**////Title: Investigating the Impact of Tannins on Gut Bacteria in Pigs**

**////Stand-first**:

Weaning is an important time in the pig lifecycle, and changes in diet and environment can lead to unbalanced gut microbiota and pathogen colonisation. Prof. Luciana Rossi, Dr. Matteo Dell’Anno from the University of Milan, and Dr. Maria Luisa Callegari from Catholic University of Sacred Heart, have been investigating the impact on gut bacteria of adding natural compounds known as tannins to piglet food. Importantly, they found that tannins do affect the gut bacteria; with increases seen in bacteria associated with improved growth and gut health, and in particular, those that produce butyrate – a substance with proven health benefits.

**////Body text:**

Across the globe, approximately 3.5 million pigs are eaten every day. It remains without a doubt that work to improve animal welfare and food production is vital. Prof. Luciana Rossi, Dr. Maria Luisa Callegari, and Dr. Matteo Dell’Anno, have published a study investigating the impact of adding chestnut and quebracho tannins to the food of piglets on the types of bacteria in the gut – the gut microbiota.

Tannins are natural compounds found in plants, including those we eat such as tea and grapes. They are a diverse group of compounds and can be split into distinct categories. As such, tannins from chestnuts are mainly hydrolysable and those from quebracho are defined as complex. Tannins are known to have antibacterial, antioxidant and antidiarrhoeal properties, and so could be added to pig feed to improve gut health.

Weaning is a stressful time for piglets due to being removed from their mother, moving to an often-crowded farm environment, and changes to their diet which can alter the gut microbiota. These stresses and changes to gut bacteria often lead to weaned piglets developing diarrhoea, which in turn leads to reduced growth.

Traditionally in farming, antibiotics have been used in the post-weaning period to reduce intestinal problems such as diarrhoea, and in various parts of the world to help growth. However, antibiotics are known to disrupt the gut microbiota which can lead to more diarrhoea, and the overuse of antibiotics in farm animals contributes to the ongoing antibiotic resistance crisis.

Previous studies have found that chestnut tannins improve feed efficiency and growth in piglets and adult pigs, as well as prevent diarrhoea in piglets infected with a pathogenic *E. coli* strain. The current study is the first to look at the effect of adding a mixture of chestnut and quebracho tannins to animal feed on gut microbiota.

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This study involved 120 weaned piglets, divided into two groups. The control group received a normal diet, and the other received the same feed but supplemented with 1.25% tannin extract from chestnut and quebracho trees for 40 days. Both diets met the dietary requirements for weaned piglets. The study aimed to highlight the microbial changes happening in the gut of weaned piglets when tannins were added to feed.

Throughout the study, the team recorded mortality and clinical symptoms of diarrhoea, and piglet faeces were collected at the end of the study for laboratory investigation. The type and amount of short-chain fatty acids (the main products of digestion in the large intestine) were also analysed. The numbers and types of bacteria in the stools were detected using bacterial genomic DNA amplification and sequencing.

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Symptoms of diarrhoea were recorded, with the most noted between days 14–28. Slightly lower levels of diarrhoea were recorded in the tannin fed piglets, but this difference was not statistically significant.

Analysis of bacterial genomes showed significant differences between the gut microbiota of piglets on the control and tannin supplemented diets. The observed species index, which measures the number of different species in a sample, and other indices reflecting the species richness and evenness, were significantly higher in the group fed additional tannins. If a bacterial population is more diverse it is likely to be more stable, as well as more resistant to environmental changes.

Several groups of bacteria were increased in samples collected from pigs fed the tannins-based additive. These included *Peptococcus*,which previous studies have shown to be more abundant in healthy pigs and also linked with healthy weight gain in piglets. Other bacteria were significantly less likely to be found in the samples taken from the tannin fed piglets, and these included *Prevotella* which have been associated with higher levels of post-weaning diarrhoea in piglets.

Laboratory analysis of short-chain fatty acids in the piglet stools showed that the total concentration of these metabolites was similar across both the control and tannin-fed piglets. However, a significant increase in butyrate was detected in the tannin-fed animals. Butyrate is known to have anti-inflammatory properties and has been shown to enhance intestinal cell health and mucosal immunity in numerous species, including pigs. Other work has shown that an increase in butyrate-producing bacteria can reduce the growth of pathogenic bacteria such as *Salmonella*.

The team then analysed the DNA sequences of the bacteria in the stool samples to predict the proteins they would produce. The analysis of bacterial genes found a significant reduction in genes involved in the production of lipopolysaccharides. LPSs are part of the Gram-negative bacterial cell wall and have been linked with inflammation due to activating the host’s immune cells. The reduction in lipopolysaccharide genes in the samples from tannin-fed pigs could be due to a reduction in Gram-negative bacteria such as *Prevotella*. This potential reduction in lipopolysaccharide could lead to a decrease in inflammation in the guts of piglets fed tannin enriched diets.

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This study by Prof. Rossi, Dr. Callegari, and Dr. Dell’Anno, is the first to investigate the effects of chestnut and quebracho tannins on performance, diarrhoea incidence and gut microbiota of piglets. The results of the work have very positive future implications, with the addition of tannins to the diet of weaned piglets increasing the levels of healthy bacteria – in particular those that produce butyrate, a substance with proven health benefits. Whilst this work shows the potential benefits of adding tannins to piglet feed, all the piglets in the study were healthy so there was no scope for improvement to be seen. The team has suggested the next step will be to test the tannin diet in experimentally infected piglets.

This SciPod is a summary of the paper ‘A mixture of quebracho and chestnut tannins drives butyrate-producing bacteria populations shift in the guy microbiota of weaned piglets’ from the open access journal, PLOS One. DOI: <https://doi.org/10.1371/journal.pone.0250874>

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