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Original Article

# The Effect of Dietary Glucose Oxidase Supplementation on Production Performance, Egg Quality and Nutrient Digestibility in Laying Hens

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■Keywords

Laying hens, glucose oxidase, feed intake, egg quality.



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## ABSTRACT

The study was conducted to investigate the effect of GOX on performance, egg quality, and nutrient digestibility in laying hens. In total, 432, 50-week-old Hy-Line brown breeder hens were assigned into four treatments, and fed a basal diet with GOX at 0, 100, 200 and 300 units for 10 weeks, respectively. A Quadratic decrease in FI in week 3 ($p$<0.05) and linear increase in egg production in week 6 to 10 and overall experiment period ($p$<0.05) and Quadratic increase in egg production in week 7 ($p$<0.05), a linear decrease in broken egg rate in week 6 ($p$<0.05) a quadratic increase in egg weight on day 14 ($p$<0.05), alinear increase in egg weight on day 28 ($p$<0.05), and linear decrease in yolk color on day 7 ($p$<0.05), a linear increase in yolk color on day 42 and day 70 ($p$<0.05), and linear increase in haugh unit on day 28 and 70 ($p$<0.05), a linear increase albumen height on day 28 and day 56 ($p$<0.05), and linear decrease in shell color on day 14 ($p$<0.05) and day 28($p$<0.05), a linear and quadratic increases in eggshell strength and eggshell thickness on day 56 ($p$<0.05), and linear increase in eggshell strength and eggshell thickness on day 70 (P0.05) were observed with the addition of GOX the the diet. Conclusion: This study suggested that the supplementation of GOX may have beneficial effects on feed intake and egg quality in laying hens.

## INTRODUCTION

Eggs can create considerable value for animal husbandry as animal products. Antibiotics as performance enhancers in layer production have primarily been applied to improve the utilization of the feed and reduce the pathogenic bacteria in the gut, and in turn, improve production performance (Bozkurt *et al.*, 2009). However, due to the increase of multiple resistance bacteria and the decrease of consumers' acceptance of antibiotics, it has been prohibited gradually (Li *et al.*, 2015). In recent years, enzymes feed additives have attracted more and more attention because of their safe and environmentally friendly nature (Li *et al.*, 2015). Some researchers have reported the positive effects of enzymes supplementation on production performance and egg quality of laying hens (Ghasemi *et al.*, 2010; Alagawany & Abd El-Hack, 2015).

GOD (β-d-glucose:oxygen 1-oxidoreductase) catalyzes the oxidation of β-d-glucose to gluconic acid by utilizing molecular oxygen as an electron acceptor with simultaneous production of hydrogen peroxide (H2O2) (Bankar *et al.*, 2009). The glucose oxidase enzyme is commercially produced from Aspergillus niger and Penicillium glaucum through a solid-state fermentation method. Muller was first to report the catalyzation of glucose oxidase and the breakdown of glucose into gluconic acid in the presence of dissolved oxygen (Singh

& Kumar, 2019). Fungal strains Aspergillus niger are able to produce notable amounts of glucose oxidase. Fungal strains Aspergillus niger are able to produce notable amounts of glucose oxidase. Glucose oxidase enzymes are used to remove small amounts of oxygen from food products or glucose from diabetic drinks. Glucose oxidase plays an important role in color development, flavor, texture, and increasing the shelf life of food products (Singh & Kumar, 2019). The enzyme has been widely used in the feed production industry, because it has been verified that GOD has effects on bacteriostasis (Zhao *et al.*, 2014), growth-promotion (Tang *et al.*, 2016), immunity (Cui *et al.*, 2015), and digestion, and it is non-toxic, low-residue, and difficult to arise resistance (Chen, 2017). This enzyme has been widely used in animal production by its characteristics of producing acid, deoxygenation, and sterilization (Kapat *et al.*, 1998). Heenkenda *et al.* (2019) have been shown that 0.025% GOD could significantly improve the BW of broilers. Wu *et al.* (2019) also indicated that dietary supplement GOD could significantly influence growth performance of broilers between days 1 to 21, and even achieve similareffects as antibiotic supplemented groups. Tang *et al.* (2016) and Mu *et al.* (2018) declared that GOD significantly improve the ADG and decrease the feed-to-gain ratio (F:G) of weaned piglets.

To our knowledge, there is little research reported on the effect of these additives in laying hens. Therefore, the current study was designed to evaluate the effect of glucose oxidase supplementation to layer diets on laying performance, egg quality, and nutrient digestibility.

## MATERIALS AND METHODS

Animal experiments were approved by the Dankook University Animal Care and Use Committee, Cheonan, Republic of Korea. (Permit number DK-1-1963).

### Sources of Gox

The commercial GOX (Bestzyme Bio-engineering Co., LTD; Jinan, China) was expressed by Aspergillus niger. According to the information provided by the manufacturer, the optimum temperature for the enzymatic function of GOX is 28-80 °C and the optimum pH is 2.0-7.0. The activity of GOX was 2000 unit/g. One unit of GOX activity is defined as the amount of enzyme which oxidizes 1 μmol β-D-glucose per minute to D-gluconic acid and hydrogen peroxide at 37 °C and pH 5.5.

### Experimental Design, Diets And Animal Management

A total of 432 Hy-line brown laying hens (50-week-age) were used in a10-week trial to evaluate the production performance, egg quality parameters, and nutrient digestibility. Laying hens were randomly allotted into four treatments. There were 9 replication pens with 12 hens per replication (1 hen/cage). Dietary treatment groups were as follows: 1) CON, Basal diet, 2) TRT1, Basal diet + 100unit Glucose oxidase, 3) TRT2, Basal diet + 200unit Glucose oxidase, 4) TRT3, Basal diet + 300unit Glucose oxidase. Feeds of corn - soybean meal were fed to the experimental diets according to the requirement of NRC (1994). The composition of the basal diet, experimental diets, and nutrient levels are presented in Table 1. All hens were

**Table 1 –** Composition of laying hen diets (as fed-basis).

| Item | Experimental diets | | | |
|---|---|---|---|---|
| | CON | TRT1 | TRT2 | TRT3 |
| Ingredients (%) | | | | |
| Corn | 53.11 | 53.09 | 53.07 | 53.05 |
| DDGS | 20.01 | 20.01 | 20.01 | 20.01 |
| Palm kernel meal | 1.85 | 1.85 | 1.85 | 1.85 |
| Soybean meal | 10.99 | 11.00 | 11.00 | 11.00 |
| Seasame meal | 2.00 | 2.00 | 2.00 | 2.00 |
| Tallow | 0.94 | 0.94 | 0.95 | 0.96 |
| MDCP | 0.06 | 0.06 | 0.06 | 0.06 |
| Limestone | 10.32 | 10.32 | 10.32 | 10.32 |
| Salt | 0.05 | 0.05 | 0.05 | 0.05 |
| Methionine (99%) | 0.05 | 0.05 | 0.05 | 0.05 |
| Lysine (50%) | 0.27 | 0.27 | 0.27 | 0.27 |
| Vitamin mix[1] | 0.10 | 0.10 | 0.10 | 0.10 |
| Mineral mix[2] | 0.10 | 0.10 | 0.10 | 0.10 |
| Choline (50%) | 0.10 | 0.10 | 0.10 | 0.10 |
| Phytase (500unit) | 0.05 | 0.05 | 0.05 | 0.05 |
| GOX | - | 0.01 | 0.02 | 0.03 |
| Total | 100.00 | 100.00 | 100.00 | 100.00 |
| Calculated value | | | | |
| Crude Protein, % | 16.02 | 16.02 | 16.02 | 16.02 |
| Crude Fat, % | 5.03 | 5.03 | 5.04 | 5.05 |
| Crude Fiber, % | 4.24 | 4.24 | 4.24 | 4.24 |
| Crude Ash, % | 4.57 | 4.57 | 4.57 | 4.57 |
| Calcium, % | 4.10 | 4.10 | 4.10 | 4.10 |
| Phosphorus, % | 0.51 | 0.51 | 0.51 | 0.51 |
| Available Phosphorus, % | 0.20 | 0.20 | 0.20 | 0.20 |
| Lysine, % | 0.75 | 0.75 | 0.75 | 0.75 |
| Methionine+Cystine, % | 0.94 | 0.94 | 0.94 | 0.94 |
| Metabolizable energy, kacl/kg | 2650 | 2650 | 2650 | 2650 |
| Linoleic Acid, % | 2.43 | 2.43 | 2.44 | 2.44 |

[1] Provided per kg of diet: vitamin A, 10,800 IU; vitamin D3, 4,000 IU; vitamin E, 40 IU; vitamin K3, 4 mg; vitamin B1, 6 mg; vitamin B2, 12 mg; vitamin B6, 6 mg; vitamin B12, 0.05 mg; biotin, 0.2 mg; folic acid, 2 mg; niacin, 50 mg; D-calcium pantothenate, 25 mg.

[2] Provided per kg of diet: Fe, 100 mg as ferrous sulfate; Cu, 17 mg as copper sulfate; Mn, 17 mg as manganese oxide; Zn, 100 mg as zinc oxide; I, 0.5 mg as potassium iodide; and Se, 0.3 mg as sodium selenite.

housed in an environmentally controlled house with the temperature maintained at approximately 18 °C to 23 °C, from 50 to 60 weeks of age. Ventilation and lighting (16L: 8D) were automatically controlled in the house. All hens were supplied with mash feed and water ad libitum. The relative humidity was maintained at 60–70% throughout the trial period. The current study lasted 10 weeks and the hens were allowed a 7-day adaptation period.

### Production Performance

The number of eggs produced was recorded daily at 13:00 h including those that were broken. Egg production rate (%) was calculated from the total number of eggs laid in 1 wk divided by the total number of hen days in that week on a replicate basis. Average egg weight was obtained by dividing the total weight of collected eggs by the number of normal eggs. We recorded feed intake weekly for each replicate.

### Egg Quality Assessment

In addition, on weeks2,4,6,8 and 10 of the experiment, 48 eggs (4 eggs per replication) were randomly collected for the egg quality measurements including egg weight, egg breaking strength, Haugh unit (HU), eggshell color, yolk color, and eggshell thickness. The egg breaking strength was measured using an egg breaking strength tester (FHK, Fujihira Co. Ltd., Tokyo, Japan). HU, a measure of the height of the albumen of the eggs broken out on a flat surface, was calculated using the formula 100×log (H+7.57–1.7W0.37), where H is the height of the egg white (mm) and W is the weight of the egg (g). Egg shell color was measured using an eggshell color fan (Samyang Co., Ltd., Seoul, Korea). Egg yolk color was measured using an egg yolk color fan of Roche. Egg shell thickness was measured at the central part of the eggshell fragments without eggshell membrane using a Digimatic micrometer (Series 293-330-30, Mitutoyo Corporation, Kawasaki, Japan).

### Nutrient Digestibility

Laying hens were fed their respective diets containing chromic oxide (Cr2 O3 at 0.20% level) for 4 days prior to the collection period to determine nutrient digestibility. Whole excreta collection was performed daily for three days in week 5 and 10 and stored at -20 ºC until further analysis. All feed and fecal samples were ground to pass through a 1-mm screen, after which they were analyzed for dry matter (DM) (method 930.15), and nitrogen (N) (method 990.03) following the procedures outlined by the Association of Official Analytical Chemists International AOAC (2000). The digestible energy was determined by measuring the heat of combustion by Parr 6400 oxygen bomb calorimeter (Parr Instrument Co., Moline, USA). Nitrogen was determined (Kjectec2300 Nitrogen Analyzer; Foss Tecator AB, Hoeganaes, Sweden), and CP was calculated as N × 6.25.

Chromium concentrations were determined via UV-absorption spectrophotometry (UV-1201, Shimadzu, Kyoto, Japan). The apparent total tract digestibility was then calculated using the following formula: Digestibility (%) = {1 — [(Nf × Cd)/(Nd × Cf)]} × 100, where Nf = nutrient concentration in feces (% DM), Cd = chromium concentration in diet (% DM), Nd = nutrient concentration in diet (% DM), and Cf = chromium concentration in feces (% DM).

### Statistical Analysis

All data were subjected to statistical analysis in a randomized complete block design using the General Linear Model procedure of the SAS (Version 9.2., SAS Institute Inc., Cary, NC, USA), with each replicate cage being defined as the experiment unit. Orthogonal contrasts were used to examine the linear and quadratic effects in response to increasing the dietary supplementation of herbal mixture extract. The results were presented as means and pooled standard error of the mean (SEM). Probability values less than 0.05 were considered significant.

## RESULTS

A Quadratic decrease in FI in week 3 ($p<0.05$) and linear increase in egg production in week 6 to 10 and overall experiment period ($p<0.05$) a Quadratic increase in egg production in week 7 ($p<0.05$), as well as a linear decrease in egg broken rate in week 6 ($p<0.05$) were observed with the dose of GOX in the diet. (Table 2). Egg weight on day 28 ($p<0.05$), and yolk color on day 42 ($p<0.05$) and day 70 ($p<0.001$), haugh unit on days 28 and 70 ($p<0.05$), albumen height on day 28 and day 56 ($p<0.05$), eggshell strength on day 56($p<0.05$) and day 70 ($p<0.001$) and eggshell thickness on days 56 and 70 ($p<0.05$) increased linearly with increasing GOX dose in the diet. However, egg weight on day 14 ($p<0.05$), eggshell strength on day 56 ($p<0.001$),and eggshell thickness on day 56 ($p<0.05$) quadratically increased by YGF251 supplementation. Supplementing GOX to the diet of laying hens linearly decreased yolk color ($p<0.05$), and shell color on day 14 ($p<0.001$),

**Table 2 –** Effects of Glucose oxidase on feed intake, egg production and egg broken rate in laying hens[1]

| Items | CON | TRT1 | TRT2 | TRT3 | SEM[2] | *p*-value[3] | |
|---|---|---|---|---|---|---|---|
| | | | | | | Linear | Quadratic |
| FI, g | | | | | | | |
| Week 1 | 95.23 | 95.59 | 93.71 | 95.62 | 0.97 | 0.8610 | 0.4030 |
| Week2 | 97.81 | 96.99 | 97.57 | 96.74 | 0.65 | 0.3136 | 0.9298 |
| Week3 | 98.38$^{a}$ | 97.89$^{ab}$ | 96.88$^{b}$ | 98.01$^{ab}$ | 0.58 | 0.2942 | 0.0520 |
| Week4 | 98.72 | 98.87 | 97.89 | 98.08 | 0.59 | 0.3737 | 0.9770 |
| Week5 | 98.74 | 98.46 | 98.56 | 98.00 | 0.63 | 0.1469 | 0.1635 |
| Week6 | 97.80 | 97.65 | 98.58 | 97.93 | 0.51 | 0.5449 | 0.5993 |
| Week7 | 97.12 | 96.61 | 96.35 | 96.98 | 1.00 | 0.8966 | 0.6123 |
| Week8 | 97.82 | 96.90 | 96.55 | 98.28 | 0.82 | 0.7949 | 0.1480 |
| Week9 | 97.91 | 97.17 | 96.08 | 96.90 | 0.95 | 0.3637 | 0.4428 |
| Week10 | 97.03 | 97.34 | 97.89 | 97.87 | 0.77 | 0.3772 | 0.8296 |
| TFI | 97.46 | 97.35 | 97.01 | 97.44 | 0.51 | 0.8480 | 0.5775 |
| Egg production, % | | | | | | | |
| Week 1 | 81.88 | 82.28 | 82.54 | 82.41 | 1.98 | 0.8427 | 0.8982 |
| Week2 | 83.86 | 84.26 | 84.79 | 85.05 | 0.95 | 0.3505 | 0.9457 |
| Week3 | 83.60 | 83.86 | 84.66 | 84.39 | 0.99 | 0.4822 | 0.7912 |
| Week4 | 84.39 | 84.52 | 84.13 | 85.32 | 0.77 | 0.4979 | 0.4992 |
| Week5 | 84.66 | 86.38 | 87.83 | 87.30 | 1.05 | 0.0639 | 0.3014 |
| Week6 | 84.79$^{b}$ | 87.17$^{ab}$ | 88.62$^{a}$ | 87.70$^{a}$ | 0.85 | 0.0170 | 0.0700 |
| Week7 | 86.64$^{b}$ | 88.89$^{a}$ | 89.55$^{a}$ | 89.29$^{a}$ | 0.51 | 0.0019 | 0.0263 |
| Week8 | 85.58$^{b}$ | 87.43$^{ab}$ | 88.23$^{a}$ | 87.96$^{ab}$ | 0.81 | 0.0454 | 0.2126 |
| Week9 | 85.58$^{b}$ | 87.04$^{ab}$ | 87.96$^{a}$ | 88.49$^{a}$ | 0.68 | 0.0060 | 0.5045 |
| Week10 | 85.85$^{b}$ | 87.30$^{ab}$ | 88.10$^{a}$ | 87.83$^{ab}$ | 0.65 | 0.0355 | 0.2081 |
| Overall | 84.70$^{b}$ | 85.91$^{ab}$ | 86.63$^{a}$ | 86.57$^{a}$ | 0.39 | 0.0023 | 0.1166 |
| Egg broken rate, % | | | | | | | |
| Week 1 | 0.32 | 0.47 | 0.17 | 0.30 | 0.24 | 0.7426 | 0.9616 |
| Week2 | 0.47 | 0.31 | 0.16 | 0.00 | 0.22 | 0.1383 | 0.9882 |
| Week3 | 0.49 | 0.31 | 0.63 | 0.47 | 0.22 | 0.7899 | 0.9910 |
| Week4 | 0.31 | 0.16 | 0.00 | 0.15 | 0.16 | 0.4001 | 0.3483 |
| Week5 | 0.47 | 0.14 | 0.30 | 0.45 | 0.20 | 0.9141 | 0.2545 |
| Week6 | 0.47 | 0.30 | 0.15 | 0.00 | 0.16 | 0.0442 | 0.9470 |
| Week7 | 0.76 | 0.59 | 0.29 | 0.45 | 0.31 | 0.3741 | 0.6008 |
| Week8 | 0.31 | 0.30 | 0.15 | 0.31 | 0.22 | 0.8864 | 0.7108 |
| Week9 | 0.78 | 0.46 | 0.31 | 0.44 | 0.32 | 0.4229 | 0.4880 |
| Week10 | 0.61 | 0.30 | 0.45 | 0.30 | 0.18 | 0.3599 | 0.6697 |
| Overall | 0.50 | 0.34 | 0.26 | 0.29 | 0.08 | 0.0709 | 0.2658 |

[1]Abbreviation: CON, Basal diet; TRT1, CON + 100unit Glucose oxidase; TRT2, CON + 200unit Glucose oxidase; TRT3, CON + 300unit Glucose oxidase.

[2]Standard error of means.

[3]Means in the same row with different superscripts differ ($p<0.05$).

and day 28($p<0.05$) (Table 3). Laying hens fed the diet supplemented with GOX had no effect on DM, N, and GE during week 5 and 10 (Table 4).

## DISCUSSION

Studies reported beneficial effects of GOX supplementation in layer's diet on laying performance (Zhao *et al.*, 2009; Wen *et al.*, 2012; Adubados, 2011). However, in the present study, hen-day egg production was improved in weeks 6 to 10 and in the overall experimental period, and broken egg rate decreased in week 6, and decreased in FI in week 3 with dietary supplementation GOX. This result was consistent with previous reports which showed no significant difference in egg production, egg weight, feed intake and the FCR when laying hens were given diet supplemented with GOX (Mathlouthi et al 2003; Wu *et al.*, 2005; Yoruk *et al.*, 2006). Vieira Filho *et al.* (2015) indicated that enzymes supplementation in the laying hen diet significantly increased the egg production rate and egg weight, but the feed consumption, and FCR were not affected. However, Zhao *et al.* (2009) and Weiping *et al.* (2019) found that the FI was reduced when the birds were fed GOX diet. Mathlouthi *et al.* (2003) found that GOX supplementation at 200 units in the diet did not affect egg production of broiler breeders from 40 weeks

**Table 3 –** Effects of Glucose oxidase on egg quality in laying hens[1]

| Items | CON | TRT1 | TRT2 | TRT3 | SEM[2] | *p*-value[3] Linear | Quadratic |
|---|---|---|---|---|---|---|---|
| Day 14 | | | | | | | |
| Egg weight, g | 64.62 | 65.88 | 65.55 | 65.43 | 0.59 | 0.7327 | 0.0444 |
| Yolk color | 6.85$^{a}$ | 6.95$^{a}$ | 6.74$^{ab}$ | 6.62$^{b}$ | 0.08 | 0.0101 | 0.1398 |
| HU | 85.52$^{b}$ | 85.82$^{ab}$ | 88.66$^{ab}$ | 87.34$^{a}$ | 1.30 | 0.2793 | 0.8836 |
| Albumen height | 7.88$^{b}$ | 8.49$^{ab}$ | 8.87$^{a}$ | 9.15$^{a}$ | 0.31 | 0.8320 | 0.9570 |
| Shell color | 10.32 | 10.58 | 9.80 | 9.53 | 0.20 | 0.0008 | 0.1946 |
| Eggshell strength, kg/cm$^{2}$ | 3.83 | 3.85 | 3.71 | 3.91 | 0.11 | 0.8820 | 0.4332 |
| Eggshell thickness, mm$^{-2}$ | 36.21 | 36.88 | 36.63 | 36.64 | 0.60 | 0.6983 | 0.5823 |
| Day 28 | | | | | | | |
| Egg weight, g | 62.82$^{b}$ | 64.78$^{ab}$ | 64.19$^{a}$ | 65.17$^{a}$ | 0.67 | 0.0312 | 0.4635 |
| Yolk color | 7.11 | 7.16 | 7.07 | 7.10 | 0.08 | 0.7903 | 0.8798 |
| HU | 82.05$^{b}$ | 83.80$^{ab}$ | 84.76$^{ab}$ | 86.34$^{a}$ | 1.37 | 0.0410 | 0.9554 |
| Albumen height | 7.13$^{b}$ | 7.61$^{ab}$ | 8.11$^{a}$ | 8.36$^{a}$ | 0.29 | 0.0002 | 0.6816 |
| Shell color | 11.00 | 10.72 | 10.80 | 10.37 | 0.21 | 0.0545 | 0.7235 |
| Eggshell strength, kg/cm$^{2}$ | 3.76 | 3.77 | 3.78 | 3.74 | 0.12 | 0.9433 | 0.8173 |
| Eggshell thickness, mm$^{-2}$ | 38.33 | 37.99 | 37.66 | 38.66 | 0.43 | 0.7438 | 0.1280 |
| Day 42 | | | | | | | |
| Egg weight, g | 65.90 | 65.81 | 65.96 | 64.91 | 0.73 | 0.3836 | 0.5109 |
| Yolk color | 7.73 | 7.88 | 7.90 | 7.93 | 0.10 | 0.0379 | 0.5255 |
| HU | 81.05$^{b}$ | 82.79$^{ab}$ | 85.72$^{a}$ | 86.06$^{a}$ | 1.54 | 0.0860 | 0.4972 |
| Albumen height | 7.33$^{b}$ | 7.47$^{ab}$ | 7.78$^{ab}$ | 7.91$^{a}$ | 0.18 | 0.8183 | 0.6665 |
| Shell color | 11.72 | 12.37 | 11.57 | 11.55 | 0.28 | 0.3044 | 0.2391 |
| Eggshell strength, kg/cm$^{2}$ | 3.95 | 4.07 | 3.96 | 4.18 | 0.14 | 0.3601 | 0.6906 |
| Eggshell thickness, mm$^{-2}$ | 41.97 | 42.38 | 42.48 | 43.11 | 0.48 | 0.1021 | 0.8256 |
| Day 56 | | | | | | | |
| Egg weight, g | 61.01 | 61.86 | 63.14 | 62.89 | 0.86 | 0.0739 | 0.5227 |
| Yolk color | 6.96 | 7.12 | 6.98 | 7.07 | 0.07 | 0.5314 | 0.6728 |
| HU | 85.38 | 87.37 | 88.36 | 89.82 | 1.20 | 0.1703 | 0.7136 |
| Albumen height | 7.81$^{b}$ | 8.50$^{ab}$ | 8.71$^{ab}$ | 8.94$^{a}$ | 0.34 | 0.0179 | 0.4971 |
| Shell color | 10.47 | 10.75 | 11.00 | 10.87 | 0.26 | 0.2144 | 0.4244 |
| Eggshell strength, kg/cm$^{2}$ | 3.78$^{b}$ | 4.35$^{a}$ | 4.23$^{a}$ | 4.15$^{a}$ | 0.12 | 0.0537 | 0.0059 |
| Eggshell thickness, mm$^{-2}$ | 42.43$^{b}$ | 44.49$^{a}$ | 44.52$^{a}$ | 43.40$^{ab}$ | 0.62 | 0.0292 | 0.0114 |
| Day 70 | | | | | | | |
| Egg weight, g | 62.89 | 63.91 | 64.29 | 64.24 | 0.61 | 0.1066 | 0.3813 |
| Yolk color | 7.18 | 7.31 | 7.47 | 7.61 | 0.18 | 0.0002 | 0.9613 |
| HU | 88.82$^{b}$ | 90.78$^{ab}$ | 91.75$^{ab}$ | 92.60$^{a}$ | 1.20 | 0.0249 | 0.7448 |
| Albumen height | 8.27$^{b}$ | 8.58$^{ab}$ | 8.87$^{a}$ | 9.11$^{a}$ | 0.20 | 0.0765 | 0.4160 |
| Shell color | 11.75 | 11.48 | 11.67 | 11.43 | 0.20 | 0.3827 | 0.9323 |
| Eggshell strength, kg/cm$^{2}$ | 3.99$^{b}$ | 4.20$^{b}$ | 4.11$^{b}$ | 4.58$^{a}$ | 0.12 | 0.0025 | 0.3049 |
| Eggshell thickness, mm$^{-2}$ | 43.46$^{b}$ | 45.32$^{a}$ | 44.22$^{ab}$ | 45.58$^{a}$ | 0.46 | 0.0124 | 0.5817 |

[1]Abbreviation: CON, Basal diet; TRT1, CON + 100unit Glucose oxidase; TRT2, CON + 200unit Glucose oxidase; TRT3, CON + 300unit Glucose oxidase.

[2]Standard error of means.

[3]Means in the same row with different superscripts differ ($p<0.05$).

of age. The inconsistent determination regarding egg production in laying hens could be due to the diverse feed ingredients, activity and concentration of GOX, or ages of the hens. Additionally, the interaction of the GOX may also contribute to the inconsistent results. Besides, in the present study, the nonconsecutive positive effects on egg production may be due to the age of the hens that during the middle and end laying period, egg production ratio increased rapidly (Guoxian *et al.*, 2006).

Egg quality is one of the factors that directly influence economic outcomes for livestock farmers in the intensive farm of laying hen (Ding *et al.*, 2016). Eggshell strength and eggshell thickness are the 2 primary indicators of eggshell quality, as they influence the storage and transportation stability of eggs. Eggshell and egg internal quality are influenced by various factors such as egg weight, shell weight, specific gravity, shell breaking strength, shell deformation, shell thickness, albumen height, and

**Table 4 –** Effects of Glucose oxidase on nutrient digestibility in laying hens[1]

| Items, % | CON | TRT1 | TRT2 | TRT3 | SEM[2] | *p*-value[3] | |
|---|---|---|---|---|---|---|---|
| | | | | | | Linear | Quadratic |
| Week 5 | | | | | | | |
| Dry matter | 72.27 | 73.05 | 73.43 | 73.81 | 0.64 | 0.0991 | 0.9713 |
| Nitrogen | 69.79 | 70.37 | 69.37 | 70.26 | 0.73 | 0.9018 | 0.8295 |
| Energy | 70.20 | 70.29 | 71.50 | 71.65 | 0.72 | 0.0970 | 0.7555 |
| Week 10 | | | | | | | |
| Dry matter | 72.87 | 73.83 | 73.67 | 74.52 | 0.64 | 0.7154 | 0.7130 |
| Nitrogen | 68.87 | 68.82 | 69.19 | 71.66 | 1.26 | 0.1384 | 0.3297 |
| Energy | 72.79 | 72.90 | 72.62 | 73.26 | 0.69 | 0.1145 | 0.9344 |

[1]Abbreviation: CON, Basal diet; TRT1, CON + 100unit Glucose oxidase; TRT2, CON + 200unit Glucose oxidase; TRT3, CON + 300unit Glucose oxidase.

[2]Standard error of means.

[3]Means in the same row with different superscripts differ ($p<0.05$).

yolk color. Our results showed that the addition of GOX into laying hen's diet difference egg weight, yolk color, albumen height, shell color, haugh unit, eggshell thickness, and eggshell strength in this overall trial, which is consistent with the findings of Guoxian *et al.* (2006). However, on days 28 and 42 of this trial, this beneficial effect was found to lose its significance. This may be attributed to the advanced age of the hens, meaning the positive gains attributable early to GOX inclusion eventually become masked by age-related performance decline. In agreement with our findings, another research has similarly found significant effect of multi-enzyme product containing xylanase and β-glucanase on eggshell strength and eggshell thickness (Khan *et al.*, 2011; Sun & Kim, 2019). In the further evaluation of eggs, their protein quality is another important judgment data of egg quality. Egg protein quality is mainly evaluated by albumen height and Haugh units (Leng *et al.*, 2014). However, the introduction of GOX to the basal diet failed to influence either albumen height or Haugh units. In further egg analysis, both yolk color and yolk relative weight are also used to examine yolk quality, while the yolk relative weight directly reflects yolk quality. Results from the current study show the effect on yolk color and yolk relative weight when our laying hen diets are included with GOX.A significant correlation between brown shell color and shell strength (Yang *et al.*, 2009) may indicate that brown eggshell pigment affects shell quality. A dark brown eggshell color has been linked to higher eggshell specific gravity, which is a shell quality indicator (Joseph *et al.*, 1999). Brown eggshell color has been positively correlated with some shell characteristics such as shell strength and hatchability (Sekeroglu & Duman, 2011), while egg internal quality has no correlation with shell color (Yang *et al.*, 2009). In brief, laying hens fed the GOX containing diet could increase the acceptance of eggs in consumers through increasing haugh unit, albumen height, eggshell color, eggshell thickness, and eggshell strength.

GOX affected gut functions by stimulating digestive secretions and enhancing enzyme activity (Manzanilla *et al.*, 2004). In our study, the supplementation of 300 units of GOX had no effect on DM, N and GE digestibility. Consistent with the results of our study, Mathlouthi *et al.* (2010) reported that enzymes supplementation in the wheat diet of broilers also had no effect on nutrient digestibility. However, Wu *et al.* (2019) and Weiping *et al.* (2019) also reported that the supplementation of GOX had increased nutrient digestibility in laying hens. Likewise, Wang *et al.* (2005) indicated that the dietary inclusion of GOX had enhanced the nutrient digestibility of broilers. The dietary supplementation of GOX improved DM on weaning piglets (Hou *et al.*, 2017). The varied response of nutrient digestibility to GOX addition among different studies may result from the differences in dietary composition, the dose of GOX in the diet and the status of gut maturation.

## CONCLUSION

Supplementing glucose oxidase to the diet of laying hens could improve the production performance and egg quality (haugh unit, egg weight, albumen height, eggshell thickness, and eggshell strength). Overall, in nutshell, GOX at the high dose of 300 units in layer diets may be beneficial and recommended.

## AUTHOR CONTRIBUTIONS

Muniyappan Madesh: Conceptualization, software, validation, visualization. Yan Jie Liu: formal analysis, project administration. Ning Bo Chen: investigation, resources. In Ho Kim: data curation, methodology, supervision, writing - original draft.

## DECLARATION OF COMPETING INTEREST

The authors have declared that they have no conflict of interest.

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