How and Why Px Works
to
Decrease Pre-Weaning Mortality
and
Increase Weight in Pigs

Scientific Summary

Updated February 2017

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Introduction

Px is the first isotonic protein drink that contains key ingredients to nourish the intestinal cells, the enterocytes. Enterocytes are the final gateway for the absorption of proteins and carbohydrates into the body. If the enterocytes are working efficiently, then the body is able to absorb more nutrients. Px contains ingredients which support the efficient function of the enterocytes.

By supporting the enterocytes, Px helps pigs overcome the key productivity hurdles of young pigs, namely pre-weaning mortality (PWM) and a smooth transition through weaning. There are many aspects to successful management of young pigs but these hurdles are always a challenge.

Various strategies are used to try to overcome these hurdles. Milk replacer, electrolyte solutions, and quick-start drenches are all used by producers to give extra energy and fluids to piglets. Antibiotics, probiotics, pre-biotics and plant extracts are all used in an attempt to modify the intestinal bacteria population.

None of those strategies actually improve the function of the enterocytes, which are the engines that are responsible for absorbing any nutrients. A more focused approach is to nourish the intestinal cells themselves and help them to do the best job they can of absorbing nutrients. This is the approach that Tonisity has taken in the development of Px.

Tonisity has taken great care to investigate how best to use Px and to demonstrate the return on investment to producers. This research has shown that Px has a positive impact on pre-weaning mortality and post-weaning weight, as well as other key parameters

Over the last two years, Tonisity has conducted over 30 controlled clinical trials involving over 760 litters and 9600 pigs on production farms in the USA, United Kingdom and Spain. Key findings from those trials are presented here. Further producer trials are ongoing or planned in Italy, Belgium, Netherlands, France, Germany, Mexico, Romania, Hungary, Serbia, Ukraine, Philippines, Denmark, China and Russia.

This version of the Scientific Summary includes new information on pre-weaning mortality and intestinal morphology, and includes new explanatory graphics.

Tonisity is committed to working with producers and vets to show a real return on investment.

Better guts, better pigs, better yields.

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Effect of Px When Given to Suckling Pigs

Effect of Px on Pre-weaning Mortality and Post-Weaning Weight (TON-USA-034)

The aim of this study was to determine the effect of Px on pre-weaning mortality and post-weaning weight when given to suckling pigs from days 2-8 of age.

Materials and Methods

The study was conducted in a farrow-to-nursery operation with 7200 sows located in Iowa, USA. The farm has an average live-born of 12.8 pigs and a historic pre-weaning mortality of 13-14%. The farm was negative for PRRS at the time of the trial.

Seventy-three sows (PIC) and their litters (968 piglets) were enrolled in the study. Sows and their litters were randomized to one of two groups. Litters in the Px group received 500 mL of Px in an open pan, once daily from day 2-8 of life. Litters in the control group received nothing. All piglets had access to an automatic drinker. The amount of Px consumed was recorded daily.

On day 2 of life, piglets were individually ear-tagged and weighed. Piglets were again weighed at day 8, at weaning (~day 19) and at day 35. ADG at all time points was calculated based on actual days of age. Mortality and apparent cause of death were recorded daily.

Dependent variables weight and ADG were analyzed by a generalized linear model with the package GLM2, including group, parity, genetics the interaction group x parity and the interaction group x genetics as fixed effects. Mortality was analysed with Fisher's exact test. Results were considered significant at $P \le 0.05$ and considered a trend at $P > 0.05$ and $P \le 0.10$. Statistical analysis was performed using R software.

Results

Three litters were withdrawn from the trial on day 2 due to sow or litter health issues.

<u>Pre-Weaning Mortality</u>

The pre-weaning mortality was significantly lower in the Px group (10.3%) compared to the control group (15.0%) (P = 0.029). The odds of survival in the Px group was 1.55 time higher in the Px group. See Table 1.

Table 1. Pre-weaning Mortality

	Survived	Died	PWM	P value
Px	454	52	10.3%	**0.029**
Control	361	64	15.1%	

<u>Weight Gain</u>

There was no significant difference in bodyweight between the groups at day 2 (baseline). At day 8, the Px group had an average daily gain of 205 gram [0.454 lb], compared to the control group that had gained an average of 186 g [0.412 lb]. The difference in body weight between groups at day 8 was statistically significant at P = 0.002.

Piglets were between 15-22 days of age when weaning weights were measured, and because of this wide range of ages, no significant differences between groups were seen at weaning. However, by *20 days post-weaning,* differences in body weight were significantly different with the Px pigs weighing 9.9 kg [21.8 lb] and control pigs weighing 9.0 kg [19.95 lb] (P = 0.006). These weights corresponded to differences in ADG of 227 grams [0.5 lb] for the Px pigs and 205 grams [0.45 lb] for the control pigs (P = 0.015).

Table 2. Weight Gain*

	Px [lb]	Px (kg)	Control [lb]	Control (kg)	P value
BW day 2	3.737 ± 0.055	1.70 ± 0.025	3.623 ± 0.058	1.64 ± 0.026	0.592
BW day 8	**7.223 ± 0.121**	**3.276 ± 0.055**	**6.875 ± 0.129**	**3.118 ± 0.059**	**0.002**
Net gain d2-d8	**3.5 lb**	**1.58 kg**	**3.2 lb**	**1.48 kg**	**0.002**
ADG d2-d8	**0.454 ± 0.016**	**0.205 ± 0.007**	**0.412 ± 0.017**	**0.186 ± 0.007**	**0.007**
BW weaning† (d15-22)	12.615 ± 0.191	5.72 ± 0.087	12.566 ± 0.205	5.70 ± 0.09	0.219
ADG d2-weaning	0.488 ± 0.009	0.221 ± 0.004	0.484 ± 0.010	0.220 ± 0.005	0.661
Wt d 20 post-weaning	21.812 ± 0.680	**9.893 ± 0.31**	19.950 ± 0.666	9.05 ± 0.30	**0.006**
ADG d2 – d20 post-weaning	**0.501 ± 0.018**	**0.227 ± 0.008**	**0.454 ± 0.017**	**0.205 ± 0.008**	**0.015**
Net gain d2 – d20 post-weaning	**18.075 lb**	**8.193 kg**	**16.327 lb**	**7.41 kg**	**0.006**

* Values are least squares means ± standard error

† Weaning occurred between 15-22 days of age. Post-weaning weights were measured from 15-20 days post-weaning. All ADG values are based on actual days of age at weighing.

Conclusions and Relevance

Px supplementation in the first week of life reduced PWM by 33% in this trial.

Px pigs weighed an extra 100 g [0.3 lb] at day 8 and an extra 783 g [1.7 lb] at day 40, showing a statistically significant impact upon weight gain.

Acknowledgements: Many thanks to Dr. Ross Kiehne of Swine Veterinary Center, St. Peter, MN and Ms. Megan Pieters of Iowa State University for performing this study.

Effect of Px on Suckling Pig Mortality and Weaning Weight (15-001)

Introduction
Improving intestinal health in piglets is important to achieving optimal productivity in the farrowing house. Px is an oral isotonic protein drink designed to provide microenteral nutrition to suckling pigs. The aim of this study was to assess the impact of Px on suckling pigs.

Materials and methods
214 sows (Landrace x Large White or Danbred) and their litters (2713 piglets) from 2 farms were used in the study. Farm A was a commercially managed farrow-to-nursery operation of 2500 sows with an average live-born of 13.1 pigs and a historic pre-weaning mortality (PWM) of 13.7%. The farm had a history of clinically significant diarrhea during farrowing with *Clostridium difficile, Clostridium perfringens, Escherichia coli* and type A rotavirus. Farm B was a small family-owned farrow-to-finish farm with 400 Landrace x Large White sows with an average live-born of 13 pigs, a historic PWM of 10-12% and batch farrowing. Farm B had a historically low incidence of diarrhea during farrowing, but documented pathogens included *E. coli* and *Clostridium difficile*, though not rotavirus A.

Sows and their litters were randomized to one of two groups. On the day of farrowing, piglets were individually ear-tagged and weighed. Starting on day 2 of age, litters in the Px group received 500 mL of Px in an open pan, once daily until day 8 of age. Litters in the control group received no extra supplementation. All litters were allowed to suckle normally and had access to an automatic drinker. The amount of Px consumed was recorded daily by weighing the administered volume and any residual liquid in the pan.

Piglets were again weighed at day 8 (D8) and at weaning (D19 Farm A, D17 Farm B) of age. Weaning day was considered the end of study (ES). Creep feed was started in all litters at day 10.

<u>Data Collection</u>
The volume of Px consumed was recorded daily. Mortality and apparent cause of death were recorded daily. The presence and severity of diarrhea in each litter was recorded three times per week during the study. Diarrhea was scored from 0 to 3 using the following scale: 0: normal, healthy; 1: a few pigs affected, pasty faeces; 2: most pigs affected, liquid faeces; 3: all pigs affected, liquid faeces, piglets gaunt.

<u>Statistical Analysis</u>
The experimental unit for mortality, scour incidence, BW and ADG was the litter. Fisher's exact test was used to assess the relationship between mortality vs treatment and scour vs treatment for each time period (D8, ES).

Scour incidence and severity was analyzed by a generalized linear model using treatment group, sow body condition and parity as fixed effects. When Farm B results were analyzed, the farrowing batch was also included as a fixed effect.

The body weight was analyzed by a linear mixed model including treatment group, gender and sow's parity as fixed effects, and litter as random effect. BW on D1 was included as a covariate (for body weight on D8 and at ES). The number of days from D1 to ES was included as covariate for the analysis of body weight at ES. When Farm B results were analyzed, the farrowing batch was also included as fixed effect.

The average daily gain was analyzed by a linear mixed model; including treatment group, gender and sow's parity as fixed effects and litter as random effect. BW on D1 was included as covariate. The number of days from D1 to ES was included as covariate for the analysis of the ADG until ES. When Farm B results were analyzed the farrowing batch was also included as fixed effect.

For mortality, scour incidence, BW and ADG, the farm and the interaction between the farm and the treatment group were also included when Farm A and Farm B combined results were analyzed. Tests were two-tailed and carried out with a risk $\alpha = 5$ %. P-values of ≤ 0.05 were considered statistically significant, while $0.05 < P \leq 0.10$ was considered a near-significant trend. All statistical analyses were performed with R software.[†]

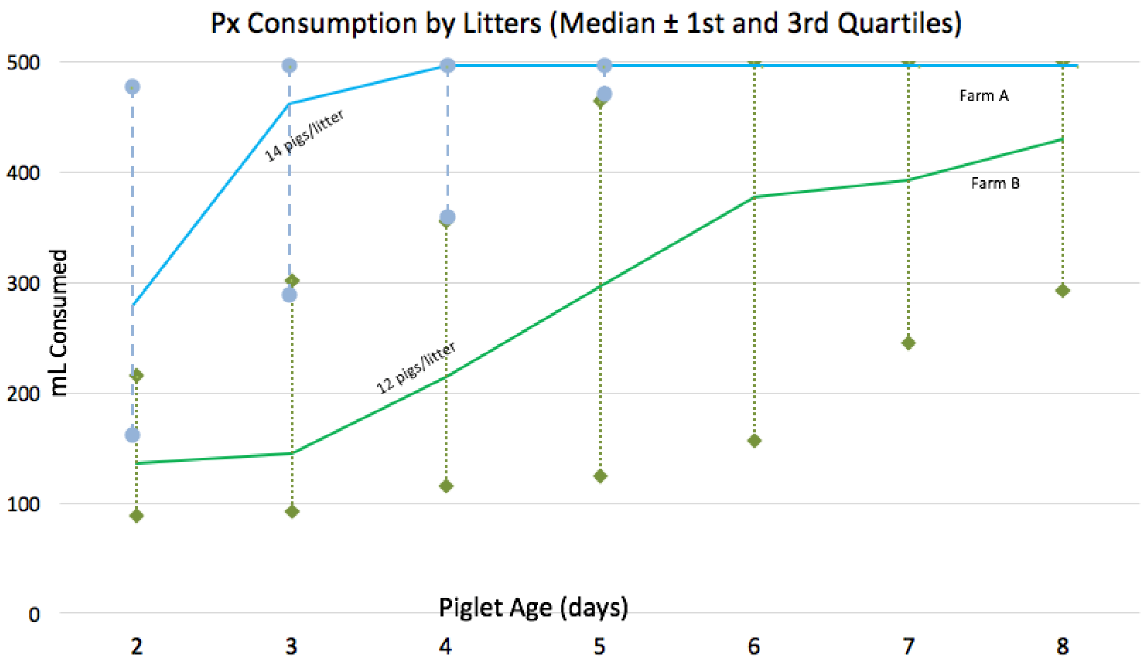
Results

Px Consumption

On Farm A, consumption of Px increased quickly during the first week of life. By day 3 of age, the median consumption of Px was 500 mL/litter/day. Litters contained an average of 14 piglets, with a median consumption of 36 mL/pig on day 3 of treatment. See Figure 1.

On Farm B, the consumption of Px also increased steadily during the first week of life, reaching a median consumption of 430 mL/litter on day 8. Litters contained an average of 12 piglets, with a median consumption of 18 mL/pig on day 3 of treatment.

Figure 1. Px Consumption by Litters



Mortality

Using the litter as the experimental unit, pre-weaning mortality was calculated at ES for both farms combined (Table 3). There was a significant difference in PWM between the two treatment groups, in which Px litters had a lower mortality by 3 percentage points (9.99% v 6.91%, P = 0.04). This equates to a 31% reduction in mortality.

Table 3. Pre-Weaning Mortality Rates

	Mean	95% CI	P value
Px	6.91%	4.6 – 9.2%	**0.0431**
Control	9.99%	7.6 – 12.3%	

Weight Gain

The body weight on day 1 was significantly different between farms. Therefore, body weight and ADG were analysed separately for each farm using the litter as the experimental unit.

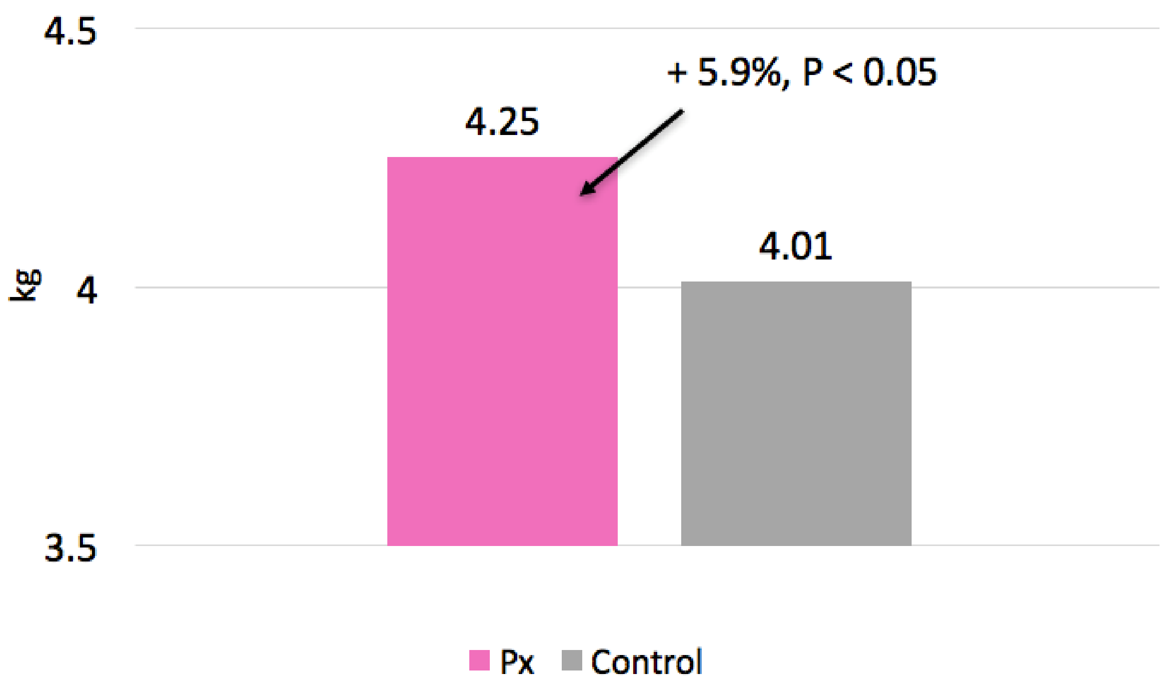
On Farm A, piglets in the Px group tended to weigh more than the control group at day 8 (P < 0.1) and weighed significantly more at day 19 (240 grams, 0.53 lb, P < 0.05). Pigs in the Px group had a higher average daily gain both in the first week (P < 0.1) and until day 19 (P < 0.05). See Table 4. Farm B results were the combination of 2 farrowing batches and showed no significant differences in body weight or ADG.

Table 4. Body Weight and ADG (Farm A)

	Total n	Px	Control	P value
Body weight d1 (kg ± SE)	1462	1.41 ± 0.04	1.37 ± 0.04	NS
Body weight d8 (kg ± SE)	1376	*2.29 ± 0.05*	*2.23 ± 0.05*	*P < 0.1*
Body weight d19 (kg ± SE)	1330	**4.25 ± 0.11**	**4.01 ± 0.11**	**P < 0.05**
ADG d1 – d8 (kg/d)		*0.123 ± 0.007*	*0.114 ± 0.007*	*P < 0.1*
ADG d1 – d19 (kg/d)		**0.158 ± 0.006**	**0.145 ± 0.006**	**P < 0.05**

Values are least-squares means ± standard error. NS = not significantly different.

Figure 2. Weight Comparison at Day 19



Incidence And Severity Of Scour In Litters

There was no significant difference in the incidence or severity of scour (scour score ≥ 2) between the control and Px litters on either farm (Table 5). However, on farm B, an abnormally high incidence of scour (24-25%) occurred in all litters during the time of the study, which was December – January.

Table 5. Litter Incidence of Scour

Site	Treatment Group	Number of litters without scour (%)	Number of litters with scour (%)	P value
Farm A	Px	47 (81.1%)	11 (19.0%)	P = 0.801
	Control	45 (83.3%)	9 (16.7%)	
Farm B	Px	38 (76.0%)	12 (24.0%)	P = 1.0
	Control	39 (75.0%	13 (25.0%)	

Discussion

Px Consumption

This study has shown that piglets less than one week old will drink significant volumes of liquid Px from an open pan. Various factors may have contributed to this, including palatability and texture of the liquid.

Delivering the product in an open pan facilitates the pigs natural curiosity and eliminates the learning and tactile barriers to drinking that are posed by mechanical nipple drinkers. Open pans do allow the possibility of faecal contamination and do require daily cleaning.

Pre-Weaning Mortality

This study demonstrated that Px had a significant impact on the pre-weaning mortality when given during the first week of life. The impact seen on pre-weaning mortality is interesting and could be explained by several different mechanisms. The 3% Px solution does not contain a significant number of calories, so extra caloric intake is not a likely explanation. The quantities consumed per piglet equate to 3-5% of a 1 kg piglets' bodyweight, so it is unlikely that correction of dehydration is the primary mode of action. It is possible however that some piglets may be subclinically dehydrated, in which case Px could be exerting an effect. The effect could also be due to the ingredient profile of Px, which contains key amino acids to support the metabolic requirements of intestinal enterocytes.

Weight Gain

Significant differences in the weights on both day 8 and at weaning were seen on Farm A. On Farm B, it is likely that the seasonal increase in scour affected the results of the study. Farm B litters also had a lower consumption of Px which may have reduced the beneficial impact of the product.

Scour

The incidence of scour in Farm B was much higher than usual for that farm but was the same in both the control and treatment groups. The increase was attributed to winter conditions. The optimal use of Px in supporting pigs with scour was not investigated in this study. A second concern with any protein source, especially before weaning, is that it may contribute to the development of scour. The protein levels in Px are very modest but it was appropriate to examine this possibility. However, there were no significant difference in scour scores between treatment groups on either farm, thus reducing this cause for concern.

Conclusion

Px was well accepted by neonatal pigs. Px administration reduced pre-weaning mortality by 33% and improved weaning weights.

Meta-Analysis of Pre-Weaning Mortality

A meta-analysis of pre-weaning mortality was performed using data from 6 studies. These studies were conducted in various sites, both in Spain and the USA, over a 12-month period. All treatment litters received Px 500 mL/day in an open pan from days 2-8 of age, while control litters received no supplementation. All piglets were allowed to suckle normally. All litters were weaned at ~ 21 days of age.

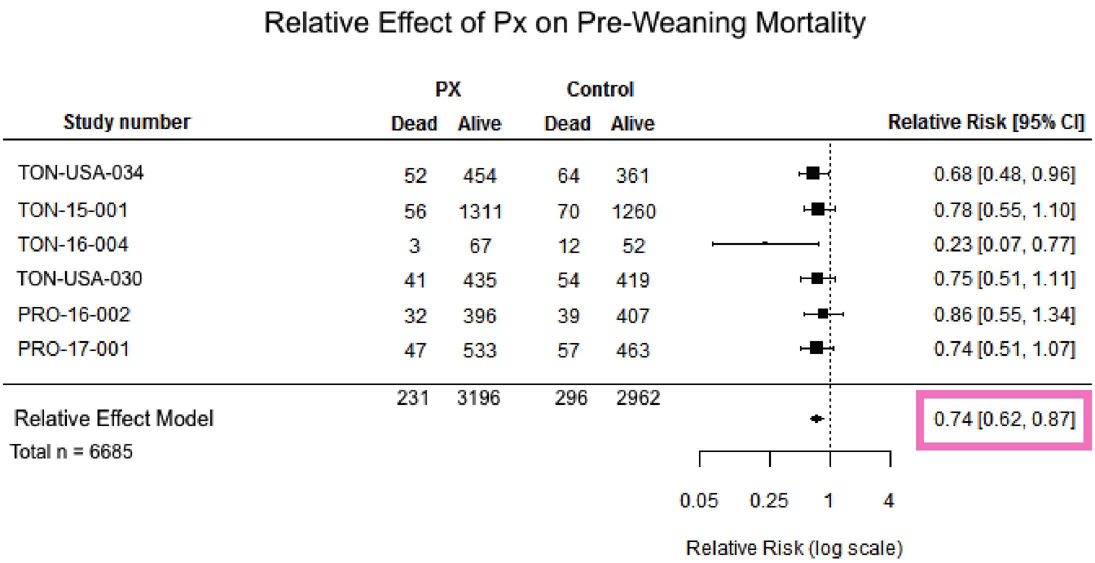
Data was analyzed using the litter as the experimental unit. All studies for which litter mortality data was available were included. Therefore, the meta-analysis was based on 6 trials, 543 litters and 6,685 piglets.

Figure 3 is a forest plot that shows the relative risk of pre-weaning mortality in the treated piglets versus the control group with corresponding 95% confidence intervals in the individual studies. The analysis is based on a random-effects model performed with R-package Metafor.[§]

Piglets who are given Px have only a 0.74 risk of pre-weaning mortality compared to the control group (95% confidence interval 0.62-0.87, P = 0.001).

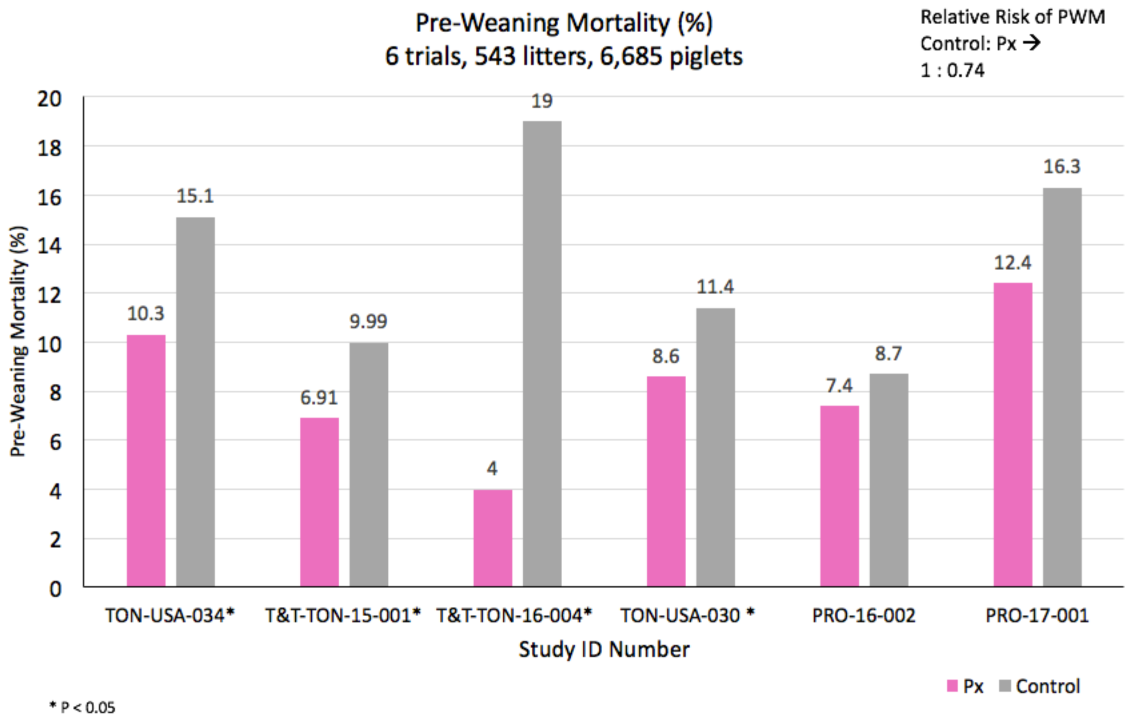
Figure 4 shows PWM data for the included trials.

Figure 3. Relative Risk of Pre-Weaning Mortality



[§] Viechtbauer, W. (2010). Conducting meta-analyses in R with the metafor package. Journal of Statistical Software, 36(3), 1-48. URL: http://www.jstatsoft.org/v36/i03/

Figure 4. Pre-Weaning Mortality in Trials



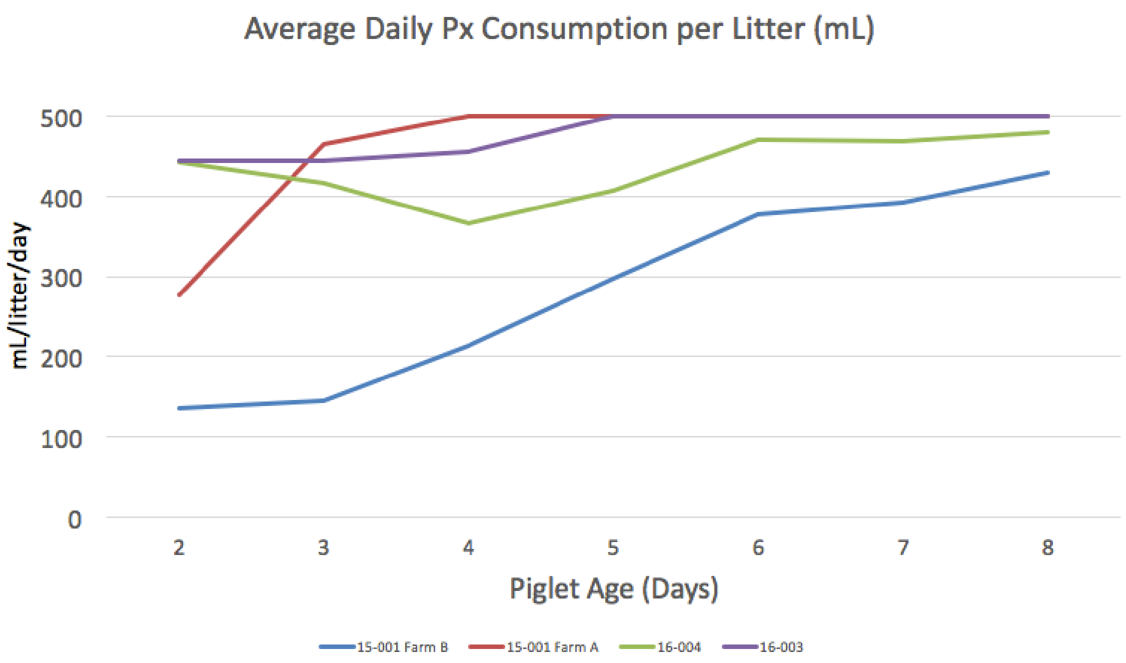
Conclusion

Px reduced pre-weaning mortality by 26%.

Px Consumption Data

The volume of Px consumed by neonatal pigs from days 2-8 of age was measured carefully in 4 studies, comprising 278 litters. By the second day of being offered Px (day 3 of age), most litters were consuming over 400 mL of Px per day.

Figure 5. Average Px Consumption (Aggregated Data)



Effect of Px in Gruel at Weaning

The palatability of Px suggested that it might be helpful in encouraging feed intake of pigs at weaning.

This section presents 3 studies …

16-003-PILOT was a simple pilot study designed to see if gruel mixed with Px would be more palatable to newly-weaned pigs than gruel made with water. ***It was.***

16-003-1 was a rather complicated, larger study designed to test Px-gruel vs water-gruel vs dry creep feed in the immediate pre-weaning and post-weaning period. ***Gruel is good****.*

TON-UK-032 was a small but simple trial that was the next logical step – give Px solution from days 2-8 of life and Px-gruel at weaning, and compare this with pigs that were given no Px and water-gruel at weaning. ***Px won.***

The fourth study in our investigation of Px in farrowing plus Px-gruel at weaning is currently in progress (Feb 2017) and will follow the pigs through till slaughter.

Effect of Px Gruel on Feed Intake and ADG Post-Weaning (16-003-PILOT)

Px is a highly palatable liquid that can be mixed with dry feed. Pigs at weaning often have reduced feed intake in the first few days after weaning.

The aim of this pilot study was to see if a gruel made with Px and creep feed would result in increased feed intake and weight gain in the immediate post-weaning period.

Materials and Methods

At weaning, 150 pigs were individually weighed and then randomly allocated to 1 of 6 pens, each containing 25 pigs. 3 pens received Px-gruel and 3 pens received water-gruel for 5 days after weaning. The gruel was mixed with the farm’s usual creep feed, using 15 L of either 3% Px solution or water to 10 kg of feed and poured into extra creep feeders. Dry creep feed was also available in each pen. The quantity of gruel and dry creep feed consumed was weighed and calculated daily.

On day 5 post-weaning, pigs were again individually weighed and ADG was calculated. The percentage of pigs achieving a positive ADG was also calculated. Gruel and dry feed consumption per pen was used to calculate gruel and feed intake per pig and per kg body weight (BW).

Results

There was wide variation in the ADG but overall, fifty of the 75 pigs (66%) in the Px-gruel group had a positive ADG over the 5 days, compared to 18 of 75 pigs (25%) in the Water-gruel group. The ADG in the Px-gruel pens is shown in the table below.

Table 6. Average Daily Gain

The Px-

	Px-Gruel (g/day)		Water-Gruel (g/day)
Pen 1	**6 ± 77**	**Pen 4**	**-21 ± 108**
Pen 2	**-29 ± 133**	**Pen 5**	**-85 ± 109**
Pen 3	**-163 ± 419**	**Pen 6**	**-137 ± 84**

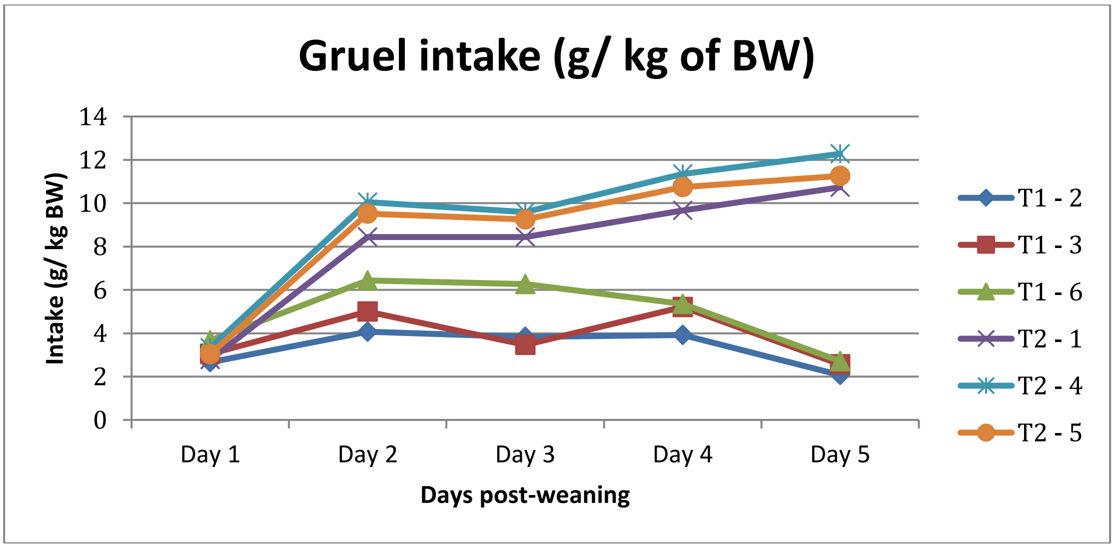
gruel pens consumed their gruel at an average of 362 g/pig (43 g/kg BW), which was approximately twice as much as the Water-gruel pens that consumed an average of 158 g/pig (20 g/kg BW). Not surprisingly, dry creep feed consumption in the Px-gruel pens averaged 9 g/pig compared to 20.6 g/pig in the Water-gruel pens.

The intake in the pens is shown in Table 7 and Figure 6 below.

Table 7. Gruel and Feed Intake

	Px-Gruel			Water-Gruel	
	Gruel intake (g/pig)	Creep Feed Intake (g/pig)		Gruel Intake (g/pig)	Creep Feed Intake (g/pig)
Pen 1	369	9	Pen 4	155	12
Pen 2	360	7	Pen 5	157	20
Pen 3	357	11	Pen 6	164	30

Figure 6. Gruel Intake Comparison



T1 = water-gruel, T2 = Px-gruel

Conclusions

Gruel made with creep feed and a 3% solution of Px is highly palatable. The consumption of gruel made with Px was twice that of water-gruel. Using Px-gruel resulted in increased average daily gain and a higher percentage of pigs that achieved positive ADG in the 5-day post-weaning period. A corresponding decrease in dry feed consumption was seen, but was offset by the increased consumption of gruel.

Px-Gruel vs Water-Gruel vs Dry Creep – Productivity (16-003-1)

The transition from suckling to weaning and dry food intake is a well-documented problem in pig management. Decreased feed intake after weaning results in a post-weaning growth check that causes significant production losses and has negative impacts on gut health Furthermore, weight gain in the first week post-weaning has a large impact upon subsequent performance and economics of the pig.[1,2] The immediate post-weaning period may also see a significant proportion of pigs that have subnormal water intake for up to 48 hours.[3]

Increased post-weaning diarrhea also results in increased antibiotic usage requirements. As producers aim to better manage their antibiotic usage, it is appropriate to investigate whether or not the use of semi-liquid feed supplementation in the period immediately before and after weaning is beneficial.

A novel isotonic protein-electrolyte solution (Px) has recently been developed and has the characteristics of being highly palatable as well as providing key amino acids for support of intestinal function. It was hypothesized that using Px solution to make a gruel with creep feed could result in improved feed intake post-weaning and improved health.

The aim of this study was to determine the effect of feeding gruel to pigs in the peri-weaning period. This study compared gruel made with Px vs. gruel made with water vs. dry creep feed.

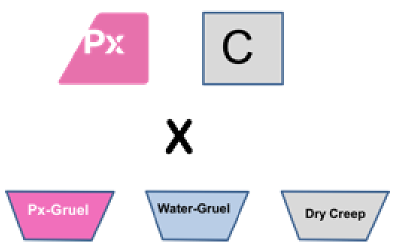
Materials and Methods

The study was performed on a 400 sow (Landrace x Large White/ Pietrain) farrow-to-finish operation with batch farrowing and weaning at 21 days of age. The farm was positive for PRRS and APP. The farm had a historically low incidence of scours in farrowing, but the most recent surveillance sample had documented *E. coli* (strains F41 and *gad*) as well as *Clostridium difficile*. Surveillance samples were negative for rotavirus A and PEDV on PCR.

In a farm located in Spain, 52 sows and their litters (608 piglets) were enrolled in the study and randomized to one of two groups, control (group A) or supplementation with 3% Px solution (group B). Groups were balanced by sow's parity, number of piglets and piglets' weight. Cross-fostering was permitted only within the treatment group and within the first 24 hrs. Starting at approximately 24 hours after birth (Day 2 of age), Px litters were given 500 mL of Px in an open pan, in their farrowing crate. Px litters continued to receive Px up through day 8 of age. Control litters were given no extra supplementation. All litters had access to fresh water through a drinker nipple and were allowed to suckle the sow normally.

Figure 7. Summary of Study Design

52 litters
Px 500 mL/litter/day on days 2-8
    vs. negative control group
Pre-weaning and post-weaning Px-gruel
    vs. negative control group with dry creep
    vs. positive control group with water-gruel
Weaned on d19
Gruel finished by d24
Histopath at d24 and d28



The pre- and post-weaning feeding plan is summarized in Table 8. Four days before weaning (approximately 15 days of age, study day -4), all pigs were weighed and litters were allocated to one of 3 new treatment groups - dry creep feed (D), water-gruel (WG) or Px-gruel (PG). Group A and B pigs were allocated equally across the 3 new treatment groups. These new groups were again balanced by sow's parity, number of piglets and piglets' weight on SD -4.

On SD -4, D litters received dry creep feed, WG litters received 500 mL of water and PG litters received 500 mL of 3% Px solution. All feeds and liquids were served in an open pan.

On SD -3, SD -2, and SD -1, D litters continued to receive dry creep feed. WG litters received a gruel of creep feed mixed with water and PG litters received a gruel of creep feed mixed with Px solution. All gruel was made using the ratio of 10 kg of dry feed to 15 L of liquid (water or 3% Px solution), i.e., a 1:1.5 w/v ratio.

On SD 0 (19 days of age), pigs were weaned, weighed and sorted into 53 pens of 10-11 pigs each. Pigs were also sorted into pens by bodyweight (heavy (H), medium (M), light (L)) while still remaining within their feed groups. Heavy was defined as > 5.8 kg, medium 4.1 – 5.8 kg and light < 4.1 kg. These weights corresponded to the upper 25% of pigs, middle 50% of pigs, and lower 25% of pigs. WG and PG pigs received their gruel on the day of weaning, while D pigs received dry creep feed.

Both WG and PG pigs continued to receive their gruel *ad libitum* for at least 1 day after weaning and were then tapered off gruel over another 3-4 days according to body weight. Light and medium pigs were tapered more slowly. All pigs also had dry creep feed available *ad lib* in separate feeders. Gruel was fed twice daily.

Table 8. Feeding Plan

Age (days)	Study Day	Individual Weights	In LITTERS		
2		x	**Group A** Normal suckling control group		**Group B** Suckling + 500 mL Px solution/ litter/ day
8		x	↓		↓
			In LITTERS		
15	SD -4	x	Dry creep feed	500 mL water	500 mL Px solution
16	-3		Dry creep feed *ad lib*	Water-Gruel *ad lib*	Px-Gruel *ad lib*
17	-2		Dry creep feed *ad lib*	Water-Gruel *ad lib*	Px-Gruel *ad lib*
18	-1		Dry creep feed *ad lib*	Water-Gruel *ad lib*	Px-Gruel *ad lib*
19	Weaning SD 0	x	**Into PEN BLOCKS* and Feed Groups****		
			Light	**Medium**	**Heavy**
			Gruel *ad lib*	Gruel *ad lib*	Gruel *ad lib*
20	1		Gruel *ad lib*	Gruel *ad lib*	Gruel *ad lib*
21	2		Gruel *ad lib*	70% of the gruel intake on SD1	60% of the gruel intake on SD1
22	3		70% of the gruel intake on SD2	50% of the gruel intake on SD1	40% of the gruel intake on SD1
23	4		50% of the gruel intake on SD2	30% of the gruel intake on SD1	20% of the gruel intake on SD1
24	5		30% of the gruel intake on SD2	10% of the gruel intake on SD1	Dry creep feed only
25	6		10% of the gruel intake on SD2	Dry creep feed only	↓
26	7	x	Dry creep feed only	Prestarter diet	Prestarter diet
29	10		Prestarter diet	↓	↓
33	14	x	↓	↓	↓
40	21		Starter diet	Starter diet	Starter diet
55	36	x	↓	↓	↓

* All pens had dry creep feed available *ad lib* in feeders, alongside the gruel feeders.

** Dry creep feed group was also blocked by size but only had dry creep feed available and is therefore not shown in this gruel feeding plan

The quantity of dry creep feed and gruel to be delivered was calculated to achieve an *ad libitum* consumption. This calculation was based on the average bodyweight (BW) of the piglets in the litter/pen calculated from individual BW measured on SD-4 and SD 0, the expected dry matter intake (DMI) based on the average BW, the number of piglets in the litter/pen, and the % dry matter (DM) of the creep feed used. The DM of the creep feed was based on proximate analysis (moisture, crude protein, crude fat and ash) as measured by near infrared spectroscopy. A margin of +20% was added in order to ensure that there was enough gruel available. During the days that gruel/feed was administered *ad libitum,* if the leftover gruel/feed of a pen was 0, the quantity of gruel/feed delivered was increased by another 20% on the next day in that pen. The total quantity of gruel or dry creep feed in open pans was divided into two doses per day.

Additionally, creep feed was offered *ad lib* in separate feeders to all groups from SD1 to at least SD7. Pre-starter feed was offered to M and H pens from SD7 and to L pens from SD10.

Data Collection

Pigs were individually weighed on day 2 and day 8 of age, then SD -4, SD 0, SD 7, SD 14 and SD 36. Feed intake was measured for each group and normalized to dry matter intake (DMI), which equalized differences between the volume and weight of the gruel and dry feed. DMI was calculated for the pre-weaning period, 7 days post-weaning and 14 days post-weaning. Average daily gain was calculated for the same periods. The number of pigs with positive ADG in the first week and second post-weaning was calculated.

The number of pigs requiring antibiotic treatment during the study period was also recorded. Antibiotic treatment for diarrhea was at the discretion of farm staff and consisted of Marbocyl 2% by injection.

The number of piglets per pen with diarrhea and their individual scour score was recorded daily from SD1 to SD14. The scale used was 0 = no scour, 1 = pasty faeces and 2 = liquid scour. The total scores per pen were then summed and divided by the number of pigs in the pen to create a pen fecal score.

Statistical Analysis

The experimental unit was the litter until weaning, and the pen after weaning. Tests were two-tailed and carried out with a risk α = 5 %. P-values of ≤0.05 were considered statistically significant, while 0.05< P ≤0.10 was considered a near-significant trend. Least squares means (LSM) ± standard error (SE) were used to calculate 95% confidence intervals. Logistic regression was used to calculate the differences between treatment groups for positive and negative ADG. All statistical analyses were performed with R software†, using packages GLM and lme4 as appropriate.

Results

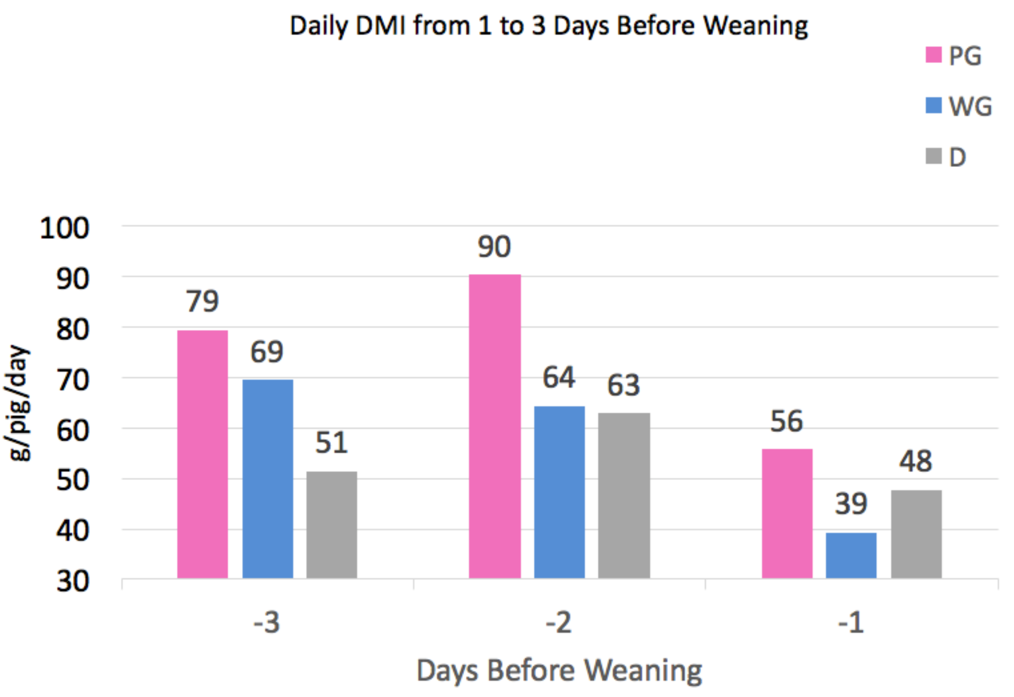
Day 2-8 Consumption of Px

The mean consumption of Px solution was 38 mL/piglet on day 2 of age, which increased to a mean of 45 mL/pig at day 8, i.e., all 500 mL was consumed by the litter. The mean consumption per kg BW was 188 mL for the week.

Dry Matter Intake

In the pre-weaning period, pigs who received Px-gruel had the greatest DMI as shown in Figure 8.

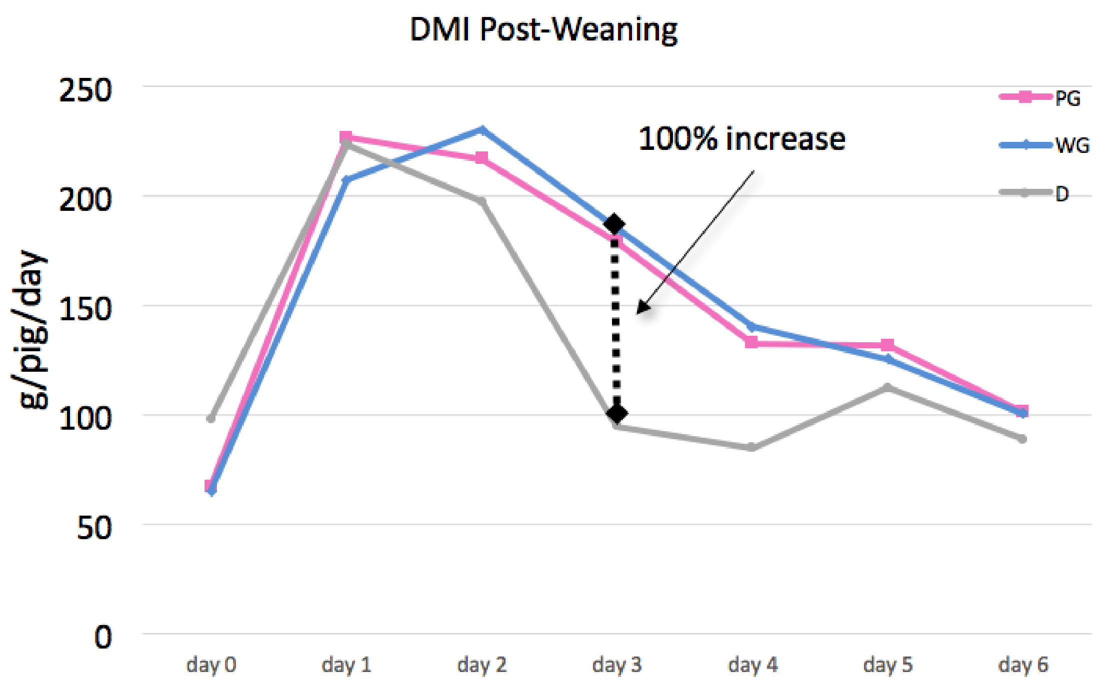
Figure 8. Daily DMI from 1 to 3 Days Before Weaning



The Px-gruel group had an average DMI of 54 ± 3.6 g/kg BW in the pre-weaning period, which was significantly higher than the water-gruel group (40 ± 3.5 g/kg BW) or the dry creep group (39 ± 4 g/kg BW) ($P < 0.05$).

This pattern of consumption continued over the next 6 days post-weaning as shown below.

Figure 9. Daily DMI intake for 6 days Post-Weaning



The DMI was also tracked for the pens of Heavy, Medium and Light pigs. Table 9 below shows total DMI of gruel and creep feed / kg BW in the first week and second weeks after weaning.

The most marked differences were seen in the Light and Medium pigs in the first week after weaning. Both gruel groups had significantly higher DMI compared to the dry feed group. Table 10 shows the average daily DMI per piglet.

Table 9. Average Daily DMI of Gruel and/or Creep Feed PER KG BW (g/kg BW), By Feed Group and Weight Class

		Px-Gruel (PG)		Water-Gruel (WG)		Dry Creep (D)	
Size	Days Post-Weaning	LSM ± SE	CI 95%	LSM ± SE	CI 95%	LSM ± SE	CI 95%
H	0 to 6	222 ± 12.4	197 - 246	233 ± 13.6	207 - 260	220 ± 13.2	194 - 245
H	7 to 14	249 ± 26.5	197 - 301	225 ± 29.1	168 - 282	236 ± 28.3	180 - 291
M	0 to 6	**198^{ab} ± 6.3**	**186 - 210**	**211^{a} ± 6.6**	**198 - 224**	**171^{b} ± 7.5**	**156 - 185**
M	7 to 14	228 ± 13.4	202 - 254	233 ± 14.1	205 - 261	214 ± 16.1	183 - 246
L	0 to 6	**254^{a} ± 11.7**	**231 - 277**	*224^{ab} ± 13.2*	*198 - 250*	***$180^{b\,t}$ ± 13.9***	***153 - 207***
L	7 to 14	201 ± 25.0	152 - 250	217 ± 28.1	162 - 272	198 ± 29.7	140 - 256

$^{a,\,b}$ different superscript in the same row indicates statistical differences ($P \le 0.05$). ^{t} in the same row indicates statistical tendency ($P \le 0.10$).

Table 10. Average Daily DMI of Gruel and/or Creep Feed PER PIGLET (g/pig/day), by Feed Group and Weight Class in the First Week Post-Weaning

		Px-Gruel (PG)		Water-Gruel (WG)		Dry Creep (D)	
Size	**Days Post-Weaning**	**LSM ± SE**	**CI 95%**	**LSM ± SE**	**CI 95%**	**LSM ± SE**	**CI 95%**
H	**0 to 6**	**148 ± 8.7**	**131 - 165**	**149 ± 9.6**	**130 - 168**	**142 ± 9.3**	**124 - 161**
M	**0 to 6**	**141[a] ± 4.4**	**133 - 150**	**150[a] ± 4.6**	**141 - 159**	**120[b] ± 5.3**	**109 - 130**
L	**0 to 6**	**164[a] ± 8.2**	**148 - 180**	**150[ab] ± 9.3**	**132 - 168**	**126[b] ± 9.8**	**107 - 145**

[a, b] different superscript in the same row indicates statistical differences (P≤0.05). [t] in the same row indicates statistical tendency (P≤0.10).

Average Daily Gain

The differences in DMI were then reflected in ADG. When all weight groups of pigs were compared, the PG group as a whole had a significantly larger number of pigs who gained weight in the first week after weaning as shown in Table 11 and Figure 10. The Px-gruel pigs were significantly more likely to have positive ADG than the water-gruel group (OR 1.79, 95CI 1.05 – 3.04, P = 0.031) and also out-performed the dry creep group (OR 1.66, 95CI 0.95 – 2.92, P = 0.076). The number of pigs with positive ADG in the water-gruel group was not significantly different from the dry creep group (OR 0.93, 95CI 0.55 – 1.58, P = 0.788).

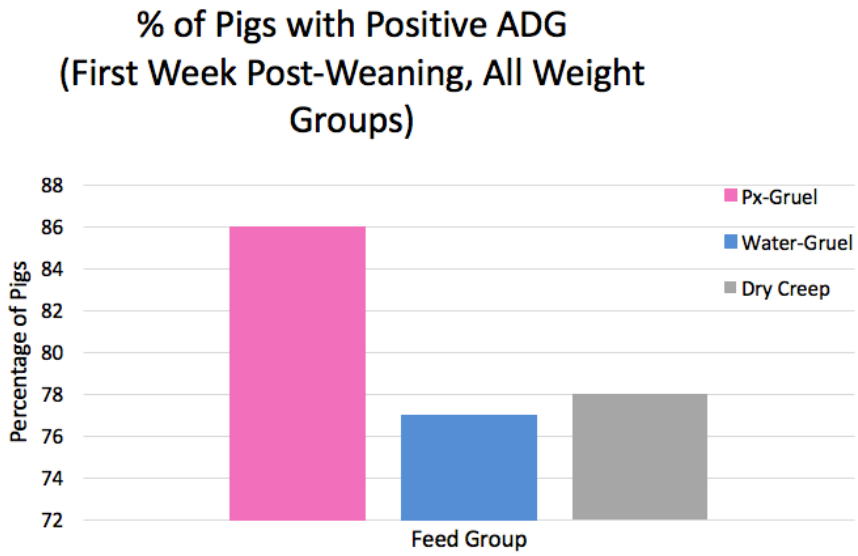
Table 11. Number and Percentage of Piglets with Positive ADG in the First Week Post-Weaning, by Feed Group

	Px-gruel (PG)	**Water-gruel (Wg)**	**Dry creep (D)**	**P value**
ADG≤0	28 (14%)	41 (23%)	31 (22%)	
ADG>0	***170[a t] (86%)***	139[b] (77%)	*113[ab t] (78%)*	
Odds ratio comparison	***1.6***		*1*	*0.076*
Odds ratio comparison	***1.79***	1		**0.031**
Odds ratio comparison		.93	*1*	0.788

[a, b] different superscript and bold in the same row indicates statistical differences (P≤0.05). [t] and italics in the same row indicates statistical tendency (P≤0.1)

Odds ratio: the likelihood of a given outcome occurring compared to another group. Can also be written as xx:1

Figure 10. Percentage of Pigs with Positive ADG



When broken down by size, the Px-gruel Medium and Light piglets also out-gained the other treatment groups. 88% of the Medium pigs and 56% of the Light pigs given Px-gruel had positive ADG in the first week post-weaning (P < 0.05). See Table 12.

Table 12. Number and Percentage of Piglets with Positive ADG in the First Week Post-Weaning, By Feed Group and Weight Class

Size		Px-gruel (PG)	Water-gruel (WG)	Dry creep (D)	P value
Light pigs (< 4.1kg)	ADG>0	**56^{a} (92%)**	31^{b} (78%)	33^{ab} (87%)	**0.05**
Medium pigs (4.1 – 5.8 kg)	ADG>0	**88^{a} (88%)**	74^{ab} (81%)	51^{b} (75%)	**0.05**
Heavy pigs (> 5.8 kg)	ADG>0	26 (70%)	34 (69%)	29 (76%)	NSD
Light pigs - Odds ratio comparison		**3.25**		1	**0.05**
Light pigs - Odds ratio comparison		**1.66**	1		0.43
Medium pigs - Odds ratio comparison		**2.44**		1	**0.032**
Medium pigs - Odds ratio comparison		1.68	1		0.202

$^{a,\,b}$ different superscript in the same row indicates statistical differences (P≤0.05).

Odds ratio: the likelihood of a given outcome occurring compared to another group. Can also be written as xx:1

Odds ratios were also calculated for each group to assess the likelihood of an effect. Light pigs in the Px-gruel group were 3.25 times more likely to have positive ADG than the dry creep group (P = 0.050) and 1.66 times more likely to have positive ADG than the water-gruel group (P = 0.43). Medium pigs in the Px-gruel group were 2.44 times more likely to have positive ADG than the dry creep group (P = 0.032) and 1.68 times more likely to have positive ADG than the water-gruel group (P = 0.202).

Antibiotic Usage

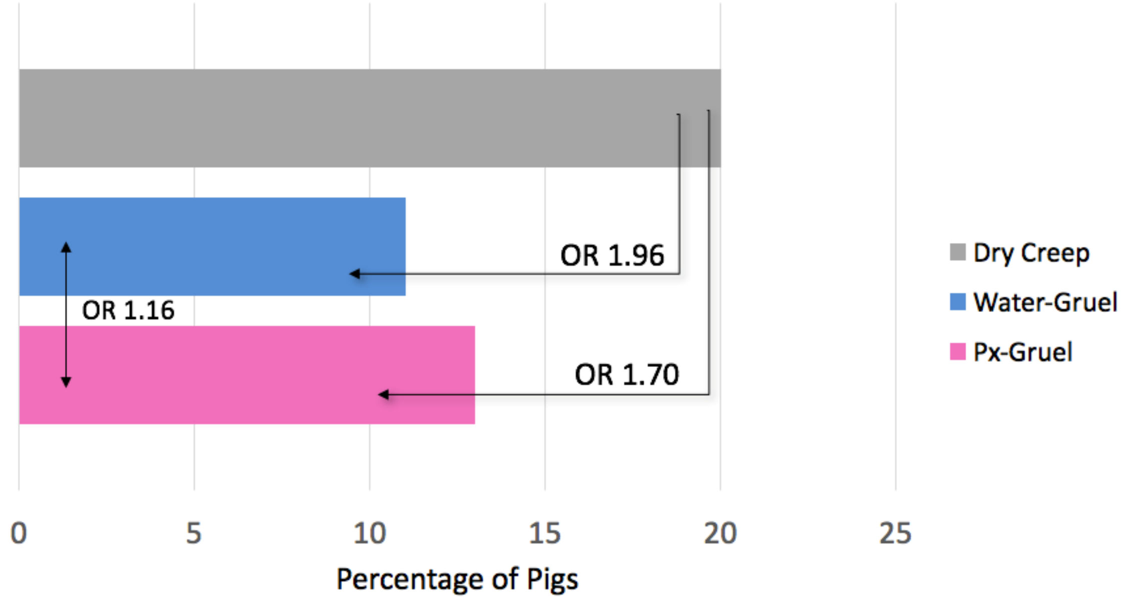
Interestingly, dry-creep pigs were 1.7 – 1.96 times more likely to be treated with antibiotics in the post-weaning period (Table 13 and Figure 11). There was no significant difference in the incidence of antibiotic treatment between WG and PG pigs (odds ratio 1.16, 95% CI 0.63 - 2.13, P = 0.645).

Table 13. Percentage of Pigs Treated With Antibiotics At Least Once

	Px-Gruel	**Water-Gruel**	**Dry Creep**	**P value**
	13%	11%	20%	-
Odds ratio comparison	1		1.70	*0.068*
Odds ratio comparison		1	1.96	**0.028**

Odds ratio: the likelihood of a given outcome occurring compared to another group. Can also be written as xx:1

Figure 11. Odds of Pigs Being Treated with Antibiotics Post-Weaning



Conclusions and Relevance

This study again showed that newborn piglets less than one week old will consume substantial volumes of 3% Px solution. It was also clear that piglets found Px-gruel more palatable than water-gruel or dry creep feed, especially in the immediate pre-weaning period.

Feeding Px-gruel and water-gruel in the peri-weaning period had a significant impact on the DMI and ADG of medium-weight and light-weight pigs. Light-weight Px-gruel pigs consumed 30 g/kg BW/day more food than the same water-gruel pigs and 74 g/kg BW/day more food than the same dry creep pigs in the first week post-weaning. This was reflected in the overall trend towards increased ADG in the Px-gruel pigs.

The reduction in antibiotic use suggests another benefit of Px-gruel during weaning and will be the subject of further investigations for Tonisity.

Px Gruel at Weaning (TON-UK-032)

The aim of this study was to build upon the results of the study 16-003-1 and compare two simple treatment groups in both farrowing and weaning.

Materials and Methods

In a farm located in Northern Ireland, 12 sows and their litters were enrolled in the study. Starting at approximately 24 hours after birth (Day 2 of life), Px litters (73 piglets) were given 500 mL of Px in an open pan, in their farrowing crate. Control litters (64 piglets) were given no extra supplementation. All litters had access to fresh water through a drinker nipple. Px litters continued to receive Px up through Day 8 of life.

On Day 25 and 26, Px litters were again given 500 ml Px solution in an open pan. On Day 27, Px litters were given a gruel consisting of Px mixed with creep feed in the ratio of 15 L Px to 10 kg dry feed, at a rate of approximately 200 g/pig. Control litters received a similar gruel but made with plain water. On Day 28, all piglets were weaned and moved into weaning pens. Px piglets continued to receive Px-gruel at a rate of approximately 200 g/pig for the next 3 days until Day 32, while Control pigs were given gruel made with water at the same rate and for the same period. Dry feed was available to both groups *ad lib.*

Table 14. Feeding Plan

	Px Group	**Control Group**
Day 2-8	Px 500 mL/litter/day	Normal suckling
Before weaning	Px liquid x 2 days, then Px-gruel x1 day	Water x 2 days, then water-gruel x 1 day
Weaning	Px gruel x 3 days	Water-gruel x 3 days

Piglet ID, Sow ID, treatment group and gender were recorded for each pig. Pigs were individually weighed on Day 2, Day 8, Day 25, Day 32, Day 63 (7 weeks) and day 94 (13 weeks).

Weight gain and average daily gain was calculated for each time interval and analysed with R software. Averages were calculated as least squares means (LSM). 95% confidence intervals were calculated from these. P values < 0.05 were considered statistically significant.

Results

The mean (LSM ± SE) weight gain per pig in the peri-weaning period (day 25-32) was 1.09 ± 0.073 kg in the control group (n = 63) and 1.46 ± 0.068 kg in the Px group (n = 49). This difference was highly significant (P < 0.001).

No clear differences were seen at day 61, but by day 94 (13 weeks of age) the Px pigs were an average of 4.72 kg heavier than controls (P = 0.0002).

Table 15. Weight Gain through Day 94

	Px-gruel	Water-gruel	Difference (kg) [lb]	P value
BW at day 2	1.62 ± 0.144	1.37 ± 0.129		0.241
BW at day 8	3.08 ± 0.249	2.94 ± 0.222		0.677
BW at 25 days of age	7.45 ± 0.316	7.24 ± 0.343		0.667
BW at 32 days of age	8.95 ± 0.259	8.35 ± 0.279		0.136
Gain d25-32	**1.46 ± 0.068**	**1.09 ± 0.073**	**0.370 [0.8]**	**< 0.001**
BW at 61 days of age	22.06 ± 0.986	21.89 ± 1.056		0.905
Gain d25-61	14.64 ± 0.884, n=73	14.63 ± 0.947, n=63		0.4256
BW at 94 days of age	46.65 ± 0.877, n=71	41.93 ± 0.933 n=62	**4.72 [10.4]**	**0.0002**
Gain d25-94	39.12 ± 0.740**, n=71**	34.66 ± 0.788**, n=62**	**4.46 [9.8]**	**<0.0001**

All weight values are reported as least squares means ± SE.

Conclusions and Relevance

The two groups of pigs did not have statistically significant differences in body weights at day 2, but pigs that were weaned with Px-gruel gained an extra 370 grams during the first week of weaning compared to those that were given water-gruel. The Px-gruel pigs also tended to be heavier at day 32. By 94 days (13 wks) of age, the Px pigs weighed 4.72 kg more than the control pigs. This confirms the theory that using Px to support young pigs gives real economic returns.

Intestinal Morphology

The usual approach to assessment of intestinal structure begins with microscopic measurements of the intestinal villi, which are the finger-like projections that line the small intestine and serve as the anchor for all intestinal cells.

Villus height is used as a marker of intestinal health and growth, and villus height decreases in the presence of inflammation or bacteria. The thickness of the intestinal mucosa is also measured as an indicator of intestinal growth, which occurs rapidly in the suckling pig.

The next 2 studies were designed to look at the effect of Px on the intestines when given in the first week of life, and then to look at the effect of gruel given in weaning.

Px from Days 2-8 of Age – Effect upon Intestinal Morphology and PWM (16-004)

The aim of this study was to determine whether or not giving Px to pigs in the first week of life had an impact on pre-weaning mortality and intestinal morphology.

Materials and Methods

This study was approved by the ethics committee of the University of Lleida, Spain.

In a farm located in Spain, 12 sows and their litters (134 piglets) were enrolled in the study. Starting at approximately 24 hours after birth (Day 2 of life), Px litters were given 500 mL of Px in an open pan, in their farrowing crate. Control litters were given no extra supplementation. All litters had access to fresh water through a drinker nipple. Px litters continued to receive Px 500 mL/litter up through Day 8 of life.

On day 9 and day 21 of age, a total of 36 piglets (18 per group) were euthanized for intestinal morphological examination. Selection of the piglets was done based on their ADG from initial weight to euthanasia time: day 2 to day 9 or day 2 to day 21. Within each litter, quartiles (25%, 50% and 75%) of the ADG were used to select three piglets per litter, from six litters (three per treatment) at day 9 and another three piglets per litter from the other six litters (three per treatment) at day 21. The piglets closest to each quartile were selected [4]

Piglets were euthanized by intracardiac injection of T-61. The abdominal cavity was opened and intestinal segments (6-8 cm in length) for microscopy were obtained at proportional distances ~10, ~50 and ~90% along the whole length of the small intestine, from the gastric pylorus to the ileo-cecal valve. These distances resulted in duodenal, jejunal and ileal sections. At each site, both ends of the tissue were opened lengthwise (1-2 cm) to allow a full contact of the formalin solution with the mucosa. Samples were fixed by immersion in 10% formalin solution.

Transverse tissue samples were cut from each segment using a stereo microscope. Samples were transferred to a slide and stained with hematoxylin and eosin. Measurements were taken using ImageJ software with a Leica ICC50 HD camera mounted on a Leica DM1000 microscope. Measurements were only taken from sections where the plane of section ran vertically from the tip of a villus to the base of an adjacent crypt. Ten of the tallest well-orientated villi (complete distance from muscle layer to tip) and 10 associated crypts (taken as the distance between the villus base and the muscular layer) were measured. Villus height (μm), crypt depth (μm), and intestinal mucosal thickness (μm) were measured. Villus height/crypt depth ratio was calculated. Villus density (number/mm) and crypt density (number/mm) were measured. The average measurements per slide were used as the experimental observation as published by Berkeveld et al.[4]

Statistical Analysis

Intestinal morphology variables were analysed with a generalized linear model using the package GLM for R software. Treatment group, intestinal section and age were considered as fixed effects. Interactions were explored but were not significant..

Pre-weaning mortality was compared by logistic regression using the package GLM for R software, with the treatment group as the fixed effect.

Results

Pre-Weaning Mortality

Pigs that received Px in the first week of life had significantly lower pre-weaning mortality (4% vs 19%, P = 0.015). This was a 75% reduction in PWM.

Intestinal Morphology

The mean villus height, crypt depth and intestinal mucosal thickness were higher in the Px group at both 9 and 21 days of age. Regardless of age at measurement, pigs that received Px in the first week of life had taller villi (P < 0.001) and tended to have a thicker intestinal mucosal layer (P = 0.87)

Figure 12. Jejunum from Px Group Pre-Weaning

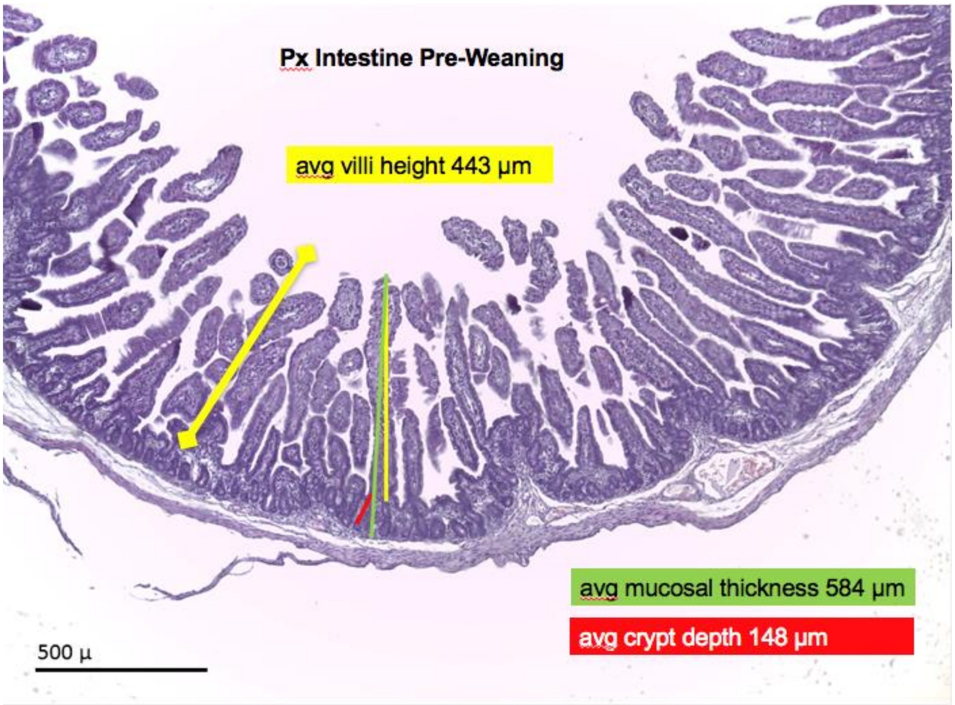


Figure 13. Jejunum from Control Group Pre-Weaning

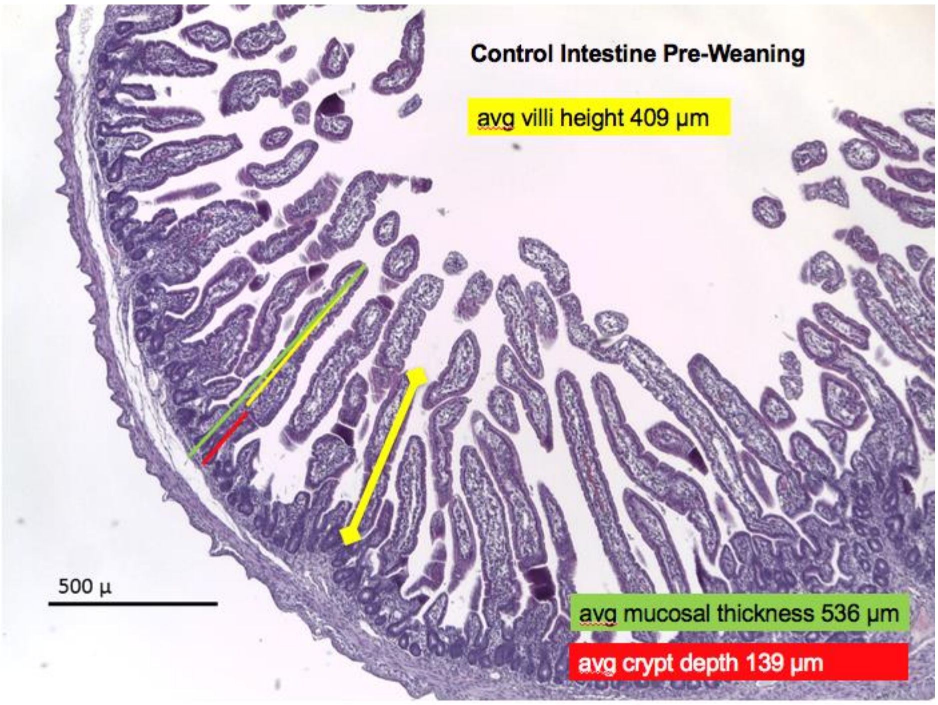


Table 16. Intestinal Morphology Pre-Weaning (d9 and d21 Combined)

	Villus height (µm)	Crypt depth (µm)	Villus height/ crypt depth ratio	Intestinal mucosal thickness (µm)	Villus density (No. /mm)	Crypt density (No. /mm)
Px	443	148	3.6	584	8.7	18.3
Control	409	139	3.6	536	9.3	20.6
SE	16.5	5.1	0.16	19.8	0.22	0.55
P-value	**<0.001**	0.199	0.962	*0.087*	**0.037**	**0.003**

Values reported are least squares means. SE: standard error.

Conclusions and Relevance

Pigs that received Px during the first week of life had significantly taller villi compared to pigs that had no Px. Px pigs also tended to have a thicker mucosal layer.

Pigs that received Px during the first week of life had significantly lower pre-weaning mortality compared to those that did not.

Px-Gruel vs Water-Gruel vs Dry Creep – Intestinal Morphology (16-003-2)

This study was part of the previous study (16-003-1) that compared gruel made with Px vs. gruel made with water vs. dry creep feed. A subset of pigs was used to assess the effect of Px supplementation upon intestinal morphology.

Materials and Methods

This study was approved by the ethics committee of the University of Lleida, Spain.

In a farm located in Spain, 52 sows and their litters (608 piglets) were enrolled in the study. Starting at approximately 24 hours after birth (Day 2 of life), Px litters were given 500 mL of Px in an open pan, in their farrowing crate. Control litters were given no extra supplementation. All litters had access to fresh water through a drinker nipple. Px litters continued to receive Px up through Day 8 of life.

Starting at 15 d of age, the litters were split into 3 subsets and given either dry creep feed, water-gruel or Px-gruel for the next 3 days (all feeds *ad lib)*. All gruel was made using the ratio of 15 L of liquid (water or 3% Px solution) to 10 kg of dry feed. At 19 d of age, pigs were weaned and sorted into pens by bodyweight (heavy, medium, light) while still remaining within their feed groups. Heavy was defined as > 5.8 kg, medium 4.1 – 5.8 kg and light < 4.1 kg.

All pigs continued to receive their gruel or dry feed *ad lib* for at least 2 days after weaning and were then tapered off gruel over another 3-4 days according to body weight. Light and medium pigs were tapered more slowly.

A total of 36 piglets (12 per group) were euthanized after weaning for intestinal morphological assay. Eighteen (18) piglets (six per group) were selected on day 24 of age and day 28 of age based on their BW at 18 d of age. Within the initial group (Px or no Px in week 1) and treatment group (Px-gruel, water-gruel or dry creep feed), quartiles (25%, 50% and 75%) of the BW at weaning were used to select six piglets per group of treatment at 5 and 9 days after weaning, corresponding to days 24 and 28 of age. The piglets closest to each quartile were selected. [4]

Piglets were euthanized by intracardiac injection of T-61. The abdominal cavity was opened and intestinal segments (6-8 cm in length) for microscopy were obtained at proportional distances ~10, ~50 and ~90% along the whole length of the small intestine, from the gastric pylorus to the ileo-cecal valve. These distances resulted in duodenal, jejunal and ileal sections. At each site, both ends of the tissue were opened lengthwise (1-2 cm) to allow a full contact of the formalin solution with the mucosa. Samples were fixed by immersion in 10% formalin solution.

Transverse tissue samples were cut from each segment using a stereo microscope. Samples were transferred to a slide and stained with hematoxylin and eosin. Measurements were taken using ImageJ software with a Leica ICC50 HD camera mounted on a Leica DM1000 microscope. Measurements were only taken from sections where the plane of section ran vertically from the tip of a villus to the base of an adjacent crypt. Ten of the tallest well-orientated villi (complete distance from muscle layer to tip) and 10 associated crypts (taken as the distance between the villus base and the muscular layer) were measured. Villus height (μm), crypt depth (μm), and intestinal mucosal thickness (μm) were measured. Villus height/crypt depth ratio was calculated. Villus density (number/mm) and crypt density (number/mm) were measured. The average measurements per slide were used as the experimental observation as published by Berkeveld et al.[4]

Statistical Analysis

Intestinal morphology variables were analysed with a generalized linear model using the package GLM for R software. Interactions between weight class, litter Px treatment, weaning feed treatment, study day and intestinal section were explored but were not significant.

Results

Villus height, villus height/crypt depth ratio and intestinal mucosal thickness were significantly higher ($P < 0.05$) in pigs that received Px in the first week of life, regardless of which type of creep feed regime they were given. The average intestinal villi and mucosal layer were greater at all time points in the group that was originally given Px from days 2-8 of age.

Figure 14. Jejunum from Px Group Post-Weaning

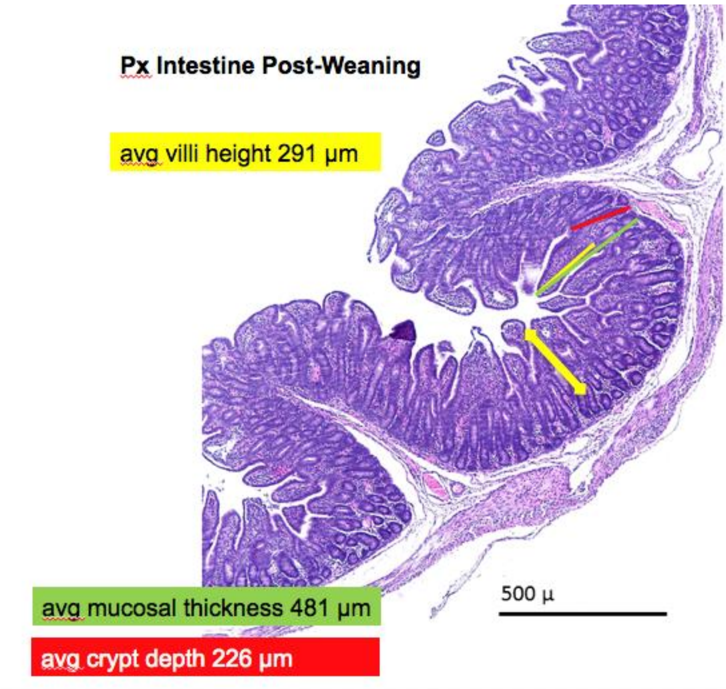


Figure 15. Jejunum from Control Group Post-Weaning

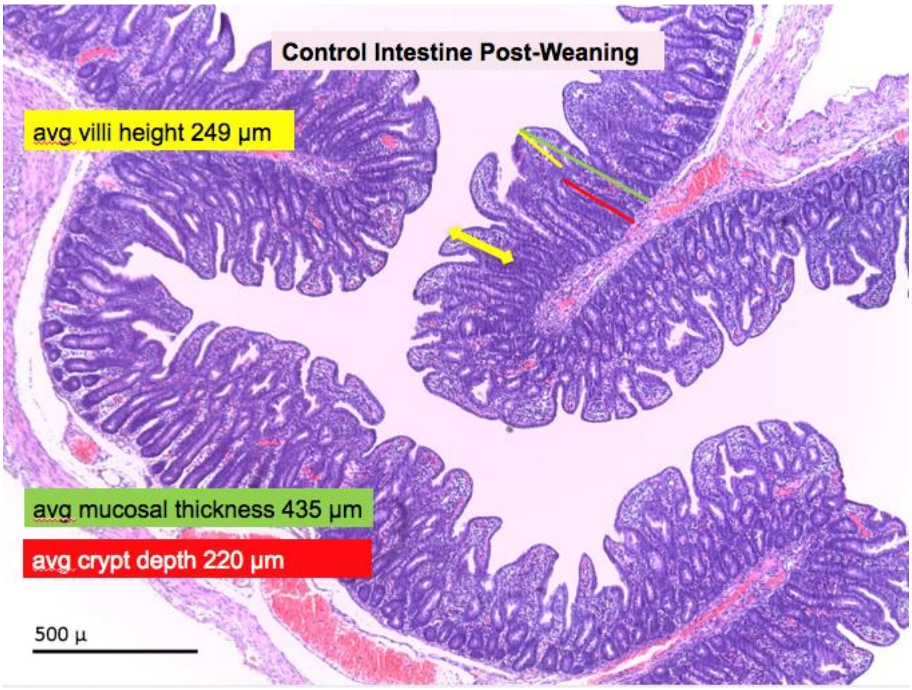


Table 17. Comparison of Intestinal Morphology Parameters Post-Weaning

	Villus height (µm)	Crypt depth (µm)	Villus height/ crypt depth ratio	Intestinal mucosal thickness (µm)	Villus density (No. /mm)	Crypt density (No. /mm)
Px	291 ± 10.4	226 ± 7.1	1.4 ± 0.06	481 ± 15.9	7.6 ± 0.17	21.8 ± 0.79
Control	249 ± 9.3	220 ± 6.4	1.2 ± 0.05	435 ± 14.2	8.0 ± 0.15	20.5 ± 0.71
P-value	**0.003**	0.512	**0.018**	**0.033**	*0.064*	0.211

Values reported are least squares means. SE: standard error.

Conclusions and Relevance

It is normal for villus height and mucosal thickness to decrease with age, which explains the differences between the results of this study and the previous study (16-004). The key finding from this study was that regardless of the feed given at weaning, pigs that received Px during the first week of life had significantly better intestinal morphology in the post-weaning period.

The development of the villi seen when Px was given from day 2-8 of age continued through to the post-weaning stage, showing the efficiency and impact of Tonisity Px formula on intestinal development.

Scour

Px in Piglets with Scour (T20)

Piglets suffering from diarrhea may become dehydrated. Px is an isotonic solution containing balanced electrolytes and protein, and may be used for rehydration and support in such situations.

The aim of this study was to determine the effect of Px on suckling pigs with scours.

Materials and Methods

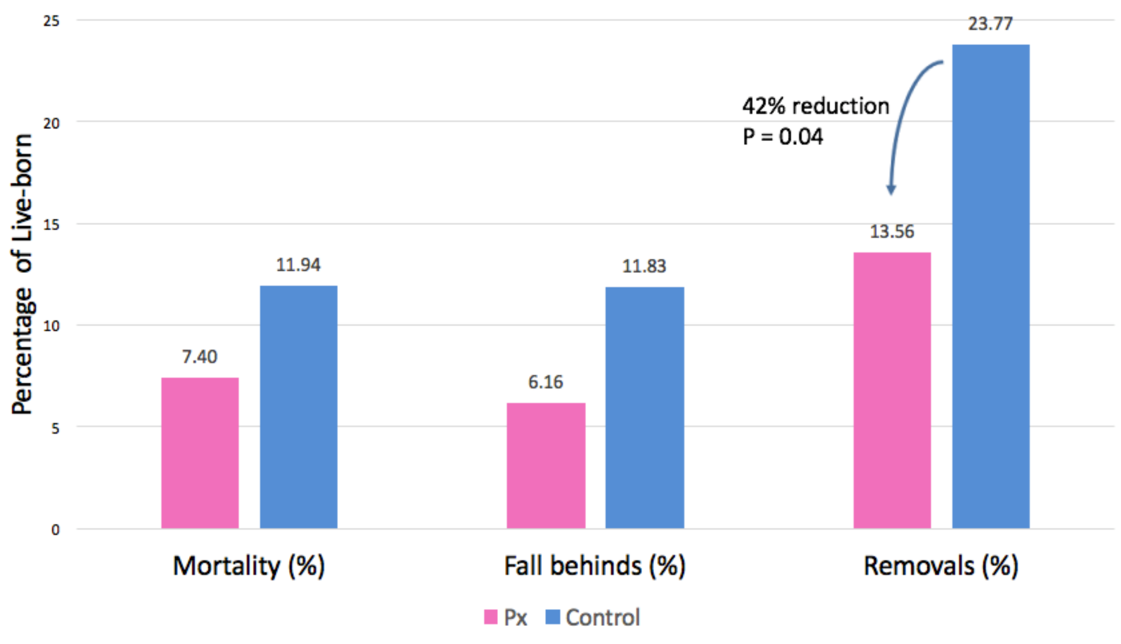
In a farm located in the midwestern USA, with historically-documented *E. coli* and rotavirus, 20 litters (268 pigs) were prospectively enrolled in the study if they developed scour between 2-4 days of age. Once enrolled, piglets were individually ear-tagged. Odd-numbered piglets within a litter were given 2 mL of Px by mouth twice daily. Even-numbered piglets were given 2 mL of water by mouth twice daily. All piglets received standard farm treatment to control secondary pathogens. All pigs were individually weighed when enrolled, and again at day 18.

Any pigs that died or were removed to a nurse sow were recorded. Data were analyzed as a randomized complete block design using the PROC MIXED procedure of SAS with piglet as the experimental unit and treatment as a fixed effect. Results were considered significant at $P \leq 0.05$ and considered a trend at $P > 0.05$ and $P \leq 0.10$.

Results

Pre-weaning mortality for the Px group was 7.40%, compared to the control group which had a mortality of 11.94% (P = 0.21). Similarly, the percentage of pigs that were removed as fall-behinds was lower in the Px group (6.16%) compared to the control group (11.83%) (P = 0.38). When mortality and fall-behinds are combined, the total percentage was significantly lower in the Px group (13.56%) compared to the water group (23.7%) (P = 0.04), which was a 42% reduction.

Figure 16. Comparison of Px v Control in Scouring Piglets



Conclusions

These results show that even small amounts of Px are helpful in the support of piglets with scour, and can have a marked impact upon both mortality and fall-behinds.

Px in Piglets with Scour (TON-USA-027)

Piglets suffering from diarrhea may become dehydrated. Px is an isotonic solution containing balanced electrolytes and protein, and may be used for rehydration and support in such situations.

The aim of this study was to determine the effect of Px on suckling pigs with scours.

Materials and Methods

In a farm located in North Carolina, USA, with documented *Clostridium perfringens, E. coli* and rotavirus, 79 litters (898 pigs) were prospectively enrolled in the study when they developed scour between 2-4 days of age. Once enrolled, piglets were individually ear-tagged. Odd-numbered piglets within a litter were given 2 mL of 3% Px solution by mouth twice daily x 5 days. Even-numbered piglets received no oral supplements. All piglets received standard farm treatment (1 mg/kg ceftiofur IM x 3 days) to control secondary pathogens. Fecal scores were recorded daily for individual pigs, where 0 = normal feces, 1 = pasty feces and 2 = liquid feces. Total fecal scores were summed for each piglet. Pigs were defined as 'recovered' if their fecal score was 0 on day 5. All pigs were individually weighed when enrolled, at 8 days after enrolment and at 18 days after enrolment. Any pigs that died or were removed to a nurse sow were recorded.

Morbidity, mortality, sick animals and recovered animals were compared by logistic regression using a generalized linear model of R software, with piglet as the experimental unit and treatment as a fixed effect. Body weight and average daily gain were analysed as a general linear model with treatment, gender, room and date of inclusion as fixed effects. Fecal scores from inclusion to study day 5 were analysed using the Wilcoxon test. Results were considered significant at $P \leq 0.05$ and considered a trend at $P > 0.05$ and $P \leq 0.10$. Odds ratios were calculated.

Results

The percentage of pigs that had recovered after 5 days was higher in the Px group (71%) compared to the control group (62%). Odds ratio calculation indicated that pigs receiving Px were 1.48 times more likely to recover in that period of time ($P = 0.112$).

The percentage of pigs that were culled as fall-behinds was higher in the control group (8%) compared to the Px group (5%). Odds ratio calculation indicated that control pigs were 1.63 times more likely to be culled ($P = 0.098$).

Conclusions and Clinical Relevance

This was the second study in a proof-of-concept series designed to investigate the role of Px in supporting piglets through episodes of neonatal scour. It is interesting that even 4 mL daily had an effect. Further studies are planned to investigate the optimum volume and timing of delivery of Px to support scouring pigs

Acknowledgements: Many thanks to Dr. Cary Sexton of Southeast Swine Research and Ms. Kathleen Wood of NCSU for performing this study.

Palatability Aid

Palatability of Water-soluble Antibiotic In Nursery Pigs (16-002)

Px is a novel isotonic solution that provides both rehydration and protein. Pilot studies have shown that it is highly palatable to both suckling and weaned pigs. Pigs are often given oral medications such as antibiotics in water, but many of those medications are poorly accepted.

The aim of this study by Tonisity was to assess whether or not Px could be used to increase the palatability of medication in weaned pigs.

Materials and Methods

One hundred and ninety eight healthy piglets of approximately 21 days of age were housed in six pens at weaning. Piglets were grouped by size in each pen. Each pen had an automatic drinker. Each pen was also provided with a bowl drinker that was connected to a 20 L carbuoy. The carbuoys were filled each day with a solution of antibiotic in water or antibiotic in a 3% solution of Px. The antibiotic used was Coliphur®, which is a mixture of neomycin and colistin.

Five pigs were randomly chosen from each pen to establish an average body weight. This average body weight was then used to calculate the dose of Coliphur® required (0.1 mL/kg BW/day) and the volume of water required (10% of body weight/day) for each pen.

Starting at 3 days after weaning, pens were allocated to receive either antibiotic in water or antibiotic in Px for 2 days. After 2 days, pens were allocated to the opposite treatment in a crossover design (Table 18).

Table 18. Allocation of Treatment Groups

Pen	No. of pigs	Treatment Day 1	2	3	4
1	19	antibiotic + water		antibiotic + 3% Px solution	
2	40	antibiotic + water		antibiotic + 3% Px solution	
3	37	antibiotic + water		antibiotic + 3% Px solution	
4	38	antibiotic + 3% Px solution		antibiotic + water	
5	40	antibiotic + 3% Px solution		antibiotic + water	
6	24	antibiotic + 3% Px solution		antibiotic + water	

All medicated solutions were made fresh each morning. The volume of unconsumed solution from the previous day was measured each morning.

Results

Pigs receiving the antibiotic in Px consumed 94% of their calculated intake volume, but the pigs receiving the antibiotic in water consumed only 33% of their calculated intake volume. Pens in the Px+antibiotic group achieved either 0.09 or 0.10 mL/kg BW of medication on 11 of the 12 treatment days, but none of the pens in the water+antibiotic group received the recommended dose of antibiotic on any day (Table 19).

Table 19. Consumption of Medicated Solutions in Pens

PEN	TREATMENT*	DAY	MEDICATED WATER (L)				NUMBER OF PIGLETS	COLIPHUR®
			ADDED	LEFTOVER	INTAKE			DOSE (mL/kg BW)
1	control	1	9.79	6.09	3.70	*38%*	19	0.04
1	control	2	9.79	6.46	3.33	*34%*	19	0.04
1	Px	3	9.83	0.00	9.83	*100%*	19	0.10
1	Px	4	9.75	0.10	9.65	*99%*	19	0.10
2	control	1	25.22	17.20	8.02	*32%*	40	0.03
2	control	2	25.22	17.85	7.37	*29%*	40	0.03
2	Px	3	25.38	3.00	22.38	*88%*	40	0.09
2	Px	4	25.38	0.00	25.38	*100%*	40	0.10
3	control	1	23.81	22.99	0.82	*3%*	37	0.00
3	control	2	23.81	9.74	14.07	*59%*	37	0.06
3	Px	3	23.64	1.44	22.19	*94%*	37	0.09
3	Px	4	23.17	0.00	23.17	*100%*	37	0.10
4	Px	1	18.01	6.42	11.59	*64%*	38	0.07
4	Px	2	17.76	0.39	17.37	*98%*	37	0.10
4	control	3	17.76	13.25	4.51	*25%*	36	0.03
4	control	4	17.30	14.25	3.05	*18%*	34	0.02
5	Px	1	24.30	2.19	22.11	*91%*	40	0.09
5	Px	2	24.30	0.40	23.90	*98%*	40	0.10
5	control	3	24.30	9.14	15.17	*62%*	40	0.06
5	control	4	23.56	10.77	12.79	*54%*	40	0.05
6	Px	1	8.00	0.18	7.82	*98%*	24	0.10
6	Px	2	8.00	0.05	7.95	*99%*	24	0.10
6	control	3	10.52	8.41	2.11	*20%*	23	0.02
6	control	4	9.47	7.26	2.21	*23%*	18	0.03

Conclusions

Px was effective at increasing the amount of medication consumed to the recommended dosing level of 0.1 mL/kg. While it is important that antibiotics are prescribed only when necessary, it is also important that the required dose be delivered. Further palatability tests using Px with other medications are warranted.

Palatability of Water-Soluble Antibiotic in Suckling Pigs (15-013)

Young piglets suffering from scour may benefit from rehydration. Some producers use oral antibiotics in the treatment of scour, but these antibiotics are sometimes unpalatable.

The objective of this study was to determine whether Px would aid the consumption of medication in suckling piglets.

Materials and Methods

Forty sows and their litters were randomly allocated to one of four treatment groups based on parity. The treatment groups were water (W), water+Coliphur® (W+C), and Px+Coliphur® (Px+C). The antibiotic used was Coliphur ®, which is a mixture of neomycin and polymyxin B. All litters received 500 mL of their designated solution in an open pan, once daily, from day 2 – 7 of life. All piglets had access to an automatic drinker. The amount of solution consumed was recorded daily for each litter, and an average intake per piglet was calculated daily.

On the day of farrowing, piglets were individually ear-tagged and weighed. Piglets were again weighed at day 7, and average daily gain was calculated.

Mortality was recorded each day.

Results

The control group receiving plain water (W) had the highest average intake of any group, consuming 180 ± 16.8 mL/pig over the 5 days of treatment. The Px+C group consumed an average of 162 ± 16.9 mL/pig, which was not significantly different from the control Water group.
The Water+C group had the lowest intake of any group, averaging 102 ± 17.7 mL/pig.

Table 20. Consumption of Medicated Solutions in Litters

Group	Mean ± SE (mL/pig)
Px+Coliphur®	162 ± 16.9
Water+Coliphur®	102 ± 17.7
Water	180 ± 16.8

Conclusions

Px+Coliphur® was significantly more palatable than Water+Coliphur®. The volume of Px+Coliphur® consumed was not significantly different from plain water, suggesting that piglets find Px a very palatable product which may be used to deliver medications

Safety Data

Dose Titration Trial (15-002)

Introduction

The purpose of this study was to assess piglets for any negative effect of Px when administered in the first week of life. The study evaluated the effect of Px on weight, scour incidence, gut bacteria populations, haematology and serum biochemistry, when it was administered to suckling piglets at different doses (2.5 mL, 25 mL, 50 mL and 100 mL) and for different durations (1 to 5 days) during first days of life.

Materials and Methods

Ten sows and their litters (~ 140 piglets) from two different farms located in central Spain were enrolled in the study.

- Farm L was a typical farrow-to-nursery farm with 2500 Danbred sows, weekly farrowing and weaning at 21-26 days. Farm L has a history of scours during lactation with the following aetiology: bacterial aetiology (Clostridium difficile, Clostridium perfringens type C and Escherichia coli) and virus aetiology (Type A Rotavirus).

- Farm A was a farrow-to-finish farm with 400 Landrace x Large White sows, with 4 weeks farrowing batches (80 sows per batch) and regular weaning at 21 days.
The farm has a low incidence of scours during lactation.

In order to minimize environmental effects, all sows were allocated to the same farrowing room on their respective farms. Each litter/sow was assigned to one of the five treatment durations (from 1 to 5 days of treatment). Within each litter, piglets were randomly allocated to one of the five treatment groups (Control: 2 piglets; 2.5 mL: 3 piglets; 25 mL: 3 piglets; 3 piglets: 50 mL: 3 piglets; 100 mL: 3 piglets) (see Table 21).

Table 21. Allocation of Piglets to Dose and Duration Groups

Litter	Day -2	Day -1	Day0	Day1	Day 2	Day 3	Day 4	Day5
Litter 1	Farrowing	Blood[1] Faeces[1] Weight$_1$	(3) 2.5 ml (3) 25 ml (3) 50 ml (3) 100 ml (2) Control	Blood[2] Faeces[2]				Weight$_2$
Litter 2	Farrowing	Blood[1] Faeces[1] Weight$_1$	(3) 2.5 ml (3) 25 ml (3) 50 ml (3) 100 ml (2) Control	(3) 2.5 ml (3) 25 ml (3) 50 ml (3) 100 ml (2) Control	Blood[2] Faeces[2]			Weight$_2$
Litter 3	Farrowing	Blood[1] Faeces[1] Weight$_1$	(3) 2.5 ml (3) 25 ml (3) 50 ml (3) 100 ml (2) Control	(3) 2.5 ml (3) 25 ml (3) 50 ml (3) 100 ml (2) Control	(3) 2.5 ml (3) 25 ml (3) 50 ml (3) 100 ml (2) Control	Blood[2] Faeces[2]		Weight$_2$
Litter 4	Farrowing	Blood[1] Faeces[1] Weight$_1$	(3) 2.5 ml (3) 25 ml (3) 50 ml (3) 100 ml (2) Control	(3) 2.5 ml (3) 25 ml (3) 50 ml (3) 100 ml (2) Control	(3) 2.5 ml (3) 25 ml (3) 50 ml (3) 100 ml (2) Control	(3) 2.5 ml (3) 25 ml (3) 50 ml (3) 100 ml (2) Control	Blood[2] Faeces[2]	Weight$_2$
Litter 5	Farrowing	Blood[1] Faeces[1] Weight$_1$	(3) 2.5 ml (3) 25 ml (3) 50 ml (3) 100 ml (2) Control	(3) 2.5 ml (3) 25 ml (3) 50 ml (3) 100 ml (2) Control	(3) 2.5 ml (3) 25 ml (3) 50 ml (3) 100 ml (2) Control	(3) 2.5 ml (3) 25 ml (3) 50 ml (3) 100 ml (2) Control	(3) 2.5 ml (3) 25 ml (3) 50 ml (3) 100 ml (2) Control	Blood[2] Faeces[2] Weight$_2$

[1]Samples collected before starting treatment.
[2]Samples collected the day after treatment ending.
(x) Number of piglets treated.
Weight$_1$: piglets' weight recorded on Day -1 (before treatment beginning).
Weight$_2$: piglets' weight recorded on Day 5.

Piglets were individually tagged, weighed and blood sampled on the day after farrowing (day 1). Pooled faecal samples were also collected on day 1. The incidence of scour and the severity of scour was recorded daily for each litter. Individual mortality was recorded daily.

Treatment commenced on day 2 after farrowing. The allocated dose of Px was administered to each piglet once daily, orally, using a volumetric pump dispenser.

Blood and faecal samples were taken from each pig on the day after their treatment ended. All blood samples were analysed for routine haematology and biochemistry (see Table 22below). All piglets were individually weighed on day 7.

Table 22. Hematology and Biochemistry Parameters Measured

Hematology Parameters	Biochemistry Parameters
• Hematocrit,	• Total protein, albumin, globulin
• Total white cell count,	• Urea, creatinine
• % neutrophils, % monocytes, % lymphocytes, % eosinophils	• ALKP, ALT, AST, cholesterol
	• Amylase, lipase
	• Calcium, Chloride, Phosphorus *

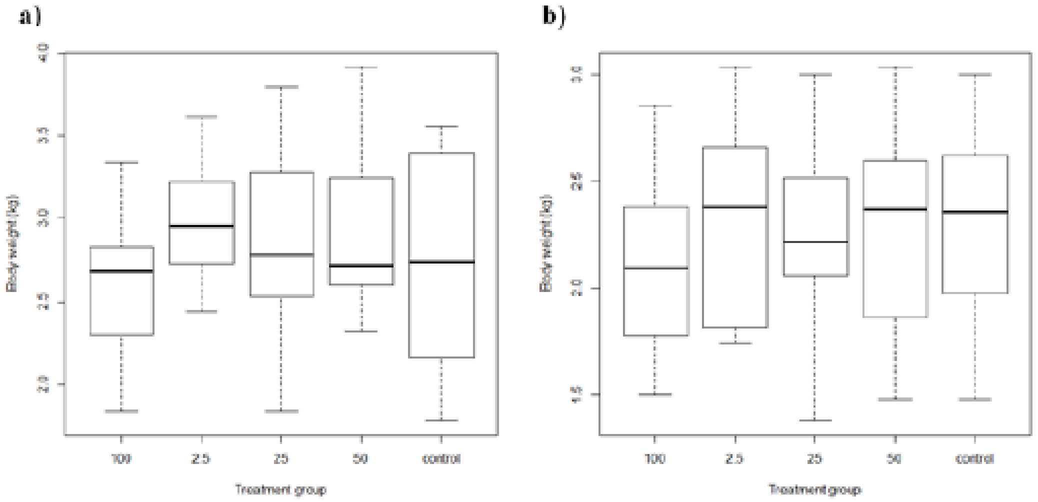
* Sodium results not available due to sample handling issues

Results

Body Weight and Average Daily Gain at day 7 of age – On both farms, pigs receiving 100 mL/day of Px tended to have decreased body weight and average daily gain (Figure 17). Other groups showed a large degree of variation and overlap in their body weights, with no detectable difference between treatment volume groups or duration of treatment.

Figure 17. Piglets Body Weight on Day 6

(a) = Farm A, (b) = Farm L



Mortality – only 4 pigs out of the 140 died. These 4 pigs were all from the same farm and same litter, and each pig was receiving a different dose of Px.

Incidence and Severity of Scour - No piglets with scour were recorded at Farm A. At Farm L, 17 of the 70 pigs developed scour. There was no association between the incidence of scour and the treatment dose or duration.

Hematology and Biochemistry – There were significant differences between the pigs from Farm A compared to Farm L. However, within each farm, there were no clinically significant differences in the hematology or biochemistry parameters between the treatment volume or treatment duration.

Conclusions

Px showed no deleterious health effects on piglets when given manually at doses that were between 3-5 times the usual intake volume. At high doses (100 /pig/day x 5 days), piglets showed decreased weight gain. This was attributed to competition for stomach capacity and milk intake, and would not be expected under normal conditions.

Statistical Analysis

Unless otherwise noted, statistical analysis was performed using R software (R Core Team (2015). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org

P values < 0.05 were considered significant, and P > 0.05 but less than 0.10 were considered a tendency.

LSM: least squares means

SE: standard error

SD: standard deviation

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