., Jung, H.J., Xuan, Z.N., Kim, J.H., Kim, D.D., Chae, B.J. and Han, I.K. (2001). Effects of feeding and processing methods of diets on performance, morphological changes in the small intestine and nutrient digestibility in growing-finishing pigs. *Asian-Australasian Journal of Animal Science*, 14(10), 1450-1459.
- Yasar, S. and Forbes, J.M. (1999). Performance and gastro-intestinal response of broiler chickens fed on cereal grain-based foods soaked in water. *British Poultry Science*, 40, 65-76.