Modulation of plasma antioxidant activity in weaned piglets by plant polyphenols

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Abstract

This study was conducted to evaluate the effect of plant polyphenols (PP) on antioxidant activity in weaned piglets. First, a uniform design, one optimising an experimental technique that can rationally arrange the concentrations of mixture components, was used to obtain the best PP mixture of apple, grape seed, green tea, and olive leaf polyphenols based on in vitro antioxidant capacity and inhibitory action on bacterial growth. Second, the optimised PP mixture was tested in vivo with an efficacy trial on piglets. The optimal effects of the mix were observed in vitro when apple, grape seed, green tea, and olive leaf polyphenols and a carrier (silicon dioxide) accounted for 16.5, 27.5, 30, 2.5 and 23.5%, respectively, of the mixture. Forty-eight weaned piglets were randomly allocated to two dietary treatments (6 replicates of 4 piglets each per treatment) and fed a control diet (CTR) or PP supplemented with 0.1% of the optimised PP mixture. Dietary PP did not affect growth performance compared to the CTR group. Plasma total protein, urea nitrogen and lysosome content were not affected by dietary treatment. No differences of E. coli or Clostridia counts in the faeces and caecum content between the CTR and PP groups were observed. A reduced malondialdehyde concentration in the PP group was observed on day 21 compared to the CTR group (P=0.02). In conclusion, the prepared PP mixture has the potential to improve plasma antioxidant activity.

Introduction

As a powerful type of naturally bioactive compound, polyphenols are found in food, such as fruits, wine and tea, as well as in wood waste, such as larch bark (Hammer et al., 1999; Heim and Tagliaferro, 2002). It has long been known that polyphenols, such as flavonoid and epicatechin, are efficient scavengers of free radicals (Sichel et al., 1991). Plant polyphenols (PP) have also exhibited antimicrobial activity against bacteria that cause food-borne diseases (Taguri et al., 2004; Percival et al., 2006; Kim et al., 2009). Previous reports have shown that polyphenols from olive leaves exert great antioxidant activity (Sudjana et al., 2009), tea polyphenols improve the balance of gut microflora in calves (Ishihara et al., 2001) and inhibit the proliferation of Chlamydia (Yamazaki et al., 2003), apple polyphenol extracts prevent damage to human gastric epithelial cells (Graziani et al., 2005), and polyphenols from grape seeds could prevent oxidative damage to cellular DNA in vitro (Fan and Lou, 2004). Thus, antioxidant and antimicrobial activity, as well as gut modulating effects of PP, have been suggested (Windisch et al., 2008). Although large studies have already demonstrated the antioxidant and antimicrobial efficacy of PP in vitro, testing the efficacy of a PP mixture of several functional polyphenols in animal trials could further support that work.

Unfortunately, it is impossible to analyse and assess the antioxidant and antimicrobial activities of all conceivable PP mixtures with different compositions experimentally. Therefore, optimal experimental design methods have been employed to arrange a set of mixtures that characterise various mixture compositions in a real environment and describe the mutual interactions of the mixture components with a limited number of experimental runs (Zhang et al., 2010). A uniform design, a type of space filling design that can be used for experiments when the underlying model is unknown, was proposed and developed by Fang and Wang (Fang, 1980; Fan and Luo, 2004). This design has been successfully used in various fields, such as chemistry and chemical engineering, pharmaceutics, quality engineering, system engineering, survey design, computer science and natural sciences. The purpose of this study was to evaluate a prepared PP mixture, optimised by a uniform design, based on in vitro antioxidant capacity and microbial modulation ability and to investigate the efficacy of the mix on the antioxidant activity of post-weaning piglets.

Materials and methods

Preparation of the plant polyphenols mixture

The complex formulations of 4 polyphenols [apple (Tianjin Jianfeng Natural Product R&D Co. Ltd., Tianjin, China), grape seed (Tianjin Jianfeng Natural Product R&D Co. Ltd.), green tea (Taiyo Green Power Co. Ltd., Wuxi, China) and olive leaf (Ningbo Sino-Taihao Herbal Science Co. Ltd., Ningbo, China)] and silicon dioxide as a carrier were tested using a uniform design to obtain the optimal PP mixture in the Key Laboratory of Feed Biotechnology of the Ministry of Agriculture of the Chinese Academy of Agricultural Sciences (Beijing, China). In this study, apple, grape seed, green tea and olive leaf were used for the PP mixture. Thus, their concentrations were chosen as the critical variables and designed as X₁, X₂, X₃...
and X4, respectively. The experimental range of each variable was equally divided into 6 levels and is presented in Table 1. According to the uniform design, only 15 tests needed to be performed. Uniform design table U15 (154) was selected to arrange the 15 tests (Table 2). For the PP mixture solution (0.1%) preparation, a 200 mg solute (polyphenols and carrier) was added to 200 mL of 0.85% saline water. The undissolved carrier served to balance the solute in the liquid.

In vitro 2,2-diphenyl-1-picrylhydrazyl radical-scavenging

The radical 2,2-diphenyl-1-picrylhydrazyl (DPPH), which is a stable and commercially available organic nitrogen radical, has a UV-visible absorption maximum at 515 nm. In its radical form, DPPH absorbs at 515 nm, but the absorption decreases due to the reduction of DPPH in the presence of an antioxidant [phenolic compounds (AH)] or a radical (R) species:

DPPH+AH→DPPH-H+A; DPPH+R→DPPH-R

For the determination of the DPPH radical-scavenging ratio, 2 mL of PP mixture were added to the same amount of a 6×10⁻⁵ mol methanol-DPPH solution. The DPPH radical was purchased from the Sigma-Aldrich Shanghai Trading Co. Ltd. (Shanghai, China). For the control, a 2 mL DPPH solution+2 mL of methanol was used and a 2 mL methanol+2 mL sample solution was added as a blank. After further mixing at 37°C for 30 min, all absorbance (A) levels were measured using a UV-visible spectrophotometer (Tongfang Inc., Shanghai, China) at 515 nm. Absorbanes were converted to the DPPH radical-scavenging rate according to the following equation:

DPPH radical-scavenging ratio (%)=[(Acontrol-Asample-Ablank)/Acontrol]×100%

In vitro bacterial growth inhibition

Three pathogenic bacteria, including Escherichia coli (E. coli), Staphylococcus aureus (S. aureus) and Salmonella typhi (S. typhi) purchased from the Chinese Institute of Veterinary Drug Control (Beijing, China), were selected to determine the antimicrobial activity of the PP mixtures. These bacterial strains were laboratory stock cultures isolated from diarrhoeic piglets’ intestines on a commercial farm. For the pathogen growth inhibition test, 100 mL of 5×10⁶ cfu/mL bacterial solution was added to 1 mL of the designed PP mixture solution and incubated at 37°C for 4 h. Afterward, 50 µL of the diluted mixture was placed on the 9 cm round flat containing the corresponding culture medium and cultured for the several hours, depending on the count of bacterium. The diluted bacteria solution with saline solution correspondingly served as the control. E. coli and S. aureus populations were assessed by using agar after being incubated at 37°C for 24 h. S. typhi populations were determined using Salmonella Shigella agar after being incubated at 37°C for 36 h. The pathogen growth inhibition ratio was calculated by applying the formula:

Growth inhibition ratio (%)={[count in control plate-count in mixture plate]/count in control plate}]×100%.

Table 1. Variable-level table constructed by 6 concentration levels of 4 polyphenols.

| Variable | Concentration levels, % |
|----------|-------------------------|
|         | 1 | 2 | 3 | 4 | 5 | 6          |
| X1       | 0.5 | 3.5 | 6.5 | 9.5 | 12.5 | 15.5       |
| X2       | 2.5 | 7.5 | 12.5 | 17.5 | 22.5 | 27.5       |
| X3       | 5 | 10 | 15 | 20 | 25 | 30         |
| X4       | 2.5 | 10 | 17.5 | 25 | 32.5 | 40         |

X1, apple polyphenol concentration; X2, grape seed polyphenol concentration; X3, green tea polyphenol concentration; X4, olive leaf polyphenol concentration.

Table 2. Uniform design U15 (154), antioxidant and antimicrobial activities of plant polyphenols mixtures.

| Test, n | PP mixture°, % | Scavenging ratio, % | Inhibition ratio, % |
|---------|----------------|---------------------|---------------------|
| X1      | X2 | X3 | X4 | Carrier° | DPPH | E. coli | S. aureus | S. typhi |
| 1       | 1 (0.5) | 3 (12.5) | 2 (10) | 5 (32.5) | 44.5 | 90.19 | 76.19 | 23.03 |
| 2       | 2 (3.5) | 3 (12.5) | 5 (25) | 4 (25) | 34 | 88.45 | 61.56 | 2.25 |
| 3       | 3 (6.5) | 5 (12.5) | 1 (5) | 1 (2.5) | 73.5 | 89.25 | 92.52 | 26.69 |
| 4       | 4 (9.5) | 1 (2.5) | 6 (30) | 3 (17.5) | 40.5 | 87.58 | 59.52 | 33.43 |
| 5       | 5 (12.5) | 2 (7.5) | 3 (15) | 3 (17.5) | 47.5 | 86.71 | 48.30 | 3.45 |
| 6       | 6 (15.5) | 6 (27.5) | 2 (10) | 5 (32.5) | 14.5 | 81.62 | 77.21 | 21.91 |
| 7       | 1 (0.5) | 4 (17.5) | 3 (15) | 2 (10) | 57 | 92.37 | 26.19 | 14.04 |
| 8       | 2 (3.5) | 2 (7.5) | 3 (15) | 4 (25) | 49 | 90.19 | 68.03 | 19.66 |
| 9       | 3 (6.5) | 5 (22.5) | 5 (25) | 6 (40) | 6 | 92.74 | 81.63 | 32.58 |
| 10      | 4 (9.5) | 6 (27.5) | 1 (5) | 3 (17.5) | 40.5 | 89.90 | 65.82 | 20.79 |
| 11      | 5 (12.5) | 2 (7.5) | 2 (10) | 6 (40) | 30 | 87.29 | 66.84 | 3.65 |
| 12      | 6 (15.5) | 4 (17.5) | 4 (20) | 2 (10) | 37 | 90.27 | 74.15 | 23.03 |
| 13      | 1 (0.5) | 5 (22.5) | 6 (30) | 1 (2.5) | 44.5 | 90.27 | 76.53 | 39.33 |
| 14      | 2 (3.5) | 1 (2.5) | 4 (20) | 1 (2.5) | 71.5 | 89.32 | 22.62 | 22.75 |
| 15      | 3 (6.5) | 1 (2.5) | 1 (5) | 2 (10) | 76 | 88.74 | 7.99 | 30.62 |

PP, plant polyphenols; X1, apple polyphenol concentration; X2, grape seed polyphenol concentration; X3, green tea polyphenol concentration; X4, olive leaf polyphenol concentration; DPPH, radical 2,2-diphenyl-1-picrylhydrazyl; E. coli, escherichia coli; S. aureus, Staphylococcus aureus; S. typhi, Salmonella typhi. °Concentration levels of X1 to X4 with their relative concentration in brackets: ViSilicon dioxide was used as a carrier.
Animals, housing and experimental design

The animal protocol for this research was approved by the Animal Care and Use Committee, University of Milan, Italy. Forty-eight crossbred weaned barrow piglets ([1×LW]×Penerland, 22 days old, 6.90±0.74 body weight (BW)) were selected for a 28-day experiment (7-days pre-feeding period+21-days regular feeding period) from a commercial swine herd and delivered to the Animal Production Research and Teaching Centre, Veterinary Department, University of Milan (Lodi, Italy). All piglets were vaccinated for *Mycoplasma hyopneumoniae* at two days old. Piglets were randomly allotted into two dietary treatments according to their initial BW (6 replicates of 4 piglets per treatment). The experimental unit was defined as one pen. All the piglets were fed a basal diet (Table 3) for one week. Then, the two dietary treatments consisted of two different diets: i) a control (CTR), basal diet; and ii) PP, basal diet+0.1% PP mixture. The plant extract mixture was standardised as 16.5% (wt/wt) apple polyphenol, 27.5% grape seed polyphenol, 30% green tea polyphenol and 2.5% olive leaf polyphenol in a silicon dioxide carrier that represented the remaining 23.5%. The basal feed was formulated according to the National Research Council requirements (1998), and the dosage of PP was based on the PP concentration of the solution in the *in vitro* test. All the diets were in mash form. Each individual PP was encapsulated before preparing the experimental diet. The PP mixture was manually mixed with a small quantity of the basal diet (10 kg) and thereafter mixed with residual basal diet. The feeds were manufactured 3 days before the start of the trial to allow time for nutritional homogeneity. Feeds were stored in a cool dry place until required. The piglets were housed in one environmentally regulated room with a slatted plastic floor (4 piglets/pen, 1.20×1.00 m). Each pen was equipped with a one sided self-feeder and a nipple waterer to allow the pig *ad libitum* access to feed and water throughout the experimental period. The temperature of the pig barn was set between 26 and 28°C with a 12 h light/dark cycle.

Sample collection, analysis and measurements

All piglets were individually weighed on day 0, 7, 14 and 21 of the trial. The average daily gain (ADG), average daily feed intake (ADFI) and gain to feed ratio (G:F) values were calculated for each pen. On day 7 and 21 of the trial, blood samples were obtained from one piglet per pen (a total of 12 samples). Blood was collected via the jugular vein in a heparinised centrifuge tube and centrifuged at 1800×g for 10 min, and then, the plasma was removed. The plasma was stored at -20°C until analysis. Faecal samples, pooled from piglets from each pen, were collected on day 11. Faecal samples (approximately 20 g) were placed in small sterile containers and immediately sent to the laboratory for the microbiological analysis. At the end of the trial, 12 animals (6 piglets per treatment), selected as being the most representative of pen performance in terms of BW, were slaughtered. Immediately after slaughtering, the gastrointestinal tract was removed from each animal. The caecum content (approximately 10 g) of each piglet was collected, placed in a small sterile container and immediately sent to the laboratory for the microbial assay. The antioxidant activities of plasma were determined using assay kits according to the manufacturers’ instructions (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Superoxide dismutase (SOD) activity was detected by monitoring the inhibition of nitro blue tetrazolium reduction. The glutathione peroxidase (GSH-Px) activity was measured with 5, 5-dithiobis-p-nitrobenzoic acid, and the change in absorbance at 412 nm was recorded. Malondialdehyde (MDA) concentration was analysed with 2-thiobarbituric acid, and the change in absorbance was read at 532 nm. The catalase (CAT) activity was determined using Aebi’s method (1984), in which the initial rate of hydrogen peroxide decomposition is determined. Total antioxidant capacity (TAC) was determined using the FRAP assay. The antioxidant activities of plasma were determined using assay kits according to the manufacturers’ instructions (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). The antioxidant activity of plasma was measured with 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging effect, and the change in absorbance at 517 nm was recorded. The radical scavenging effect of plasma was measured with 2,2-diphenylpicrylhydrazyl (DPPH) radical scavenging effect, and the change in absorbance at 517 nm was recorded. The antioxidant activities of plasma were determined using assay kits according to the manufacturers’ instructions (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). The antioxidant activity of plasma was measured with 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging effect, and the change in absorbance at 517 nm was recorded. The radical scavenging effect of plasma was measured with 2,2-diphenylpicrylhydrazyl (DPPH) radical scavenging effect, and the change in absorbance at 517 nm was recorded.

### Table 3. Composition and calculated nutrient content of the basal diet.

| Ingredients, g/kg | Pre-starter (22-36 d of age) | Starter (36-50 d of age) |
|-------------------|-----------------------------|--------------------------|
| Wheat micro       | 290.0                       | -                        |
| Corn micro        | 130.0                       | 91                       |
| Barley micro      | 130.0                       | 100                      |
| Full-fat soybean  | 40                          | 75                       |
| Wheat             | 70                          | 410                      |
| Whey soluble (80% lactose) | 110.0            | 75.0                     |
| Milk powder       | 70                          | 25.0                     |
| Herring meal      | 60                          | 60.0                     |
| Soy protein concentrate (Soycomil) | 40.0            | 25.0                     |
| Potato protein    | 40                          | 15.0                     |
| Dried whey        | 50                          | 22.3                     |
| Soy oil           | 40                          | 8.0                      |
| Dicalcium phosphate | 3.0                       | 3.0                      |
| Calcium formate   | 3.0                         | 3.0                      |
| Calcium sulphate  | 4.0                         | 4.0                      |
| L-lysine HCl 7B   | 6.4                         | 6.8                      |
| DL-methionine     | 3.0                         | 2.8                      |
| L-threonine       | 2.3                         | 2.9                      |
| Tryptophane       | 1.0                         | 0.9                      |
| Premix             | 13.3                        | 13.3                     |

Calculated nutrient composition:

| Nutrient          | Pre-starter (22-36 d of age) | Starter (36-50 d of age) |
|-------------------|-----------------------------|--------------------------|
| Crude protein, g/kg | 200.0                       | 190.0                     |
| Crude fibre, g/kg  | 17.0                        | 26.0                      |
| Ether extract, g/kg | 82.5                       | 55.0                      |
| Lysine, g/kg       | 15.2                        | 14.0                      |
| Methionine, g/kg   | 6.5                         | 6.0                      |
| Met-Cys, g/kg      | 9.0                         | 8.4                      |
| Threonine, g/kg    | 9.8                         | 9.1                      |
| Tryptophane, g/kg  | 2.9                         | 2.7                      |
| Ca, g/kg           | 10.4                        | 10.5                     |
| Available P, g/kg  | 4.5                         | 4.4                      |
| DE, Mcal/kg        | 3,925                       | 3,757                     |

DE, digestible energy; “Inclusion per kg in the diet: vitamin A, 3,600,000 IU; vitamin D$_3$, 360,000 IU; vitamin E (dl-α-tocopherol acetate), 24,000 mg; thiamine (vitamin B$_1$), 800 mg; riboflavin (vitamin B$_2$), 1,200 μg; pyridoxine (vitamin B$_6$), 1,000 mg; cyanocobalamin (vitamin B$_12$), 3.0 μg; vitamin K$_3$, 100 mg; niacin (nicotinic acid), 50 mg; pantothenic acid, 300 mg; folic acid, 400 mg; biotin, 60,000 μg; choline, 90,000 mg; Mn (MnO), 3,000 mg; Zn (ZnO), 29,000 mg; Fe (FeSO$_4$·7H$_2$O), 46,000 mg; Cu (CuSO$_4$·5H$_2$O), 30,000 mg; Co (CoSO$_4$·7H$_2$O), 200 mg. 1 (R) 300 mg; Sr (NaSeO$_3$), 70 mg.”
plant polyphenols in weaned piglets

Table 4. Stepwise regression analysis results for the plant polyphenols mixtures.

| Regression equations | R²   | P   |
|----------------------|------|-----|
| Y1=5.58-0.78*X1-0.85*X2+0.38*X3-0.39*X4+0.39*X5+0.03*X1+0.02*X1*X3-0.02*X1*X4+0.05*X22 | 0.9672 | 0.01 |
| Y2=78.39-12.37*X1+16.33*X2-2.89*X3+3.09*X4-1.30*X1+0.31*X1*X2+0.31*X1*X3+0.07*X1*X4 | 0.8859 | 0.02 |
| Y3=114.43-8.13*X1-4.35*X2+5.60*X3-0.96*X4+0.15*X1*X2+0.30*X1*X3+0.03*X1*X4+0.12*X22 | 0.9523 | 0.07 |
| Y4=-86.14+13.53*X1+5.98*X2-5.92*X3+3.15*X4+0.62*X1-0.09*X1*X2-0.68*X1*X3-0.37*X1*X4 | 0.9996 | 0.03 |

| X1, apple polyphenol concentration; X2, grape seed polyphenol concentration; X3, green tea polyphenol concentration; X4, olive leaf polyphenol concentration; Y1, DPPH radical-scavenging ratio; Y2, growth inhibition ratio of Escherichia coli; Y3, growth inhibition ratio of Staphylococcus aureus; Y4, growth inhibition ratio of Salmonella typhi.

Results

In vitro 2,2-diphenyl-1-picrylhydrazyl radical-scavenging and bacterial growth inhibition

Table 2 reports the results of the DPPH radical-scavenging ratio and the inhibition effects of PP mixtures on E. coli, S. aureus and S. typhi. The mixtures exhibited excellent antioxidant capacity, with more than an 80% DPPH radical-scavenging ratio. The inhibition effects of PP mixtures against E. coli and S. typhi were steady and effective, except that two mixtures had stimulating effects on S. typhi growth. However, the antimicrobial activity against S. aureus was somewhat less effective and unstable compared to the other two pathogens, and the highest inhibition ratio was less than 40%.

Selection of the optimal plant polyphenols mixture

By fitting apple, grape seed, green tea and olive leaf polyphenol concentrations as independent variables X1, X2, X3 and X4, and DPPH radical-scavenging ratio and growth inhibition ratio of E. coli, S. aureus and S. typhi as dependent variables Y1, Y2, Y3 and Y4, the regression equations were obtained (Table 4). The results indicated that the regression relationship of antioxidant capacity, or the inhibitions of E. coli, S. aureus or S. typhi equation, was effective and also that the parameters adopted here were feasible for the optimisation of the PP mixture because each P value of the regression was less than 0.05.

Table 5. Integrated optimal solutions for superb antioxidant and antimicrobial activities from the regression equations.

| X1 | X2 | X3 | X4 | Ymax/Ymin | Ki |
|----|----|----|----|-----------|----|
| Y1 | 0.5 | 2.5 | 26 | 40 | 115 | 1 |
| Y2 | 8.7 | 19.0 | 5 | 40 | 2.50 | 1 |
| Y3 | 16.5 | 27.5 | 30 | 2.5 | 140 | 1 |
| Y4 | 0.5 | 11.1 | 30 | 40 | 2.84 | 1 |

| Integrated optimal solution | 16.5 | 27.5 | 30 | 2.5 |
|-----------------------------|------|------|----|-----|

X1, apple polyphenol concentration; X2, grape seed polyphenol concentration; X3, green tea polyphenol concentration; X4, olive leaf polyphenol concentration; Y1, DPPH radical-scavenging ratio; Y2, growth inhibition ratio of Escherichia coli; Y3, growth inhibition ratio of Staphylococcus aureus; Y4, growth inhibition ratio of Salmonella typhi; Ki, weight coefficient.

Discussion

In the present study, we employed a uniform design for testing a PP mixture based on in vitro antioxidant capacity and microbial modu-
Although our insight into possible antioxidant and antimicrobial properties of the optimised PP mixture, to obtain a superb mixture with the expectation that synergistic action would occur, irre-

spective of the solo activity of any of the single plant extracts. This study confirms that the PP possesses in vitro antimicrobial activity and antioxidant capacity, and the optimised solution of the PP mixture was obtained by using the uniform design based on the antimicrobial and antioxidant activities. Phytobiotics is a term used to describe plant-derived natural bioactive compounds that promote livestock health and well-being, but their bioavailability is not yet clear (Wu and Wu, 2012). Many studies have employed the in vitro and in vivo screening models to evaluate the effects of plant extracts on pigs (Calvo et al., 2006; Kumar et al., 2010; Verhelst et al., 2014). In vitro studies directly evaluate product performance, while in vivo tests can suffer from complications due to the indirect approach (Polli, 2008). Moreover, there may be a high variability in the in vivo studies due to the environment in which animals are raised and the type of diet they are fed (Calvo et al., 2006). Although our in vitro study provides useful insight into possible antioxidant and antimicrobial properties of the optimised PP mixture,

| Table 6. Effect of dietary plant polyphenols on the growth performance of 48 piglets (4 piglets/6 replicates/treatment). |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Item            | CTR | PP | SEM  |  P   |
| BW, kg          |     |     |      |      |
| d 0             | 7.43 | 7.92 | 0.207 | ns   |
| d 7             | 9.99 | 10.36 | 0.239 | ns   |
| d 14            | 13.36 | 13.73 | 0.293 | ns   |
| d 21            | 17.24 | 17.82 | 0.357 | ns   |
| ADG, g/d        |     |     |      |      |
| d 0-7           | 365  | 349  | 17.0  | ns   |
| d 7-14          | 482  | 482  | 12.6  | ns   |
| d 14-21         | 554  | 555  | 13.4  | ns   |
| d 0-21          | 467  | 462  | 11.6  | ns   |
| ADFI, g/d       |     |     |      |      |
| d 0-7           | 441  | 431  | 21.5  | ns   |
| d 7-14          | 658  | 634  | 20.6  | ns   |
| d 14-21         | 707  | 721  | 20.1  | ns   |
| d 0-21          | 602  | 595  | 17.3  | ns   |
| G:F             |     |     |      |      |
| d 0-7           | 0.831 | 0.811 | 0.0228 | ns   |
| d 7-14          | 0.736 | 0.762 | 0.0183 | ns   |
| d 14-21         | 0.785 | 0.770 | 0.0150 | ns   |
| d 0-21          | 0.778 | 0.776 | 0.0102 | ns   |

CTR, control diet; PP, basal diet+0.1% plant polyphenols mixture; ns, not significant; ADG, average daily gain; ADFI, average daily feed intake; G:F, gain to feed ratio; ns, not significant.

| Table 7. Effect of dietary plant polyphenols on the plasma biochemical parameters of 48 piglets (4 piglets/6 replicates/treatment). |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Item            | CTR | PP | SEM  |  P   |
| TP, mg/mL       |     |     |      |      |
| d 0             | 35.5 | 37.9 | 2.0  | ns   |
| d 21            | 37.69 | 38.58 | 0.963 | ns   |
| BUN, mg/L       |     |     |      |      |
| d 0             | 68.0 | 53.2 | 7.5  | ns   |
| d 21            | 52.8 | 43.6 | 6.2  | ns   |
| Lysozyme, μg/mL |     |     |      |      |
| d 0             | 6.53  | 6.40  | 0.217 | ns   |
| d 21            | 6.66  | 6.33  | 0.249 | ns   |

CTR, control diet; PP, basal diet+0.1% plant polyphenols mixture; ns, not significant; TP, total protein; BUN, urea nitrogen.

| Table 8. Effect of dietary plant polyphenols on the plasma antioxidant activity of 48 piglets (4 piglets/6 replicates/treatment). |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Item            | CTR | PP | SEM  |  P   |
| MDA, nmol/mL    |     |     |      |      |
| d 0             | 5.08  | 3.64  | 0.692 | ns   |
| d 21            | 3.64a | 1.93b | 0.422 | 0.02  |
| T-AOC, U/mL     |     |     |      |      |
| d 0             | 18.1 | 18.8 | 1.10  | ns   |
| d 21            | 18.2 | 22.1 | 1.41  | 0.08  |
| CAT, U          |     |     |      |      |
| d 0             | 16.3 | 18.9 | 4.1   | ns   |
| d 21            | 16.4 | 25.1 | 3.8   | ns   |
| SOD, U/mL       |     |     |      |      |
| d 0             | 103  | 103  | 10.4  | ns   |
| d 21            | 97.0 | 102.7 | 5.24  | ns   |
| GSH-Px, U       |     |     |      |      |
| d 0             | 404  | 381  | 26.2  | ns   |
| d 21            | 397  | 410  | 26.3  | ns   |

CTR, control diet; PP, basal diet+0.1% plant polyphenols mixture; ns, not significant; MDA, malondialdehyde; T-AOC, total antioxidant; CAT, catalase; SOD, superoxide dismutase; GSH-Px, glutathione peroxidase. *Values within the same row with different superscripts are significantly different (P<0.05).
the in vivo study should be employed to investigate if the candidate compound possesses the antioxidant or antimicrobial efficacy on weaned piglets when high variability is present.

In the current work, feeding diets containing PP did not affect animal performance from day 0 to 21 of the trial. Similar to our observation, Rossi et al. (2013) did not observe changes of growth performance in piglets fed control diet or diet supplemented with either vitamin E or plant extract. The absence of a PP mixture effect on growth performance in this study may be due to the anti-nutritional properties of certain polyphenols. Verhelst et al. (2014) observed that PP from high molecular weight hydrolysable tannins and grape seeds did not affect growth performance, whereas the polyphenol extract from cocoa beans that may contain an anti-nutritional factor depressed the ADG. Moreover, the lack of an effect from the PP mixture on growth performance may be due to the inactivation of certain polyphenols by dietary protein (Verhelst et al., 2010). In our study, plasma TP, BUN concentration and lysozyme activity were not affected by the PP supplementation, which may be related to the absence of any effect of the PP mixture on growth performance. Blood BUN concentration was used to monitor the adequacy of dietary amino acids in growing animals and has been suggested as a potential indicator of efficiency of lean tissue growth in pigs (Coma et al., 1995). In our studies, although PP mixtures showed excellent inhibitory activity on bacterial growth in the laboratory, no antimicrobial activity occurred in the faeces or caecum content of piglets throughout the in vivo experimental period. The lack of response to PP supplementation from the populations of E. coli and Clostridia is likely due to the aforementioned inactivation of certain polyphenols by proteins (Verhelst et al., 2010). In addition, the absence of an antimicrobial effect might be ascribed to the nutritionally balanced diet and the health status of the piglets. The absence of diarrhoea episodes might suggest that the piglets were in good health.

An organism’s health is closely related to the antioxidant defence system, which consists of enzymatic and non-enzymatic reactions. In the enzymatic reaction system, active sites of many enzymes are composed of micro elements, such as SOD, GSH-PX, CAT, etc. In contrast, the non-enzymatic reaction system is mainly composed of vitamins, amino acids and metal-binding proteins, such as vitamin C, vitamin E, cysteine, histidine, serine, methionine, glucose, transferrin, lactoferrin and ceruloplasmin. In our in vivo study, the reduced MDA concentration after 21 days in the piglets fed the PP mixture suggested that the PP mixture may improve the non-enzymatic reactions of the antioxidant defence system. The roles of free radicals, potent scavengers of oxygen, have been observed in polyphenols from apple (Lu and Foo, 2000), green tea (Unno et al., 2000), grape seed (Bagchi et al., 1997) and olive leaves (Lee et al., 2009) in vitro. The present in vitro and in vivo work confirmed the effects of a PP mixture on the improvement of the antioxidant defence system, in accordance with the results from Rossi et al. (2013), who observed that long-term supplementation with plant extracts tended to increase total antioxidant activity in the blood.

Conclusions

The results demonstrated that an optimised PP mixture supplementation favourably affected plasma antioxidant activity by decreasing the production of MDA. However, the inclusion of the PP mixture did not substantially improve piglets’ growth performance and had no inhibitory activity on the counts of E. coli and Clostridia in the faeces and caecum of piglets, which suggests that in vitro pre-selection is not always predictive for in vivo studies. To better evaluate the potential for the use of the PP mixture as a protective feed ingredient against infection, future research including experimental challenge with enterotoxigenic Escherichia coli would be highly recommended.

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