# Effect of Tonisity $Px^{TM}$ administration on intestinal morphology

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# Introduction

Intestinal morphology measurements, particularly increased villus height, are closely correlated with post-weaning weight gain. <sup>1,2</sup> The protein and amino acid profile of Px delivers key energy-producing substrates to the enterocyte which could in turn have an impact upon intestinal morphology. The aim of this study was to assess the impact of Px on intestinal morphology.

## Materials and methods

This study was approved by the ethics committee of the University of Lleida, 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). From days 2-8 of age, group B litters were given 500 mL of 3% Px solution daily in an open pan. Pigs were individually weighed at 1, 9 and 19 days of age. At 9 and 19 days of age, 18 pigs from each group were euthanized for gut morphological histopathology. Selection of the piglets was done based on their ADG from initial weight to slaughtering time: SD1 to SD8 or SD1 to SD20. Within litters, quartiles (25%, 50% and 75%) of the ADG were used to select three piglets per litter, from six litters (three per treatment) at SD8 and another three piglets per litter from the other six litters (three per treatment) at SD20. Remaining pigs from the litters were then split into 3 treatment groups and given either dry creep feed (D), gruel made with creep feed and water (WG) or gruel made with 3% Px solution and creep feed (PG). A full description of that trial is published elsewhere in these proceedings. From those pigs, 6 pigs per treatment group were euthanized at 5 days after weaning (~24 days of age) and a further 6 pigs per treatment group were euthanized 9 days after weaning (~28 days of age). Selection of those pigs was based on quartiles of the BW at weaning.

Intestinal morphology measurements were performed as published by Berkeveld et al.<sup>3</sup> in which sections were cut from 10%, 50% and 90% along the length of the small intestine. Villus height, crypt depth, villus and crypt density, and intestinal mucus thickness were measured. The average measurements per slide were used as experimental observation.

### Statistical analysis

The experimental unit for morphologic assay was the piglet. Gut morphology variables were analysing by a generalized linear model with the package GLM including the group, the intestine section and the study day as fixed effects. Interactions were explored but they were not significant so they were not included in the model. Tests were two-tailed and carried out with a risk  $\alpha = 5$  %. P-values of  $P \le 0.05$  were considered statistically significant, while  $0.05 < P \le 0.10$  was considered a near-significant trend. All statistical analyses were performed using R software.†

## Results

Histopathology analysis showed that pigs which received Px from day 2-8 of age had significantly greater (P < 0.05) villus height, villus density, and crypt density that persisted until at least 28 days of age, regardless of what creep feed they were given. Px pigs also tended to have greater intestinal mucus thickness (P = 0.08) (Table 1).

### Discussion

The results of this study were consistent with previously published studies, in which villi height was reduced by 50% at 5 days after weaning and the villus height/crypt depth ratio was lower in weaned piglets than in unweaned piglets. 1,4,5 However, in this study, Tonisity Px, when given in the first week of life, had a significant and favourable impact upon villus height, villus height/crypt depth ratio and villus density that persisted until at least 9 days after weaning. This effect was seen across all creep feed groups and ages. This has favourable implications for both pre-weaning and post-weaning weight gain.

The Px pigs also had a significantly thicker intestinal mucus layer post-weaning. This has clinical relevance because the intestinal mucus layer has been shown to be critical to maintenance of normal gut permeability and restoration of gut barrier function in inflammation and ischaemic processes.6,7 Further studies in pigs are indicated to examine the relationship between Px, intestinal mucus thickness and the development of post-weaning diarrhea in pigs.

<sup>†</sup> 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.

**Table 1:** Morphologic measurements of small intestinal sections.

	Villus height (μm)	Crypt depth (µm)	Villus height/ crypt depth ratio	Intestinal mucus thickness (µm)	Villus density (No./mm)	Crypt density (No./mm)
Day 9 and 19 measurements combined						
Control	409 ± 16.5	139 ± 5.1	$3.6 \pm 0.16$	536 ± 19.8	$9.3 \pm 0.22$	$20.6 \pm 0.55$
Px	$443 \pm 16.5$	$148 \pm 5.1$	$3.6 \pm 0.16$	584 ± 19.8	$8.7 \pm 0.22$	$18.3 \pm 0.55$
P-value	< 0.001	0.199	0.962	0.087	0.037	0.003
Day 24 and 28 measurements combined						
Control	$249 \pm 9.3$	$220 \pm 6.4$	$1.2 \pm 0.05$	$435 \pm 14.2$	$8.0 \pm 0.15$	$20.5 \pm 0.71$
Px	291 ± 10.4	$226 \pm 7.1$	$1.4 \pm 0.06$	481 ± 15.9	$7.6 \pm 0.17$	$21.8 \pm 0.79$
P-value	0.003	0.512	0.018	0.033	0.064	0.211

# References

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