Australasian Pork Research Institute Ltd APRIL

PROJECT SUMMARY

Project Number and Title: A1-103 Improving enteric health, understanding impact on gut microbiome and weaner performance through the use of protease enzymes.

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Aims and Objectives: This project aimed to investigate how a protease affects digestibility, its role in reducing the production of potential toxic breakdown products, and ultimately how weaner and grow-finisher pig performance may be improved and how protease could be included as part of an integrated health strategy. The study also sought to understand the impact of protease inclusion on the microbial community of weaner and finisher pigs, and the influence that targeted antibiotic use has on the microbiome and performance in grow-finisher pigs.

Experimental design: This research project initially involved two studies, one in weaner pigs and one in grower pigs, to look at the role of protease as part of an integrated health strategy. As a result of the outcomes observed, the project has resulted in four studies – two identical weaner studies where diet analysis suggested a repeat was necessary, a grower study as proposed, and another grower study looking at a higher rate of protease use offsetting a down-specification in amino acids.

The weaner experiments were a 2 x 2 factorial design with diet protein content (standard, 22.5% *vs* low, 18.5%) and protease inclusion (0 *vs* 150 ppm) were the factors over a 28-day experimental period, with growth performance and blood, digesta and faecal samples collected to assess markers of metabolism and digestion and microbiota assessment.

The first grower study was also a 2 x 2 factorial design with protease inclusion (0 *vs* 125 ppm) and medication (nil *vs* Tiamulin) as the factors over a 7-week period, with growth performance and faecal samples collected to assess markers of digestion and microbiota assessment.

The second grower study assessed the performance of pigs over the last 8 weeks of production when protease was included at a higher rate, 250 ppm, and its ability to offset total amino acid content of the diet.

Key Findings: The weaner studies within this project showed that protease had little impact on performance at low inclusion rates on diets that are formulated with generally highly digestible sources of amino acids. When the inclusion rate of protease was doubled to 250 ppm in the second grow-finisher study and the matrix values associated with the uplift in amino acid digestibility were taken into account, the ability to maintain performance indicates that proteases likely have a role in profitable pork production, and that the levels used in the weaner studies were likely too low to illicit a response.

Evidence generated suggests that improvements in the intestinal health of the weaner pig were able to be achieved, with a decreased level of calprotectin observed at the end of the weaner trial, indicating a reduction in inflammation in the gut after 28 days. The addition of protease most likely reduced the amount of substrate available for the proliferation of pathogenic bacteria. Other factors such as differences in plasma urea nitrogen, digesta ammonia-N and total volatile fatty acid production were in agreement with previous studies that have investigated the role of low protein diets in weaner pig production.

Similarly, the reduced relative abundance of *Enterobacteriaceae* and increased abundance of *Prevotellaceae* in weaner pigs fed lower protein diets in this study agrees with previous studies, with a higher abundance of *Prevotellaceae* having been reported as a dominant feature of the faecal microbiota in healthy pigs as compared to pigs suffering diarrhoea after weaning. However, as the weaner pigs in this study were not subjected to a post-weaning diarrhoea challenge, these apparent improvements in intestinal health did not result in an increase in the efficiency of growth.

Lower levels of protease (125 ppm) were not able to enhance the performance of grower pigs fed adequate diets. However, there was some evidence of protease being able to modify protein metabolism such that a lower immune response was seen through a reduced level of calprotectin. When a higher level of protease (250 ppm) was applied to a diet below amino acid specifications, performance was able to be restored. Whilst it was not able to be determined if the higher level of protease inclusion could enhance an adequate diet, the application of a protease to better utilize protein could allow for the inclusion of lower levels of protein when access to feedstuffs is challenging, at a lower cost per unit gain.

The largest impact on the microbiome of the grower pig was neither the antibiotic treatment nor the differing levels of protease, but rather simply the effect of sampling time. The ratio of *Bacteroidetes*:*Firmicutes* observed at day 14, was significantly altered from that at day 47, as well as that observed in the weaner study that was more reflective of the day 47 measure. It is possible that the shift in food and housing that occurred to these pigs at day 0 of the experiment caused a major, but temporary shift, in their microbiome with similar findings being observed in tonsil microbiome communities.

This study showed that determining the correct inclusion rate of protease is important to achieve the desired outcome of improved performance.

Applications to Industry: The evidence generated in this project suggests that improvements in the intestinal health of the weaner pig were able to be achieved, whilst other factors such as differences in plasma urea nitrogen, digesta ammonia-N and total volatile fatty acid production were in line with previous studies that have shown the benefits of low protein diets, supporting the use of low protein diets when pigs are subject to a postweaning diarrhoea challenge.

The largest changes in microbiome were not a result of treatment, but sampling time, with the moving of the pigs to the research finisher facility and the sudden transition to a new shed and new diets resulting in disturbance of the relative abundance of the most common bacteria phyla. Understanding the impact of change on the microbiome, and developing strategies to minimize this in commercial environments, is likely to result in decreased gut disturbance and reduce performance checks.

Finally, the project was able to show that the inclusion of a higher dose of protease in a diet fed to grow-finish pigs was able to positively influence their performance, adding another management tool to deal with increasingly scarce sources of protein.