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Dietary glucose oxidase supplementation improves growth performance, apparent nutrient digestibility, and serum antioxidant enzyme parameters in growing pigs

De Xin Dang[a], Md Raihanul Hoque[a], Yanjie Liu[b], Ningbo Chen[b] and In Ho Kim[a]

[a]Department of Animal Resource and Science, Dankook University, Cheonan, South Korea; [b]Jinan Bestzyme-Bio Engineering Co., LTD, Jinan, China

ABSTRACT

This study was conducted to investigate the effects of supplementing glucose oxidase (GOX; 1000 unit/g) to the diet of growing pigs on growth performance, apparent nutrient digestibility, faecal bacteria counts, and serum antioxidant enzyme parameters. A total of 200 growing pigs [(Yorkshire × Landrace) × Duroc] with an initial body weight of 23.3 ± 0.33 kg were used in a 42-day trial. Pigs were randomly allotted to four dietary treatments with 10 replicate pens per treatment and five pigs (mixed sex) per pen based on the initial body weight. The dietary treatments consisted of the basal diet (control), control + 0.01% GOX, control + 0.02% GOX, or control + 0.03% GOX. One unit of GOX concentration is defined as the amount of enzyme which oxidises 1 μmol β-D-glucose per minute to D-gluconic acid and hydrogen peroxide at 37 °C and pH5.5. Body weight on day 42 ($p = .035$), average daily gain during days 22–42 ($p = .001$) and 1-42 ($p = .001$), average daily feed intake during days 22–42 ($p = .008$) and 1–42 ($p = .020$), apparent nitrogen digestibility on day 42 ($p = .029$), apparent energy retention on day 42 ($p = .032$), and the concentrations of serum glutathione peroxidase ($p = .044$) and glutathione ($p = .032$) on day 42 increased linearly at graduated doses of GOX increased in the diet. Therefore, supplementing GOX to the diet of growing pigs could improve the apparent nutrient digestibility and serum antioxidant enzyme parameters in a dose-dependent manner, thus improving growth performance. In this study, the suitable dosage of GOX used in the diet of growing pigs was 0.03%.

HIGHLIGHTS

- Feeding growing pigs with GOX containing diet has beneficial effects on the growth performance
- Dietary supplementation of GOX could improve the apparent nutrient digestibility in growing pigs
- The antioxidant status of growing pigs has improved by GOX supplementation.

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Growing pig; glucose oxidase; antioxidant enzyme; growth performance; nutrient digestibility

Introduction

It is reported that supplementing exogenous enzymes with antioxidant and intestinal bacteria regulation properties to the diet of pigs could improve growth performance. This is achieved by improving antioxidant status and intestinal health in pigs (Oliver and Wells 2015; Long et al. 2021). Indeed, any factors that affect intestinal health and/or antioxidant status will undoubtedly affect the overall performance of animals (Attia et al. 2015, 2017; Pluske et al. 2018).

Glucose oxidase (GOX) as an exogenous enzyme has intestinal bacteria regulation properties (Tang et al. 2016; Zhao et al. 2021). It could utilise the oxygen to oxidise glucose into gluconic acid and produce hydrogen peroxide (Yu et al. 2017). Hydrogen peroxide is a key role in regulating the development of intestinal beneficial bacteria (Shigeno et al. 2019). In addition, GOX supplementation also has been reported to play an important role in improving the antioxidant status (Zhang et al. 2020) and immune response (Noguera-Machado et al. 2018; Liu et al. 2020).

When used in swine husbandry, plenty of studies have reported that feeding weaning pigs with GOX containing diet had positive effects on growth performance, nutrient digestibility, intestinal bacteria

CONTACT Dr In Ho Kim inhokim@dankook.ac.kr Dankook University, Cheonan, South Korea

communities, circulating growth hormone concentration, and serum antioxidant enzyme parameters (Chen et al. 2015; Wu et al. 2015; Tang et al. 2016; Mu et al. 2018; Dang et al. 2021).

However, the application potential of GOX in growing pigs was still unknown. We hypothesised that supplementing 0.01, 0.02, or 0.03% GOX (1000 unit/g) to the diet of growing pigs could increase the counts of faecal beneficial bacteria, decrease the counts of faecal harmful bacteria, and/or serum antioxidant enzyme concentration in a dose-dependent manner, thus improving apparent nutrient digestibility, and further enhancing growth performance. The objective of this study was to investigate the effects of GOX supplementation on growth performance, apparent nutrient digestibility, faecal bacteria counts, and serum antioxidant enzyme parameters in growing pigs.

Material and methods

The protocol (DK-2-2016) used for the conduction of this research was approved by the Dankook University Animal Care and Use Committee (Cheonan, South Korea).

Information of glucose oxidase (GOX)

The commercial GOX (Bestzyme Bio-engineering Co., LTD; Jinan, China) is expresses by *Aspergillus niger*. According to the information provided by the manufacturer, the optimum temperature for the enzymatic function of GOX is 20–80 °C and the optimum pH is 2.0–7.0. The concentration of GOX is 1000 unit/g. One unit of GOX concentration is defined as the amount of enzyme which oxidises 1 μmol β-D-glucose per minute to D-gluconic acid and hydrogen peroxide at 37 °C and pH 5.5.

Animals and housing

A total of 200 crossbred growing pigs [(Landrace × Yorkshire) × Duroc] with an initial body weight of 23.3 ± 0.33 kg were used in a 42-day trial. Based on the initial body weight, pigs were allotted to one of four treatments in a completely randomised block design with 10 replicate pens per treatment and five pigs per pen (mixed sex). Dietary treatments consisted of a basal diet and a basal diet supplemented with 0.01, 0.02, or 0.03% GOX (1000 unit/g). Each of the treatments were added to the basal diet at the expense of corn and all diets were formulated to meet the nutrient requirements recommended by the National Research Council (NRC 2012; Table 1).

Table 1. Experimental diet compositions (as fed-basis).

	Glucose oxidase, %			
Items	0.00	0.01	0.02	0.03
Ingredients, %				
Corn	75.05	75.04	75.03	75.00
Soybean meal	19.88	19.88	19.88	19.88
Tallow	1.88	1.88	1.88	1.90
Dicalcium phosphate	1.28	1.28	1.28	1.28
Limestone	0.73	0.73	0.73	0.73
Salt	0.20	0.20	0.20	0.20
Methionine (99 %)	0.08	0.08	0.08	0.08
Lysine	0.52	0.52	0.52	0.52
Threonine (99 %)	0.13	0.13	0.13	0.13
Tryptophan (99 %)	0.02	0.02	0.02	0.02
Choline (25 %)	0.03	0.03	0.03	0.03
Mineral mix[a]	0.10	0.10	0.10	0.10
Vitamin mix[b]	0.10	0.10	0.10	0.10
Glucose oxidase	–	0.01	0.02	0.03
Total	100.00	100.00	100.00	100.00
Calculated value, %				
Metabolizable energy (MJ/kg)	13.82	13.82	13.82	13.82
Analysed composition, %				
Crude protein	16.00	16.00	16.00	16.00
Calcium	0.66	0.66	0.66	0.66
Phosphorus	0.56	0.56	0.56	0.56
Lysine	1.16	1.16	1.16	1.16
Methionine	0.32	0.32	0.32	0.32
Crude fat	4.75	4.75	4.75	4.77
Crude fibre	2.48	2.48	2.48	2.48
Crude ash	4.47	4.47	4.47	4.47

[a]Provided per kg diet: Fe, 138 mg as ferrous sulphate; Cu, 84 mg as copper sulphate; Mn, 24 mg as manganese oxide; Zn, 72 mg as zinc oxide; I, 0.6 mg as potassium iodide; and Se, 0.36 mg as sodium selenite.

[b]Provided per kilograms of diet: vitamin A, 15,600 IU; vitamin D_3, 2,040 IU; vitamin E, 72 IU; vitamin K_3, 6 mg; vitamin B_1, 5.04 mg; vitamin B_2, 22.8 mg; vitamin B_6, 8.04 mg; vitamin B_{12}, 0.06 mg; biotin, 0.408 mg; folic acid, 2.52 mg; niacin, 66 mg; D-calcium pantothenate, 54 mg.

All pigs were housed in an environmentally controlled room. The ambient temperature and humidity were controlled at 24 °C and 60%, respectively. The room was equipped with a mechanical ventilation system and the floor was slatted plastic. The pen size was 1.43 m × 1.67 m. Providing light for 16 h daily throughout the experiment. The one-side stainless steel self-feeder and nipple drinker were installed for pigs to give the animals free access to feed and water.

Sample collection and measurements

Body weight of pigs weighed individually on the 1st, 21st, and 42nd days. Pen-based average body weight was used to measure the average daily gain (ADG). Feed consumption was recorded daily on a pen basis to calculate the average daily feed intake (ADFI). The feed conversion ratio (FCR) was measured using ADG and ADFI values.

During days 14–21 and 35–42 of this experiment, 0.20% chromium oxide was supplemented to the diet to determine the apparent total tract digestibility

(ATTD) of dry matter (DM) and nitrogen, and the apparent energy retention. Representative feed samples in each dietary treatment were taken after mixing. On the 21st and 42nd days, two pigs were randomly selected from each pen to take the faecal samples via the rectal massage method. All feed and faecal samples were stored at −20 °C until analysis. Before chemical analysis, samples were dried for 72 h in a 60 °C oven. After that, samples were ground into powder, which can pass through a 1 mm sieve, and be collected. Following the procedure established by the Association of Official Analytical Chemists (AOAC 2000), diet samples were analysed for DM (method 930.15), crude protein (nitrogen × 6.25; method 968.06), crude fibre (method 991.43), calcium (method 984.01), phosphorus (method 965.17), and crude fat (method 954.02), and crude ash (method 942.05). Faecal powder samples were also analysed for DM (method 930.15) and crude protein (method 968.06) following the procedures established by AOAC (2000). The lysine and methionine content of the diets were measured using an AA analyser (Beckman 6300; Beckman Coulter, Inc., Fillerton, CA, USA). The combustion heat was measured by a bomb calorimeter (Parr 6100; Parr Instrument Co., Moline, IL, USA) to determine the gross energy content of the feed and excreta powder samples. The chromium levels were analysed via UV absorption spectrophotometry (UV-1201, Shimadzu, Kyoto, Japan). The equation for calculating digestibility was as follows: digestibility (%) = (1 − ((Nf × Cd)/(Nd × Cf))) × 100, where Nf = nutrient concentration in excreta (% DM), Cd = chromium concentration in diet (% DM), Nd = nutrient concentration in diet (% DM), and Cf = chromium concentration in excreta (% DM).

On the 21st and 42nd days, faecal samples were collected via the same method as above for measuring the counts of coliform bacteria and lactic acid bacteria in faeces from two randomly selected pigs. Samples were pooled on a pen basis and placed on ice for transportation to the laboratory, where analysis was immediately carried out. One gram of the composite faecal sample from each pen was diluted with 9 mL of 1% peptone broth (Becton, Dickinson and Co., Franklin Lakes, NJ, USA), and homogenised. The counts of microbial in the faecal samples were calculated through plating serial 10-fold dilutions (in 1% peptone solution) onto MacConkey agar plates (Difco Laboratories, Detroit, MI) or Lactobacilli medium III agar plates (Medium 638, DSMZ, Braunschweig, Germany) to isolate coliform bacteria and lactic acid bacteria, respectively. The lactobacilli medium III agar plates were incubated for 48 h at 39 °C with anaerobic conditions. Besides, the MacConkey agar plates were incubated for 24 h at 37 °C under aerobic conditions. The colonies of coliform bacteria and lactic acid bacteria were counted immediately after removal from the incubator.

On the 21st and 42nd days, blood samples were collected from randomly selected from two pigs per pen via anterior vena cava puncture into non-heparinized vacuum tubes (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ, USA). The blood samples were collected during 11:00 to 12:00 h in order to exclude the circadiurnal fluctuations in hormone concentrations. Blood samples were centrifuged (3,000 × *g*) for 15 min at 4 °C to obtain serum samples and then stored at −20 °C until analysis. The glutathione (GSH), glutathione peroxidase (GPX), and superoxide dismutase (SOD) in serum were measured using GSH Activity Colorimetric Assay Kit (Catalog Number #K261-100, Biovision, Milpitas, CA, USA), GPX Activity Colorimetric Assay Kit (Catalog Number #K762-100, Biovision, Milpitas, CA, USA), and SOD Colorimetric Activity Kit (Catalog Number #K028-H1, DetectX, Ann Arbour, MD, USA), respectively. The blood glucose concentrations were analysed by an automatic biochemistry blood analyser (HITACHI 747; Hitachi, Tokyo, Japan).

Statistical analysis

All data were subjected to statistical analysis in a randomised completely block design using the General Linear Model procedure (SAS Inst. Inc., Cary, NC, USA). The replicate pen was used as the experimental unit. Orthogonal contrasts were used to examine the linear, quadratic, and cubic effects in response to increasing the dietary supplementation of GOX. Variability in the data was expressed as the standard error of means (SEM), $P < .05$ was considered to be statistically significant.

Results

Body weight on day 42 ($p = .035$), ADG during days 22–42 ($p = .001$) and 1–42 ($p = .001$), and ADFI during days 22–42 ($p = .008$) and 1–42 ($p = .020$) increased linearly with the increasing dose of GOX. The decreased tendency regarding FCR during days 1–42 ($p = .062$) was observed in pigs consuming graded levels of GOX containing diet (Table 2).

Growing pigs fed diet with increasing level of GOX linearly increased apparent nitrogen digestibility

Table 2. Effect of dietary supplementation of glucose oxidase (GOX) on growth performance in growing pigs[a].

	GOX, %					*p* Value		
Items	0.00	0.01	0.02	0.03	SEM	Linear	Quadratic	Cubic
Body weight, kg								
Day 1	23.3	23.3	23.3	23.3	0.33	.989	.993	.995
Day 21	36.1	36.3	36.2	36.4	0.39	.558	.903	.708
Day 42	51.7	52.3	52.6	53.1	0.47	.035	.997	.789
ADG, g								
Days 1–21	606	619	615	624	8.29	.186	.797	.398
Days 22–42	746	759	776	794	9.99	.001	.827	.919
Days 1–42	676	689	696	709	6.50	.001	.997	.644
ADFI, g								
Days 1–21	1332	1343	1336	1345	12.33	.547	.933	.547
Days 22–42	1840	1867	1888	1896	15.52	.008	.533	.939
Days 1–42	1586	1605	1612	1621	10.50	.020	.610	.767
FCR								
Days 1–21	2.20	2.17	2.18	2.16	0.02	.113	.757	.455
Days 22–42	2.48	2.47	2.44	2.40	0.04	.158	.636	.968
Days 1–42	2.35	2.33	2.32	2.29	0.02	.062	.814	.814

ADG: average daily gain; ADFI: average daily feed intake; FCR: feed conversion ratio; SEM: standard error of the mean.
[a]Values represent the means of 10 pens with five pigs per replicate pen ($n = 50$) per treatment for body weight and 10 pens ($n = 10$) per treatment for ADG, ADFI, and FCR.

Table 3. Effect of dietary supplementation of glucose oxidase (GOX) on apparent total tract digestibility in growing pigs[a].

	GOX, %					*p* Value		
Items, %	0.00	0.01	0.02	0.03	SEM	Linear	Quadratic	Cubic
Dry matter								
Day 21	79.0	80.0	79.3	80.2	0.62	.333	.867	.252
Day 42	78.2	78.3	78.5	78.9	0.73	.402	.891	.885
Nitrogen								
Day 21	76.4	77.6	76.7	77.8	0.67	.317	.945	.167
Day 42	75.2	75.9	76.4	76.8	0.52	.029	.770	.954
Energy								
Day 21	78.0	78.9	78.0	79.0	0.45	.262	.887	.057
Day 42	76.0	76.6	77.1	77.5	0.50	.032	.920	.995

SEM: standard error of the mean.
[a]Values represent the means of 10 pens with two pigs per replicate pen ($n = 20$) per treatment.

($p = .029$) and apparent energy retention ($p = .032$) on day 42, but had no effect on the apparent DM digestibility (Table 3).

Supplementing GOX to the diet of growing pigs had no effects on the counts of faecal lactic acid bacteria and faecal coliform bacteria (Table 4).

The serum GPX ($p = .044$) and GSH ($p = .032$) concentrations on day 42 increased linearly at graduated doses of GOX increased in the diet. Supplementing GOX to the diet did not affect the glucose and SOD concentrations (Table 5).

Discussion

GOX has been reported to improve the growth performance of weaning pigs through increasing serum growth hormone levels, ameliorating stress response, improving nutrient digestibility, and regulating

Table 4. Effect of dietary supplementation of glucose oxidase (GOX) on faecal bacteria counts in growing pigs[a].

	GOX, %					*p* Value		
Items, log_{10}cfu/g	0.00	0.01	0.02	0.03	SEM	Linear	Quadratic	Cubic
Coliform bacteria								
Day 21	7.20	7.18	7.19	7.17	0.04	.680	.955	.744
Day 42	7.16	7.13	7.15	7.12	0.05	.595	1.000	.692
Lactic acid bacteria								
Day 21	9.28	9.30	9.31	9.33	0.03	.198	.863	.904
Day 42	9.25	9.26	9.29	9.31	0.03	.142	.892	.680

SEM: standard error of the mean.
[a]Values represent the means of 10 pens with two pigs per replicate pen ($n = 20$) per treatment.

Table 5. Effect of dietary supplementation of glucose oxidase (GOX) on serum antioxidant enzyme parameters in growing pigs[a].

	GOX, %					*p* Value		
Items	0.00	0.01	0.02	0.03	SEM	Linear	Quadratic	Cubic
Glucose, mg/dL								
Day 21	85.8	86.3	86.5	88.0	1.56	.335	.754	.833
Day 42	86.3	87.5	87.8	89.0	1.40	.201	1.000	.755
Superoxidase dismutase, U/mL								
Day 21	0.49	0.64	0.59	0.74	0.12	.192	1.000	.480
Day 42	0.82	0.80	0.86	0.85	0.04	.420	.923	.389
Glutathione peroxidase, μmol/L								
Day 21	10.0	10.1	10.1	10.4	0.47	.532	.874	.810
Day 42	13.4	16.8	15.1	17.9	1.17	.044	.799	.098
Glutathione, ug/μL								
Day 21	0.91	1.18	1.02	1.22	0.11	.147	.731	.125
Day 42	0.79	0.85	0.86	0.94	0.05	.032	.785	.560

SEM: standard error of the mean.
[a]Values represent the means of 10 pens with two pigs per replicate pen ($n = 20$) per treatment.

intestinal microbiota communities (Tian et al. 2012; An et al. 2014; Wu et al. 2015; Chen et al. 2015; Tang et al. 2016; Mu et al. 2018). The results from the present study showed that supplementing 0.01, 0.02, or 0.03% GOX (1000 unit/g) to the diet of growing pigs increased body weight, ADG, apparent nitrogen digestibility, apparent energy retention, and serum GPX and GSH concentrations, however, did not affect the faecal lactic acid bacteria and coliform bacteria counts. The improvement of apparent nutrient digestibility means that animals get more nutrient ingredients from feed, which was helpful to improve growth performance (Attia et al. 2015, 2017; Dang et al. 2021). Antioxidant enzymes could remove the reactive oxygen species, thus avoiding the occurrence of various diseases and physiological and behavioural changes caused by oxidase stress which was beneficial to growth performance (Yuan et al. 2007). In addition, Dowarah et al. (2018) reported that the improvement of the antioxidant status of pigs has corresponded to the enhancement of nutrient digestibility and the subsequent improvement in growth performance parameters. Therefore, supplementing 0.01, 0.02, or 0.03% GOX to the diet of growing pigs had a positive effect

on growth performance, which was related to the increase of apparent nutrient digestibility and serum antioxidant enzyme concentrations. In addition, the increase in feed intake means that animals have taken in much energy (Dang et al. 2021). The improvement of ADFI observed in this study was also helpful to improve growth performance. Therefore, the application of GOX to the diet of growing pigs has the potential to be used as a growth promoter.

The carbohydrates in the feed are converted into glucose by digestive enzymes in the intestine and then absorbed by the intestine. It is reported that the increase in absorbable glucose concentration in the intestine can be inhibited by inhibiting the secretion of digestive enzymes, so as to avoid the increase in blood glucose (Yu et al. 2019). Therefore, the fluctuation of intestinal absorbable glucose concentrations may affect the blood glucose level. GOX is known to specifically oxidise glucose into gluconic acid and produce hydrogen peroxide, and consume large amounts of oxygen (Yu et al. 2017), thus affecting the intestinal bacteria communities. Therefore, the existence of GOX in the intestine was beneficial to reduce the concentration of absorbable glucose, thereby affecting blood glucose levels. However, the glucose concentration was not affected in growing pigs fed the diet supplemented with GOX, which probably means that the biological activity of GOX was not exerted in the intestine. Tang et al. (2016) shown that supplementing 0.02% GOX (1000 unit/g) to the diet of weaning pigs had no effects on the concentration of glucose in the blood, and the counts of caecal lactic bacteria and coliform bacteria counts. In addition, Dang et al. (2021) reported that feeding weaning pigs with GOX-containing diet did not affect the blood glucose levels, also had no effects on faecal lactic bacteria and coliform bacteria counts. Although supplementing GOX to the diet of weaning pigs has been reported to increase the intestinal beneficial bacteria (lactic acid bacteria) counts and decrease the intestinal harmful bacteria (coliform bacteria) counts (Chen et al. 2015). However, they did not report the change in the concentration of glucose in the blood. More researches are needed to explore the mechanism of dietary supplementation of GOX on improving intestinal bacteria counts and to explore the indicators of its activity.

Regarding the effect of GOX supplementation on the nutrient digestibility of pigs, Chen et al. (2015) reported that weaning pigs fed with 0.02% GOX (1000 unit/g) containing diet increased the apparent nutrient digestibility, which was suggested to be due to an increase in caecal lactic acid bacteria counts and a decrease in caecal coliform bacteria counts. However, the improvement of faecal bacteria counts was not observed by GOX addition in this study. Ali et al. (2011) mentioned that the increase of serum antioxidant enzyme concentrations was helpful to reduce the production of intestinal free radicals, thus improving intestinal health and promoting the absorption of nutrient ingredients. Moreover, Cui et al. (2019) also observed the increase of circulating antioxidant enzyme concentrations corresponding with the high nutrient digestibility in pigs. In this study, growing pigs fed the diet supplemented with GOX increased nutrient digestibility and serum antioxidant enzyme concentrations. The nutrient digestibility of pigs improved by GOX addition was demonstrated by Chen et al. (2015), they noted that supplementing 0.02% GOX (1000 unit/g) to the diet of weaning pigs increased apparent total tract digestibility of dry matter and nitrogen. Moreover, An et al. (2014) observed that the apparent energy retention increased in weaning pigs fed with 0.07, 0.13, or 0.20% GOX (15 unit/g) containing diet. However, they did not investigate the effects of dietary supplementation of GOX on the relationship between nutrient digestibility and serum antioxidant enzyme concentrations. More studies are needed to evaluate the relationship between nutrient digestibility and serum antioxidant enzyme concentration in pigs consuming a GOX containing diet. In this study, the nutrient digestibility improved by GOX addition was considered due to the antioxidant enzyme concentrations increased, which was probably beneficial to the nutrient absorption environment, thereby improving the nutrient digestibility.

Regarding the effects of supplementing GOX to the diet of pigs on the serum antioxidant enzyme concentrations, Mu et al. (2018) reported that supplementing 0.04% GOX (250 unit/g) to the diet of weaning pigs increased serum GSH and SOD concentrations. The oxidative stress alleviation capacity of GOX has been reported by Cruz et al. (2012) and Zhang et al. (2020). Moreover, it has been reported that supplementing GOX to the diet of broiler chicks leads to an increase in serum antioxidant enzyme concentrations (Wang et al. 2018; Wu et al. 2019). In this study, the improvement of GPX and GSH concentrations in serum were observed by GOX addition. GPX and GSH are considered the most representative oxidation status indicators in *vivo* (Olofsson et al. 2005). As mentioned by Dang et al. (2021), raising the levels of antioxidant enzyme in animals to avoid oxidative stress was a way to improve the performance of pigs. Therefore, supplementing GOX to the diet of growing pigs increased

the concentrations of serum antioxidant enzyme, which was probably beneficial to the performance and production traits of growing pigs.

Conclusion

In brief, dietary supplementation of 0.01, 0.02, or 0.03% GOX (1000 unit/g) improved growth performance, apparent nutrient digestibility, and serum antioxidant enzyme parameters of growing pigs in a dose-dependent manner, which partially confirmed our hypothesis. Therefore, the application of GOX in the diet of growing pigs has the potential to be used as the growth promoter as it can enhance nutrient digestibility and serum antioxidant levels. In this study, the suitable dosage of GOX used in the diet of growing pigs was 0.03%.

Ethical approval

The protocol (DK-2-2016) used for the conduction of this research was approved by the Dankook University Animal Care and Use Committee (Cheonan, South Korea).

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Disclosure statement

The authors confirm that there were no conflicts of interest associated with the publication.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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