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Assessment of Quillaja saponin as a feed supplement in maize-soybean-oilseed rape meal-based diet for enhanced growing pig performance

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ABSTRACT. The present study evaluated the effects of Quillaja saponin (QS) supplementation on growth efficiency, nutritional digestibility, gas emissions, and faecal score in growing pigs. In a 42-day experiment, 80 growing pigs [(Yorkshire × Landrace) × Duroc] were randomly assigned (29.17 ± 0.23 kg initial average body weight) to 2 different trial groups. Each group had 8 replicates, consisting of 3 male and 2 female pigs in each pen. The nutritional treatments included a control group (CON) receiving a basal diet and a treatment group (TRT1) receiving a basal diet supplemented with 0.01% QS. The results of this study indicated that pigs consuming the QS-supplemented diet exhibited significantly higher ($P < 0.05$) body weight on day 42 compared to the CON group. Furthermore, dietary QS led to higher ($P < 0.05$) average daily gain and showed a tendency to reduce ($P < 0.10$) the feed conversion ratio throughout the study period, as compared to the CON diet. Growing pigs that consumed the QS-supplemented diet also showed higher ($P < 0.05$) nutrient utilisation of dry matter and nitrogen compared to the CON group. At day 42, QS supplementation reduced ($P < 0.05$) NH_3 and H_2S emissions, along with a trend to reduce ($P < 0.10$) CH_4 generation in the initial stage in comparison to the CON diet. Furthermore, the faecal score in the experimental group was lower ($P < 0.05$) on day 42 compared to control animals. In conclusion, QS proved to be a suitable supplement for growing pigs as it improved growth efficiency and nutrient digestibility, as well as reduced gas emissions and faecal score.

Introduction

Phytogenic feed additives (PFAs), often referred to as phytobiotics or botanicals, are organic, bioactive compounds derived from plants. They are added to livestock feed to increase animals' productivity (Windisch et al., 2008). The main components of phytogenic supplements for livestock include natural acids, volatile oils, saponins, flavonoids, as well as inorganic compounds, including antibacterial and growth stimulants. Due to bacterial susceptibility to antimicrobial agents, the use of phytogenic feed to improve performance has become increasingly popular as an alternative to antibiotics (Wallace et al., 2002; Chaudhary et al., 2018).

Quillaja saponin (QS) is a substitute for antibiotics that is obtained from the wood and bark of the soap tree and contains a triterpenoid as a structural component (Francis et al., 2002). As a PFA, it consists of readily available, naturally occurring compounds with a variety of physiological and biochemical properties, including antioxidant, antibacterial, antiviral, antifungal, and immunomodulatory properties. It has been demonstrated that it can increase the productivity, boost the immune system, promote intestinal health, and improve meat quality in monogastric animals without changing production costs (Chaudhary et al. 2018; Liu et al., 2019). The pharmacological effects of QS have been extensively studied and include strengthening biological membranes, lowering serum cholesterol levels, and influencing ammonia production (Francis et al. 2002). As a natural glycosidic compound, QS exert numerous effects on the gastrointestinal tract, including nutrient digestion and absorption, which are closely linked to the bioactivity of saponins (del Hierro et al., 2018). The long persistence time of saponins in the intestinal lumen affects crucial bioactive processes through the hypocholesterolemic effect and interference with cholesterol absorption from the intestinal lumen (Vinarova et al., 2015; Zhao, 2016). The inclusion of QS in the diets of weaned pigs at a concentration of 125 mg/kg led to improved feed intake and conversion rates, as observed in the study by Vaclavkova and Beckova (2008). Another study found that supplementing the diet of growing pigs with 200 mg/kg of QS resulted in improved growth, reduced levels of infectious microbes, and a more favourable barn atmosphere due to decreased faecal gas emissions (Dang and Kim, 2021). PFAs containing QS can improve performance by enhancing nutrient intake, improving growth efficiency through enhanced flavour qualities, and reducing ammonia emissions from pig barns (Bartos et al., 2016). Dang and Kim (2020) reported that the addition of 400 mg/kg QS to the diet of finishing pigs improved meat quality and faecal gas emission without affecting growth efficiency and nutrient retention. Moreover, nutritional treatment involving a combination of saponins (Quillaja and Yucca) led to substantial improvements in growth and productivity, nutrient utilisation, and improved villus height in broilers (Bafundo et al., 2021). Bioactive components of QS, known as saponins and polyphenols, have been shown to enhance the stability of biological membranes, function as antioxidants, and minimise the impact of parasitic infections (Sparg et al., 2004).

Given our existing knowledge, there is a limited body of research on the use of QS supplements to enhance the performance and overall health of growing pigs. Our hypothesis postulated that providing growing pigs with a diet enriched in QS would lead to improvements in their performance. Therefore, the aim of the experiment was to assess the effects of QS addition to growing pigs' diet on growth, nutrient absorption, gas emission, and faecal score.

Material and methods

The experimental protocol was approved by the Animal Care and Use Committee of Dankook University (approval number: DK-2-2142).

QS source

The commercially available QS (Delacon Inc., Steyregg, Austria) utilised in this study was derived from the *Quillaja saponaria* Molina tree from the Andes region of Chile. QS was provided in the form of an enriched biomass powder (99% content).

Animal husbandry and dietary regimens

Before analysis, all instruments and pens used were disinfected. Based on initial body weight (BW) (average 29.17 ± 0.23 kg) and gender, a total of 80 pigs [(Landrace × Yorkshire) × Duroc] were randomly allocated to 1 of 2 nutritional treatments for 42 days (8 replicates per treatment, 2 females, and 3 males per pen). Both dietary treatments were maize-soybean-oilseed rape meal-based basal diets, which contained 0 and 0.01% QS. All pig diets were prepared according to the standards of the National Research Council (NRC) (2012) for the nutritional needs of pigs (Table 1). Additives and feed were properly mixed using a feed mixer (Daedong Tech, DDK801F, Anyang-si, South Korea). Throughout the whole study period, the housing room underwent routine weekly sterilisation and cleaning. All pigs were housed in clean facilities equipped with slatted plastic flooring, mechanical ventilation and environmental controls. The room's temperature and humidity were set at 25 °C and 60%, respectively. Stainless steel self-feeders and nipple drinkers were provided in each enclosure, allowing the pigs unrestricted access to both feed and water.

Sampling and measurements

To calculate average daily gain (ADG), each pig was weighed on days 1 and 42. Average daily feed intake (ADFI) was evaluated every day, pen by pen. Feed conversion ratio (FCR) was measured using ADG and ADFI values.

Chromium oxide (Cr_2O_3, 0.2%) was selected as an indigestible marker and mixed with feed on days 3541 to measure dry matter (DM), nitrogen (N), and energy (E) values. Rectal massage was used to collect faecal samples from two pigs in each pen (one male and one female) on day 42. After pooling the samples from each pen, selected samples were stored at −20 °C in a freezer until analysis. Faecal samples were dried at 60 °C for 72 h and crushed

Table 1. Composition of experimental diets (%, as feeding basis)

Items	Content
Ingredients	
maize	60.01
soybean meal (crude protein 45%)	16.07
oilseed rape meal	2.50
distillers dried grains with soluble	6.50
wheat	6.00
tallow	3.00
molasses	3.00
dicalcium phosphate	1.08
limestone	0.65
salt	0.30
lysine, 98%	0.19
methionine, 99%	0.14
threonine, 98.5%	0.14
tryptophan, 20%	0.08
mineral premix[a]	0.10
vitamin premix[b]	0.20
choline, 50%	0.04
total	100.00
Calculated composition, %	
crude protein	15.50
crude fat	5.78
lysine	0.91
calcium	0.65
phosphorus	0.55
digestible energy, kcal/kg	3428
crude fibre	3.43
crude ash	4.59

[a] provided per kg of complete diet: mg: Fe (as $FeSO_4 \times 7H_2O$) 80, Cu (as $CuSO_4 \times 5H_2O$) 12, Zn (as $ZnSO_4$) 85, Mn (as MnO_2) 8, I (as KI) 0.28, Se (as $Na_2SeO_3 \times 5H_2O$) 0.15; [b] provided per kg of complete diet: IU: vit. A 11 025, vit. D_3 1 750, vit. E 44; mg: vit. K 4.4, riboflavin 8.3, niacin 50, thiamine 4, d-pantothenic acid 29, choline 166, vit. B_{12} 33

to pass through a 1-mm sieve. The AOAC (2010) approach was used to evaluate feed and faecal samples for DM, E, and N contents. The combustion heat in the specimen was measured using a Parr 6100 bomb calorimeter to determine E content. The chromium content in the samples was measured using atomic absorption spectroscopy (UV-1201, Shimadzu, Kyoto, Japan). The calculation of apparent total tract digestibility (ATTD) of nutrients was performed using the method described by Biswas and Kim (2022).

On days 1 and 42, fresh faecal samples (300 g) were collected from two randomly selected pigs (one male and one female) in each pen to measure the content of faecal ammonia (NH_3), hydrogen sulphide (H_2S), CH_4, CO_2, acetic acid, and methyl mercaptans (R-SH). The samples were placed in a 2.6 ml sealed plastic containers with a small aperture in the centre of one side, which was carefully sealed with tape and allowed to ferment for 24 h at 25 °C. A 100-ml sample was removed from the headspace (approx. 2.0 cm) to allow for air circulation. To assess crust formation, the container was resealed and manually shaken for 30 sec. After fermentation, NH_3, H_2S, methyl mercaptan, CO_2, and acetic acid gases were quantified using a gas sampling pump kit (model GV100S, Gastec Corp.). Several measurement tubes (No. 3L, No. 4LT, and No. 70L; Gastec) were used to determine the amount of total mercaptans.

On days 1 and 42, the faecal score was determined at 8:00 and 20:00. The average value of five pigs from each pen served as the basis for determining the faecal score using a 5-grade scoring system. The standard method of stool scoring is as follows: 1. hard, dry pellets in a small, hard mass; 2. firm, formed, remaining solid and soft; 3. soft, formed, and moist, maintaining its shape; 4. loose, unformed, taking the shape of the container; 5. watery, liquid, pourable faeces (Biswas et al., 2023).

Statistical analysis

The pen was considered as a test unit, and the t-test implemented in SAS software (SAS Institute, Cary, NC, USA) was employed for statistical analysis. The standard error of the mean is a method of expressing data variability. A *P*-value of 0.05 indicated significant differences, and *P*-value of 0.10 was regarded as a trend.

Results

Growth performance

Pigs on the QS-supplemented diet had higher ($P = 0.010$) BW on day 42 and ADG ($P = 0.028$) throughout the study period and showed a tendency towards reduced ($P = 0.057$) FCR over the entire trial compared to the CON group. However, dietary treatment showed no significant effect ($P > 0.05$) on ADFI (Table 2).

Table 2. Effect of dietary Quillaja saponin on growth performance in growing pigs

Items	CON	TRT1	SEM	*P*-value
Body weight, kg				
initial	29.10	28.70	0.77	0.689
at day 42	55.39	59.16	0.77	0.010
Overall				
ADG, g	689	725	9.64	0.028
ADFI, g	1755	1759	14.98	0.836
FCR	2.52	2.42	0.03	0.057

CON – basal diet, TRT1 – basal diet + 0.01% Quillaja saponin, ADG – average daily gain, ADFI – average daily feed intake, FCR – feed conversion ratio, SEM – standard error of the mean. $P < 0.05$ indicates that data are significantly different

Nutrient digestibility

According to Table 3, the incorporation of QS into the diet of growing pigs resulted in a higher DM (P = 0.004) and N (P = 0.029) digestibility compared to the CON group. Nevertheless, no significant treatment effect (P > 0.05) was found on E retention.

Table 3. Effect of dietary Quillaja saponin on nutrient digestibility in growing pigs, %

Items	CON	TRT1	SEM	*P*-value
Dry matter	74.17	76.55	0.37	0.004
Nitrogen	72.04	74.12	0.65	0.029
Energy	72.62	73.50	0.22	0.262

CON – basal diet, TRT1 – basal diet + 0.01% Quillaja saponin, SEM – standard error of the mean, P < 0.05 indicates that data are significantly different

Gas emissions

Table 4 presents the effect of dietary QS on gas emissions of growing pig. The dietary treatment caused a decrease in NH_3 (P = 0.012) and H_2S (P = 0.048) production on day 42 and a trend towards a reduction in CH_4 emission (P = 0.057) at baseline compared to the CON diet. However, R-SH, CH_4, and CO_2 gas emissions did not differ significantly (P > 0.05) between treatments.

Table 4. Effect of dietary Quillaja saponin on gas emissions in growing pigs, ppm

Items,	CON	TRT1	SEM	*P*-value
Initial				
NH_3	3.87	4.25	0.74	0.694
H_2S	3.02	4.92	1.08	0.144
methyl mercaptans	5.62	6.62	0.31	0.521
acetic acid	8.50	7.25	1.92	0.583
CO_2	11400	11275	381.61	0.898
CH_4	13.00	11.12	0.47	0.057
At day 42				
NH_3	8.12	6.12	0.37	0.012
H_2S	4.47	5.12	0.14	0.048
methyl mercaptans	6.37	5.75	0.31	0.426
acetic acid	11.37	10.62	1.65	0.755
CO_2	14125	13175	510.51	0.461
CH_4	17.87	17.37	0.96	0.696

CON – basal diet, TRT1 – basal diet + 0.01% Quillaja saponin, SEM – standard error of the mean, P < 0.05 indicates that data are significantly different

Faecal score

The results of dietary QS treatments on faecal scores of growing pigs are summarised in Table 5. The dietary administration of QS reduced faecal scores (P = 0.006) on day 42 compared to the CON group.

Table 5. Effect of dietary Quillaja saponin on faecal score in growing pigs

Items	CON	TRT1	SEM	*P*-value
Faecal score				
initial	3.11	3.13	0.03	0.712
at day 42	3.20	3.08	0.02	0.006

CON – basal diet, TRT1 – basal diet + 0.01% Quillaja saponin, SEM – standard error of the mean, P < 0.05 indicates that data are significantly different

Discussion

According to Dang and Kim (2020), finishing pig's diet mixed with QS (400 mg/kg) did not exert any substantial effect on growth performance (ADG, ADFI, and FCR), as opposed to our study. However, Dang and Kim (2021) observed in their work that the diet supplemented with QS (200 mg/kg) enhanced ADG and ADFI indices, while decreasing FCR in growing pigs. The inclusion of QS (500 mg/kg) in the diet reduced the gain to feed (G/F) ratio to a higher extent than in other treatment groups, but did not significantly affect ADG and ADFI values in weaning piglets (Turner et al., 2002). Similarly, Ilsley et al. (2005) stated that the addition of QS to the diet (300 mg/kg) of weaning pigs decreased ADFI and the G/F ratio. Vaclavkova and Beckova (2008) found that feeding weaned pigs a feed containing 125 mg QS per kg did not affect their body weight or average daily weight gain, but improved feed consumption and conversion compared to CON animals. Weaning pigs fed a diet enriched with 500 mg/kg QS did not show any changes in ADG, ADFI or the G/F ratio (Turner et al., 2000). Interestingly, the combination of QS and fructooligosaccharide (FOS) was found to improve growth performance of growing pigs in a study of Muniyappan et al. (2022). Since nutrient utilisation is closely linked with growth efficiency (Cai et al., 2018), improved nutrient digestibility likely contributed to the enhanced growth performance in our trial. Moreover, the improvement in feed efficiency observed in our study can be attributed to multiple factors, with major ones involving an interaction of QS with the gastrointestinal environment, such as enzymes, nutrients, and the gut microbiota. Many studies have reported that saponins can affect the composition of the gut. Firstly, modification of the intestinal microflora by QS can lead to enhanced nutrient digestibility and absorption. Beneficial bacteria supported by the saponin can help break down complex nutrients, making them more available for absorption. Secondly, saponin supplementation can lead to a reduction in harmful bacteria, thereby contributing to a healthier gut environment, potentially reducing competition for nutrients among different microorganisms and, consequently, promoting better nutrient utilisation by the host. However, it is important to note that inconsistencies in the findings of various studies may arise from variations in factors such as the type and quality of the supplement used, as well as the species and age of the animals.

It is essential to increase digestibility to improve feed efficiency and reduce the amount of undigested material, which could potentially promote the development of gastrointestinal disorders (Abdelli et al., 2021). In the present trial, growing pigs on a diet containing QS (0.01%) showed improved DM nutrient retention and N content compared to the animals on the CON diet. Similarly, Yan et al. (2012) demonstrated that weaning piglets fed diets containing plant extract combinations had increased ATTD of DM and N on days 7 and 42 of their growth. Our research team previously reported that the inclusion of QS into the diet of growing and finishing pigs had no significant effect on DM nutrient retention, and N, and E contents (Dang and Kim, 2020; 2021). In contrast, Muniyappan et al. (2022) showed improved digestibility of DM and N when QS was included in the growing pigs' diet. As reported by Devi et al. (2015), including PFAs in the diet of weaning pigs led to increased DM digestibility, while showing no effect on N and E retention. In the case of broilers, a combination of Quillaja and Yucca saponin supplements improved both apparent gastrointestinal absorption of DM and N retention (Bafundo et al., 2021). Oh et al. (2018) observed that pigs given a diet enriched with PFA, known for its antioxidant properties, showed enhanced nutritional digestibility due to reduced counts of faecal pathogenic microbes, ultimately leading to improved productivity. PFA was also found to stimulate the secretion of natural and pancreatic proteolytic enzymes, primarily trypsin, protease, and bile, thereby contributing to increased feed conversion efficiency and feed utilisation (Gopi et al., 2014). Further comparisons of the effect of QS supplementation on nutrient digestibility of growing pigs are not possible due to the lack of available literature. The effects of dietary QS supplementation on the absorption of nutrients in developing pigs require further research.

The principal airborne contaminants in the swine industry, including NH_3, R-SH, and H_2S, can have serious negative effects on the immediate pig environment and the environment in general. Our previous studies demonstrated that the incorporation of QS in the diets of growing (200 mg/kg) and finishing pig (400 mg/kg) reduced NH_3 emissions in faecal gasses, contributing to the improvement of barn environments (Dang and Kim, 2020; 2021). This finding is consistent with the results of our current study. Additionally, as reported by Muniyappan et al. (2022), pigs fed diets containing a combination of QS and fructooligosaccharide (FOS) exhibited decreased emissions of faecal NH_3 and CO_2 gases compared to pigs on the CON diet. The study of Bartos et al. (2016) highlighted the significant reduction in NH_3 emissions associated with the use of QS-containing PFAs, suggesting their potential as a viable strategy to mitigate NH_3 emissions in pig production. Conversely, Devi et al. (2015) reported that the inclusion of PFA had no impact on harmful gas emissions in weaning pigs. The reduction in emissions of harmful gases can be attributed to the improvements in the composition of faecal microbes and enhanced nutrient utilisation, as indicated by Zhao et al. (2016). Quillaja saponin is known for its ability to impact the gut microflora by promoting the growth of beneficial bacteria, while suppressing the development of harmful microorganisms. These alterations in microbial populations can lead to changes in fermentation processes, potentially affecting CO_2 production. Moreover, the mechanism behind the reduction in ammonia production is related to QS's ability to modify gut microbial populations. A lower abundance of harmful bacteria that contribute to ammonia production may result in reduced ammonia emissions. The decrease in both ammonia and CO_2 emissions is important not only due to the odour, but also because it indicates more efficient utilisation of nutrients by pigs.

Reducing the incidence of diarrhoea is an important approach to enhancing the growth efficiency of pigs. Chen et al. (2015) reported that triterpenoid saponins could increase the microbial counts in the animal gastrointestinal tract. However, Devi et al. (2015) found that weaning pigs challenged with *Escherichia coli* did not show significant changes in faecal scores after PFA administration. In contrast, the addition of fermented medicinal plants to the diet of weaning piglets resulted in a reduced diarrhoea score in comparison to the negative control diet (Zhao et al., 2016). A study by Muniyappan et al. (2022) found that the incorporation of a combination of QS and FOS improved the abundance of lactobacilli, while reducing the counts of *E. coli*, thereby improving gastrointestinal health. Dang and Kim (2021) suggested that the reduced activity of coliform bacteria caused by the addition of QS to growing pig feeds was the reason for their better performance. Thus, the inclusion of QS into the diets of growing pig could be a suitable strategy to improve the faecal score and performance of pigs by reducing harmful bacterial counts. In our research, we recorded a reduced faecal score on day 42 as a result of QS (0.01%) inclusion in the growing pig's diet compared to the CON group. However, further research is needed to assess the effects of QS supplementation on faecal scores in pigs at different growth stages.

Conclusions

In the present study, growing pigs receiving a diet containing Quillaja saponin (QS) (0.01%) showed improved growth efficiency, as indicated by increased body weight gain, average daily gain and reduced feed conversion ratio compared to the animals receiving a control diet. Additionally, the treated pigs exhibited enhanced nutrient utilisation of dry matter and nitrogen and reduced faecal scores. Furthermore, the inclusion of QS in the diet had the added benefit of decreasing the emission of harmful gases such as NH_3, H_2S, and CH_4, which can improve barn environmental conditions. Therefore, QS can be used as a stimulant of performance and may also play a role in mitigating environmental pollution caused by the swine industry.

Conflict of interest

The Authors declare that there is no conflict of interest.

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