Possibility of using glucose oxidase in the diet to improve selected indicators of blood antioxidant defense, digestibility and growth performance of broiler chicken

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ABSTRACT
We conducted this experiment to establish a consistent result of dietary glucose oxidase (GOX) supplementation in broilers’ growth performance, nutrient digestibility, gas emission, caecum bacterial count, and antioxidant status. A total of 792 broilers of 42.38 ± 0.72 g average body weight (BW) at one-day old were fractionated into four treatment groups (18 birds/pen; 11 pens/treatment) named as 0%, Basal diet; 0.01%, basal diet +100 mg/kg GOX; 0.02%, basal diet + 200 mg/kg GOX; 0.03%, basal diet + 300 mg/kg GOX. For growth performance, this 35 days’ experimental period was divided into three phases (d 1 to 7, d 7 to 21, d 22 to 35, overall). For the final phase, nutrient digestibility, gas emission, caecum bacterial count, and blood parameters were measured. GOX supplementation (0.02%, 0.03%) showed increased body weight gain (BWG) and reduced feed conversion ratio (FCR) during days 22 to 35 and in overall period. At the same time, linear increase of BWG and linear decrease of FCR were observed. Increasing doses of GOX showed linear improvement in dry matter digestibility and a tendency for a gradual increase in energy digestibility. On day 7, GOX supplementation (0.02%, 0.03%) reduced drip loss in meat with a gradual decrease. Blood antioxidant parameters of glutathione peroxidase (GSSG) and glutathione (GSH) were linearly increased by rising doses of GOX where 0.03% GOX had the highest value. In short, GOX supplementation brought improvements in digestibility and antioxidant capacity, which helped to increase body weight gain in broilers.

HIGHLIGHTS
1. Glucose oxidase enzyme supplementation could benefit growth performance in broilers
2. Dietary addition of glucose oxidase could increase apparent nutrient digestibility in broilers
3. As a health benefit, antioxidant status of broilers were improved with glucose oxidase supplementation

Introduction
The poultry industry has a long history of supplying protein to the human population. Antibiotic growth promoters (AGPs) have been used for several years in livestock feed since they helped with growth performance, efficient feed conversion, digestibility, and disease reduction (Gadde et al. 2017). However, after a few years, it was realised that the continuous use of antibiotics as a growth promoter has adverse effects on livestock and human health due to the resistance of microbial to antibiotics along with antibiotic residues in food and the environment. This has led to the imposition of a ban on the use of antibiotics as a growth promoter in animal feed. The World Health Organisation predicted that 10 million deaths could be related to these antibiotic concerns by 2050 (WHO 2019). To find an alternative to AGPs, pre/pro-biotics, phytopgenic extracts, organic acids, oils, and enzymes have been tested (Gadde et al. 2017) in animal diets. Results obtained from these experiments have shown inconsistent and partial effectiveness depending on doses, species, and environment (Oladokun and Adewole 2020). Also, it looks like a single additive cannot replace the whole AGP usage.

Glucose oxidase (GOX) is an enzyme that can be extracted from different fungal strains. \textit{Asperillus niger} and \textit{Penicillium} are the most common sources of GOX (Danielle et al. 2017). This enzyme catalyses glucose...
oxidation and produces gluconic acid and hydrogen peroxide (Wong et al. 2008). As the process uses free oxygen to produce hydrogen peroxide ($\text{H}_2\text{O}_2$), it may have an antioxidant function in farm animals by inactivating free radicals. Danielle et al. (2017) reported glucose oxidase to help in glucose utilisation by oxidation of glucose to gluconolactone. This gluconolactone becomes gluconic acid through further breakdown. Tang et al. (2016) mentioned $\text{H}_2\text{O}_2$ to inhibit salmonella growth in the intestine. In the food and feed industry, GOX has a reputation for food preservation, bacteriostasis (Zhao et al. 2014), growth promoting, and digestibility increasing capabilities (Tang et al. 2016). Asano et al. (1994) mentioned that gluconic acid increased bifidobacteria and reduced Clostridium perfringes. Growth promoting effects have been linked to changes in digestibility, bacterial population and antioxidant status in previous studies (Sun et al. 2014; Tang et al. 2016; Wu et al. 2019). All these characteristics are found in various organisms and conditions. From the above-mentioned literature, we hypothesised that glucose oxidase supplementation might improve nutrient digestibility, selective bacterial population, and antioxidant status, which might help to improve growth performance in broilers. However, inconsistent results on growth, digestibility, meat quality, and antioxidant activity in broilers have been presented by using GOX added diets (Sun et al. 2014; Wu et al. 2019; Heenkenda et al. 2019).

So, we have planned this study to evaluate the possibility of GOX supplementation in the broiler diet to improve growth performance through better digestion and antioxidant capacity while maintaining a beneficial bacterial population with no negative effect on meat quality.

Materials and methods

The planned protocols for the management and care of animals were approved (DK-1-1962) by the Animal Care and Use Committee of Dankook University, South Korea.

Experimental design, animals, and diets

A total of 792 Ross 308 male broilers of 42.38 ± 0.42 g were used in a 35 days trial to evaluate the growth performance, meat quality and organ weight, caecum microbial, gas emission, nutrient digestibility, and blood antioxidant profile. Broilers were randomly allotted into four treatments according to their initial body weight (BW). There were 11 replication pens in each treatment where each pen had 18 chickens. The experimental diets (Table 1) were prepared following Aviagen (2019) requirements for poultry. Dietary treatment groups were as follows: 1) 0%, Basal diet; 2) 0.01%, basal diet + 100 mg/kg GOX; 3) 0.02%, basal diet + 200 mg/kg GOX; 4) 0.03%, basal diet + 300 mg/kg GOX. The GOX was added by replacing corn in the diet. The commercial GOX (Bestzyme bio-engineering Co., LTD; China) was produced from Aspergillus niger. According to manufacturer information, GOX had an optimum functioning temperature of 20–80°C with a pH range of 2.0–7.0. The commercial GOX (solid form) was mixed with basal broiler diet to prepare the treatment diets. First the birds were randomly allocated to 44 pens (18 birds/pen). Then, the experimental diets were assigned in a randomised block design to the pens.

The test was conducted at a research farm of Dankook University. Feeds of corn - soybean meal were fed to the experimental diets according to the requirement of Aviagen (2019). ROSS 308 chicks were raised in a three – stage cage, and the position of the treatments was adjusted, and the feed and water were ad libitum.

Growth performance

Body weight was measured by pen at seven days, 21 days, and at the last day (35 days). Feed intake (FI)

| Table 1. Composition of broiler diets (as fed-basis). |
|------------------------------------------------------|
| Item | Starter | Grower | Finisher |
|------|---------|---------|----------|
| Ingredients, % | | | |
| Corn | 43.63 | 47.45 | 53.78 |
| Corn gluten meal | 35.08 | 31.28 | 28.18 |
| Soybean meal | 13 | 13 | 10 |
| Wheat bran | 3 | 3 | 3 |
| Soyoil | 1.76 | 1.74 | 1.51 |
| Tri calcium phosphate | 1.81 | 1.81 | 1.81 |
| Limestone | 0.94 | 0.94 | 0.94 |
| Salt | 0.36 | 0.36 | 0.36 |
| Methionine | 0.19 | 0.19 | 0.19 |
| Lysine | 0.03 | 0.03 | 0.03 |
| Mineral mixa | 0.1 | 0.1 | 0.1 |
| Vitamin mixb | 0.1 | 0.1 | 0.1 |
| Total | 100 | 100 | 100 |
| Calculated value | | | |
| Crude protein, % | 23 | 21.5 | 20 |
| Ca, % | 1.1 | 1.08 | 1.07 |
| P, % | 0.83 | 0.82 | 0.79 |
| Available P, % | 0.54 | 0.53 | 0.52 |
| Lys, % | 1.26 | 1.15 | 1.06 |
| Met, % | 0.54 | 0.52 | 0.5 |
| ME, kcal/kg | 3200 | 3200 | 3200 |
| Fat, % | 4.45 | 4.51 | 4.52 |
| Fibre, % | 3.55 | 3.48 | 3.3 |
| Ash, % | 6.76 | 6.57 | 6.3 |

Provided per kg of complete diet: 37.5 mg ZnSO4; 37.5 mg MnO2; 37.5 mg FeSO4·7H2O; 3.75 mg CuSO4·5H2O; 0.83 mg I; and 0.23 mg Na2SeO3. Provided per kg of complete diet: 15,000 IU of vitamin A, 3,750 IU of vitamin D3, 3.75 IU of vitamin E, 2.53 mg of vitamin K3, 3 mg of Thiamine, 7.5 mg of Rivoflavin, 4.5 mg of vitamin B6, 24 ug of vitamin B12, 51 mg of Niacin, 1.5 mg of Folic acid, 0.2 mg of Biotin and 13.5 mg of Ca-Pantothenate. Ca: calcium; P: phosphorus; Lys: Lysine; Met: Methionine; Me: Metabolizable energy.
was calculated by subtracting the remaining amount from the feed amount during body weight gain (BWG), and the feed conversion ratio (FCR) was calculated by dividing the feed intake by the body weight gain (BWG). For growth performance, each pen was used as a unit.

**Nutrient digestibility**

Chromium oxide (Cr$_2$O$_3$, 0.5%), an indigestible marker was added to the diets seven days prior to faecal collection to calculate the nutrient digestibility at the end of experiment. Compound faecal samples were collected from each pen (11 samples/treatment). Based on treatment, all the feed and faecal samples were dried (70 °C for 72 h) and finely ground to be able to pass through a 1 mm screen. Then, all the feed and faecal samples were analysed for dry matter (DM) (method 930.15), nitrogen (N) (method 990.03) following the procedures outlined by the Association of Official Analytical Chemists International AOAC (2000). Chromium was analysed via UV/VIS spectrophotometer (Optizen POP, Korea) Williams et al. (1962). For calculating the ATTD of the nutrients, we used the following formula:

\[
\text{Digestibility} = 1 - \frac{(N_f \times C_d)}{(N_d \times C_f)} \times 100,
\]

where \(N_f\) = concentration of nutrient in faecal (% DM), \(N_d\) = concentration of nutrient in the diet, \(C_d\) = concentration of chromium in the diet, and \(C_f\) = concentration of chromium in the faecal. The gross energy was determined by measuring the heat of combustion by Parr 6400 oxygen bomb calorimeter (Parr Instrument Co., USA). The apparent total tract digestibility (ATTD) of dry matter, nitrogen and energy were calculated using indirect methods described by Williams et al. (1962).

**Meat quality**

At the end of the experiment (35 days), 11 birds per treatments (1 bird per each replication pen) were randomly selected and slaughtered. Breast meat samples were collected and taken to the laboratory for meat quality tests. Immediately meat colour of the lightness (L*), redness (a*), and yellowness (b*) was determined using a Minolta Chromameter (CR-210, Minolta, Japan) to evaluate the freshly cut surface. To estimate the water holding capacity (WHC), we used pressure method. From each sample a 0.2 gm meat was wrapped with a filter paper (125 mm) and put under 3000 psi force for 3 minutes. The original sample area and later expressed moisture area were marked and measured with a digitising area-line sensor (MT-105; M. T. Precision Co. Ltd, 123, Japan). To determine cooking loss, meat samples (4 g/sample) were cooked in plastic bags in a water bath (75 °C) for 30 min (Kauffman et al. 1986). After cooling down, the samples were weighed. The weight loss was expressed as percentage to calculate the cooking loss. Cumulative drip loss was measured by putting 4 g sample in a plastic bag at 4 °C (Honikel 1998). On d 1, d 3, d 5, and d 7, sample weight was recorded and weight loss was expressed in percentage.

**Antioxidant status in blood**

Blood samples (11 samples/treatment) were collected by venipuncture and collected using a K3EDTA Vacuum tube (Becton Dickson Vacutainer Systems, Franklin Lakes, NJ) on week 5. For each sample, blood from two different chickens were collected and mixed in one tube. Blood samples were centrifuged (3000 x g) for 15 min at 4 °C to obtain serum samples and then stored at −20 °C until analysis. The glutathione (GSH), glutathione peroxidase (GSH-PX), 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.

**Statistical analyses**

All data were statistically analysed using the GLM procedure of the SAS program (SAS 2014). Orthogonal contrast and Tukey’s multiple range test were conducted. Growth performance data represents 11 replication pens per treatments. Caecum microbial...
represents 11 samples per treatment and meat quality represents 11 sample per treatments. Blood profile data represents 11 samples per treatment. Data variability was expressed as the standard error of mean (SEM) and the level of significance was set at $p < .05$.

### Results

#### Growth performance

GOX supplementation on broiler growth performance showed positive responses in between groups and a gradual increase (Table 2). GOX supplementation improved ($p < .05$) BWG in groups supplemented with 0.02% and 0.03% GOX compared to the basal diet group during days 21 to 35 and in the overall period. At the same time, FCR was reduced ($p < .05$) in the same groups compared to the group without GOX supplementation. Increasing doses of GOX supplementation brought linear improvement ($p < .05$) in BWG and FCR during days 21 to 35 and in the overall period. However, GOX supplementation did not manipulate ($p > .05$) FI in this experiment.

| Items | 0 % | 0.01 % | 0.02 % | 0.03 % | SEM | Linear | Quadratic | Cubic |
|-------|-----|--------|--------|--------|-----|--------|-----------|-------|
| BWG, g | 110 | 113 | 113 | 107 | 2 | .373 | .083 | .898 |
| FI, g | 137 | 138 | 138 | 136 | 3 | .877 | .709 | .943 |
| FCR | 1.247 | 1.224 | 1.233 | 1.276 | 0.03 | .462 | .276 | .985 |
| BWG, g | 584 | 600 | 613 | 605 | 10 | .095 | .241 | .693 |
| FI, g | 854 | 862 | 887 | 871 | 15 | .263 | .436 | .392 |
| FCR | 1.467 | 1.436 | 1.452 | 1.441 | 0.03 | .658 | .751 | .601 |
| BWG, g | 1010$^b$ | 1049$^{ab}$ | 1056$^a$ | 1073$^a$ | 21 | .048 | .610 | .666 |
| FI, g | 1702 | 1720 | 1733 | 1747 | 23 | .143 | .919 | .956 |
| FCR | 1.689$^a$ | 1.624$^{ab}$ | 1.598$^b$ | 1.558$^b$ | 0.02 | .003 | .602 | .634 |
| Overall BWG, g | 1705$^b$ | 1762$^{ab}$ | 1782$^a$ | 1785$^a$ | 22 | .011 | .225 | .818 |
| FI, g | 2694 | 2721 | 2759 | 2755 | 32 | .120 | .613 | .693 |
| FCR | 1.599$^a$ | 1.573$^a$ | 1.558$^{ab}$ | 1.503$^b$ | 0.02 | .049 | .351 | .472 |

| Items | 0 % | 0.01% | 0.02% | 0.03% | SEM | Linear | Quadratic | Cubic |
|-------|-----|-------|-------|-------|-----|--------|-----------|-------|
| Dry matter | 72.48 | 73.39 | 74.06 | 74.22 | 0.66 | .047 | .578 | .926 |
| Nitrogen | 70.39 | 71.46 | 73.00 | 72.95 | 1.86 | .280 | .765 | .804 |
| Digestible energy | 73.36 | 73.55 | 74.74 | 74.61 | 0.63 | .083 | .806 | .419 |

There was no significant difference ($p > .05$) in DM, nitrogen, and energy digestibility among the groups (Table 3). However, GOX doses showed a linear improvement ($p < .05$) in excreta dry matter digestibility and a trend to increase (linear, $p = .083$) in energy digestibility.

#### Microbial count

In this experiment, none of the bacterial populations differed ($p > .05$) (Table 4). Moreover, increasing doses of GOX did not influence the *Lactobacillus*, *E. coli* and *Salmonella* population in broilers’ caecum.

#### Meat quality

GOX supplementation impacted on drip loss only in the meat quality (Table 5). Drip loss on day 7 was reduced (linear, $p < .05$) by GOX supplementation in broilers’ meat compared to the group without GOX supplementation. Meat pH, colour, water holding

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**Table 2. Effect of dietary glucose oxidase supplementation on growth performance in broiler**

| Items | 0 % | 0.01 % | 0.02 % | 0.03 % | SEM | Linear | Quadratic | Cubic |
|-------|-----|--------|--------|--------|-----|--------|-----------|-------|
| BWG, g | 110 | 113 | 113 | 107 | 2 | .373 | .083 | .898 |
| FI, g | 137 | 138 | 138 | 136 | 3 | .877 | .709 | .943 |
| FCR | 1.247 | 1.224 | 1.233 | 1.276 | 0.03 | .462 | .276 | .985 |
| BWG, g | 584 | 600 | 613 | 605 | 10 | .095 | .241 | .693 |
| FI, g | 854 | 862 | 887 | 871 | 15 | .263 | .436 | .392 |
| FCR | 1.467 | 1.436 | 1.452 | 1.441 | 0.03 | .658 | .751 | .601 |
| BWG, g | 1010$^b$ | 1049$^{ab}$ | 1056$^a$ | 1073$^a$ | 21 | .048 | .610 | .666 |
| FI, g | 1702 | 1720 | 1733 | 1747 | 23 | .143 | .919 | .956 |
| FCR | 1.689$^a$ | 1.624$^{ab}$ | 1.598$^b$ | 1.558$^b$ | 0.02 | .003 | .602 | .634 |
| Overall BWG, g | 1705$^b$ | 1762$^{ab}$ | 1782$^a$ | 1785$^a$ | 22 | .011 | .225 | .818 |
| FI, g | 2694 | 2721 | 2759 | 2755 | 32 | .120 | .613 | .693 |
| FCR | 1.599$^a$ | 1.573$^a$ | 1.558$^{ab}$ | 1.503$^b$ | 0.02 | .049 | .351 | .472 |

$^a$0 %, Basal diet; 0.01%, basal diet + 100 mg/kg Glucose oxidase; 0.02%, basal diet + 200 mg/kg Glucose oxidase; 0.03%, basal diet + 300 mg/kg Glucose Oxidase. Data represents 11 replication pens per treatment with 18 birds per pen. Tukey’s multiple range test and orthogonal contrast was tested.

$^b$Standard error of means.

$^c$Means in the same row with different superscripts differ ($p < .05$).

BWG: body weight gain; FI: feed intake; FCR: feed conversion ratio.

**Table 3. Effect of dietary glucose oxidase supplementation on nutrient digestibility in broiler**

| Items | 0 % | 0.01% | 0.02% | 0.03% | SEM | Linear | Quadratic | Cubic |
|-------|-----|-------|-------|-------|-----|--------|-----------|-------|
| DM | 72.48 | 73.39 | 74.06 | 74.22 | 0.66 | .047 | .578 | .926 |
| Nitrogen | 70.39 | 71.46 | 73.00 | 72.95 | 1.86 | .280 | .765 | .804 |
| Digestible energy | 73.36 | 73.55 | 74.74 | 74.61 | 0.63 | .083 | .806 | .419 |

$^a$0 %, Basal diet; 0.01%, basal diet + 100 mg/kg Glucose oxidase; 0.02%, basal diet + 200 mg/kg Glucose oxidase; 0.03%, basal diet + 300 mg/kg Glucose Oxidase. Data represent 11 samples per treatment. Tukey’s multiple range test and orthogonal contrast was tested ($p < .05$).

$^b$Standard error of means.

$^c$Level of significance $p < .05$.
capacity, and cooking loss did not show any influence (p > .05) of GOX supplementation.

**Blood antioxidant parameters**

GOX supplementation showed a noticeable impact on specific indicators of the antioxidant status of broilers (Table 6). SOD and GPx activity did not vary (p > .05) among the groups. GSSG and GSH were higher (p < .05) in the 0.03% GOX supplemented group compared to the basal group, with a linear increase (p < .05) by GOX supplementation. GSH:GSSG did not vary (p > .05) among the groups.

**Discussion**

GOX is an enzymatic supplement that can reduce the amount of AGP used in the broiler. It breaks down glucose into gluconic acid and H₂O₂. Gluconic acid and H₂O₂ are supposed to reduce the pH of the digestive chime (semi fluid mass of partially digested feed materials with digestive system secretions in the stomach and intestine) and prevent bacterial growth.
As a result, GOX has been found to improve the growth performance of the broiler (Huo et al. 2015). Our study showed a significant linear increase in ADG by GOX supplementation, which is supported by other GOX studies (Sun et al. 2014; Wu et al. 2019; Heenkenda et al. 2019). Sun et al. (2014) used 0.2% combined glucose oxidase with selenium yeast, Wu et al. (2019) used 60 units of GOX in diet and Heenkenda et al. (2019) used 0.025% GOX in drinking water. In our study, ADFI was not different, but FCR was reduced by GOX supplementation. Wu et al. (2019) presented similar results in broilers at a young age (1 to 21 days). Interestingly, Sun et al. (2014) found both ADFI and FCR improved when GOX was combined with selenium yeast. It could have been due to a synergistic effect. In contrast, Wang et al. (2017) reported no effect of GOX supplementation on broiler growth performance. In that study, 75 units of GOX were supplemented, which failed to show a positive response in broiler growth performance. This improved BWG and FCR in the present study may be due to increased digestibility of DM and energy. Wu et al. (2019) mentioned that GOX supplementation in broilers enhanced nutrient digestibility. Biggs and Parsons (2008) mentioned this produced gluconic acid as an important organic acid that increased mineral digestibility. It also helped to improve the growth performance of young chicks. Gluconic acid breaks down into acetic, propionic and mainly butyric acid. Roediger (1980) explained that butyric acid provides principal energy to the epithelial cells of the large intestine, which may result in better nutrient absorption. Again, Wu et al. (2019) found that GOX supplementation increased digestive enzyme activity, which could be related to some specific class of bacteria. In humans, bifidobacteria are the main fermenters of gluconic acid, which may release increased digestive enzymes (Asano et al. 1994). In addition, Liu et al. (2020) reported that GOX supplementation increased villus height and decreased crypt depth, which helped to repair intestinal damage and increased absorption in ducks. In short, GOX supplementation increases gluconic acid break down to butyric acid which can improve intestinal integrity and absorptive capability (Ma et al. 2012).

In the present study, GOX supplementation had insignificant effects on the Lactobacillus, E. coli, or Salmonella populations in caecum digesta. Though GOX is known for reducing bacterial growth in food and feed, it did not work similarly in vivo. Glucose oxidase produces H₂O₂ as a natural oxidant while catalysing glucose (Lee et al. 2020). H₂O₂ is renowned for being used as a natural biocide in agriculture and food storage without any toxic effects (Linley et al. 2012; Dubey et al. 2017). It reduces the availability of oxygen and glucose to bacteria, which are essential for bacterial growth. (Tiina and Sandholm 1989). Asano et al. (1994) and Biagi et al. (2006) previously reported that gluconic acid does not affect the Lactobacillus or E. coli population in the digestive system. It can only increase the bifidobacterial population. In a study, Tang et al. (2016) found that GOX reduces the intestinal Salmonella population in piglets without affecting Lactobacillus and E. coli. That means the antibacterial effect of H₂O₂ is showing different performance in vitro and in vivo conditions. Furthermore, activation of antioxidant properties in broilers could have reduced the availability of H₂O₂ as they deactivate peroxides (Wu et al. 2004).

With regards to meat quality, we have found only drip loss to be linearly reduced. Wang et al. (2017) found a similar result and explained it by the increased antioxidant activity of GOX. Sun et al. (2014) found some positive effects of GOX combined with selenium yeast in meat colour, which is also due to the stress reducing antioxidant mechanism. Precisely, GOX has some antioxidant properties that have a positive effect on meat quality.

SOD and GPx are the primary antioxidant enzymes in animals. SOD neutralises the reactive oxygen and produces H₂O₂, whereas GPx breaks down H₂O₂ into water (Ighodaro and Akinloye 2018). As no changes in blood SOD or GPx concentrations were observed by the inclusion of GOX in the broiler diet in the present study, we can assume that there was no significant oxidative stress produced by GOX supplementation. Though GOX breaks down glucose and produces H₂O₂, GPx did not work to neutralise it. Kryukov et al. (2003) mentioned that GPx activity depends on selenium presence and that it can be impaired by selenium absence. On the other hand, GSH and GSSG concentrations in the blood were linearly increased by GOX supplementation. GSH is a non-enzymatic antioxidant that converts H₂O₂ into water and produces GSSG. The increase in GSH raised the possibility of GOX’s antioxidant mechanism. In the case of SOD inactivation, GSH can be a beneficial antioxidant (Mladenov et al. 2015). Limited supplementation of H₂O₂ or GOX can activate and increase the number of antioxidants, including GSH (Cholia et al. 2017). Some of this GSH must have reacted with the H₂O₂ produced by glucose breakdown and increased the amount of GSSG. It also explains the absence of the antimicrobial effect of H₂O₂ in our experiment.
Conclusion
In brief, 0.02% and 0.03% of GOX supplementation showed clear improvement in growth performance and antioxidant status in broilers. Our study presented a good possibility of GOX being used as a growth promoter in a broiler diet to improve growth performance, partial nutrient digestibility, and antioxidant capacity.

Ethical approval
The planned protocols for the management and care of animals were approved (DK-1-1962) by the Animal Care and Use Committee of Dankook University, South Korea.

Acknowledgements
The Department of Animal Science and Resources was supported through the Research-Focused Department Promotion Project as a part of the University Innovation Support Program for Dankook University in 2021 and the authors gratefully acknowledge Center for Bio-Medical Engineering Core-Facility at Dankook University for providing critical reagents and equipment.

Disclosure statement
The authors declare that there is no conflict of interest.

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