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Running Head: Lysozyme for weaned pigs

Effects of lysozyme on the growth performance, nutrient digestibility, intestinal barrier and microbiota of weaned pigs fed diets containing spray-dried whole egg or albumen powder

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ABSTRACT

Two experiments were conducted to evaluate energy value of spray-dried whole egg (SWE), albumen powder (AP) and effects of lysozyme in diets containing these two protein sources. Exp. 1: 18 barrows were allotted to 1 of 3 diets (Basal, SWE and AP). Both digestible (DE) and metabolizable energy (ME) were lower ($P < 0.01$) in AP than SWE. Exp. 2: 120 piglets weaned at 21-day were allotted in a 2×2 factorial experiment. Diets contained either 8.0% SWE or 4.67% AP, supplying the same amount of crude protein and were fed with or without 1 g/kg lysozyme. Supplementation of lysozyme in SWE and AP diets improved ($P < 0.01$) G:F than the diets without lysozyme. Lysozyme decreased ($P < 0.01$) *E. coli* counts in cecum, and *Lactobacilli* counts in both cecum and colon ($P < 0.05$). Lysozyme reduced serum IgM ($P < 0.05$), d-lactic acid ($P < 0.01$) and diamine oxide ($P < 0.05$). Piglets fed AP had lower ($P < 0.05$) serum urea nitrogen levels than those fed SWE. In conclusion, SWE contains a higher DE and ME content than AP. Lysozyme improved the performance of piglets by regulating the gut microflora, protecting the intestinal barrier and lowering immune activation.

Keywords: lysozyme, weaned pig, growth performance, nutrient digestibility, intestinal microflora, intestinal barrier

Abbreviations: **ADFI**, average daily feed intake; **ADG**, average daily weight gain; **G:F**, feed efficiency expressed as ADG/ADFI; **ATTD**, apparent total tract digestibility; **DM**, dry matter; **SWE**, spray-dried whole egg; **AP**, albumen powder; **LZ**, lysozyme; **DE**, digestible energy; **ME**, metabolizable energy

INTRODUCTION

Antibiotics are applied in domestic animals for three major purposes: therapy to treat an identified bacterial infection, prevention of bacterial infections in animals at risk, or as feed additives to enhance performance (Anthony and Ellen, 1999). In particular, antibiotics have been widely used in swine productions at subtherapeutic levels for promoting growth (Cromwell et al., 2002), improving feed efficiency and decreasing morbidity (Verstegen and Williams, 2002). However, resistance against antibiotics selected in animals might be transmitted to humans to the detriment of their health (Casewell et al., 2003). Lysozyme (LZ) is a suitable alternative to antibiotics in swine feed (May et al., 2012). Thus, nutritionists have been driven to explore alternatives to the use of antibiotics such as organic acids, pro- or pre-biotics, and essential oils (Thacker et al., 2013; Zeng et al., 2015). The current U.S. FDA guidelines will be taking effects in January 2017 on antibiotic use in feed.

Lysozyme (LZ) derived from egg products, has the ability to cleave the N-acetylmuramic acid and N-acetylglucosamine residues in gram-positive bacteria but has a limited ability to restrict gram-negative bacteria (Schmidt et al., 2003). The enzyme has been reported to modulate the immune response and maintain gut barrier

function in weaned pigs (Lee et al., 2009), improve small intestinal morphology, regulate intestinal microbes (May et al., 2012) and alleviate stress during *E. coli* K88 oral challenge (Nyachoti et al., 2012). These findings indicate that LZ may prove to be a viable alternative to the use of antibiotics in swine feeds.

Spray-dried whole egg (SWE) and albumen powder (AP) are major egg byproducts manufactured in the food industry. They possess several nutritional benefits, such as a desirable energy content and amino acid profile, as well as functional substances such as IgY (Vilà et al., 2010; Heo et al., 2012;) and lysozyme (Schmidt et al., 2003). However, it has not been determined whether or not additional supplemental LZ will improve the nutritional value of SWE and AP when fed to weanling pigs. Our hypothesis was that LZ supplementation would improve the nutritional utilization of SWE and AP. The purpose of this study was to determine the apparent DE and ME content of SWE and AP, applying their energy content values to the formulation in the subsequent experiment, to evaluate the effect of LZ on performance, nutrient digestibility, intestinal microflora, intestinal barrier and immune response of piglets fed SWE or AP.

MATERIAL AND METHODS

All procedures used in these experiments were approved by the China Agricultural University Institutional Animal Care and Use Committee (Beijing, China), and pigs were cared for according to the guidelines of the Canadian Council on Animal Care (CCAC, 2009). The SWE, AP and LZ (15,000 units/mg) used in these

experiments were supplied by the Shendi Company (Hubei, China). The analyzed nutrient composition (as dry matter basis) of the protein ingredients is presented in Table 1.

Animals and Experimental Design

Exp. 1 was conducted to determine the apparent DE and ME content of SWE and AP. Eighteen barrows (initial BW 20.47 ± 2.50 kg) were individually placed in metabolism cages ($1.44 \times 0.66 \times 1.22$ m³) equipped with a feeder and a nipple drinker and used in a randomized complete block design with 3 diets and 6 pigs per diet. The basal diet contained 97% corn as the sole energy source to determine the apparent DE and ME content of added corn with the remainder of the diet comprising 0.5% minerals and vitamins. The test diets were formulated to contain 19.4% SWE or AP respectively added at the expense of corn (Table 2). The calculation of apparent DE and ME content in spray-dried whole egg and albumen powder was conducted using the difference procedure of Adeola (2001).

Two equal sized meals were fed twice daily at 08:00 and 15:30 h and water was freely accessed throughout the trial. The daily feed allowance was equivalent to 4% of body weight and fed in mash form. The experiment comprised a seven-day adaptation to the diets followed by a five-day total collection of urine and feces. During the 5-d collection period, all feces were freshly collected into plastic bags and stored at -20°C . At the end of each period, the 5-d collection of feces from each pig was pooled and weighed and a 350-g sample was taken and dried in a forced-draft oven at 65°C for 72 h (She et al., 2015). After drying and grinding through a 1-mm screen,

subsamples were stored at -20°C until used for further chemical analysis. Total urine production was collected into plastic buckets attached to funnels located under the metabolic crates at the same time as the fecal collection was conducted. Approximately 50 mL of 6 N HCl was added to the buckets to limit microbial growth and to reduce the loss of ammonia (Li et al., 2015). Urine volume was recorded daily and a subsample of 10% of the urine excreted from each pig was collected and stored at -20°C . At the end of the collection period, urine samples were pooled for each pig and a subsample (about 45 mL) was saved for further analysis. Urine samples (4 mL) were dried at 65°C for 8 h with quantitative filter paper in crucibles for energy determination (Li et al., 2015). Two sheets of quantitative filter paper from each box were used to calibrate the energy content of the paper.

Exp. 2 was conducted to evaluate the growth performance, nutrient digestibility, intestinal microflora, intestinal barrier and immune function of weanling pigs. A total of 120 pigs [Duroc $\times$ (Landrace $\times$ Large White)] with an average BW of 6.83 ± 1.19 and weaned at 21 d of age were used in a 2×2 factorial experiment. The experimental diets contained either 8.0% SWE or 4.67% AP and were fed with or without 1 g/kg LZ. Both ingredients contributed 3.42% crude protein so as to better compare the two protein sources. All diets (Table 3) met or exceeded recommendations for required nutrients (NRC, 2012). The nutrients levels were kept consistent among diets. Chromic oxide (0.25%) was included in all diets as an indigestible marker. Each treatment consisted of 5 pens with 6 piglets (3 barrows and 3 gilts) per pen. All pigs were housed in a temperature-controlled nursery room (25 to 27°C) and were allowed

to consume their diets ad libitum for 21 d. Fresh fecal grab samples were obtained once daily from at least 2 pigs in each pen on the last 2 d of the 3rd wk. After collection, all feed and fecal samples were stored freshly at -20°C until analysis (Lei and Kim, 2014). Feed bags and pigs were weighed at the beginning and end of the experiment to determine ADG, ADFI, and G:F.

Chemical Analysis of Feed and Feces

Feed samples were collected at the beginning of each experiment. Fecal samples were dried in an oven (65°C for 72 h) and ground to pass through a 1-mm sieve. Chemical analysis were conducted according to the methods of the AOAC (2007). Feed and fecal samples were analysed in terms of dry matter (AOAC 2007, procedure 930.15), acid hydrolyzed ether extract (AOAC 2007, procedure 954.02), Kjeldahl N (Thiex et al., 2002), ash (AOAC 2007, procedure 942.05), calcium (AOAC 2007, procedure 927.02) and phosphorus (AOAC 2007, procedure 984.27). Gross energy was determined using an Automatic Isoperibol Oxygen Bomb Calorimeter (Parr 6400 Automatic Energy Analyzer, Moline, IL). The chromium content in the diets and feces was measured using an Atomic Absorption Spectrophotometer (Hitachi Z-5000 Automatic Absorption Spectrophotometer, Tokyo, Japan) according to the procedures of Williams et al. (1962).

Amino acids were assayed using ion-exchange chromatography with an Automatic Amino Acid Analyzer (L-8900 Hitachi Automatic Amino Acid Analyzer, Tokyo, Japan) after hydrolysing with 6 N HCl at 110°C for 24 h. Tryptophan was determined after LiOH hydrolysis for 22 h at 110°C using High Performance Liquid

Chromatography using an Amino Acid Analyzer (Hitachi L-8800). Methionine and cysteine were determined as methionine sulfone and cysteic acid (Agilent 1200 Series, Santa Clara, CA) after cold performic acid oxidation overnight and hydrolyzing with 7.5 N HCl for 24 h at 110°C, following the procedures of Li et al. (2014).

Assay of lysozyme activity

Lysozyme activity was determined with a modified method developed by Weaver and Kroger (1978). Lysozyme sample (> 20 mg powder) was mixed in 0.1 mol/L of phosphate buffer (pH = 6.2), and diluted to approximately 100 U/mL~200U/mL. A suspension of micrococcus lysodeikticus cells was diluted to a concentration until the absorbance reached 0.65~0.75 at 450 nm. 2.5 ml of lysodeikticus suspension in a cuvette was read at 450 nm in a spectrophotometer as a result (A_0) at 0 second. Lysozyme sample (0.5 ml) was then added to the cuvette and stirred immediately. Optical density (OD) reading (A_{60}) was taken at 60 s. Phosphate buffer was used as a blank. A decrease in absorbance of 0.001 per minute was taken as one unit of enzyme activity (U) and the results were expressed as units/mg calculated as:

$$X_D = (A_0 - A_{60}) \times N \times 2 / M$$

X_D — Lysozyme activity in the sample, U/mg

A_0 — absorbance at 0 second

A_{60} — absorbance at 60 second

N — Dilution of the sample

M — Mass of the sample, mg

Microbiological Analysis

At the end of Exp. 2, one male piglet selected from each pen (n=5) was killed by a lethal injection of sodium pentobarbital to obtain intestinal digesta samples. The cecal and colonic digesta were immediately placed in sterile 50 ml capped tubes and stored on ice until laboratory analysis was conducted within 12 h. A modification of the method as described by Orban et al. (1997) was used to determine the populations of *Lactobacilli* and *E. coli*. Cecal and colonic contents (1 g) were serially diluted 1:9 in sterile resazurin solution (KH_2PO_4 0.3 mol/L, NaOH 0.22 mol/L and resazurin 4.3 mmol/L). Appropriate serial dilutions were used to enumerate the two bacteria (10^{-5} , 10^{-6} , 10^{-7} for *Lactobacilli* and 10^{-2} , 10^{-3} , 10^{-4} for *E. coli*). *E. coli* (Mac Conkey agar) was incubated aerobically at 37°C for 12 to 18 h. *Lactobacilli* (MRS agar) was incubated anaerobically at 37°C for 12 to 18 h. The microbial enumerations of digesta are expressed as $\log_{10}$ Colony-Forming Units per gram.

Serum Metabolites and Antibody Titers

On d 21 (Exp. 2), blood samples (10 ml) were collected via jugular vein puncture from all pigs into uncoated vacuum container tubes (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ), and immediately centrifuged at $2,000 \times g$ for 10 min at 5°C to recover serum, which was immediately stored at -20°C until required for analysis. Serum levels of urea nitrogen (SUN), immunoglobulins (IgA, IgG), d-lactic acid and diamine oxidase activity, were quantified using a Biological Analyzer (7160 Hitachi Automatic Biological Analyzer, Tokyo, Japan) located in the Sino-UK Institute of Biological Technology (Beijing, China).

Statistical Analysis

All data were analyzed using SAS (SAS inst. Inc., Cary, NC). In Exp. 1, data were analyzed as a completely randomized design with pig as the experimental unit using the GLM program. In Exp. 2, all data were analyzed as a 2×2 factorial design using the Mixed Model. Each pen was the experimental unit for performance and digestibility. The factors in the model included the different protein sources, LZ supplementation and their interaction. Results are expressed as least square means and standard error of the mean (SEM). In all analysis, probability values less than 0.05 were used as the criterion for statistical significance.

RESULTS

Chemical Analysis of Assay Proteins

The analyzed chemical composition of the assay protein ingredients (SWE and AP) are presented in Table 1. AP (78.49%) was higher in CP than SWE (44.46%). However, SWE had a higher acid hydrolyzed ether extract content (37.22%) compared with AP (8.25%). The concentration of lysine in AP (9.36%) was much higher than in SWE (2.79%).

Energy Concentration

Concentrations of apparent DE and ME in SWE and AP are presented in Table 4 and concentrations of GE in SWE and AP are presented in Table 1. On a DM basis, both the apparent DE and ME were significantly lower ($P < 0.01$) in AP (4067 and 3043 kcal/kg) compared with SWE (4882 and 4606 kcal/kg) and the GE of AP (5476 kcal/kg) was also lower compared with SWE (6554 kcal/kg).

Animal Performance and Digestibility of Nutrients

Dietary supplementation of LZ in SWE and AP diets improved ($P < 0.01$) G:F compared with the corresponding diets without LZ during 8-14 day and 0-21 day (Table 5). There were no statistical differences in the ADG and ADFI in dietary supplementation of LZ in SWE and AP diets compared with the corresponding diets without LZ. No significant interaction was observed between dietary protein source and LZ supplementation for piglet performance.

Lysozyme supplementation did not increase the apparent digestibility of gross energy, dry matter and crude protein. In addition, digestibility of nutrients did not vary between the two protein ingredients (Table 5). No significant interaction was noted between dietary animal protein source and LZ supplementation for these parameters.

Microbiology of Digestive Contents

Lysozyme significantly decreased ($P < 0.01$) the population of *E. coli* in the cecum. Populations of *Lactobacilli* in both cecum and colon were also decreased ($P < 0.05$) by LZ supplementation (Table 6). No difference was noted between two protein sources in the population of *E. coli* and *Lactobacilli*. No significant interaction was observed between dietary protein source and LZ supplementation for the population of *E. coli* and *Lactobacilli* in the cecum and colon.

Serum Metabolites and Antibody Titers

Lysozyme significantly decreased serum IgM ($P < 0.05$), d-lactic acid ($P < 0.01$) and diamine oxidase ($P < 0.05$) (Table 7). Albumen powder resulted in lower ($P < 0.05$) serum urea nitrogen (SUN) level compared with SWE. No significant interaction was observed between dietary protein source and LZ supplementation for

these parameters.

DISCUSSION

Chemical Analysis of Assay Proteins

Chemical characteristics of SWE and AP were generally in accordance with previous reports (Schmidt et al., 2003; Heo et al., 2012; NRC, 2012), indicating the assay proteins were representative of similar ingredients. Admittedly, some minor differences were present, such as, a relatively higher lysine, glutamic acid and a lower methionine level in AP compared with the report by Schmidt et al. (2003).

All these discrepancies were probably due to difference in processing technique (Caboni et al., 2005) and storage conditions (Caboni et al., 2005). In addition, Walker et al. (2012) pointed out three main factors influencing the composition of eggs including hen age, breed and diet. They emphasized that the lipid fraction can be modified and controlled through the feeding of hens (Fredriksson et al., 2006; Fraeye et al., 2012). Moreover, differences in one nutrient component can lead to variance in the content of other nutrients. Mazalli and Bragagnolo (2009) indicated an increase in the unsaturation of fatty acids in egg yolk through dietary manipulation could favor lipid oxidation, resulting in a change in the overall nutrient profile. Spray-dried whole egg assessed in the present study contained a well-balanced amino acid profile, even though its total amino acids were relatively lower than AP, partly due to its high fat content.

Energy Concentration

To our knowledge, there are no published data for the apparent DE and ME of

SWE (4882, 4606 kcal/kg of DM) and AP (4067, 3043 kcal/kg of DM) in pigs. In the present experiment, the apparent ME:DE ratio in SWE and AP was 94.35% and 74.82%, respectively and the apparent ME:GE ratio in SWE and AP was 70.28% and 55.57%, respectively. NRC (2012) pointed that high protein content with unbalanced amino acid profile may result in a decreased apparent ME content. In the current trial, lysine in AP was an astonishingly high level. Furthermore, the high fat content (37.22%) in SWE may partly contribute to the narrower gap between apparent ME and DE in SWE.

Animal Performance and Digestibility of Nutrients

In this study, dietary supplementation of LZ tended to improve performance of nursery pigs by improving feed efficiency (G:F). Positive effects of LZ on nursery pigs have been reported by May et al. (2012) and Oliver and Wells (2013). Oliver and Wells (2012) reported that lysozyme improved feed efficiency in weaning pigs only during late nursery phase, which was similar with the results of the current trial. Dietary LZ significantly increased feed efficiency (G:F) probably due to its regulating effect on gut microbiota, protection for the intestinal barrier and attenuation of unnecessary immune responses. However it may take time to be reflected in terms of G:F. Reasons of lysozyme effect decreased during the third week is unclear, but it might be due to improved overall immune status as pigs were growing which partially masked the effect of lysozyme. Results from May et al. (2012) only demonstrated positive effects from lysozyme on ADG not G:F, probably because the 10-day weaning piglets and liquid diet were used which were different from current trial.

ADG of piglets fed LZ was numerically larger than those fed without LZ, however no statistical difference was observed, which may be related to our limited statistical power to detect pen differences, so further study will be needed for validation.

Microbiology of Digestive Contents

The gut microbiota of pigs plays a pivotal role in body health and can be influenced by dietary differences (Anderson et al., 2000). Our results demonstrate that dietary LZ can significantly decrease the population of *E. coli* in the cecum and *Lactobacilli* in both the cecum and colon. Lysozyme is usually considered as a promising alternative to antibiotics that can inhibit the growth of pathogenic bacteria, with a beneficial impact on host health (Maga et al., 2006; Brundige et al., 2008; May et al., 2012). The results from the current study are consistent with those of Nyachoti et al. (2012) and Brundige et al. (2008), who reported decreased numbers of coliforms and *E. coli* as a result of LZ addition. Also, May et al. (2012) and Wells et al. (2015) reported that *Campylobacter* as the gram-negative bacteria could be decreased in the gastrointestinal tract as the result of LZ addition in the diet. It is interesting to note that LZ acts mainly on gram-positive bacteria compared with gram-negative organisms. *E. coli* is a gram-negative bacteria, and it is not intuitive why this bacteria in the digesta should be decreased by LZ. There is no indication whether *E. coli* and *Lactobacillus* are susceptible or resistant to LZ. In fact, this largely depends on a range of factors, such as culture substrate (Chassy and Giuffrida, 1980), LZ concentration (Akashi, 1972) and thermal treatment (Schmidt et al., 2003). Thus, it is possible that the decrease in both *E. coli* and *Lactobacillus* were due, in part, to

changes in the composition of gut microflora which indirectly reduced *E. coli*. and *Lactobacillus* counts.

Albumen powder demonstrated an increased capacity in restricting intestinal *E. coli* counts which is consistent with a previous study (Schmidt et al., 2003) where investigators found that AP had a more effective capacity in inhibiting Enterobacteriaceae than SWE. Spray-dried whole egg consists of a nutrient-rich yolk and the albumen where LZ mainly exists. The impaired bacteriostasis due to SWE can be interpreted according to a previous review (Cunningham et al., 1991), extrapolating that electrostatic interaction between LZ and yolk components may give rise to decreased activity of LZ when albumen was contaminated with yolk. Schmidt et al. (2003) proposed a possible explanation of bacteriostasis resulting from high fat content. However, it disagrees with the current trial. Thus, an electrostatic effect (Cunningham et al., 1991) may play a role rather than a nutritional effect.

Serum Metabolites and Antibody Titers

Given that AP and SWE are both protein ingredients, it is reasonable to evaluate SUN, which is related to the bioavailability of dietary amino acids in piglets (Oliver and Wells, 2013). Lysozyme did not markedly influence SUN in piglets. Similarly, research conducted by Oliver and Wells (2013) did not demonstrate a difference in plasma urea nitrogen (PUN) for LZ-treated piglets. However, SUN for piglets offered AP was significantly lower than for piglets fed SWE, indicating a more efficient nitrogen utilization and better performance (Coma et al., 1995).

Diamine oxidase and d-lactic acid are both effective markers for mucosal integrity. A rise in diamine oxidase or d-lactic acid indicates dysfunction in the intestinal barrier (Luk et al., 1980; Vella and Farrugia, 1998). In the current study, LZ supplementation reduced both serum d-lactic acid and diamine oxidase in piglets, indicating the intestinal barrier is well-maintained, probably because of the protective effect of LZ in gut development and morphology (May et al., 2012; Oliver and Wells, 2013). The present results show that piglets offered LZ had a significantly reduced serum IgM, which is likely due to a lower immune activation caused by LZ supplementation. This result is similar to a study conducted by Namkung et al. (2004), with the assumption that LZ shares analogous bacteriostasis with antibiotics. Accordingly, more available protein can be used for pig growth as a result of less protein requirement for antibody production.

CONCLUSION

Our findings suggest that SWE contained a higher apparent DE and ME content than AP. In addition, supplementation of 1 g/kg of LZ improved performance of piglets most likely by regulating gut microflora, protecting intestinal barrier and lowered immune activation.

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REFERENCES

- Adeola, O. 2001. Digestion and balance techniques in pigs. In: Lewis, A.J., Southern, L.L. (Eds.), *Swine Nutrition*. CRC Press, NY, USA, pp. 903-916.
- Akashi, A. 1972. The effect of egg-white lysozyme on the growth of cheese starter organisms. *J. Fac. Agr. Kyushu. Univ.* **17**: 67-73.
- Anderson, D.B., McCracken, V.J., Aminov, R.I., Simpson, J.M., Mackie, R.I., Verstegen, M.W.A. and Gaskins, H.R. 2000. Gut microbiology and growth-promoting antibiotics in swine. *Nutr Abstr. Rev. Ser B.* **70**: 101-108.
- Anthony, E., van den, B. and Ellen, E.S. 1999. Antibiotic Usage in Animals: Impact on Bacterial Resistance and Public Health. *Drugs.* **58**: 589-607.
- AOAC. 2007. *Official Methods of Analysis*. 18th Ed. Association of Official Analytical Chemists, Washington, USA.
- Brundige, D.R., Maga, E.A., Klasing, K.C. and Murray, J.D. 2008. Lysozyme transgenic goats' milk influences gastrointestinal morphology in young pigs. *J. Nutr.* **138**: 921-926.
- Caboni, M.F., Boselli, E., Messina, M.C., Velazco, V., Fratianni, A., Panfili, G. and Marconi, E. 2005. Effect of processing and storage on the chemical quality markers of spray-dried egg. *Food Chem.* **92**: 293-303.
- Canadian Council on Animal Care. 2009. *Guide to the Care and Use of Experimental Animals*. 2nd ed. Vol. 1. CCAC, Ottawa, Ontario, Canada.
- Casewell, M., Friis, C., Marco, E., McMullin, P. and Phillips, I. 2003. The European ban on growth-promoting antibiotics and emerging consequences for human and animal health. *J. Antimicrob Chemother.* **52**: 159-161.
- Chassy, B.M. and Giuffrida, A. 1980. Method for the lysis of gram-positive, asporogenous bacteria with lysozyme. *Appl Environ Microbiol.* **39**: 153-158.
- Coma, J., Carrion, D. and Zimmerman, D.R. 1995. Use of plasma urea nitrogen as a rapid response criterion to determine the lysine requirement of pigs. *J. Anim.*

- Sci. **73**: 472-481.
- Cromwell, G.L. 2002. Why and how antibiotics are used in swine production. Anim Biotechnol. **13**: 7-27.
- Cunningham, F.E., Proctor, V.A. and Goetsch, S.J. 1991. Egg-white lysozyme as a food preservative: A review. Worlds Poult Sci J. **47**: 141-163.
- Fraeye, I., Bruneel, C. and Lemahieu, C. 2012. Dietary enrichment of eggs with omega-3 fatty acids: a review. Food Res Int. **48**: 961-969.
- Fredriksson, S., Elwinger, K. and Pickova, J. 2006. Fatty acid and carotenoid composition of egg yolk as an effect of microalgae addition to feed formula for laying hens. Food Chem. **99**: 530-537.
- Heo, J.M., Kiarie, E., Kahindi, R.K., Maiti, P., Woyengo, T.A. and Nyachoti, C.M. 2012. Standardized ileal amino acid digestibility in egg from hyperimmunized hens fed to weaned pigs. J. Anim Sci. **90**: 239-241.
- Lee, M., Kovacs-Nolan, J., Yang, C., Archbold, T., Fan, M.Z. and Mine, Y. 2009. Hen egg lysozyme attenuates inflammation and modulates local gene expression in a porcine model of dextran sodium sulfate (DSS)-induces colitis. J Agric Food Chem. **57**: 2233-2240.
- Lei, Y and Kim, I.H. 2014. Effect of *Phaffia rhodozyma* on performance nutrient digestibility, blood characteristics, and meat quality in finishing pigs. J. Anim Sci. **92**: 171-176.
- Li, P., Li, D.F., Zhang, H.Y., Li, Z.C., Zhao, P.F., Zeng, Z.K., Xu, X. and Piao, X.S. 2015. Determination and prediction of energy values in corn distillers dried grains with solubles sources with varying oil content for growing pigs. J. Anim. Sci. **93**:3458– 3470.
- Li, Q.Y., Piao, X.S., Liu, J.D., Zeng, Z.K., Zhang, S. and Lei, X.J. 2014. Determination and prediction of the energy content and amino acid digestibility of peanut meals fed to growing pigs. Arch Anim Nutr. **68**: 196-210.

- Luk, G.D., Bayless, T.M. and Baylin, S.B. 1980. Diamine oxidase (histaminase): a circulating marker for rat intestinal mucosal maturation and integrity. *J Clin Investig.* **66**: 66-70.
- Maga, E.A., Walker, R.L., Anderson, G.B. and Murray, J.D. 2006. Consumption of milk from transgenic goats expressing human lysozyme in the mammary gland results in the modulation of intestinal microflora. *Transgenic Res.* **15**: 515-519.
- May, K.D., Wells, J.E., Maxwell, C.V. and Oliver, W.T. 2012. Granulated lysozyme as an alternative to antibiotics improves growth performance and small intestinal morphology of 10-day-old pigs. *J. Anim. Sci.* **90**: 1118-1125.
- Mazalli, M.R. and Bragagnolo, N. 2009. Increase of cholesterol oxidation and decrease of PUFA as a result of thermal processing and storage in eggs enriched with n-3 fatty acids. *J Agric Food Chem.* **57**: 5028-5034.
- NRC. 2012. *Nutrient Requirements of Swine*. 11th Ed. Washington (DC): National Academy Press.
- Namkung, H., Li, M., Gong, J., Yu, H., Cottrill, M. and de Lange, C.F.M. 2004. Impact of feeding blends of organic acids and herbal extracts on growth performance, gut microbiota and digestive function in newly weaned pigs. *Can. J. Anim. Sci.* **84**: 697-704.
- Nyachoti, C.M., Kiarie, E., Bhandari, S.K., Zhang, G. and Krause, D.O. 2012. Weaned pig responses to *Escherichia coli* K88 oral challenge when receiving a lysozyme supplement. *J. Anim. Sci.* **90**: 252-260.
- Oliver, W.T. and Wells, J.E. 2013. Lysozyme as an alternative to antibiotics improves growth performance and small intestinal morphology in nursery pigs. *J. Anim. Sci.* **91**: 3129-3136.
- Orban, J.I., Patterson, J.A., Adeola, O., Sutton, A.L. and Richards, G.N. 1997. Growth performance and intestinal microbial populations of growing pigs fed diets containing sucrose thermal oligosaccharide caramel. *J. Anim. Sci.* **75**: 170-175.

- Schmidt, L.S., Nyachoti, C.M. and Slominski, B.A. 2003. Nutritional evaluation of egg byproducts in diets for early-weaned pigs. *J. Anim. Sci.* **81**: 2270-2278.
- She, Y., Su, Y.B., Liu, L., Huang, C.F., Li, J.T., Li, P., Li, D.F. and Piao, X.S. 2015. Effects of microbial phytase on coefficient of standardized total tract digestibility of phosphorus in growing pigs fed corn and corn co-products, wheat and wheat co-products and oilseed meals. *Anim. Feed Sci. Technol.* **208**:132–144.
- Thacker, P.A. 2013. Alternatives to antibiotics as growth promoters for use in swine production: a review. *J Anim Sci Biotechnol.* **4**: 35.
- Thiex, N.J., Manson, H., Anderson, S. and Persson, J. 2002. Determination of crude protein in animal feed, forage, grain, and oilseeds by using block digestion with a copper catalyst and steam distillation into boric acid: Collaborative study. *J AOAC Int.* **85**: 309-317.
- Vella, A. and Farrugia, G. 1998. D-lactic acidosis: Pathologic consequence of saprophytism. *Mayo Clin Proc.* **73**: 451-456.
- Verstegen, M.W. and Williams, B.A. 2002. Alternatives to the use of antibiotics as growth promoters for monogastric animals. *Anim Biotechnol.* **13**: 113-127.
- Vilà, B., Peris, S., Calafat, F., Fontgibell, A., Esteve-Garcia, E. and Brufau, J. 2010. Strategies of use of a specific immunoglobulin-rich egg yolk powder in weaning piglets. *Livest Sci.* **134**: 228-231.
- Walker, L.A., Wang, T., Xin, H. and Dolde, D. 2012. Supplementation of laying-hen feed with palm tocos and algae astaxanthin for egg yolk nutrient enrichment. *J Agric Food Chem.* **60**: 1989-1999.
- Weaver, G.L. and Kroger, M. 1978. Lysozyme Activity of High-Leucocyte-Count Milk and the Effect of Heat and Potassium Dichromate on Lysozyme Activity. *J Dairy Sci.* **61**:1089-1092.
- Wells, J.E., Berry, E.D., Kalchayanand, N., Rempel, L.A., Kim, M. and Oliver, W.T.

2015. Effect of lysozyme or antibiotics on faecal zoonotic pathogens in nursery pigs. *J Appl Microbiol.* **118**:1489-1497.
- Williams, C.H., David, D.J. and Iismaa, O. 1962. The determination of chromic oxide in feces samples by atomic absorption spectrophotometry. *J Agric Sci.* **59**: 381-385.
- Zeng, Z.K., Zhang, S., Wang, H.L. and Piao, X.S. 2015. Essential oil and aromatic plants as feed additives in non-ruminant nutrition: a review. *J Anim Sci Biotechnol.* **6**: 1-7.
- Zhang, S., Piao, X.S., Ma, X.K., Xu, X., Zeng, Z.K., Tian, Q.Y. and Li, Y. 2015. Comparison of spray-dried egg and albumen powder with conventional animal protein sources as feed ingredients in diets fed to weaned pigs. *Anim Sci J.* **86**: 772–781.

**Table 1. Analyzed nutrient composition of spray-dried whole egg and albumen powder
(%, dry matter basis)**

	Ingredients	
	Spray-dried whole egg	Albumen powder
Dry matter	96.18	93.30
Crude protein	44.46	78.49
Ash	3.54	5.14
Calcium	0.16	0.13
Total phosphorus	0.57	0.72
Acid hydrolyzed ether extract	37.22	8.25
Gross energy (kcal/kg)	6554	5476
Indispensable amino acids		
Arginine	3.03	5.19
Histidine	0.99	1.78
Isoleucine	2.02	3.32
Leucine	3.47	5.64
Lysine	2.79	9.36
Methionine	0.90	0.99
Phenylalanine	2.24	3.72
Threonine	1.75	2.72
Tryptophan	0.53	0.79
Valine	2.20	3.37
Dispensable amino acids		
Alanine	2.04	3.07
Aspartate	4.62	7.93
Cystine	0.79	1.01
Glutamate	6.65	12.25
Glycine	1.65	2.85
Proline	2.00	3.59
Serine	2.52	3.76
Tyrosine	1.49	2.59

Table 2. Ingredient and nutrient content of experimental diets for Exp. 1 (% as-fed)

	Diets		
	Basal diet	Spray-dried whole egg	Albumen powder
Ingredients			
Ground corn	97.00	77.60	77.60
Spray-dried whole egg	-	19.40	-
Albumen powder	-	-	19.40
Dicalcium phosphate	1.63	1.63	1.63
Ground limestone	0.57	0.57	0.57
Sodium chloride	0.30	0.30	0.30
Vitamin mineral premix ^a	0.50	0.50	0.50
Analyzed Nutrient levels			
Dry matter	88.58	89.71	89.34
Crude protein	7.74	14.52	20.03
Acid hydrolyzed ether extract	2.43	8.68	3.22
Gross energy (kcal/kg)	3,790	4,276	4,024

^aVitamin and mineral premix provided the following per kg diet: 12,000 IU of vitamin A; 2,500 IU of vitamin D₃; 30 IU of vitamin E; 12 µg of vitamin B₁₂; 3 mg of vitamin K₃; 4 mg of riboflavin; 15 mg of d-pantothenic acid; 40 mg of nicotinic acid; 400 mg of choline chloride; 0.7 mg of folacin; 1.5 mg of vitamin B₁; 3 mg of vitamin B₆; 0.1 mg of biotin; Mn, 40 mg as manganous oxide; Zn, 100 mg as zinc oxide; Fe, 90 mg as iron sulfate; Cu, 8.8 mg as copper oxide; Mg, 22 mg; I, 0.35 mg as ethylenediamine dihydroiodide; and Se, 0.3 mg as sodium selenite.

Table 3. Ingredient and nutrient content of experimental diets^a for Exp. 2 (% as-fed)

Protein source	Spray-dried whole egg		Albumen powder	
	—	+	—	+
Lysozyme				
Corn, yellow	61.50	61.50	64.07	64.07
Soybean meal, dehulled	21.32	21.32	21.16	21.16
Spray-dried whole egg, 42.76% CP	8.00	8.00	-	-
Albumen powder, 73.23% CP	-	-	4.67	4.67
Glucose	4.00	4.00	4.00	4.00
Soybean oil	0.60	0.60	1.67	1.67
Dicalcium phosphate	1.70	1.70	1.75	1.75
Limestone	0.70	0.70	0.68	0.68
Salt	0.30	0.30	0.30	0.30
L-Lysine·HCl	0.44	0.44	0.20	0.20
DL-Methionine	0.16	0.16	0.20	0.20
L-Threonine	0.19	0.19	0.20	0.20
L-Tryptophan	0.06	0.06	0.07	0.07
Zinc oxide	0.28	0.28	0.28	0.28
Chromic oxide	0.25	0.25	0.25	0.25
Vitamin mineral premix ^b	0.50	0.50	0.50	0.50
Analysed nutrient levels (%)				
Dry matter	88.88	88.88	88.27	88.84
Crude protein	19.01	19.01	19.03	19.03
Ash	5.08	5.08	5.14	5.14
Calcium	0.67	0.67	0.69	0.69
Total phosphorus	0.57	0.57	0.58	0.58
Lysozyme (U/mg)	6.38	21.52	8.69	23.83
Calculated nutrient levels (%)				
SID ^c Lys	1.20	1.20	1.20	1.20
SID ^c Met+Cys	0.68	0.68	0.68	0.68
SID ^c Thr	0.78	0.78	0.78	0.78
SID ^c Trp	0.24	0.24	0.24	0.24
DE ^c (kcal/kg)	3,400	3,400	3,400	3,400

^a Diets were formulated so that each protein source contributed the same amount of CP

^b Vitamin and mineral premix provided the following per kg diet: 12,000 IU of vitamin A; 2,500 IU of vitamin D₃; 30 IU of vitamin E; 12 µg of vitamin B₁₂; 3 mg of vitamin K₃; 4 mg of riboflavin; 15 mg of d-pantothenic acid; 40 mg of nicotinic acid; 400 mg of choline chloride; 0.7 mg of folacin; 1.5 mg of vitamin B₁; 3 mg of vitamin B₆; 0.1 mg of biotin; Mn, 40 mg as manganous oxide; Zn, 100 mg as zinc oxide; Fe, 90 mg as iron sulfate; Cu, 8.8 mg as copper oxide; Mg, 22 mg; I, 0.35 mg as ethylenediamine dihydroiodide; and Se, 0.3 mg as sodium selenite

^c SID values and DE values for corn and soybean meal were obtained from NRC (2012). SID values for spray-dried whole egg and albumen powder were obtained from Zhang et al. (2015), and DE values for spray-dried whole egg and albumen powder were from the experiment 1. The SID AAs levels and DE

in the diets were calculated by multiplying the SID AA and DE of the individual ingredients by their inclusion level in the diets and then summing the products.

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Table 4. Concentrations of apparent DE and ME (kcal/kg DM) in spray-dried whole egg and albumen powder for Exp. 1^a

	Spray-dried whole egg	Albumen powder	SEM ^b	<i>P</i> -value
DE	4882 <i>a</i>	4067 <i>b</i>	34.67	<0.01
ME	4606 <i>a</i>	3043 <i>b</i>	37.22	<0.01

Note: Means within a column not sharing a lowercased italic letter differ significantly at the $P < 0.05$ level.

^a Data are least squares means of 6 observations for all treatments.

^b SEM, Standard error of the mean.

Table 5. Effects of dietary lysozyme and protein source on performance and apparent digestibility of nutrients in weanling pigs for Exp. 2^a

	Spray dried egg		Albumen powder		Protein source		Lysozyme		SEM ^b	P-values		
	-	+ lysozyme	-	+ lysozyme	Spray dried egg	Albumen powder	-	+		Protein	Lysozyme	Interaction
Performance												
0-7 d												
ADG, g	194	218	216	232	206	224	205	225	33.49	0.60	0.56	0.92
ADFI, g	265	274	285	291	270	288	275	283	45.03	0.69	0.86	0.97
G:F	0.74	0.80	0.77	0.80	0.77	0.78	0.75	0.80	0.03	0.52	0.10	0.66
8-14 d												
ADG, g	311	349	303	360	330	331	307	355	25.39	0.97	0.08	0.70
ADFI, g	447	464	465	476	455	471	456	470	36.81	0.69	0.72	0.94
G:F	0.70 ^b	0.74 ^a	0.65 ^b	0.74 ^a	0.73	0.70	0.68 ^b	0.76 ^a	0.01	0.17	<0.01	0.12
15-21 d												
ADG, g	506	521	519	532	513	526	512	527	28.31	0.66	0.63	0.97
ADFI, g	790	794	794	802	792	798	792	798	43.47	0.88	0.89	0.96
G:F	0.64	0.66	0.65	0.66	0.65	0.66	0.65	0.66	0.01	0.11	0.06	0.65
0-21 d												
ADG, g	337	363	346	375	350	361	342	369	27.76	0.71	0.34	0.96
ADFI, g	501	511	515	523	506	519	508	517	40.70	0.75	0.82	0.98
G:F	0.68 ^b	0.70 ^a	0.67 ^b	0.71 ^a	0.69	0.69	0.67 ^b	0.71 ^a	0.01	0.57	<0.01	0.57
ATTD of nutrients (%)												
Gross energy	83.31	83.01	81.06	81.63	83.16	81.35	82.18	82.32	0.98	0.08	0.89	0.66
Dry matter	83.92	83.66	82.18	82.36	83.79	82.27	83.05	83.01	0.83	0.08	0.96	0.80
Crude protein	77.08	75.41	73.23	74.61	76.25	73.92	75.16	75.01	1.33	0.09	0.91	0.26

Note: Means within a column not sharing a lowercased italic letter differ significantly at the $P < 0.05$ level.

^aData are least squares means of 5 observations for all treatments.

^bSEM, Standard error of the mean.

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Table 6. Effects of dietary lysozyme and protein source on microbial concentrations in the cecum and colon (log₁₀ cfu/g of digesta)^a

	Spray dried egg		Albumen powder		Protein source		Lysozyme		SEM ^b	P-values		
	-	+ lysozyme	-	+ lysozyme	Spray dried egg	Albumen powder	-	+		Protein	Lysozyme	Interaction
Cecum												
<i>Escherichia coli</i>	5.32a	4.58b	4.94a	4.43b	4.95	4.69	5.13a	4.51b	0.15	0.11	<0.01	0.45
<i>Lactobacilli</i>	7.82a	7.19b	7.81a	7.30b	7.51	7.56	7.82a	7.25b	0.23	0.83	0.04	0.80
Colon												
<i>Escherichia coli</i>	5.57	5.26	5.16	5.08	5.42	5.12	5.37	5.17	0.16	0.11	0.26	0.50
<i>Lactobacilli</i>	7.91a	7.55b	7.87a	7.43b	7.73	7.65	7.89a	7.49b	0.14	0.58	0.02	0.79

Note: Means within a column not sharing a lowercased italic letter differ significantly at the $P < 0.05$ level.

^aData are least squares means of 5 observations for all treatments

^bSEM, Standard error of the mean.

Table 7. Effects of dietary lysozyme and protein source on serum urea nitrogen, intestinal barrier parameters and immune level in weanling pigs^a

	Spray dried egg		Albumen powder		Protein source		Lysozyme		SEM ^b	P-values		
	-	+ lysozyme	-	+ lysozyme	Spray dried egg	Albumen powder	-	+		Protein	Lysozyme	Interaction
N-metabolite												
Serum urea nitrogen (mmol/l)	4.69 ^a	4.48 ^a	3.67 ^b	3.55 ^b	4.59 ^a	3.61 ^b	4.18	4.02	0.41	0.03	0.69	0.91
Intestinal barrier parameters												
D-lactic acid (mmol/l)	1.33 ^a	1.24 ^b	1.33 ^a	1.23 ^b	1.29	1.28	1.33 ^a	1.24 ^b	0.03	0.77	<0.01	0.99
Diamine oxidase (U/l)	2.12 ^a	1.68 ^b	1.77 ^a	1.61 ^b	1.90	1.69	1.95 ^a	1.65 ^b	0.11	0.08	0.02	0.24
Immunoglobulin levels												
IgG (g/l)	8.56	8.47	8.02	8.01	8.51	8.02	8.29	8.24	0.24	0.06	0.81	0.85
IgM (g/l)	0.90 ^a	0.82 ^b	0.89 ^a	0.71 ^b	0.86	0.80	0.90 ^a	0.77 ^b	0.05	0.26	0.02	0.37

Note: Means within a column not sharing a lowercased italic letter differ significantly at the $P < 0.05$ level.

^aData are least squares means of 5 observations for all treatments.

^bSEM, Standard error of the mean.