

Effects of Tannins on Diarrhea and Growth of Weaned Piglets

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Abstract

Tannins, found in many plants, have traditionally been used to treat diarrhea in pigs, but few *in vivo* studies have evaluated their effects in piglets. The objective of this study was to evaluate the effects of tannin supplementation on post-weaning diarrhea and the growth performance of weaned piglets. Eighty-one Duroc x (Landrace x Yorkshire) piglets were randomly allocated to treatment based on age and sex-balanced groups: 27 piglets for the control group with basal feed (CON) and 54 piglets for the treatment group with a supplementation of a 0.25-g tannin blend for every 1.0kg of the basal feed (TAN). At weaning, each piglet was individually identified by ear tag and had their diarrhea status evaluated for 30 days. Body weights were recorded at weaning (IBW) and at the end of the nursery period (FBW) to calculate the average daily gain (ADG). Diarrhea was evaluated by scoring fecal consistency twice per day. From this trial, the odds-ratio for the treatment and for IBW were 3.014 and 0.841, respectively, showing positive effects that the TAN treatment and increased IBW had on the diarrhea rate. Increasing the IBW by one kilogram led to a decrease in the diarrhea rate by 0.841 times. The FBW and ADG in TAN were higher ($P = 0.0089$ and $P = 0.0044$, respectively) than those in CON. It was concluded that tannin supplementation at 0.25g per 1.0kg of feed reduces post-weaning diarrhea and improves ADG in weaned piglets. Higher body weight at weaning also lowers the incidence of diarrhea.

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Keywords

Plant extraction, piglet, disease, growth rate

Introduction

Weaning is one of the most challenging periods because piglets are subjected to many substantial nutritional, physiological, environmental, and social challenges, causing a high proportion of post-weaning diarrhea and poor growth performance or even death. The nutritional change from milk to solid feed causes a decline in

colostrigenic immunity and the disappearance of lactogenic immunity (Fu *et al.*, 1990; Poonsuk & Zimmerman, 2018). Further exacerbating the situation, the immature gut, impaired intestinal epithelial barrier function, and undeveloped mucosal-immunity (Gresse *et al.*, 2017; Moeser *et al.*, 2017; Pluske *et al.*, 2018) make these organs more susceptible to pathogenic agents that can cause diarrhea. In addition, the changing environment from farrowing pens to weaning pens, where piglets are mixed with unfamiliar pigs, may expose them to new pathogens, such as *Escherichia coli* (Van Beers-Schreurs *et al.*, 1992), rotavirus (Pettersson *et al.*, 2019), porcine epidemic diarrhea virus and porcine delta coronavirus (Niederwerder & Hesse, 2018). Post-weaning diarrhea may have negative effects on the growth performance of pigs and the economic efficiency of the pig production sector. Therefore, finding an effective strategy for prevention of post-weaning diarrhea is essential.

Various strategies to prevent post-weaning diarrhea in piglets have been studied and applied to reduce the use of antibiotics in the pig industry (Canibe *et al.*, 2022). These authors reported that the major feed strategies for post-weaning diarrhea prevention include changes in feed ingredients, feed additives, or feed forms, but that the efficacy of these measures depends on many factors including the animal's health status, as well as the level of feed intake and composition of the feed. Among these strategies, the use of plants and plant extracts are considered promising methods that can potentially reduce the use of antibiotics and zinc oxide for post-weaning diarrhea prevention and control in piglets (López-Gálvez *et al.*, 2021). The supplementation of botanicals or their extracts may have both antimicrobial effects and immune-regulating and antioxidant effects (Yang *et al.*, 2015).

Tannins are known as a traditional medicine used for the treatment of chronic diarrhea in humans (Lewis & Elvin-Lewis, 2003) and animals due to their antimicrobial, antioxidant and anti-inflammatory properties (Huang *et al.*, 2018). Tannins are polyphenolic compounds that can be classified as hydrolysable, condensed (Ma *et al.*, 2021) or complex (Canibe *et al.*, 2022).

Tannins are commonly found in many plants, including forages (Barry, 1989), cereals (Ayyagari *et al.*, 1989), medicinal herbs (Neumann *et al.*, 2022), fruits (Mercurio & Smith, 2008; Tee & Ding, 2010), and tea (Savolainen, 1992). Several studies have demonstrated the efficacy of tannins in preventing diarrhea in piglets (Liu *et al.*, 2013; Girard *et al.*, 2018; Dell'anno *et al.*, 2021), but these studies have generally involved small sample sizes, and few *in vivo* trials have directly examined their effects on post-weaning diarrhea (Huang *et al.*, 2018). Therefore, this study was conducted to evaluate the effects of dietary tannin supplementation on post-weaning diarrhea occurrence and growth performance of weaned piglets.

Materials and Methods

Experimental design

This study was conducted at a commercial pig farm in the Dong Nai province in Southern Vietnam, and was over 30 days from September 29 to October 29, 2022. Eighty-one weaned, DLY piglets (27-42 days of age) from Duroc boars and Landrace × Yorkshire sows, were used in this trial. There were 31 females and 50 castrated males.

On weaning day, each individual animal was given an ear tag and was weighed to obtain their initial body weight (IBW, 6.60 ± 1.39 kg, Mean \pm SD). After stratifying the piglets according to age and sex, the animals were randomly allocated to one of three pens. One pen was a control group where 27 piglets were fed a basal diet (CON). The two other pens (27 pigs pen⁻¹) were the treatment group (TAN), and those pigs were fed a basal diet supplemented with a 0.25-g Silvafeed Nutri P powder blend with 75% tannin (tannin blend) produced by Silvateam (San Michele Mondovi, Italy) for each 1.0 kg of feed. The numbers of piglets in the CON and TAN groups were 27 (11 females and 16 male castrated males) and 54 (20 females and 34 castrated males), respectively.

The powder blend used in this experiment contained 75% hydrolysable ellagitannins extracted from Chestnut wood (*Castanea sativa*)

by using hot water. Two types of feed were provided to the piglets: commercial feed in pellet form (ME 3,200 Kcal kg⁻¹, crude protein 20%, lysine 1.2%, calcium 0.5-0.8% and phosphorus 0.4-0.5%) and on-farm mixed feed in powder form (ME 3,300 Kcal kg⁻¹, crude protein 19%, lysine 1.2%, calcium 0.5-0.8% and phosphorus 0.4-0.5%).

The tannin blend was added to the powder on-farm mixed feed during feed processing at the farm. For transition from one feed to another according to the stages, during the first 20 days of the experiment, the mixture contained 70% commercial feed and 30% on-farm mixed feed. The commercial feed in pellet was crushed before mixing with powder feed. The last 10 days of this study, the proportions of commercial and on-farm mixed feed were 60% and 40%, respectively. The feed was supplied twice per day (at 07:30 and 14:00) at the rate of 130g per day per piglet during the first three days, and then increasing up to 400g by the end of the experiment. Before our study, diarrhea was observed on the weaning pigs in all pens on the farm.

The diarrhea status was recorded individually for all piglets every day at 09:00 and 15:00 during the 30-day trial. Some of the piglets were lost in the last stages of the experiment, therefore we actually obtained only 2,342 measures instead of 2,430 from the 81 piglets monitored for the 30 experimental days. The health status of each animal was evaluated according to the methods of Thomas *et al.* (2021) based on a scoring grid, namely D0 = normal state - healthy animals, D1 = onset of diarrhea - soft feces, and D2 = marked diarrhea - liquid feces. Animals with scores of D1 and D2 were considered diarrheic. The diarrhea status determined d for the 30 experimental days. The diarrhea rate was calculated based on the methods of Yu *et al.* (2020) as follows: diarrhea rate (%) = total cumulated number of piglets with diarrhea divided by the total cumulated number of piglets and multiplied by 100.

At the end of the experiment, individual body weights were recorded y as the final body weight (FBW, 10.64 ± 1.95kg). Average daily

gain (ADG) was calculated based on the FBW, IBW and duration of the experiment.

The experiment was carried out according to National Technical Regulation QCVN 01-14: 2010/BNNPTNT (Conditions for biosecurity of pig farms) and TCVN 1547:2020 (Animal feeding stuffs - Compound feeds for pigs).

Statistical analysis

Analyzing the effects of diarrhea rate, growth performance and statistical parameters were performed using SAS® OnDemand for Academics.

Diarrhea status

The effect of tannin supplementation on the diarrhea status was assessed using logistic regression with the LOGISTIC procedure in SAS. Two fixed factors (tannin supplementation and sex) and two covariates (weaning age and body weight at weaning) were included in the initial model. The backward method was applied to remove the non-significant effects ($P = 0.15$). The weaning age was removed from the initial. The final logistic regression model was:

$$\log[p_i / (1 - p_i)] = \beta_0 + \beta_1 \times TA + \beta_2 \times IBW + \beta_3 \times S$$

where p_i = probability of a diarrhea if piglet i (0 = healthy and 1 = diarrhea), β_0 = intercept, TA = effect of tannin supplementation (CON and TAN), IBW = covariate effect of body weight at weaning, S = effect of sex (female and castrated male), and β_1 , β_2 and β_3 = slopes for TA, IBW, and S, respectively. The odd ratio (OR) was calculated to compare the effect of the treatments (CON and TAN) on the diarrhea rate.

Growth performance

Data on the growth performance was analyzed using the following statistical model with the GLM procedure in SAS. The weaning age varied among the piglets and was therefore added in the model as a covariate.

$$y_{ijkl} = \mu + TA_i + S_j + \beta \times D_k + \varepsilon_{ijkl}$$

where y_{ijkl} = variable of growth performance (IBW, FBW and ADG), μ = overall mean, TA_i = effect of tannin supplementation (CON and TAN), S_j = effect of sex (female and castrated male), β = regression coefficient, D_k =

covariate effect of weaning age, and ε_{ijkl} = residual. The number of observations (n), least square mean (LSM), and standard error (SE) are presented in the result section. Pairwise comparisons between the LSMs were made using Tukey's test and were considered statistically significant when $P < 0.05$.

Results

Diarrhea rate

During the experiment, 12 out of the 81 piglets died (4 from the CON group and 8 from TAN group). The overall mortality rate was 14.81%, with no significant difference between the CON and TAN groups (exact fit). The cumulative counts of diarrhea status during the 30 days of the experiment are shown in **Table 1**. The diarrhea rates were 15.39% in CON and 5.14% in TAN.

The regression parameters of tannin supplementation and body weight at weaning (BWW) were 1.1034 ($P < 0.0001$) and -0.1737 ($P = 0.0043$) respectively (**Table 2**). The diarrhea rate in the piglets was significantly affected by tannin supplementation ($P < 0.0001$) and the BWW ($P = 0.0043$).

The diarrhea rate of the piglets in CON was 3.014 times (95% confidence interval [CI]: 2.223 to 4.087) higher than that in TAN (**Table 3**). Similarly, an increase of one kilogram in the IBW was associated with a 0.841-fold decrease in the diarrhea rate (95% CI: 0.746 to 0.947) (**Table 3**).

Growth performance

The effects of tannin supplementation and sex on the growth performance of piglets are shown in **Table 4**. The coefficient of determination of the statical models, namely the fixed effects (tannin supplementation and sex) and covariate effect (weaning age), for the IBW, FBW and ADG were 0.14, 0.13, and 0.14, respectively. The weaning day was also correlated to the IBW ($P = 0.0082$), but it did not affect the FBW ($P = 0.4974$) and ADG ($P = 0.0838$). The FBW and ADG were significantly higher ($P = 0.0089$ and $P = 0.0044$, respectively) for the pigs in the in TAN treatment compared to those pigs in CON (**Table 4**).

Inversely, the IBW ($P = 0.5931$), FBW (0.9004) and ADG ($P = 0.2865$) were observed to be similar between females and castrated male pigs (**Table 4**).

Table 1. Effect of tannin supplementation on diarrhea rate in weaned piglets

| Diarrhea status | Control (CON) | Tannin (TAN) | Total |
|-----------------|---------------|----------------|--------------------|
| Diarrhea | 121 (15.39%) | 80 (5.14%) | 201 |
| No diarrhea | 665 (84.61%) | 1,476 (94.86%) | 2,141 |
| Total | 786 | 1,556 | 2,342 ¹ |

Note: Control (CON), Tannin (TAN); In each cell, the first value is a cumulative observed count and the second value in parentheses is a diarrhea rate; ¹Accumulated count of diarrhea status of 81 piglets monitored for 30 experimental days.

Table 2. Estimated parameters from logistic regression

| Parameter | Estimate | SE | χ^2 | P-value |
|------------------------|----------|--------|----------|---------|
| Intercept | -1.7436 | 0.4173 | 17.4591 | <0.0001 |
| Tannin supplementation | 1.1034 | 0.1553 | 50.4572 | <0.0001 |
| Body weight at weaning | -0.1737 | 0.0609 | 8.1440 | 0.0043 |

Table 3. Odds ratio estimation (OR)

| Effect | OR | 95% confidence interval |
|------------------------|-------|-------------------------|
| CON:TAN | 3.014 | 2.223-4.087 |
| Body weight at weaning | 0.841 | 0.746-0.947 |

In the present study, only 81 piglets and 3 pens were available. The animals were allocated 27 piglets per pen to have a similar density. One pen was for CON, and two other pens were for TAN. The feed intake was recorded for a group of 27 piglets. Consequently, 3 sample sizes for the feed intake were observed, including one for CON and 2 for TAN. The body weight gains over the 30 day experiment, were 142.6 and 56.9kg for TAN and CON, respectively. Correspondingly, total feed intake was 388.9kg and 179.6kg for TAN and CON, respectively. As a result, feed to gain ratios were 2.73kg for TAN and 3.15kg for CON. Pigs on the TAN treatment were numerically more efficient than the CON pigs; however, we did not make a statistical comparison due to the limitation of number of observation (one pen for CON, two pens for CON).

Discussion

Diarrhea rate

Post-weaning diarrhea remains a significant challenge in pig production, adversely affecting growth and economic outcomes. In order to reduce antibiotic use in the pig industry, alternative strategies such as botanicals are increasingly being investigated to prevent post-weaning. In our study, tannins, a compound commonly found in many plants, were effective in reducing post-weaning diarrhea and improving piglet growth performance. The proportion of piglets affected by diarrhea was lower when tannins were supplemented in the diet. This result is consistent with previous studies. Girard *et al.* (2018) reported that supplementation of 1% chestnut extract significantly reduced diarrhea incidence and duration in piglets. Song *et al.* (2021) found that supplementation of 0.05%, 0.1%, 0.2%, or 0.4% of tannic acid in the diet of weaned piglets linearly reduced the diarrhea rate and diarrhea index. Tannins have a wide range of effects on inhibiting or slowing down diarrhea-related pathogens. Girard & Bee (2020) summarized the effects of tannins on the prevention of microbial infections, including the prevention of bacterial adhesion to the intestine, inhibition of bacterial enterotoxins and channels in electrolytes and

water secretion into the lumen, and the prebiotic effects in the gut that benefit the resistance ability of animals to diarrhea-related pathogens. In our study, hydrolysable tannins were used and this form has been demonstrated to inhibit the total activity of cecal bacteria (Bee *et al.*, 2017).

In our study, the diarrhea rate was reduced when the initial body weight at weaning increased. Ming *et al.* (2021) reported that piglets weaned at a later age had a higher weaning body weights and lower diarrhea rates than those weaned at an earlier age. This can be explained by a lower level of digestive enzymes, especially the pepsin concentration in the stomach of earlier weaning piglets due to the lower pH in the stomach of younger animals (Suiryanrayna & Ramana, 2015). This may cause the excess nutrients passing through the stomach and intestine to enter the hindgut for the proliferation of harmful bacteria, and thus resulting diarrhea. Therefore, increasing body weight at weaning may help prevent post-weaning diarrhea.

The effect of tannic acid on diarrhea in piglets depends on its dose in the diet. In our study, at 0.25g tannin blend per kg, the diarrhea percentage was 5.14% during the 30 days of the experiment. This is consistent with the previous study by Song *et al.* (2021) who reported a diarrhea rate of 7.34% at 0.2% tannic acid dose during the 28 days of their experiment. An increase in the dose of tannic acid in the diet was shown to linearly decrease the diarrhea rate (Song *et al.*, 2021), but it may have negative impacts on growth performance due to the affinity of tannins with proteins, digestive enzymes, and polysaccharides in monogastric animals (Mueller-Harvey, 2006; Viveros *et al.*, 2011; Ebrahim *et al.*, 2015) unless more than 1% of tannic acid was supplemented their diets (Rezar & Salobir, 2014).

In our study, increasing the body weight at weaning led to a reduction in the diarrhea rate in weaning piglets. The study of Richard *et al.* (2013) confirmed that diarrhea rate was highly correlated with body weight. Pigs with a lower weight had a higher diarrhea rate. Having body weight at birth below 1.1kg increased the diarrhea rate by 2.3 times (Eriksen *et al.*, 2021). Increasing body weight at weaning

Table 4. Effect of tannin supplementation and sex on growth performance of DYL piglets

| Variable | Treatment | | | | | | | Sex | | | | | | | WA (<i>P</i> -value) | R ² |
|----------|-----------|-------------------|------|-----|--------------------|------|-----------------|--------|------|------|----------------|-------|------|-----------------|-----------------------|----------------|
| | CON | | | TAN | | | <i>P</i> -value | Female | | | Castrated male | | | <i>P</i> -value | | |
| | n | LSM | SE | n | LSM | SE | | n | LSM | SE | n | LSM | SE | | | |
| IBW | 27 | 6.26 | 0.26 | 54 | 6.74 | 0.20 | 0.1426 | 31 | 6.42 | 0.24 | 50 | 6.58 | 0.20 | 0.5931 | 0.0082 | 0.14 |
| FBW | 23 | 9.75 ^b | 0.40 | 46 | 11.10 ^a | 0.28 | 0.0089 | 29 | 10.4 | 0.35 | 40 | 10.44 | 0.31 | 0.9004 | 0.4974 | 0.13 |
| ADG | 23 | 111 ^b | 8.12 | 46 | 140 ^a | 5.72 | 0.0044 | 29 | 131 | 7.23 | 40 | 121 | 6.25 | 0.2865 | 0.0838 | 0.14 |

Note: Number of records (*n*), Least square means (LSM), Standard error, Control (CON), Tannin (TAN), Weaning age (WA), Initial bodyweight (IBW, kg), Final bodyweight (FBW, kg), Average daily gain (ADG, g day⁻¹), Coefficient of determination (*R*²). Within a row and according to factor (Treatment, Sex), LSMs followed by different letters are significantly different (*P* < 0.05).

could also be a solution to reduce post-weaning diarrhea in pig production.

Growth performance

Although tannins are known to have an anti-nutritional factor (Pathaw *et al.*, 2022), in some cases, especially at an appropriate dose, they may have positive effects on pig performance. In this study, supplementation of 0.25g tannin blend per kg in the diet improved average daily gain. This is similar to a previous study that reported that 0.45% hydrolysable tannin supplementation helped increase the ADG of piglets (Biagi *et al.*, 2010). Similarly, Girard *et al.* (2020) found that 2% of hydrolysable tannins/condensed tannin supplementation increased the ADG of piglets. According to Caprarulo *et al.* (2021), the mechanism for improving pig performance by tannin supplementation is not fully understood, but the literature data indicates that low (<1%) or medium (≥ 1 -2%) dietary tannin rates benefited growth performance of post-weaning piglets. In this study, we used a low tannin dose and thus it resulted in an improved ADG of piglets compared to those in the control group.

The sex of the piglets did not affect the IBW, FBW and ADG. This is in agreement with Vázquez-Gómez *et al.* (2020) and Škorjanc *et al.* (2007). The body weight and ADG of the pre-weaning piglets increased gradually through the weeks of age, nevertheless the effect of sex was not observed (Škorjanc *et al.*, 2007). Similarly, these traits of post-weaning piglets were not different between females and castrated males (Vázquez-Gómez *et al.*, 2020).

Conclusions

The supplementation of tannins at a 0.25-g tannin blend per kg in the diet had positive effects on the diarrhea incidence and average daily gain of weaned piglets. An increase in body weight at weaning also reduced the diarrhea percentage of the pigs. Further studies should be conducted with different levels of tannins supplementation in weaned piglets to identify the optimal dose that benefits post-weaning diarrhea while having no negative impacts on growth performance.

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