Effects of glucose oxidase on the growth performance, serum parameters and faecal microflora of piglets

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Abstract

The experiment was conducted to investigate the effects of diets supplemented with glucose oxidase (GOD) on growth performance, serum parameters and faecal microflora of piglets. One hundred and twelve piglets (35 days old) were randomly assigned to two groups (four replicates per group, half male and half female, and 14 piglets per replicate) and fed a diet with or without 100 U GOD per kg, for 35 days. Feeding GOD caused a higher average daily weight gain and feed intake, and lower feed conversion ratio (FCR) of piglets. No significant difference was observed in the reference values of serum biochemical parameters between the groups fed with or without GOD. The contents of triiodothyronine, thyroxine and growth hormone of piglets fed GOD were higher than those of the control. Moreover, GOD supplementation suppressed the concentration of faecal *Salmonella*. The results showed that supplementation of GOD to diets promoted growth performance, increased the contents of growth and development-related hormones, and improved the faecal microflora of growing piglets.

Keywords: Glucose oxidase, intestinal health, performance, swine
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Introduction

Glucose oxidase (GOD) is a flavoprotein, which catalyses the oxidation of β-D-glucose to D-glucono-δ-lactone and hydrogen peroxide (H_2O_2), using molecular oxygen as an electron acceptor (Hatzinikolaou *et al*., 1996; Pluschkell *et al*., 1996). Glucose oxidase has several commercial applications, for example improving colour, flavour and shelf-life of food materials, removing oxygen from fruit juices and canned beverages, inhibiting the growth of pathogens and preventing rancidity in mayonnaise (Tiina *et al.,* 1989; Malherbe *et al*., 2003; Seifu *et al*., 2004; Opwis *et al*., 2008; Bankar *et al*., 2009). Glucose oxidase can catalyse the formation of gluconic acid, and has been used as a food additive to regulate the acidity of sterilization solutions and bleaching agents in food manufacturing and as a salt in chemical components for medication (Nakao *et al*., 1997; Klein *et al*., 2002). Since 1999, GOD has been supplemented into 12 feed preparations, according to the Catalogue of Feed Additive Varieties released by the Ministry of Agriculture, People's Republic of China. There are broad prospects for GOD application in the feed industry. However, owing to low fermentation capacity and high cost, the application of GOD is limited as a feed additive. Recently, the Laboratory for Food Technology developed a new recombinant GOD with high yield (615 U/mL and 2.5 g protein/L in a 3 L fermenter) and favourable properties (Gao *et al*., 2012).

Various commensal and pathogenic bacterial species are present in the gastrointestinal tracts of pigs, especially pathogenic, which represent a major source of economic loss to the pig industry worldwide (Pluske *et al*., 2002; Namroud *et al*., 2010). Of these, haemolytic enterotoxigenic, *Escherichia coli*, is the primary infectious agent of post-weaning colibacillosis, which provokes hypersecretory diarrhoea through the release of specific enterotoxins (Argenzio, 1992; Jones *et al.*, 2001). *Salmonella* is associated with diarrhoeic piglets, with lesions more typical of enterotoxigenic diarrhoeal disease than salmonellosis (Reed *et al.*, 1985). Schwartz (1999) remarked that most diarrhoeic piglets were associated with poor hygiene, concurrent enteric pathogens, inappropriate diets and a poor environment. It is common for haemolytic *E. coli* and *Salmonella* to appear in the faeces of pigs in increased numbers in the first week after weaning in both healthy and diarrhoeic pigs (Hampson *et al*., 1985). Serum biochemical parameters are good indicators of animal health.

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Numerous studies have been conducted to determine fundamental values for serum biochemical and haematological parameters of pigs. For example, triiodothyronine (T_3) and thyroxine (T_4) are tyrosine-based hormones produced by the thyroid gland, which are primarily responsible for regulation of the pig's metabolism (Decuypere *et al.*, 1983; 2005). However, no experiment has been designed to evaluate the interrelationships between blood constituent changes and GOD. Therefore, the present study was designed to evaluate the effects of GOD as a feed additive on growth performance, serum parameters and faecal microflora of piglets.

Materials and Methods

The GOD preparation and millrun were provided by the Challenge Group, Beijing, People's Republic of China. All pigs used in this study were cared for in accordance with the FASS Guide for the Care and Use of Agricultural Animals in Research and Teaching (2012).

The GOD derived from *Penicillium notatum* was modified and produced in *Pichia pastoris* with a high yield (Gao *et al.*, 2012). Recombinant GOD had optimal activity at 35 - 40 ºC and pH 6.2, and was stable at pH 3.0 to 7.0 at 50 ºC. The optimized recombinant GOD yielded 615 U/mL (2.5 g protein/L) in a 3 L fermenter. One GOD unit is defined as that quantity of enzyme that liberates 1 µmol of H_2O_2 per minute under the conditions of 37 ºC and pH 5.5.

All pigs were fed a commercial starter diet for an adaptation period of one week prior to the start of the experiment. The diets consisted of the control (no enzyme) and a control supplemented with GOD (100 U/kg diet). The control diet was formulated to meet or exceed the requirements of the Feeding Standard of Swine of the People's Republic of China (2004) and was composed of maize, soybean meal, soybean oil, extruded soybean, fermented soybean meal, fish meal, glucose, etc. (Table 1).

Table 1 Ingredient composition and nutrient contents of experimental diet (g/kg) (as fed)[a]

Item	Content
Ingredient	
Maize	600.0
Soybean meal	155.0
Soybean oil	16.0
Extruded soybean	80.0
Fermented soybean meal	20.0
Fish meal	34.0
Whey powder	15.0
Glucose	40.0
Premix [b]	40.0
Nutrient content	
Digestible energy (MJ/kg) (calculated)	14.19
Crude protein	185.0
Calcium	7.2
Total phosphorus	6.0
Available phosphorus	3.7
Lysine	13.7
Methionine	5.4
Threonine	9.0
Tryptophan	2.4

[a] The basic diet was formulated with/without addition of glucose oxidase (100 U/kg of diet).
[b] Premix (per kg of diet) contained 30 glysine; 10 g choline; 75 g salt; 200 g Ca; 240 g total P; 4500 mg Fe; 4000 mg Cu; 7500 mg Mn; 3000 mg Zn; 60 mg I; 10 mg Se; 750 mg nicotinic acid; 16 mg folic acid; 375 mg calcium pantothenate; 7.5 mg biotin; 75000 IU vitamin A; 25000 IU vitamin D; 375 IU vitamin E; 14 mg vitamin K; 37.5 mg vitamin B_1; 120 mg vitamin B_2; 78 mg, vitamin B_6; 0.4 mg vitamin B_{12}.

One hundred and twelve piglets, weaned at 21 ± 1 d old, were obtained from a local livestock breeding farm in Beijing, China, and housed 14 in a pen with plastic-covered expanded metal floors. The average weight of the piglets was recorded at the end of the adaptation period. The piglets were then randomly assigned in two groups for control and treatment based on sex and bodyweight. Each group contained four pens (half male and half female), and each pen contained 14 pigs. Pigs had unlimited access to feed and water throughout the five-week study. The room temperature was initially maintained at 28 ºC on week 1, and gradually decreased at about 1.5 ºC per week.

Bodyweight and feed intake were monitored at d 35 to determine average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR). Blood samples were collected from two pigs/pen via jugular venipuncture on d 35. Samples (approximately 6 mL/tube) were collected into plastic tubes without anticoagulant. The blood was allowed to clot at room temperature, followed by centrifugation at 1, 155 × *g* for 5 min at room temperature. Serum was collected and stored at −20 ºC until further analysis. The contents of albumin, globulin and total protein were determined by automatic biochemical analyser (7600; Hitachi Medical (Guangzhou) Co., Ltd., Guangzhou, China), and the coefficient of serum proteins was calculated. The concentrations of T_3, T_4, thyroid-stimulating hormone, growth hormone (GH), insulin and glucagon were determined using radioimmunoassay (Hunter, 1986).

Fresh faecal samples were collected immediately after defecation from three pigs per pen on d 14 and d 35, and subjected to microflora analysis right away. The total counts of bacteria and *E. coli* and *Salmonella* were determined by the dilution method on LB agar (peptone 10.0 g/L, yeast power 5.0 g/L, NaCl 9.0 g/L, agar 15.0 g/L, pH 7.2 and MacConkey agar plates (peptone 20.0 g/L, lactose 10.0 g/L, bile salt 5.0 g/L, NaCl 5.0 g/L, toluylene red 0.03 g/L, and agar 14.0 g/L, pH 7.1)), respectively. *Lactobacilli* were enumerated by using MRS agar plates (peptone 10.0 g/L, beef extract 10.0 g/L, yeast powder 5.0 g/L, diammonium citrate 2.0 g/L, sodium acetate 5.0 g/L, glucose 20.0 g/L, twain 80 1.0 mL/L, $MgSO_4.7H_2O$ 0.58 g/L, K_2HPO_4 2.0 g/L, $MnSO_4.7H_2O$ 0.25 g/L, agar 15.0 g/L, pH 6.8). Approximately 1.0 g of fresh faecal sample was suspended in 99.0 mL of sterile 0.9% normal saline solution, vibrated for 10 min (35 × *g*, 20 ºC), and serially diluted 1.0 mL to 9.0 mL in the same saline solution. Dilutions of 10^4 to 10^6 (0.1 mL) were plated on agar plates and incubated at 37 ºC for 24 h. The total counts of bacteria *E. coli*, *Salmonella* and *Lactobacilli* were then determined. All plates were done in triplicate, and the results were reported as cfu per gram of fresh faecal sample.

Bacterial enumeration data were transformed into lgcfu/g before statistical analysis. The sex effect was insignificant, and was excluded from the model. A pen of 14 pigs was treated as an experimental unit for growth performance data analysis, and each pig was a unit for the serum parameter analysis. The data were submitted to ANOVA using the GLM procedure of SAS (SAS, 2004). Significant differences (P <0.05) were determined by Duncan's multiple range test.

Results and Discussion

The growth performance of pigs in the various groups is summarized in Table 2. No mortality was observed in this experiment. Piglets on control and treatment groups were similar in ADFI (P >0.05), but showed significant differences in the final weight, ADG and FCR (P <0.05). Pigs fed GOD-supplemented diet had higher ADG (382 g/d vs. 341 g/d) and lower FCR (1.76 vs. 1.94) values than those fed the control diet. The natural function of GOD is closely related to its catalytic activity. It catalyses the oxidation of β-D-glucose to gluconic acid by utilizing molecular oxygen as an electron acceptor with simultaneous production of H_2O_2 (Hatzinikolaou & Macris, 1995). Two products of GOD hydrolysis are H_2O_2 and gluconic acid, which may act as a bacteriocide (Crueger & Crueger, 1990; Rasiah *et al*., 2005; Costa *et al*., 2013) and acidity regulator (Nakao *et al*., 1997; Klein *et al*., 2002) to reduce the gastric pH, inhibit harmful bacteria and promote the growth of beneficial bacteria. Biagi *et al.* (2006) reported that feeding gluconic acid can improve the growth performance of piglets after weaning. With large-scale production of GOD by fermentation, it is possible to achieve commercial application of GOD in the feed industry (Gao *et al*., 2012). The researchers' previous study showed that the best dosage of GOD for growth performance improvement of pigs was 100 U/kg diet (Tang *et al*., 2013). Therefore, in the present study 100 U GOD/kg was supplemented into a maize-soybean meal diet. The results confirmed that GOD has positive effects on the weight gain and feed conversion efficiency of piglets.

The effects of GOD supplementation in diets on blood parameters are presented in Table 3. The researchers compared 11 parameters between the control and treatment groups and found significant differences in T_3, T_4 and GH concentrations (P <0.05). Pigs fed GOD-supplemented diets had higher concentrations of T_3 (0.97 ng/mL vs. 0.82 ng/mL), T_4 (6.79 μg/dL vs. 5.45 μg/dL) and GH (3.81 ng/mL vs. 3.35 ng/mL) in the serum than those fed the control diet. Other blood parameters had a few improvements by supplementing the control diet with GOD. However, no significant differences (P >0.05) were observed.

Table 2 Effects of glucose oxidase (GOD) supplementation on the growth performance of piglets[a]

Items	Diet [b]		SEM	*P*-value
	Control	Control + GOD		
Initial weight (kg)	7.2	7.2	0.118	0.832
Final weight (kg)	19.3[c]	20.6[d]	0.416	0.039
ADG (g/d)	341[c]	382[d]	18	0.031
ADFI (g/d)	666	671	32	0.712
FCR (g/g)	1.94[c]	1.76[d]	0.039	0.008

[a] Average initial weight of pigs was weighed after the 7-d adaptation period, and the trial lasted 5 weeks. Data are means of four replicates of 14 pigs per replicate pen.
ADG: average daily gain; ADFI: average daily feed intake on a DM basis; FCR: feed conversion ratio.
[b] Glucose oxidase provided 100 U per kg diet
[c,d] Mean values within a row with different superscripts are significant difference (P <0.05).

Triiodothyronine and thyroxine are tyrosine-based hormones produced by the thyroid gland, and plasma T_4 can be converted into T_3 (Sterling *et al*., 1970). Thyroid hormones have profound effects on important physiological processes such as development and growth (Visser, 1990; Namroud *et al*., 2010) and regulation of metabolism (Decuypere *et al*., 1983; 2005). Another key factor is GH, which mainly stimulates growth, cell reproduction and regeneration (Sorensen *et al*., 1992). Pigs are uniquely sensitive to high levels of GH, which could improve feed conversion and carcass composition (Machlin, 1972). In this experiment, serum T_3, T_4 and GH concentrations were increased by 13.7% after GOD supplementation. The mechanism of this effect is not clear. It might be due to gluconic acid or H_2O_2 produced by GOD. Brookes *et al*. (2005) reported that gluconic acid, as an additive, can be used as an acidity regulator, raising agent, antioxidant and chelating agent in food, feed, etc. In the current study, supplementation of GOD increased the concentrations of T_3, T_4 and GH in serum and promoted the growth and development of piglets, suggesting the beneficial role of GOD in piglet growth.

Table 3 Effects of glucose oxidase (GOD) on the serum parameters of piglets[a]

Items	Diet		SEM	*P*-value
	Control	Control + GOD		
Glucose (mmoL/L)	4.24	4.92	0.87	0.35
Albumin (g/L)	30.31	31.26	1.03	0.54
Globulin (g/L)	13.76	14.22	0.95	0.42
Albumin/globulin	2.15	2.22	0.51	0.68
Total protein (g/L)	44.15	45.33	2.18	0.46
Triiodothyronine (T_3, ng/mL)	0.82[c]	0.97[d]	0.06	0.04
Thyroxine (T_4, µg/dL)	5.45[c]	6.79[d]	0.49	0.03
Thyroid-stimulating hormone (µIU/mL)	2.84	3.20	0.40	0.11
Growth hormone (GH, ng/mL)	3.35[c]	3.81[d]	0.22	0.04
Insulin (µIU/mL)	21.49	22.10	3.28	0.81
Glucagon (pg/mL)	258.7	271.0	26.3	0.33

[a] Data are means of four replicates of two pigs per replicate pen.
[c,d] Mean values within a row with different superscripts differ significantly (P <0.05).

The effects of GOD on faecal microflora of piglets are summarized in Table 4. Pigs fed dietary GOD had lower counts of faecal *Salmonella* (5.3 lgcfu/g vs. 6.0 lgcfu/g, P <0.05) than pigs fed the non-GOD diet

on day 35, but not on day 14 (P >0.05). The counts of total bacteria and *E. coli* in the treatment group were lower than those of the control group, but the differences were not significant (P >0.05). *Lactobacilli* counts showed no difference between the groups fed with or without GOD. In the current experiment, faecal pathogenic bacteria counts, especially *Salmonella,* were lower in the GOD-supplemented diet. The reason is probably that the function of GOD is to act as an antibacterial agent through the production of gluconic acid and H_2O_2. Biagi *et al*. (2006) demonstrated that feeding gluconic acid positively influenced the composition and activity of the intestinal microflora of piglets after weaning. It has been reported that sustained oxidative stress through the maintenance of low concentration of H_2O_2 by GOD's continued catalytic activity to be effective against pathogenic bacteria (Dobbenie *et al*., 1995; Wu *et al*., 1995). Etemadzadeh *et al*. (1985) reported that the ability of GOD to kill *Streptococcus mutans* appears to be enhanced, because the H_2O_2 produced by GOD acts as a useful bacteriocide. Tiina & Sandholm (1989) reported that the application of the GOD-glucose system in food products inhibited the growth of pathogens such as *Salmonella*, *Bacillus cereus*, and *Yersinia*. *Lactobacilli* are lactic acid bacteria and commonly considered to have probiotic effects in the gastrointestinal tract of animals (Zhang *et al*., 2011). Few studies have been performed concerning the effect of GOD on *Lactobacilli*. The effects of GOD on fermented sausages have shown inhibition of growth of *Staphylococcus aureus,* whereas GOD did not affect the growth of *Lactobacilli*. *Lactobacilli* themselves are known to produce H_2O_2 and therefore they are rather resistant to H_2O_2 (Klaenhammer, 1982; Attaie *et al*., 1987). In this study, except for *Lactobacilli*, pigs fed GOD showed decreased concentrations of bacteria enumerated in contrast to the control. It is likely that GOD and their associated physicochemical effects play a beneficial role in maintaining the intestinal microflora.

Table 4 Effects of glucose oxidase (GOD) on the faecal microflora of piglets[a]

Items	Diet		SEM	*P*-value
	Control	Control + GOD		
Total plate count (lgcfu/g)				
d 14	8.155	8.081	0.095	0.57
d 35	8.899	8.847	0.115	0.52
Escherichia coli (lgcfu/g)				
d 14	6.203	6.127	0.112	0.48
d 35	7.287	7.230	0.091	0.54
Salmonella (lgcfu/g)				
d 14	5.263	5.115	0.107	0.22
d 35	5.954[c]	5.300[d]	0.190	0.03
Lactobacilli (lgcfu/g)				
d 14	6.189	6.175	0.036	0.71
d 35	6.864	6.851	0.040	0.65

[a] Data are means of four replicates of three pigs per replicate pen.
[c,d] Mean values within a row with different superscripts differ significantly (P <0.05).

Conclusion

The results showed that supplementation of GOD to diets promoted growth performance (ADG and feed efficiency), increased the concentrations of growth and development-related hormone (T_3, T_4 and GH), and improved the faecal microflora of growing piglets. These changes might be attributed to the functional activities of GOD in the gastrointestinal tract, which utilizes O_2 and produces H_2O_2 and gluconic acid. Further research is needed to determine the effect of dietary GOD supplementation on other parameters of intestinal health.

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