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Running Title (within 10 words)	Effect of glucose oxidase on lactating sows
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Ethics approval and consent to participate	The experiment protocols used in the study were approved by the Anima Care and Used Committee of Dankook University (DK-2-1942).

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# Abstract

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The objective of this study is to investigate the effect of dietary glucose oxidase (GOx) supplementation on the reproductive performance, litter performance, total tract digestibility, and blood profile of lactating sows fed corn- wheat-based diet. A total of twenty multiparous sows (Landrace × Yorkshire) were allocated into one of four treatments with five replicates per treatment. The dietary treatments were as follows: CON (Basal diet), GO1(basal diet + 200U GOx/kg), GO2 (basal diet + 300U GOx/kg), GO3 (basal diet + 400U Gox/kg). Dietary GOx supplementation did not affect lactating sow's reproduction performance as well as body weight, backfat thickness, and body condition score during pre and post farrowing, and at weaning (p > 0.05). However, after farrowing to weaning period lactating sow's fed GOx supplement has linearly (p = 0.0196) decreased the bodyweight loss. While, there were no effects (p > 0.05) observed on sows backfat thickness loss, average daily feed intake, and estrus interval among treatment groups. Dietary supplementation of GOx has linearly improved the body weight gain (p = 0.049) and average daily gain (p = 0.040) of suckling piglets. The total tract digestibility of dry matter and nitrogen was linearly increased with the graded level of GOx supplement. Also, a linear effect was observed on the glucose and superoxide dismutase of blood profile with the dietary inclusion of GOx. In summary, our finding indicates that the dietary inclusion of GOx supplement with corn- wheat-based diet had a beneficial effect on the nutrient digestibility and blood profile of lactating sows and improved the growth performance of suckling piglets.

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Keywords: glucose oxidase, lactating sows, suckling piglets, reproduction performance

### INTRODUCTION

Modern livestock industries aim to increase the production performance through the selection of sows with large litter sizes. However, during the gestation and lactation period sows have to tackle various stress factors such as body weight loss and changes in their housing [1,2]. Besides, these anxieties may reduce their immune function and increase the risk of pathogenic disease susceptibility in their intestinal tract, which leads to huge economic loss in swine production [3]. Furthermore, the biological and immunological health status of highly producing sows, not only affects their progeny performance but also affects their reproductive performance. To defeat this situation antibiotic growth promoters (AGP) have been used in swine feed for many years to improve their health and feed intake but the use of AGP in livestock feed causes various health issues to animals as well to consumers due to its antimicrobial resistance [4]. Owing to the demand for high quality livestock products, it is important to explore effective and natural feed additives that can stimulate productivity as well improve the health status of swine. Consequently, many researchers were a quest to find a suitable nutritional strategy that can enhance the health status of sows as well as livestock production. In such research, glucose oxidase (GOx) was found to be an excellent alternative

GOx is a small stable enzyme that could oxidizes gluconolactone, oxygen into hydrogen peroxide [5]. They are predominantly produced from Aspergillus niger and Penicilliumglaucum through the fermentation method [6] and found on the surface of fungi. It plays a vital role in a commercial application such as increasing the texture and color of food materials, palate, and shelf life of food products [7] and helps to prevent bacterial infection. During the fermentation processes, GOx could be used as a biosensor to determine D-glucose content in body fluids, foodstuffs, and beverages [8,9]. Due to the high production cost and low fermentation capacity, the use of GOx in livestock feed is not vigorously implemented. Previously, Biagi et al. [10] and Tang et al. [11] pointed out that GOx supplement had a beneficial effect on the growth performance of piglets. Also, Wu et al. [12] demonstrated that the GOx supplement has significantly increased the growth performance of average daily gain (ADG) and feed and gain ratio (G: F) of broilers. Earlier studies have shown that GOx supplement could reduce oxidative stress and intestinal mycotoxin poisoning thereby, improving the immunity to ameliorate the growth performance and productivity of animals [13-15]. Wheat contains variable amounts of non-starch polysaccharides (NSP), main arabinoxylans which can interfere with nutrient digestibility, feed efficiency, and growth performance of poultry and piglets [16,17] (Lordelo et al., 2008; Tsai et al., 2017), such wheat has not been commonly used in sows diet. Though previous literature showed a positive effect on the application of GOx in the livestock industry, still now there was no information presented on the supplementation of GOx with cornwheat-based diet. Therefore, we initiate this study to evaluate the effect of GOx with corn- wheat diet on the growth performance, nutrient digestibility, and blood profile of lactating sows. We hypothesized that the GOx supplement with corn-wheat-based diet could improve the nutrient digestibility and blood profile of lactating sows and the growth performance of piglets.

# MATERIALS AND METHODS

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The protocol of this experiment was reviewed and approved by the Animal Care and Use Committee of Dankook University (DK-2-1942) Cheonan, Republic of Korea. The source of GOx supplement was obtained from the commercial company (Jinan Bestzyme-Bio Engineering Co., LTD, China) and expressed by *Aspergillus niger*. One unit (U) of GOx activity is defined as the amount of enzyme which oxidates 1 μmol β-D-glucose per minute to D-gluconic acid and H<sub>2</sub>O<sub>2</sub> at 37°C and pH5.5.

A total of 20 multiparous sows [Landrace × Yorkshire] with an average parity of 2.8 (SD = 0.89) (In brief, Parity 1 consist of 1 sow, whereas parity 2 and 3 consists 7 sows, and parity 4 consist- 5 sows) were randomly allocated into one of four treatments with five replicates per treatment. The dietary treatments were as follows: CON (Basal diet), GO1(basal diet + 200U GOx/kg), GO2 (basal diet + 300U GOx/kg), GO3 (basal diet + 400U Gox/kg). During gestation period, the sows were caged in separate stalls, which had partially slatted and specific strips floor consisting of a 0.80 × 1.05 m. Experimental diets were fed from day 100 to day 135 of the feeding trial, sows were weighed and transferred to farrowing room at 107th day of gestation, and fed 2.5 kg per day feed to allow for adjustment to the lactation diet before parturition. However, sows were not fed on the day of farrowing. The nutrient diets were formulated to meet or exceed the nutrition criteria of NRC, 2012 (Table 1 (gestation) & Table 2 (lactation)). The farrowing crate contained an air-conditioned for newborn pigs at the same time temperature of the farrowing house was maintained at least 20 °C with additional ventilation generated by heat lamps. Within 24 h of birth, all piglets were treated with 1 mL of iron injection, ear notching, needle teeth clipping and tail docking, within 5 days of postpartum male piglets were castrated. During lactation the feed intake of sow had raised up to 7 kg, until day 21 the piglets were continued to weaned in the farrowing room. Water was available ad libitum to both the sows and piglets throughout the experimental. Piglets were not offered creep feed. Sow milk was the only feed available to the piglets during lactation.

At the beginning of the experiment, before farrowing, after farrowing, and at the weaning period individual sows body weight and backfat thickness were measured using real-time ultrasound instruments (Pig lot 105; SFK Technology, Denmark). After farrowing litter size was recorded according to numbers of alive piglets or dead litter to calculate the survival ratio. Feed consumption and residual were measured after feeding to calculate the

daily feed intake of sows' Body condition score was recorded within a few hours, a day after farrowing, after farrowing to weaning, and weaning to 21-day of lactation. Each piglet body weight was measured at initial birth and 21 days of lactating (weaning). During gestation and lactation, the consumption of feed was recorded on each pen to calculate the average daily gain (ADG), average daily feed intake (ADFI). In order to determine the survival rate, the piglets were recorded to report on farrowing day to weaning day. Each sow weaning estrus interval was noted after weaning. After weaning, sows were transferred to pens, which is very near to mature boar and also, they have direct exposer two times a day (08:00 and 16:00 h) for estrus detection. The presence of a boar, a sow was assumed to be in estrus when displaying a standing reaction caused by a back-pressure test.

To calculate total tract digestibility of dry matter (DM), nitrogen (N), and energy (E), 0.20% chromium oxide was added to the diet as an indigestible marker for 7 days prior to fecal collection at end of the lactation period. Sows rectum was gently massaged by the trainer and fresh fecal samples were collected, pooled (pen basis) and stored at -20°C until analyzed. All feed and fecal samples were freeze-dried and finely ground to pass through a 1 mm screen. DM and N digestibility were determined using methods established by the Association of Official Analytical Chemists [18]. UV absorption spectrophotometry (UV-1201, Shimadzu, Kyoto, Japan) was used to determine the chromium absorption in the diets and feces. Parr 6100 oxygen bomb calorimeter (Parr Instrument Co., Moline, IL, USA) was used to analyze energy by measuring the heat of combustion in the samples. Nitrogen was analyzed using Kjeltec 8600 (Foss Tecator AB, Hoeganaes, Sweden). The calculation of total tract digestibility formula was used according to Sales and Janssens [19]. The blood samples were collected via puncturing the vena cava of sows before feeding at farrowing, after farrowing, and at weaning. For serum analysis, approximately 3 mL blood samples were centrifuged at 4,000(rpm) × g for 15 min at 4°C to obtain serum samples and then stored at -20°C until analysis. The serum antioxidant activities of glutathione peroxidase (GPX), glutathione (GSH), and glutathione disulfide (GSSG) were detected using the ELISA kit (R & D Systems, Minneapolis, MN, USA). Superoxide dismutase (SOD) in serum was measured using a commercial kit from Cayman Chemical Company (Cayman Chemical Co., Ann Arbor, MI, USA). The blood glucose concentrations were analyzed by an automatic biochemistry blood analyzer (HITACHI 747; Hitachi, Tokyo, Japan).

# Statistical Analysis

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All data in this experiment were analyzed in accordance with a completely randomized design using the general linear models (GLM procedure) in SAS 9.4 software (SAS Institute, Cary, NC, USA). The significance of differences between treatment means was determined using Linear and quadratic polynomial contrasts to

examine the responses of supplemental graded levels of GOx in the basal diet. P < 0.05 was considered significant, P < 0.1 was considered a trend.

# RESULTS AND DISCUSSION

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Dietary inclusion of GOx supplement in the diet of lactating sows failed to affect the reproductive performance as well as body weight, backfat thickness, and body condition score before farrowing, after farrowing, and weaning (21 days) (p > 0.05) (Table 3). However, from farrowing to weaning, lactating sow's bodyweight loss was linearly decreased in the GOx treatment groups (p = 0.020). Moreover, there were no effects on sow backfat thickness loss, ADFI, and estrus interval among the treatments (p > 0.05). Previously, Wu et al. [12] reported that dietary inclusion of GOx supplementation had significantly enhanced growth performance and gut health of chicken. Moreover, Baudon and Hancock [20] demonstrated that low feed intake results sows with excessive body weight loss. Furthermore, Bergsma and co-authors [21] pointed out that decreased feed intake during the lactation period may leads to excessive body weight loss of sows that can decrease milk production and subsequent litter development. Whilst, Eissen et al. [22] demonstrated that enhanced feed intake during lactation can help the sow to reduce back fat thickness loss and body weight loss. However, in this study, there were no significant difference (P<0.05) observed on the ADFI and BWL in sows before farrowing and after farrowing, and at weaning (21 days). To date, no literature has been presented on the application of GOx in sow diet. However, Tang et al. [23] noted that the dietary inclusion of GOx supplementation has increased growth hormone content, and modified the fecal microbiota, thereby improving he growth efficiency in weaned piglets fed a corn-based diet. Previously Gatrell et al. [24] pointed out the supplementation of corn and wheat as a dominant energy sources in poultry diets due to their high energy content. However, the presence of NSP in wheat and corn may negatively affect nutrient utilization and performance of monogastric animal [25, 26]. Apart from this, the NSP-degrading enzymes in diets has been shown to improve apparent ileal digestibility of NSP constituents, other than galactose [27] (Nitrayová et al. 2009), and this, in turn, results in improved digestibility of organic matter, amino acids and energy [28, 29] (Nortey et al. 2008; Kiarie et al. 2010). However, with an opposing result, Han et al., (2005) [30] stated that pigs fed wheat- based diet has improved growth performance and meat quality of pigs. Similarly, Seerly et al (1988) [31] and Van Lunen and Schulze (1996) [32] reported that pigs fed wheat-based diet had a significantly higher ADFI than those fed a corn-based diet. Besides, some researchers reported that there was no significant difference between corn and wheat diets (Carr et al., 2005) [33]. We assume that the discrepancies results might be due the difference on animals age or due to the concentration of wheat-based diet.

In an earlier study Park et al. (2008) [34] stated that a high-energy diet has reduced the BW of sows and enhance the growth performance of piglets until weaning period. The present study indicates that GOx supplementation had no linear effects on the initial number and final number of suckling piglets, and their survival rate (p > 0.05). However, sows fed GOx supplementation has a beneficial effect on the growth performance of their piglets by linearly increasing their BW during the weaning period (p = 0.049). In addition, linearly enhanced ADG (p = 0.04) was observed on piglets during the overall experiment period (Table 4) was agreed with Biagi et al. [10] who reported that GOx supplementation has enhanced the growth performance of piglet's especially after weaning. Likewise, Tang et al. [15] reported that adding 100U/1kg GOx supplement to soybean meal-based diet had enhanced the BWG and feed conversion ratio of piglets. Also, Mu et al. [35] stated that the dietary inclusion of 0.04% GOx (250 U/g) had enhanced the superoxide dismutase concentrations, serum glutathione, and growth performance of weaning pigs. According to Cabrera et al. [36] weaning weight of piglet has a direct effect on their post weaner performance. After weaning, pigs with high weaning weight can grow faster than those with lighter weaning weight [37]. The growth performance can represent a beneficial impact on the digestibility of nutrients. However, the present study did not assess the nutrient digestibility of suckling piglets.

At the end of this experiment, the apparent total digestibility of DM (p = 0.045), and N (p = 0.084) in lactating sows showed a linear, and trend to increase with a graded level of GOx. However, there were no effects observed on energy (p > 0.05) digestibility (Table 5). Previously, many researchers had reported that the pigs fed cellulose or enzyme mixture supplementation has improved the apparent nutrient digestibility of DM, energy, and crude protein, thereby enhancing the growth performance of pigs [38-40]. In addition, Wu et al. [12] demonstrated that total tract digestibility was regulated by the intestinal microbiota. As reported by Liu et al. [41], effective nutrient absorption and better performance are usually associated with enhanced villus height and decreased crypt depth. However, the present study has failed to measure intestinal morphology. Therefore, we assume that the enhanced nutrient digestibility (with GOx) may be associated with intestinal microflora. There were limited research results available on the enhanced performance of lactating sows' nutrient digestion with GOx supplement, so we could not able to make more comparison with other studies.

Dietary supplementation of GOx linearly improved SOD (p = 0.050) after farrowing. Similarly, weaning period lactating sows had a linear effect on observed SOD, and Glucose (p = 0.049, and 0.042, respectively), without effects on GPx, GSSG, and GSH in GOx- supplementation diets (p > 0.05) (Table 6). Yoon et al. [42] reported that dietary inclusion of 0.05%  $\beta$ - mannanase enhanced the blood profile glucose level in finishing pigs. In contrast, Tan et al. [43] stated that dietary inclusion of corn starch supplementation significantly reduced the

serum glucose in the sow's gestation and lactation period and improved ADFI. In, 2019, Yang et al [44] demonstrated that glucose receptors are present in the brains center, and a reduce in the plasma glucose concentration of corn-starch fed sows possibly means the decline of neuronal signaling for the metabolism of glucose, thereby promoting sow feed intake. During normal respiration in mitochondria, the biological system could produce superoxide, which might have strong damage to the organism and cells. Fan et al. [45] demonstrated that the anti-oxidant ability of sows at farrowing was mainly modulated by SOD, which consists of sows' defense system with other anti-oxidant enzymes in order to manage the oxidative damage caused by excessive activists. Blood profiles of SOD, GPX, GSH, and GSSG is considered the most representative markers of oxidation status in vivo [46]. Waisundara, [47] reported that an increase in antioxidant enzyme level could reduce oxidative stress. An early study, Michiels et al. [48] demonstrated that the oxidative state of the weaned piglet has been found to influence factors such as birth weight. GSH acts as a major endogenous antioxidant with respect to the gut, GPX transforms GSH to its GSSG oxidized type. Oxidative stress will increase the GSSG level along with a corresponding decrease in SOD, GPX, and GSH concentrations [49,50]. Therefore, we believe that dietary inclusion of GOx has been shown to improve blood profile glucose and SOD of lactating sows by enhancing the immune system. However, there was no information presented on the effects of GOx supplementation on the blood profile of lactating sows' diets, so adequate justifications could not be made. Thus, more trials are needed to know the exact cause for the lack of results in this study.

# **CONCLUSIONS**

Our findings demonstrated that the inclusion of GOx supplement to corn- wheat-based diet had a beneficial effect on the reproductive performance of lactating sows, as well as it enhanced the growth performance of body weight and ADG of suckling piglets. In addition, gradually increased levels of GOx supplementation in the diet of lactating sows had linearly improved dry matter, nitrogen digestibility, and blood profile. Besides, current results will provide a novel insight on the applications of the GOx diet as an excellent alternative solution to promote the growth efficiency of lactating sows in the future.

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### DECLARATION OF COMPETING INTEREST

The authors declare that they have no conflict of interest.

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### **AUTHOR'S CONTRIBUTIONS**

208	Conceptualization: Sureshkumar S, Liu YJ, Chen NB, Park JH; Data curation: Sureshkumar S, Liu YJ;
209	Formal analysis: Chen NB; Investigation: Shanmugam S, Chen NB, Kim IH; Writing - original draft:
210	Sureshkumar S; Writing - review & editing: Chen NB, Liu YJ, Kim IH
211	ETHICS APPROVAL AND CONSENT TO PARTICIPATE
212	The experiment protocols used in the study were approved by the Animal Care and Used Committee of
213	Dankook University (DK-2-1942).



# 214 REFERENCES

- 215 1. Spoolder HAM, Geudeke MJ, Van der Peet-Schwering CMC, Soede NM. Group housing of sows in early
- pregnancy: A review of success and risk factors. Livest Sci. 2009; 125:1–14.
- 217 https://doi.org/10.1016/j.livsci.2009.03.009
- 218 2. Van Kranendonk MJ, Smithies HR, Hickman AH, Champion DC. Review: Secular tectonic evolution of
- Archean continental crust: Interplay between horizontal and vertical processes in the formation of the
- 220 Pilbara Craton, Australia. Terra Nova. 2007;19(1):1–38. https://doi.org/10.1111/j.1365-
- 221 3121.2006.00723.x
- Nowland TL, Plush KJ, Barton M, Kirkwood RN. Development and function of the intestinal microbiome
- and potential implications for pig production. Animals. 2019;9(3):76. https://doi.org/10.3390/ani9030076
- 224 4. Cheng G, Hao H, Xie S, Wang X, Dai M, Huang L, Yuan Z. Antibiotic alternatives: the substitution of
- 225 antibiotics in animal husbandry? Front Microbiol. 2014;5:217. https://doi.org/10.3389/fmicb.2014.00217
- Viswanathan S, Li P, Choi W, Filipek S, Renugopalakrishnan V. Chapter Nine Protein–Carbon Nanotube
- Sensors: Single Platform Integrated Micro Clinical Lab for Monitoring Blood Analytes. Methods in
- 228 Enzymology, 2012;509:165-194. https://doi.org/10.1016/B978-0-12-391858-1.00010-1
- Hatzinikolaou DG, Hansen OC, Macris BJ, Tingey A, Kekos D, Goodenough P, Stougaard P. A new
- glucose oxidase from Aspergillus niger: Characterization and regulation studies of enzyme and gene. Appl
- 231 Microbiol Biotechnol. 1996;46:371-381. https://doi.org/10.1007/BF00166232
- 232 7. Khurshid S, Kashmiri MA, Quershi Z, Ahmad W. Optimization of glucose oxidase production by
- 233 Aspergillus niger. Afr J Biotechnol. 2011;10(9):1674-1678.
- 8. Bankar SB, Bule MV, Singhal RS, Ananthanarayan L. Glucose oxidase An overview. Biotechnol Adv.
- 235 2009;27:489-501. https://doi.org/10.1016/j.biotechadv.2009.04.003
- 236 9. Rauf S, Ihsan A, Akhtar K, Ghauri MA, Rahman M, Anwar MA, Khalid AM. Glucose oxidase
- 237 immobilization on a novel cellulose acetate-polymethylmethacrylate membrane. J Biotechnol. 2006;
- 238 10:121(3):351-60. https://doi.org/10.1016/j.jbiotec.2005.08.019.
- 239 10. Biagi G, Piva A, Moschini M, Vezzali E, Roth FX. Effect of gluconic acid on piglet growth performance,
- intestinal microflora, and intestinal wall morphology. J Anim Sci. 2006;84:370–378.
- 241 https://doi.org/10.2527/2006.842370x
- Tang H, Gao X, Yao B, Li X, Wang X. Study on feed effects of glucose oxidase applied in piglets. China
- 243 Feed. 2013;19:21-23.

- 244 12. Wu S, Li T, Niu H, Zhu Y, Liu Y, Duan Y. Effects of glucose oxidase on growth performance, gut function,
- and cecal microbiota of broiler chickens. Poult. Sci. 2019;98:828-841. https://doi.org/10.3382/ps/pey393
- 246 13. Zhao Y, Cui Y, Chen B, Sun H, Li S, Li T. Glucose oxidase: effects on body weight and intestinal flora of
- 247 mice with moldy feed. Anim Nutr. 2014;26:3531-3536.
- 248 14. Cui YL, Zhao YJ, Sun HY, Chen BJ, Liu SX. Effects of glucose oxidase on serum indexes, antioxidative
- ability and histological structure of livers in mice. Anim Husbandry Vet Med. 2015;47:47-51.
- 250 15. Tang H, Gao X, Yao B, Zhang G, Wang Z, Li X. Effects of glucose oxidase on growth performance, serum
- parameters and slaughter performance of meat ducks and its antidotal effect on aflatoxin B. Anim Nutr.
- 252 2015;27:2361-2367.
- Lordelo MM., Cunha LF, and Freire JPB. Effects of dietary fibre source and enzyme supplementation on
- faecal apparent digestibility, short chain fatty acid production and activity of bacterial enzymes in the gut
- 255 of piglets. Anim Feed Sci Technol. 2008;146:124–136. https://doi.org/10.1016/j. anifeedsci.2007.12.001
- 256 17. Tsai T, Dove C. R., P. M. Cline, A. Owusu-Asiedu, M. C. Walsh, and M. Azain. 2017. The effect of
- 257 adding xylanase or  $\beta$ -glucanase to diets with corn distillers dried grains with solubles (CDDGS) on growth
- performance and nutrient digestibility in nursery pigs. Livest. Sci. 197:46–52.
- 259 https://doi.org/10.1016/j.livsci.2017.01.008
- 260 18. AOAC, 2000. Official Methods of Analysis. 17th ed. Assoc. Off. Anal. Chem. Gaithersburg, MD, USA..
- 261 19. Sales J, Janssen GPJ. Methods to determine metabolizable energy and digestibility of feed ingredients in
- the domestic pigeon (Columba livia domestica). Poult Sci. 2003;82(9):1457-61.
- 263 https://doi.org/10.1093/ps/82.9.1457
- 264 20. Baudon EC, Hancock JD, Pelleted diets for lactating sows. Swine Day. 2003;33-35.
- 265 http://hdl.handle.net/2097/2089
- 266 21. Bergsma R, Kanis E, Verstegen MWA, van der Peet–Schwering CMC, Knol EF. Lactation efficiency as a
- result of body composition dynamics and feed intake in sows. Livest Sci. 2009;125:208-222.
- 268 https://doi.org/10.1016/j.livsci.2009.04.011
- 269 22. Eissen JJ, Apeldoorn EJ, Kanis E, Verstegen MWA, de Greef KH. The importance of a high feed intake
- during lactation of primiparous sows nursing large litters. J Anim Sci. 2003;81:594–603.
- 271 https://doi.org/10.2527/2003.813594x
- 272 23. Tang H, Yao B, Gao X, Yang P, Wang Z, Zhang G. Effects of glucose oxidase on the growth performance,
- serum parameters and faecal microflora of piglets. S Afr J Anim Sci. 2016;46:14-20.

- 274 https://doi.org/10.4314/sajas.v46i1.2
- 275 24. Gatrell S, Lum K, Kim J, Lei XG. Nonruminant Nutrition Symposium: Potential of defatted microalgae
- from the biofuel industry as an ingredient to replace corn and soybean meal in swine and poultry diets. J
- 277 Anim Sci. 2014;92:1306–1314. https://doi.org/10.2527/jas.2013-7250
- 278 25. Yegani M, Korver DR. Factors affecting intestinal health in poultry. Poult. Sci. 2008;87:2052–2063.
- 279 https://doi.org/10.3382/ps.2008-00091
- 280 26. Jahanian R, Golshadi M. Effect of dietary supplementation of butyric acid glycerides on performance,
- immunological responses, ileal microflora, and nutrient digestibility in laying hens fed different basal diets.
- 282 Livest. Sci. 2015;178:228–236. https://doi.org/10.1016/j.livsci.2015.05.038
- 283 27. Nitrayová S, Heger J, Patras P, Kluge H, Broz J. Effect of xylanase on apparent ileal and total tract
- digestibility of nutrients and energy of rye in young pigs. Arch Anim Nutr. 2009;63: 281–291.
- 285 https://doi.org/10.1080/17450390903020455
- 286 28. Nortey TN, Patience JF, Sands JS, Trottier NL, Zijlstra RT. Effects of xylanase supplementation on the
- apparent digestibility and digestible content of energy, amino acids, phosphorus, and calcium in wheat and
- wheat by-products from dry milling fed to grower pigs. J Anim Sci. 2008;86:3450–3464.
- 289 https://doi.org/10.2527/jas.2007-0472
- 290 29. Kiarie E, Owusu-Asiedu A, Simmins PH, Nyachoti CM. Influence of phytase and carbohydrase enzymes
- on apparent ileal nutrient and standardized ileal amino acid digestibility in growing pigs fed wheat and
- 292 barley-based diets, Livest Sci. 2010; 134: 85–87. https://doi.org/10.1016/j. livsci.2010.06.105.
- Han Y, Soita JW, Thacker PA. Performance and carcass composition of growing-finishing pigs fed wheat
- 294 or corn-based diets. Asian-Australas J Anim Sci 2005;18:704-10. https://doi.org/10.5713/ajas.2005.704
- 295 31. Seerley RW, Vandergrift WL, Hale OM. Effect of particle size of wheat on performance of nursery,
- 296 growing and finishing pigs. J Anim Sci 1988;66:2484-9. https://doi.org/10.2527/jas1988.66102484x
- 297 32. Lunen TV, Schulze H. Influence of Trichoderma longibrachiatum xylanase supplementation of wheat and
- corn-based diets on growth performance of pigs. Can J Anim Sci 1996;76:271-3.
- 299 https://doi.org/10.4141/cjas96-041
- 300 33. Carr SN, Rincker PJ, Killefer J, Baker DH, Ellis M, McKeith FK. Effects of different cereal grains and
- ractopamine hydrochloride on performance, carcass characteristics, and fat quality in late-finishing pigs. J
- 302 Anim Sci 2005;83:223-30. https://doi.org/10.2527/2005.831223x
- 303 34. Park M., Yang Y, Choi J, Yoon S, Ahn S, Lee S, Yang B, Lee J, Chae B. Effects of dietary fat inclusion at

- two energy levels on reproductive performance, milk compositions and blood profiles in lactating sows.
- 305 Acta Agric Scandinavica A Anim Sci. 2008;58:121–128. https://doi.org/10.1080/09064700802400803
- 306 35. Mu S, Li N, Yan J, Zheng Z, Ma Y, Li Q. et al. Effect of Glucose Oxidase on Growth Performance and
- 307 Serum Biochemical Indexes of Piglets. China Anim Husban Veteri Medic. 2018;45: 2212-2218.
- 308 36. Cabrera RA, Boyd RD, Jungst SB, Wilson ER, Johnston ME, Vignes JL, Odle J. Impact of lactation length
- and piglet weaning weight on long-term growth and viability of progeny. J Anim Sci. 2010;88:2265–2276.
- 310 https://doi.org/10.2527/jas.2009-2121
- 311 37. Klindt J. Influence of litter size and creep feeding on pre-weaning gain and influence of preweaning growth
- 312 to slaughter in barrows. J Anim Sci. 2003;81:2434–2439. https://doi.org/10.2527/2003.81102434x
- 313 38. Varel VH, Pond WG, Yen JT. Influence of dietary fiber on the performance and cellulase activity of
- 314 growing-finishing swine. J Anim Sci. 1984;59:338 https://doi.org/10.2527/jas1984.592388x
- 315 39. Caine WR, Suaer WC, Tamminga S, Verstegen MWA, Schulze H. Apparent ileal digestibilities of amino
- acids in newly weaned pigs fed diets with protease-treated soybean meal. J Anim Sci. 1997;75: 2962-2969.
- 317 https://doi.org/10.2527/1997.75112962x
- 318 40. Yin YL, McEvoy JDG, Schulze H, Hennig U, Souffrant WB, McCracken KJ. Apparent digestibility (ileal
- and overall) of nutrients and endogenous nitrogen losses in growing fed wheat (var. Soissons) or its by-
- products without or with xylanase supplementation. Livest Prod Sci. 2000;62:119-132.
- 321 https://doi.org/10.1016/S0301-6226(99)00129-3
- 322 41. Liu X, Lee SI, Kim IH. Achyranthes japonica extracts supplementation to growing pigs positively
- influences growth performance, nutrient digestibility, fecal microbial shedding, and fecal gas emission.
- 324 Asian-Australas. J. Anim. Sci. 2020; https://doi.org/10.5713/ajas.20.0012
- 325 42. Yoon SY, Yang YX, Shinde PL, Choi JY, Kim JS, Kim YW, Yun K, Jo JK, Lee JH, Ohh SJ, Kwon IK,
- 326 Chae BJ. Effects of mannanase and distillers dried grain with solubles on growth performance, nutrient
- digestibility, and carcass characteristics of grower-finisher pigs. J Anim Sci. 2010;88:181–191.
- 328 https://doi.org/10.2527/jas.2008-1741
- 329 43. Tan C, Zhai Z, Ni X, Hao W, Ji Y, Tang T, Ren W, Long H, Deng B, Deng J. Metabolomic Profiles Reveal
- Potential Factors that Correlate with Lactation Performance in Sow Milk. Sci Rep. 2018;8:10712-.
- 331 https://doi.org/10.1038/s41598-018-28793-0
- 332 44. Yang Y, Hu CJ, Zhao X, Xiao K, Deng M, Zhang L, Qiu, X, Deng J, Yin Y, Tan C. Dietary energy sources
- during late gestation and lactation of sows: effects on performance, glucolipid metabolism, oxidative status

334		of sows, and their offspring. J Anim Sci. 2019;97(11):4608-4618. https://doi.org/10.1093/jas/skz297
335	45.	Fan Z, Xiao Y, Chen Y, Wu X, Zhang G, Wang Q, Xie C. Effects of catechins on litter size, reproductive
336		performance and antioxidative status in gestating sows. Anim. Nutri. 2015;1:271e275.
337		https://doi.org/10.1016/j.aninu.2015.11.002
338	46.	Olofsson EM, Marklund SL, Karlsson K, Brännström T, Behndig A. In vitro glucose-induced cataract in
339		copper–zinc superoxide dismutase null mice. Exp Eye Res. 2005;81:639-646.
340	47.	Waisundara VY. Evaluation of the Antioxidant Activity of Scutellaria baicalensis and its Constituents in
341		Diabetic Rats. [Doctor Degree Thesis Dissertation]. National University of Singapore; 2010.
342	48.	Michiels J, De Vos M, Missotten J, Ovyn A, De Smet S, Van Ginneken C. Maturation of digestive function
343		is retarded and plasma antioxidant capacity lowered in fully weaned low birth weight piglets. Br. J. Nutr.
344		2012;109 (1):65-75. https://doi.org/10.1017/S0007114512000670
345	49.	Wen YF, Zhao JQ, Bhadauria M, Nirala SK. Baicalin prevents cadmium induced hepatic cytotoxicity,
346		oxidative stress and histomorphometric alterations. Exp Toxicol Pathol. 2013;65:189-196.
347		https://doi.org/10.1016/j.etp.2011.08.005
348	50.	Wang J, Zhu H, Zhang C, Wang H, Yang Z. Baicalein ameliorates cadmium-induced hepatic and renal
349		oxidative damage in rats. Indian. J Anim Res. 2019;53:523-527. https://doi.org/10.18805/ijar.B-853
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352 TABLES

Table 1. Composition of gestation sow diets (as fed-basis)<sup>1</sup>

T(		Gest	ation	
Items	CON	GO1	GO2	GO3
Ingredients (%)				
Corn	56.35	56.33	56.31	56.3
Wheat	20.00	20.00	20.00	20.00
Soybean meal (48%)	5.82	5.82	5.82	5.82
Rapeseed meal	10.00	10.00	10.00	10.00
Tallow	2.82	2.82	2.83	2.83
Molasses	2.00	2.00	2.00	2.00
DCP	0.75	0.75	0.75	0.75
Limestone	1.22	1.22	1.22	1.22
Salt	0.30	0.30	0.30	0.30
Lysine (78%)	0.12	0.12	0.12	0.12
Threonine (99%)	0.14	0.14	0.14	0.14
Tryptophan (99%)	0.05	0.05	0.05	0.05
Mineral mix <sup>2</sup>	0.20	0.20	0.20	0.20
Vitamin mix <sup>3</sup>	0.20	0.20	0.20	0.20
Choline (25%)	0.03	0.03	0.03	0.03
GOX		0.02	0.03	0.04
Total	100.00	100.00	100.00	100.00
Calculated value				
Crude protein, %	13.00	13.00	13.00	13.00
Ca, %	0.75	0.75	0.75	0.75
P, %	0.50	0.50	0.50	0.50
LYS, %	0.68	0.68	0.68	0.68
MET, %	0.24	0.24	0.24	0.24
ME, kcal/kg	3200	3200	3200	3200
FAT, %	5.39	5.39	5.40	5.40
Fiber, %	3.34	3.34	3.33	3.33
Ash, %	4.56	4.56	4.56	4.56

<sup>&</sup>lt;sup>1</sup>Abbreviation: CON (Basal diet), GO1(basal diet + 200U GOx/kg), GO2 (basal diet + 300U GOx/kg), GO3 (basal diet + 400U Gox/kg).

<sup>&</sup>lt;sup>2</sup> Provided per kg diet: Fe, 150 mg as ferrous sulfate; Cu, 12 mg as copper sulfate; Mn, 24 mg as manganese oxide; Zn, 60 mg as zinc oxide; I, 0.6 mg as potassium iodide; and Se, 0.4 mg as sodium selenite.

 $<sup>^3</sup>$  Provided per kilograms of diet: vitamin A, 16,800 IU; vitamin D<sub>3</sub>, 2,400 IU; vitamin E, 108 IU; vitamin K<sub>3</sub>, 7.2 mg; vitamin B<sub>1</sub>, 2.7 mg; vitamin B<sub>2</sub>, 18 mg; vitamin B<sub>6</sub>, 6.6 mg; vitamin B<sub>12</sub>, 0.06 mg; biotin, 0.8 mg; folic acid, 6.6 mg; niacin, 81 mg; D-calcium pantothenate, 46 mg.

Table 2. Composition of lactation sow diets (as fed-basis)<sup>1</sup>

Itama	Lactation								
Items	CON	GO1	GO2	GO3					
Ingredients (%)									
Corn	42.77	42.74	42.73	42.72					
Wheat	23.00	23.00	23.00	23.00					
Soybean meal (48%)	21.71	21.71	21.71	21.71					
Bakery byproduct	3.00	3.00	3.00	3.00					
Tallow	4.89	4.90	4.90	4.90					
Molasses	2.00	2.00	2.00	2.00					
DCP	0.18	0.18	0.18	0.18					
Limestone	1.52	1.52	1.52	1.52					
Salt	0.30	0.30	0.30	0.30					
Lysine (78%)	0.01	0.01	0.01	0.01					
Threonine (99%)	0.14	0.14	0.14	0.14					
Tryptophan (99%)	0.05	0.05	0.05	0.05					
Mineral mix <sup>2</sup>	0.20	0.20	0.20	0.20					
Vitamin mix <sup>3</sup>	0.20	0.20	0.20	0.20					
Choline (25%)	0.03	0.03	0.03	0.03					
GOX		0.02	0.03	0.04					
Total	100	100	100	100					
Calculated value									
Crude protein, %	16.50	16.50	16.50	16.50					
Ca, %	0.75	0.75	0.75	0.75					
P, %	0.54	0.54	0.54	0.54					
LYS, %	0.93	0.93	0.93	0.93					
MET, %	0.30	0.30	0.30	0.30					
ME, kcal/kg	3400	3400	3400	3400					
FAT, %	7.12	7.13	7.12	7.12					
Fiber, %	2.47	2.47	2.47	2.47					
Ash, %	4.51	4.51	4.51	4.51					

<sup>&</sup>lt;sup>1</sup>Abbreviation: CON (Basal diet), GO1(basal diet + 200U GOx/kg), GO2 (basal diet + 300U GOx/kg), GO3 (basal diet + 400U Gox/kg).

<sup>&</sup>lt;sup>2</sup> Provided per kg diet: Fe, 150 mg as ferrous sulfate; Cu, 12 mg as copper sulfate; Mn, 24 mg as manganese oxide; Zn, 60 mg as zinc oxide; I, 0.6 mg as potassium iodide; and Se, 0.4 mg as sodium selenite.

 $<sup>^3</sup>$ Provided per kilograms of diet: vitamin A, 16,800 IU; vitamin D<sub>3</sub>, 2,400 IU; vitamin E, 108 IU; vitamin K<sub>3</sub>, 7.2 mg; vitamin B<sub>1</sub>, 2.7 mg; vitamin B<sub>2</sub>, 18 mg; vitamin B<sub>6</sub>, 6.6 mg; vitamin B<sub>12</sub>, 0.06 mg; biotin, 0.8 mg; folic acid, 6.6 mg; niacin, 81 mg; D-calcium pantothenate, 46 mg.

Table 3. The effect of dietary glucose oxidase supplementation on reproduction performance in lactating sows<sup>1</sup>

Itama	CON	GO1	GO2	GO3	SEM <sup>2</sup> -	P - Value <sup>3</sup>	
Items	CON	GOI	GO2			Linear	Quadratic
Parity	2.8	2.8	2.8	2.8	0.4	1.000	1.000
Litter size							
Total birth, head	12.4	11.8	12.0	12.4	0.7	0.953	0.512
Total alive, head	11.8	11.6	11.8	11.6	0.8	0.917	1.000
Stillbirth, head	0.6	0.2	0.2	0.6	0.3	1.000	0.219
Mummification, head	0.0	0.0	0.0	0.2	0.1	0.205	0.337
SUR1 <sup>4</sup> , %	94.8	98.3	98.5	93.6	2.9	0.791	0.177
Body weight, kg							
Before Farrowing	225.9	224.8	223.8	222.9	4.7	0.638	0.986
After Farrowing	208.4	206.1	205.6	205.5	4.9	0.681	0.824
Weaning	192.6	190.7	190.3	191.2	5.0	0.836	0.784
Body weight difference loss 15	17.5	18.7	18.3	17.4	1.5	0.907	0.518
Body weight difference loss 2 <sup>5</sup>	15.8a	15.4 <sup>ab</sup>	15.3ab	14.3 <sup>b</sup>	0.4	0.020	0.470
Backfat thickness, mm							
Before Farrowing	18.8	18.6	18.4	18.6	0.9	0.847	0.829
After Farrowing	17.8	17.4	17.6	17.6	1.0	0.929	0.843
Weaning	15.8	16.0	16.0	16.2	0.9	0.758	1.000
Backfat thickness difference- loss 1 <sup>6</sup>	1.0	1.2	0.8	1.0	0.2	0.641	1.000
Backfat thickness difference loss 2 <sup>6</sup>	2.0	1.4	1.6	1.4	0.3	0.309	0.563
Body condition score							
Before Farrowing	3.1	3.4	3.3	3.2	0.2	0.790	0.2476
After Farrowing	3.1	3.3	3.3	3.0	0.1	0.629	0.090
Weaning	2.7	2.8	2.8	2.7	0.1	1.000	0.469
ADFI, kg	Y						
Pregnant	2.7	2.7	2.7	2.8	0.03	0.752	0.410
Lactation	7.1	7.2	7.1	7.3	0.05	0.261	0.436
Estrus interval, d	4.0	3.8	3.8	3.4	0.4	0.350	0.813

<sup>&</sup>lt;sup>1</sup>Abbreviation: CON (Basal diet), GO1(basal diet + 200U GOx/kg), GO2 (basal diet + 300U GOx/kg), GO3 (basal diet + 400U Gox/kg).

<sup>&</sup>lt;sup>2</sup>Standard error of means.

<sup>&</sup>lt;sup>3</sup>Means in the same row with different superscript differ significantly (P<0.05).

<sup>&</sup>lt;sup>4</sup>SUR1: Survival rate of number of alive pigs per number of totals born pigs.

<sup>&</sup>lt;sup>5</sup>Body weight difference: 1, 2 weeks Before farrowing to After farrowing; 2, After farrowing to Weaning.

<sup>&</sup>lt;sup>6</sup>Backfat thickness difference: 1, 2 weeks Before farrowing to After farrowing; 2, After farrowing to Weaning.

Table 4. The effect of dietary glucose oxidase supplementation on growth performance in suckling piglets<sup>1</sup>

Items	CON (	GO1	GO2	GO3	SEM <sup>2</sup>	P - Value <sup>3</sup>	
items		GOI				Linear	Quadratic
INO <sup>4</sup>	11.8	11.6	11.8	11.6	0.8	0.917	1.000
FNO <sup>5</sup>	11.2	11.2	11.0	10.8	0.8	0.698	0.901
SUR2 <sup>6</sup> , %	95.2	96.8	94.0	92.7	3.1	0.475	0.667
Body weight, kg							
Birth weight	1.40	1.46	1.44	1.42	0.10	0.913	0.707
Weaning	6.47	6.67	7.02	7.74	0.10	0.049	0.675
Average daily gain, g							
Overall	241 <sup>b</sup>	$247^{ab}$	$245^{ab}$	251 <sup>a</sup>	3	0.040	0.940

<sup>&</sup>lt;sup>1</sup>Abbreviation: CON (Basal diet), GO1(basal diet + 200U GOx/kg), GO2 (basal diet + 300U GOx/kg), GO3 (basal diet + 400U Gox/kg).

<sup>&</sup>lt;sup>2</sup>Standard error of means.

<sup>&</sup>lt;sup>4</sup>INO: the number of initial suckling piglet

<sup>&</sup>lt;sup>5</sup>FNO: the number of finish suckling piglet

<sup>&</sup>lt;sup>6</sup>SUR2: survival rate during lactation.

 $<sup>^{</sup>a,b,3}$ Means in the same row with different superscripts differ (P < 0.05).

Table 5. The effect of dietary glucose oxidase supplementation on nutrient digestibility in lactating sows<sup>1</sup>

Itama 0/	CON	GO1	GO2	GO3	SEM <sup>2</sup>	P - Value <sup>3</sup>	
Items, %	CON	GOI				Linear	Quadratic
Weaning							
Dry matter	71.64 <sup>b</sup>	$72.93^{ab}$	73.88 ab	74.13 a	2.97	0.045	0.864
Nitrogen	70.15 b	70.21 ab	70.3 ab	71.52 a	2.81	0.084	0.839
Digestible energy	70.84	71.58	72.04	72.37	2.72	0.514	0.914

<sup>&</sup>lt;sup>1</sup>Abbreviation: CON (Basal diet), GO1(basal diet + 200U GOx/kg), GO2 (basal diet + 300U GOx/kg), GO3 (basal diet + 400U Gox/kg).

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<sup>&</sup>lt;sup>3</sup>Means in the same row with different superscript differ significantly (P<0.05).



<sup>&</sup>lt;sup>2</sup>Standard error of means.

Table 6. The effect of dietary glucose oxidase supplementation on blood profile in lactating sows<sup>1</sup>

Itama	CON	GO1	GO2	GO3	SEM <sup>2</sup>	P - Value <sup>3</sup>		
Items	CON	GOI		GUS	SEM	Linear	Quadratic	
Before farrowing								
Glucose, mg/dL	89.5	86.75	95	90.5	2.76	0.386	0.758	
SOD, U/mL	0.54	0.29	0.55	0.55	0.13	0.620	0.339	
GPx, μmol/L	8.95	9.18	9.98	9.41	0.38	0.231	0.327	
GSSG, ug/ $\mu$ L	0.92	1.14	0.96	1.19	0.09	0.144	0.989	
GSH, $ng/\mu L$	8.19	10.21	9.14	10.31	0.7	0.128	0.559	
After farrowing								
Glucose, mg/dL	82	83.5	88.75	85.75	2.25	0.136	0.344	
SOD, U/mL	$0.76^{\mathrm{b}}$	$0.87^{ab}$	1.1 a	$1.08^{\mathrm{ab}}$	0.12	0.050	0.580	
GPx, μmol/L	8.86	8.86	9.46	9.43	0.47	0.301	0.973	
GSSG, ug/ $\mu$ L	0.93	1.04	1.14	0.98	0.08	0.503	0.101	
GSH, $ng/\mu L$	10.54	9.89	11.46	9.39	0.76	0.596	0.374	
Weaning				. </td <td></td> <td></td> <td></td>				
Glucose, mg/dL	100.25 <sup>b</sup>	103.5 ab	110.5 a	109.25 ab	3.35	0.049	0.518	
SOD, U/mL	0.75 ab	$0.73^{\mathrm{b}}$	0.88 ab	0.99 a	0.08	0.042	0.450	
GPx, μmol/L	9.53	9.07	9.33	10.3	0.67	0.411	0.311	
GSSG, $ug/\mu L$	0.98	1.1	0.95	1.04	0.08	0.964	0.875	
GSH, $ng/\mu L$	9.13	10.23	9.29	10.03	0.62	0.548	0.777	

<sup>&</sup>lt;sup>1</sup>Abbreviation: CON (Basal diet), GO1(basal diet + 200U GOx/kg), GO2 (basal diet + 300U GOx/kg), GO3 (basal diet + 400U Gox/kg).

<sup>2</sup>Standard error of means.

<sup>&</sup>lt;sup>3</sup>Means in the same row with different superscript differ significantly (P<0.05).