Canadian Science Publishing | Canadian Journal of Animal Science

# Evaluation of the effect of beta-mannanase supplementation and mannans on nursery pig growth performance and serum acute phase protein concentrations



| | |
|---|---|
| Journal: | *Canadian Journal of Animal Science* |
| Manuscript ID | CJAS-2018-0248.R2 |
| Manuscript Type: | Article |
| Date Submitted by the Author: | 01-Sep-2019 |
| Complete List of Authors: | Huntley, Nichole; Iowa State University, Dept. of Animal Science<br>Gould, Stacie; Iowa State University, Dept. of Animal Science<br>Patience, John; Iowa State University, Dept. of Animal Science |
| Keywords: | β-mannan, β-mannanase, C-reactive protein, feed efficiency, haptoglobin |
| | |

Running Head: β-Mannanase effect on growth and inflammation

# Evaluation of the effect of beta-mannanase supplementation and mannans on nursery pig growth performance and serum acute phase protein concentrations[1]

**Nichole F. Huntley*, Stacie. A. Gould*, John F. Patience*[2]**

*Department of Animal Science, Iowa State University, Ames, 50011, USA



[1]The authors would like to thank Elanco Animal Health for financial and in kind support of this research.

[2]Correspondance: jfp@iastate.edu; current address: Department of Animal Science, Iowa State University, 201 Kildee Hall, Ames IA 50011

**ABSTRACT:**

The objective was to evaluate the effects of dietary mannan and β-mannanase supplementation on growth performance and serum acute phase proteins in nursery pigs. Pigs (n = 480) were blocked by initial BW (6.6 ± 0.4 kg) and 12 pens per treatment were randomly assigned to 1 of 4 treatments in a 2 x 2 factorial arrangement for 28 d. Two levels of dietary mannan (0.4% vs 2.8%) were offered with and without 0.05% endo-1,4-β-mannanase. Serum was collected (1 pig/pen) for haptoglobin and C-reactive protein (**CRP**) analysis on d 0 and d 28. There were no significant interactions between mannan level or β-mannanase supplementation. High mannan decreased ADG ($P = 0.027$) and ADFI ($P = 0.024$) compared to low mannan diets with no effect of β-mannanase ($P > 0.10$). Haptoglobin and CRP were not affected by mannan level or β-mannanase supplementation ($P \geq 0.160$). Therefore, dietary β-mannans as high as 2.8% did not induce a systemic innate immune response. Thus the levels of β-mannan found in practical diets (< 2%) are unlikely to induce an immune response, so supplementation with β-mannanase to avoid this response is not warranted.

**Key words:** β-mannan, β-mannanase, C-reactive protein, feed efficiency, haptoglobin, swine

## INTRODUCTION

Certain dietary carbohydrate components, such as β-mannans, have been hypothesized to induce innate immune system activation by mimicking bacterial cell wall structures (Zhang and Tizard 1996; Duncan et al. 2002; Gazi and Martinez-Pomares 2009). Beta-mannans are found in feed ingredients such as soybean meal (**SBM**), palm kernel meal, and copra meal in varying concentrations (Shastak et al. 2015). If feed-induced immune stimulation occurs, it may partition less dietary energy toward productive processes such as growth (Huntley et al. 2018). Exogenous β-mannanase enzymes have been developed to inhibit this innate immune stimulation. In poultry, β-mannanase supplementation decreased serum acute phase proteins (**APP**), thus ameliorating systemic inflammatory stress in broilers (Anderson et al. 2008). However, this response has yet to be demonstrated in pigs.

Beta-mannanase supplementation responses in pigs have been variable (Pettey et al. 2002; Yoon et al. 2010; Lv et al. 2013; Carr et al. 2014). Previous research in our laboratory evaluated β-mannanase supplementation in a corn, SBM, and soy hulls-based diet and found no effects on diet digestibility, nitrogen balance, maintenance energy requirements, or systemic innate immune response variables (Huntley et al. 2018). These results did not support the hypothesis, supported by studies in poultry (Arsenault et al., 2017), that β-mannanase supplementation ameliorates an innate immune response induced by soybean β-mannans at a concentration of 1.3% of the diet. It is possible, however, that this concentration was insufficient to either effectively generate a systemic inflammatory response or to be able to measure the effects of β-mannanase supplementation. To test this hypothesis, the objectives of this study were to 1) evaluate the hypothesis that β-mannans induce an innate immune response, and 2) evaluate the impact of the

dietary concentration of β-mannans and β-mannanase supplementation on serum APP, as indicators of systemic immune activation, and on growth performance in nursery pigs.

## MATERIALS AND METHODS

All experimental procedures adhered to guidelines for the ethical and humane use of animals for research according to the Guide for the Care and Use of Laboratory Animals (8th edition, National Academies Press) and were approved by the Iowa State University Institutional Animal Care and Use Committee (IACUC #5-16-8263-S).

### *Animals, Housing, and Experimental Design*

A total of 480 weanling pigs (6.6 ± 0.4 kg BW; Genetiporc 6.0 × Genetiporc F25, PIC, Inc., Hendersonville, TN) were purchased and transported to the Iowa State University Swine Nutrition farm. Upon arrival, pigs were individually weighed, ear-tagged, and vaccinated for porcine reproductive and respiratory syndrome virus, and *Escherichia coli*. Pigs were blocked by initial weight and pens were randomly assigned to 1 of 4 dietary treatments. There were 10 pigs per pen and 12 pens per treatment. Sexes were not separated but there were the same number of barrows and gilts per treatment within each block.

### *Diets and Feeding*

Pens (1.2 m × 2.4 m) had a four-space self-feeder and 2 nipple waterers to provide *ad libitum* access to feed and water. Pigs were fed a common diet for 7 d post weaning; the experimental diets were then fed in mash form in 3 phases over 28 d. Phases 1 and 2 each were offered for 7 d each and phase 3 for 14 d. Diets were formulated to meet or exceed NRC (2012) nutrient recommendations and were formulated to provide equal ME:SID lysine concentrations. Treatments were arranged as a 2 × 2 factorial of dietary β-mannan level (low or high) and β-

mannanase supplementation (positive or negative). Two levels of dietary mannan were achieved by replacing a proportion of the corn and soybean meal used in the low mannan diet (0.4% β-mannan) with copra meal for a high mannan diet (2.8% β-mannan; Table 1).

The nutrient profile of the experimental diets is presented in Tables 2 through 4. To avoid confounding, diets were formulated to be isocaloric on an ME basis and also to contain the same levels of essential amino acids as well as calcium and phosphorus.

Diet β-mannan concentration was determined based on ingredient β-mannan concentrations (Bach Knudsen 1997; Hsiao et al. 2006; Sundu et al. 2012). Beta-mannanase (Hemicell HT-1.5, Elanco Animal Health, Greenfield, IN; endo-1,4-β-mannanase (EC 3.2.1.78; 237 x $10^6$ units/kg) from *Bacillus lentus*) was supplemented at 0.05% of the diet, slightly above the manufacturer's recommendations. One β-mannanase unit of activity was defined as the amount of enzyme that generates 0.72 μg of reducing sugars per min from a mannose-containing substrate at pH 7.0 and 40°C.

***Sample Collection***

Pig BW and feed intake were measured on d 0and 28 of the experiment to calculate overall ADG, ADFI, and G:F. On d 0 (baseline) and d 28, serum was collected from 1 barrow per pen (the same pig at each time point) via jugular venipuncture. Blood was allowed to clot and serum was separated by centrifugation (2000 × *g* for 15 min at 4°C), collected and divided into two subsamples, and stored at -80°C until analysis. Diet subsamples were collected during manufacture.

***Analytical Methods***

Diets were ground to 1 mm particle size and analyzed in duplicate for DM (method 930.15) and acid-hydrolyzed ether extract (**aEE**; method 2003.06) using standard methods (AOAC 2007);

and in triplicate for NDF (Van Soest and Robertson 1979) and ADF (Goering and Van Soest 1970). Diets were analyzed in duplicate for nitrogen (**N**; method 990.03 (AOAC 2007); TruMac; LECO Corp., St. Joseph, MI). An EDTA sample (9.56% N) was used as the standard for calibration and was determined to contain 9.55 ± 0.01% N. Crude protein was calculated as N x 6.25. Gross energy was determined in duplicate using an isoperibol bomb calorimeter (model 6200; Parr Instrument Co., Moline, IL). Benzoic acid (6318 kcal GE/kg; Parr Instrument Co., Moline, IL) was used as the standard for calibration and was determined to contain 6316 ± 0.9 kcal GE/kg. Diets were analyzed in duplicate for β-mannanase concentration (colorimetric determination, Elanco Animal Health, Gaithersburg, MD).

Serum subsamples were analyzed in duplicate for haptoglobin and C-reactive protein (**CRP**) using porcine specific commercially available ELISA kits according to the manufacturer's instructions (Immunology Consultants Laboratory, Inc., Portland, OR). The intra- and inter-assay coefficients of variation for haptoglobin and CRP were 4.9 and 16.0%, and 3.6 and 9.9%, respectively. Haptoglobin and CRP have been identified as primary acute phase proteins in swine and increases in their serum concentrations are correlated with systemic inflammation and response to disease (Chen et al. 2003; Eckersall and Bell 2010).

***Statistical Analysis***

Data were analyzed as a 2 × 2 factorial in a randomized complete block design to test the main effects and interaction of dietary mannan level and β-mannanase supplementation. Data were analyzed with pen as the experimental unit and block as a random variable using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC). Differences among treatments were determined using ANOVA and means were separated using the least square means statement and the PDIFF option. Serum APP data were log transformed and analyzed as repeated measures with compound

symmetry covariance structure. Differences were considered significant if $P$ was $\leq 0.05$ and a tendency if $P$ was $> 0.05$ and $\leq 0.10$.

## RESULTS AND DISCUSSION

There were no significant interactions between mannan level and β-mannanase supplementation. Pigs fed low mannan diets had greater ADG and ADFI compared to pigs fed high mannan diets ($P \leq 0.027$), although feed efficiency did not differ ($P = 0.653$; Table 5). Beta-mannanase inclusion did not impact BW or growth performance ($P \geq 0.106$). There is no way to differentiate between the effect of mannans and the effect of copra meal; this is the nature of research in which changes in ingredient composition of experimental diets is required to achieve compositional differences between diets. The lower rate of gain and feed intake could have been due to errors in the estimated energy and nutrient component of copra meal. If this is the case, it is unlikely that energy content was incorrect due to the equivalence of feed efficiency across mannan levels. While errors in the estimated digestibility of amino acids could be involved, a possibly more likely explanation would be the physical bulk of the diet due to the high fiber content, which could have exceeded the capacity of the pigs to eat more volume of feed to maintain energy and nutrient intake. The fact that rate of gain and feed intake was impacted, but not feed efficiency, suggests this is the most likely explanation (NRC, 2012).

In this experiment, β-mannanase supplementation did not improve growth performance in either the high or low mannan diets. Similarly, other studies have reported no β-mannanase effects on rate or efficiency of gain (Yoon et al. 2010; Carr et al. 2014; Upadhaya et al. 2016), or nutrient and energy digestibility (Mok et al. 2015; Upadhaya et al. 2016; Huntley et al. 2018). Yet, others have reported improvements due to β-mannanase supplementation (Pettey et al. 2002; Yoon et al.

2010; Lv et al. 2013). Kim et al. (2017) reported that β-mannanase supplementation effectively improved DM, GE, and β-mannan digestibility as well as growth performance, regardless of the amount of β-mannan in the diet. More research is clearly needed to better understand the circumstances in which β-mannanase supplementation is effective.

Serum APP concentrations were similar among treatments at baseline ($P \geq 0.20$). On d 28, haptoglobin concentrations were lower ($P \leq 0.0001$; Fig. 1) and CRP concentrations were greater ($P \leq 0.0001$; Fig. 2) compared to baseline values, but neither were affected by mannan level or β-mannanase supplementation ($P \geq 0.160$). Beta-mannanase supplementation tended to increase serum CRP in the high mannan diet (291 ± 20 μg/ml) compared to the low mannan diet (243 ± 20 μg/ml) with no differences compared to when the enzyme was not supplemented (avg. = 274 ± 20 μg/ml; $P = 0.0791$).

This experiment evaluated the interaction of dietary β-mannan level and β-mannanase supplementation on nursery pig growth performance and serum acute phase protein concentrations and tested the hypotheses that dietary β-mannans induce an innate immune response in nursery pigs and that β-mannanase supplementation alleviates this response (Anderson et al. 2008; Ferreira et al. 2014). Understanding if, or how, this response occurs in pigs is important because when an immune challenge is perceived, it can partition energy and nutrients away from productive processes such as muscle growth and negatively impact the efficiency and cost of meat production (Lochmiller and Deerenberg, 2000; Gabler and Spurlock, 2008; Huntley et al., 2018). β-mannan in soybean, copra, and palm kernel meals resemble carbohydrate structures on pathogen surfaces (Badia et al. 2012) and may induce an unproductive and undesirable innate immune response, termed a feed-induced immune response (**FIIR**).

Beta-mannans are components of hemicellulose in the plant cell wall and are linear polysaccharides comprised of repeating β-(1,4)-linked D-mannose units with galactose and/or glucose substitutions (Moreira and Filho 2008). Specifically, galactomannans (α-(1,6)-linked D-galactose residues attached to mannose residues within the β-(1,4)-mannan backbone) are the type of mannans present in the endosperm of legume plant seeds such as soybeans (Moreira and Filho 2008). As a constituent of hemicellulose, β-mannans are not digested by mammalian endogenous enzymes. Thus, intact β-mannans may be available to bind carbohydrate recognition domains of pattern recognition receptors on innate immune cells surveying the intestinal epithelium for potential pathogens (Kraehenbuhl and Corbett 2004; Hardison and Brown 2008; Gazi and Martinez-Pomares 2009). It is hypothesized that in this way, β-mannans may be capable of stimulating innate immune cells resulting in a nonproductive, energy-demanding, inflammatory immune response (Zhang and Tizard 1996; Duncan et al. 2002; Anderson et al. 2008). One component of innate immune activation is the acute phase response, which is characterized by dramatic changes in the concentration of APP, such as haptoglobin and CRP.

The initial FIIR descriptions were in poultry and reported increased macrophage and lymphocyte proliferation (Adibmoradi et al. 2010) and plasma APP concentrations (Anderson et al. 2008) due to increasing dietary SBM inclusion. This response was then attenuated by the addition of β-mannanase and was supported by improvements in feed efficiency (Anderson et al. 2008; Adibmoradi et al. 2010). It was hypothesized that the hydrolyzed manno-oligosaccharides no longer retained the degree of polymerization necessary to stimulate and cross-link multiple mannose receptors, thus reducing innate immune stimulation.

However, there is a paucity of comparable data regarding immune responses of pigs when dietary β-mannan and β-mannanase are supplemented, and a β-mannan-derived FIIR has yet to be

demonstrated. This study provides novel data on the interaction of dietary β-mannan level and β-mannanase enzyme supplementation on nursery pig serum APP concentrations, as a measure of systemic immune stress. If an immune response to β-mannan had occurred, both CRP and haptoglobin concentrations would have been expected to increase (Cray et al. 2009). However, this did not occur. Plus, the changes in APP concentrations from baseline to d 28 were likely due to time, age of the pig, or other environmental factors that equally affected each treatment (Wright et al. 2000).

Similarly, there was no interaction between the effects of mannan level and β-mannanase supplementation on serum ACP concentrations. Had an innate immune response occurred in either the low or high-mannan diets, it would have been expected that β-mannanase supplementation would prevent immune stimulation and decrease serum haptoglobin and CRP. This response would have been exaggerated in the high-mannan diets due to the greater amount of substrate for the enzyme. Since this was not observed, it was concluded that dietary β-mannan concentrations as high as 2.8% do not induce a systemic innate immune response in nursery pigs. However, because diets with different β-mannan concentrations were achieved by replacing corn and SBM with copra meal, a specific ingredient effect, separate from a β-mannan nutrient effect, cannot be ruled out.

The results of this experiment corroborate data from Huntley et al. (2018) which indicated that β-mannanase supplementation did not impact nursery pig serum proinflammatory cytokines, haptoglobin, or mannose-binding lectin concentrations. Furthermore, there were no differences in maintenance energy requirements between control and β-mannanase supplemented pigs, supporting the conclusion that β-mannanase did not prevent an energy demanding immune response. Interpreted together, the data from Huntley et al. (2018) and the current study indicate

that the levels of β-mannan found in practical, commercial-type swine diets are not likely to induce a FIIR and supplementation of β-mannanase for this purpose is not warranted.

In conclusion, the high-mannan diet decreased ADFI and thus, decreased ADG by 2.7% compared to the low-mannan diet with no apparent effect on serum haptoglobin or CRP concentrations. This experiment provides immune data previously lacking in pig growth trials assessing β-mannanase efficacy. Beta-mannanase supplementation had no impact on growth performance and did not affect haptoglobin or CRP concentrations differently in high compared to low mannan diets. Thus, these data do not support the hypothesis that the level of β-mannan found in practical swine diets induces an innate immune response or that β-mannanase supplementation may prevent such a response.

## LITERATURE CITED

Adibmoradi, M., Mehri, M., Samie, A., Shivazad, M., Adibmoradi, M., Samie, A., and Schivazad, M. 2010. Effects of β-mannanase on broiler performance, gut morphology and immune system. African J. Biotechnol. **9**: 6221–6228. doi:10.5897/AJB09.1371.

Anderson, D.M., Hsiao, H.Y., and Dale, N.M. 2008. Identification of an inflammatory compound for chicks in soybean meal. Poult. Sci. **88**: 153. doi:10.1017/CBO9781107415324.004.

AOAC 2007. Official methods of analysis of AOAC international. *Edited By*W. Horwitz and G.W. Latimer 18th edition. Association of Official Analytical Chemists International, Gaithersburg, MD.

Bach Knudsen, K.E. 1997. Carbohydrate and lignin contents of plant materials used in animal feeding. Anim. Feed Sci. Technol. **67**: 319–338. doi:10.1016/S0377-8401(97)00009-6.

Badia, R., Brufau, M.T., Guerrero-Zamora, A.M., Lizardo, R., Dobrescu, I., Martin-Venegas, R., Ferrer, R., Salmon, H., Martínez, P., Brufau, J., Martinez, P., and Brufau, J. 2012. β-galactomannan and Saccharomyces cerevisiae var. boulardii modulate the immune response against Salmonella enterica serovar typhimurium in porcine intestinal epithelial and dendritic cells. Clin. Vaccine Immunol. **19**: 368–376. doi:10.1128/CVI.05532-11.

Carr, S.N., Allee, G.L., Rincker, P.J., Fry, R.S., and Boler, D.D. 2014. Effects endo-1,4-β-D-mannanase enzyme (Hemicell HT 1.5x) on the growth performance of nursery pigs. Prof. Anim. Sci. **30**: 393–399. doi:10.15232/pas.2014-01326.

Chen, H.H., Lin, J.H., Fung, H.P., Ho, L.L., Yang, P.C., Lee, W.C., Lee, Y.P., and Chu, R.M. 2003. Serum acute phase proteins and swine health status. Can. J. Vet. Res. **67**: 283–290.

Cray, C., Zaias, J., and Altman, N.H. 2009. Acute phase response in animals: A review. Comp.

Med. **59**: 517–526. doi:10.1002/ece3.1939.

Duncan, C.J.G., Pugh, N., Pasco, D.S., and Ross, S.A. 2002. Isolation of a galactomannan that enhances macrophage activation from the edible fungus Morchella esculenta. J. Agric. Food Chem. **50**: 5683–5685. doi:10.1021/jf020267c.

Eckersall, P.D., and Bell, R. 2010. Acute phase proteins: Biomarkers of infection and inflammation in veterinary medicine. Vet. J. **185**: 23–27. doi:10.1016/j.tvjl.2010.04.009.

Ferreira, M., Schinckel, A.P., Ferrel, J.E., Richert, B.T., Radcliffe, J.S., and Stewart, T.S. 2014. Impact of level of soybean meal and B-mannanase in nursery diets on two genetic populations of pigs selected for soybean meal allergic response. Page 68 *in* Proceedings of the ADSA-ASAS Midwestern Meetings.

Gabler, N., and Spurlock, M. 2008. Integrating the immune system with the regulation of growth and efficiency. J. Anim. Sci. **86**: E64–E74. doi:10.2527/jas.2007-0466.

Gazi, U., and Martinez-Pomares, L. 2009. Influence of the mannose receptor in host immune responses. Immunobiology **214**: 554–561. doi:10.1016/j.imbio.2008.11.004.

Goering, H.K., and Van Soest, P.J. 1970. Forage fiber analyses (Apparatus, reagents, procedures, and some applications). Agricultural Handbook. Agricultural Research Service, USDA.

Hardison, S.E., and Brown, G.D. 2008. C-type lectin receptors in antifungal immunity. Trends Microbiol. **16**: 27–32. doi:10.1016/j.tim.2007.10.012.

Hsiao, H.Y., Anderson, D.M., and Dale, N.M. 2006. Levels of beta-mannan in soybean meal. Poult. Sci. **85**: 1430–1432. doi:10.1093/ps/85.8.1430.

Huntley, N.F., Nyachoti, C.M., and Patience, J.F. 2018. Lipopolysaccharide immune stimulation but not β-mannanase supplementation affects maintenance energy requirements in young weaned pigs. J. Anim. Sci. Biotech. **9**:47. doi: 10.1186/s40104-018-0264-y.

Kim, J.S., Ingale, S.L., Hosseindoust, A.R., Lee, S.H., Lee, J.H., and Chae, B.J. 2017. Effects of mannan level and beta-mannanase supplementation on growth performance, apparent total tract digestibility and blood metabolites in growing pigs. Anim. **186**: 64–70. doi:10.1017/S1751731116001385.

Kraehenbuhl, J.-P., and Corbett, M. 2004. Keeping the gut microflora at bay. Science. **303**: 1624–1625. doi:10.1126/science.1096222.

Lochmiller, R.L., and Deerenberg, C. 2000. Trade-offs in evolutionary immunology: Just what is the cost of immunity? Oikos **88**: 87–98. doi:10.1034/j.1600-0706.2000.880110.x.

Lv, J.N., Chen, Y.Q., Guo, X.J., Piao, X.S., Cao, Y.H., and Dong, B. 2013. Effects of supplementation of β-mannanase in corn-soybean meal diets on performance and nutrient digestibility in growing pigs. Asian-Australasian J. Anim. Sci. **26**: 579–587. doi:10.5713/ajas.2012.12612.

Mok, C.H., Kong, C., and Kim, B.G. 2015. Combination of phytase and β-mannanase supplementation on energy and nutrient digestibility in pig diets containing palm kernel expellers. Anim. Feed Sci. Technol. **205**: 116–121. doi:10.1016/j.anifeedsci.2015.04.012.

Moreira, L.R.S., and Filho, E.X.F. 2008. An overview of mannan structure and mannan-degrading enzyme systems. Appl. Microbiol. Biotechnol. **79**: 165–178. doi:10.1007/s00253-008-1423-4.

NRC 2012. Nutrient requirements of swine. 11th revis. National Academy Press, Washington, DC.

Pettey, L.A., Carter, S.D., Senne, B.W., and Shriver, J.A. 2002. Effects of beta-mannanase addition to corn-soybean meal diets on growth performance, carcass traits, and nutrient digestibility of weanling and growing-finishing pigs. J. Anim. Sci. **80**: 1012–1019.

doi:10.2527/2002.8041012x.

Shastak, Y., Ader, P., Feuerstein, D., Ruehle, R., and Matuschek, M. 2015. ß-Mannan and mannanase in poultry nutrition. Worlds. Poult. Sci. J. **71**: 161–174. doi:10.1017/S0043933915000136.

Van Soest, P.J., and Robertson, J.B. 1979. Systems of analysis for evaluating fibrous feeds. Pages 49–60 *in* W.J. Pigden, C.C. Balch, and M. Graham, eds. Workshop standardization of analytical methodology for feeds. International Development Research Centre, Ottawa, Canada.

Sundu, B., Hatta, U., and Chaudhry, A.S. 2012. Potential use of beta-mannan from copra meal as a feed additive for broilers. Worlds. Poult. Sci. J. **68**: 707–716. doi:10.1017/S0043933912000839.

Upadhaya, S.D., Park, J.W., Lee, J.H., and Kim, I.H. 2016. Efficacy of β-mannanase supplementation to corn-soya bean meal-based diets on growth performance, nutrient digestibility, blood urea nitrogen, faecal coliform and lactic acid bacteria and faecal noxious gas emission in growing pigs. Arch. Anim. Nutr. **70**: 33–43. doi:10.1080/1745039X.2015.1117697.

Wright, K.J., Balaji, R., Hill, C.M., Dritz, S.S., Knoppel, E.L., and Minton, J.E. 2000. Integrated adrenal, somatotropic, and immune responses of growing pigs to treatment with lipopolysaccharide. J. Anim. Sci. **78**: 1892–1899. doi: 10.2527/2000.7871892x.

Yoon, S.Y., Yang, Y.X., Shinde, P.L., Choi, J.Y., Kim, J.S., Kim, Y.W., Yun, K., Jo, J.K., Lee, J.H., Ohh, S.J., Kwon, I.K., and Chae, B.J. 2010. Effects of mannanase and distillers dried grain with solubles on growth performance, nutrient digestibility, and carcass characteristics of grower-finisher pigs. J. Anim. Sci. **88**: 181–191. doi:10.2527/jas.2008-1741.

Zhang, L., and Tizard, I.R. 1996. Activation of a mouse macrophage cell line by acemannan: The major carbohydrate fraction from Aloe vera gel. Immunopharmacology **35**: 119–128. doi:10.1016/S0162-3109(96)00135-X.

**Table 1.** Ingredient composition (as-fed basis) of the experimental diets for phases 1 – 3[a]

| Ingredient, % | Phase 1 | | Phase 2 | | Phase 3 | |
|---|---|---|---|---|---|---|
| | Low Mannan | High Mannan | Low Mannan | High Mannan | Low Mannan | High Mannan |
| Corn | 51.69 | 45.79 | 51.04 | 45.30 | 49.75 | 44.23 |
| Reduced-oil corn DDGS | 10.00 | 10.00 | 12.50 | 12.50 | 15.00 | 15.00 |
| Fish meal | 5.00 | 5.00 | 4.00 | 4.00 | 3.00 | 3.00 |
| Soybean meal | 25.00 | 20.00 | 25.00 | 20.00 | 25.00 | 20.00 |
| Copra meal | - | 10.00 | - | 10.00 | - | 10.00 |
| Soybean oil | 4.20 | 4.80 | 3.50 | 4.10 | 3.20 | 3.70 |
| Limestone | 1.10 | 1.20 | 1.15 | 1.15 | 1.20 | 1.10 |
| Monocalcium phosphate | 0.60 | 0.70 | 0.55 | 0.55 | 0.55 | 0.50 |
| Lysine-HCl | 0.50 | 0.60 | 0.43 | 0.55 | 0.45 | 0.57 |
| DL-Methionine | 0.08 | 0.07 | 0.06 | 0.07 | 0.10 | 0.09 |
| L-Threonine | 0.15 | 0.17 | 0.15 | 0.15 | 0.11 | 0.15 |
| L-Tryptophan | 0.05 | 0.06 | 0.04 | 0.05 | 0.04 | 0.06 |
| L-Valine | 0.05 | 0.03 | 0.00 | 0.00 | 0.00 | 0.00 |
| Vitamin premix[b] | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| Trace mineral premix[c] | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 | 0.20 |
| Denagard[d] | 0.18 | 0.18 | 0.18 | 0.18 | 0.00 | 0.00 |
| Aureomycin[e] | 0.40 | 0.40 | 0.40 | 0.40 | 0.00 | 0.00 |
| Mecadox 2.5[f] | 0.00 | 0.00 | 0.00 | 0.00 | 0.20 | 0.20 |
| Salt | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| Choline chloride, 60% | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 |
| Titanium dioxide | 0.00 | 0.00 | 0.00 | 0.00 | 0.40 | 0.40 |

For the enzyme positive diet, β-mannanase (Hemicell HT 1.5, Elanco Animal Health, Greenfield, IN; endo-1,4-β-D-mannanase from *Bacillus lentus* with not less than 237 million units per kg of product) was included at 0.05% at the expense of corn.

[a]Phase 1 = d 0 – 7; Phase 2 = d 8 – 14; Phase 3 = d 15 - 28

[b]Provided 6,614 IU vitamin A, 827 IU vitamin D, 26 IU vitamin E, 2.6 mg vitamin K, 29.8 mg niacin, 16.5 mg pantothenic acid, 5.0 mg riboflavin, and 0.023 mg vitamin B12 per kg of diet.

[c]Provided 165 mg Zn (zinc sulfate), 165 mg Fe (iron sulfate), 39 mg Mn (manganese sulfate), 17 mg Cu (copper sulfate), 0.3 mg I (calcium iodate), and 0.3 mg Se (sodium selenite) per kg of diet.

[d]Denagard 10 (tiamulin, 0.22 g per kg), Elanco Animal Health, Greenfield, IN

[e]Aureomycin (chlortetracycline (0.44 g per kg diet), Zoetis Inc., Kalamazoo, MI

[f]Mecadox 2.5 (carbadox), Phibro Animal Health Corp., Ridgefield Park, NJ

**Table 2**. Phase 1 diet formulated and analyzed energy and nutrient composition (as-fed basis)

| | Low mannan diet | | High mannan diet | |
|---|---|---|---|---|
| | - enzyme | + enzyme | - enzyme | + enzyme |
| Formulated composition | | | | |
| ME, Mcal/kg | 3.46 | 3.46 | 3.46 | 3.46 |
| SID amino acid, % | | | | |
| Lys | 1.38 | 1.38 | 1.37 | 1.37 |
| Met | 0.42 | 0.42 | 0.40 | 0.40 |
| Total sulfur AA | 0.74 | 0.74 | 0.74 | 0.74 |
| Thr | 0.83 | 0.83 | 0.81 | 0.81 |
| Trp | 0.26 | 0.26 | 0.26 | 0.26 |
| Ca, % | 0.81 | 0.81 | 0.85 | 0.85 |
| STTD P, % | 0.41 | 0.41 | 0.44 | 0.44 |
| Analyzed composition | | | | |
| DM, % | 90.43 | 89.99 | 90.93 | 90.91 |
| GE, Mcal/kg | 4.16 | 4.16 | 4.32 | 4.33 |
| CP, % | 22.70 | 22.15 | 21.77 | 20.85 |
| aEEa, % | 7.62 | 7.41 | 8.78 | 8.37 |
| NDF, % | 9.21 | 8.88 | 12.90 | 13.01 |
| ADF, % | 3.58 | 3.47 | 5.04 | 5.89 |
| β-mannanase[b,c], U/kg | < detection limit | 345,000 | < detection limit | 355,000 |

[a]Acid-hydrolyzed ether extract (**aEE**)

[b]Endo-1,4-β-mannanase (EC 3.2.1.78); 1 U = the amount of enzyme which generates 0.72 µg of reducing sugars per min from a mannose-containing substrate at pH 7.0 and 40°C

[c]The limit of detection for feed samples was 45,000 U/kg

**Table 3**. Phase 2 diet formulated and analyzed energy and nutrient composition (as-fed basis)

| | Low mannan diet | | High mannan diet | |
|---|---|---|---|---|
| | - enzyme | + enzyme | - enzyme | + enzyme |
| Formulated composition | | | | |
| ME, Mcal/kg | 3.42 | 3.42 | 3.42 | 3.42 |
| SID amino acid, % | | | | |
| Lys | 1.30 | 1.30 | 1.30 | 1.30 |
| Met | 0.40 | 0.40 | 0.40 | 0.40 |
| Total sulfur AA | 0.71 | 0.71 | 0.71 | 0.71 |
| Thr | 0.82 | 0.82 | 0.79 | 0.79 |
| Trp | 0.25 | 0.25 | 0.24 | 0.24 |
| Ca, % | 0.78 | 0.78 | 0.77 | 0.77 |
| STTD P, % | 0.39 | 0.39 | 0.40 | 0.40 |
| Analyzed composition | | | | |
| DM, % | 89.50 | 89.79 | 90.82 | 90.88 |
| GE, Mcal/kg | 4.10 | 4.11 | 4.23 | 4.24 |
| CP, % | 21.52 | 21.88 | 21.06 | 20.57 |
| aEE[a], % | 6.61 | 7.19 | 8.59 | 8.53 |
| NDF, % | 9.62 | 9.91 | 13.89 | 14.43 |
| ADF, % | 3.04 | 3.19 | 4.92 | 4.92 |
| β-mannanase[b,c], U/kg | < detection limit | 304,000 | < detection limit | 316,000 |

[a]Acid-hydrolyzed ether extract (**aEE**)

[b]Endo-1,4-β-mannanase (EC 3.2.1.78); 1 U = the amount of enzyme which generates 0.72 μg of reducing sugars per min from a mannose-containing substrate at pH 7.0 and 40°C

[c]The limit of detection for feed samples was 45,000 U/kg

**Table 4**. Phase 3 diet formulated and analyzed energy and nutrient composition (as-fed basis)

| | Low mannan diet | | High mannan diet | |
|---|---|---|---|---|
| | - enzyme | + enzyme | - enzyme | + enzyme |
| Formulated composition | | | | |
| ME, Mcal/kg | 3.39 | 3.39 | 3.39 | 3.39 |
| SID amino acid, % | | | | |
| Lys | 1.28 | 1.28 | 1.28 | 1.28 |
| Met | 0.43 | 0.43 | 0.41 | 0.41 |
| Total sulfur AA | 0.72 | 0.72 | 0.70 | 0.70 |
| Thr | 0.78 | 0.78 | 0.78 | 0.78 |
| Trp | 0.25 | 0.25 | 0.25 | 0.25 |
| Ca, % | 0.75 | 0.75 | 0.70 | 0.70 |
| STTD P, % | 0.38 | 0.38 | 0.38 | 0.38 |
| Analyzed composition | | | | |
| DM, % | 90.60 | 90.35 | 90.94 | 91.15 |
| GE, Mcal/kg | 4.17 | 4.15 | 4.27 | 4.27 |
| CP, % | 21.60 | 21.61 | 21.72 | 21.11 |
| aEE[a], % | 6.98 | 6.99 | 8.16 | 8.47 |
| NDF, % | 10.38 | 10.83 | 14.87 | 15.00 |
| ADF, % | 3.27 | 3.39 | 5.70 | 5.80 |
| β-mannanase[b,c], U/kg | < detection limit | 327,000 | < detection limit | 365,000 |

[a]Acid-hydrolyzed ether extract (**aEE**)

[b]Endo-1,4-β-mannanase (EC 3.2.1.78); 1 U = the amount of enzyme which generates 0.72 µg of reducing sugars per min from a mannose-containing substrate at pH 7.0 and 40°C

[c]The limit of detection for feed samples was 45,000 U/kg

**Table 5.** Effects of dietary mannan level and β-mannanase supplementation on nursery pig BW, growth rate, feed intake, and efficiency

| | Mannan level[a] | | Enzyme inclusion[b] | | SEM | *P*-value | | |
|---|---|---|---|---|---|---|---|---|
| | High | Low | - | + | | Mannan ×Enzyme | Mannan | Enzyme |
| Overall, d 0 - 28 | | | | | | | | |
| d 0 BW, kg | 6.64 | 6.65 | 6.64 | 6.65 | 0.36 | 0.583 | 0.736 | 0.790 |
| d 28 BW | 17.05 | 17.43 | 17.14 | 17.33 | 0.67 | 0.954 | 0.051 | 0.317 |
| ADG, kg | 0.37 | 0.38 | 0.37 | 0.38 | 0.01 | 0.852 | 0.027 | 0.354 |
| ADFI, kg | 0.52 | 0.54 | 0.53 | 0.53 | 0.02 | 0.922 | 0.024 | 0.922 |
| G:F | 0.71 | 0.71 | 0.70 | 0.71 | 0.01 | 1.000 | 0.653 | 0.106 |

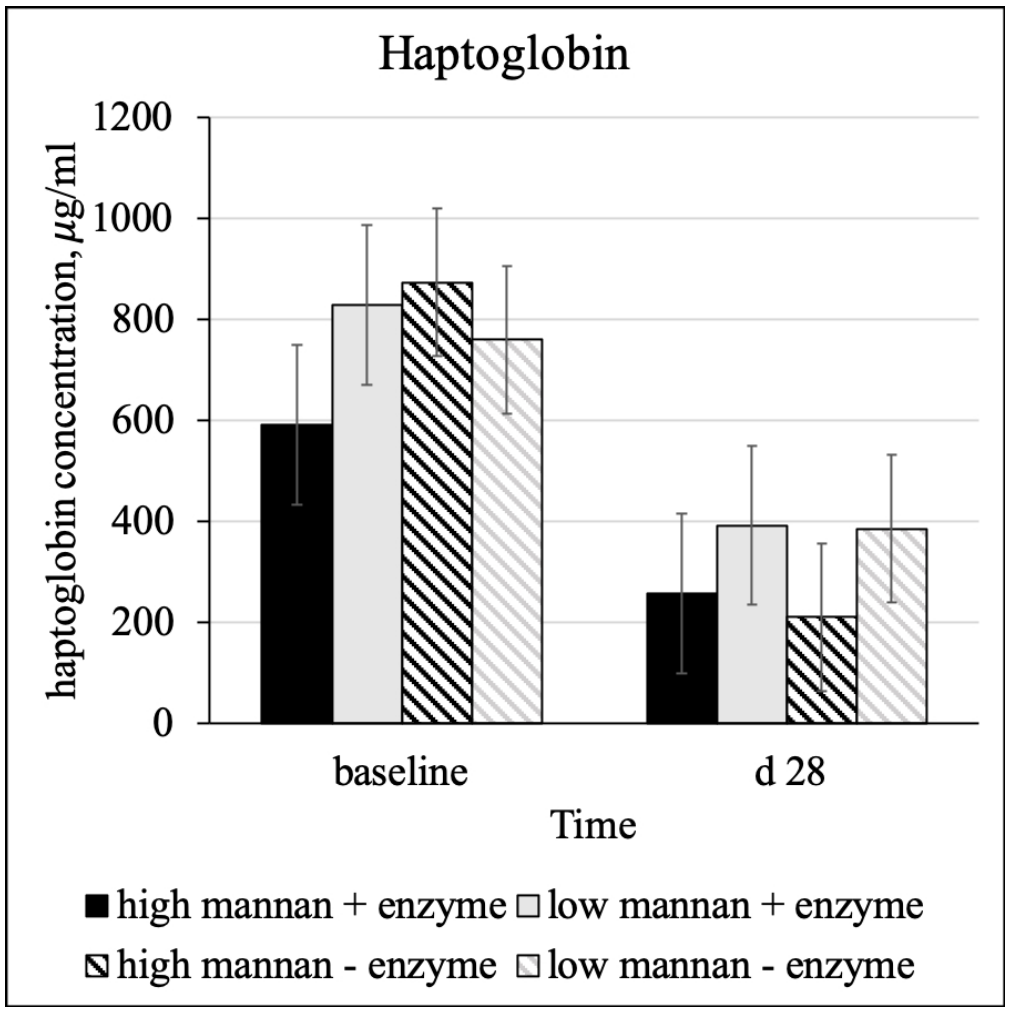
[a] wo levels of dietary mannan were achieved by replacing a proportion of the corn and soybean meal in the low mannan diet (0.4% β-mannan) with copra meal for a high mannan diet (2.8% β-mannan)

[b]For the enzyme positive diet, β-mannanase (Hemicell HT 1.5, Elanco Animal Health, Greenfield, IN; endo-1,4-β-D-mannanase from *Bacillus lentus* with not less than 237 million units per kg of product) was included at 0.05%

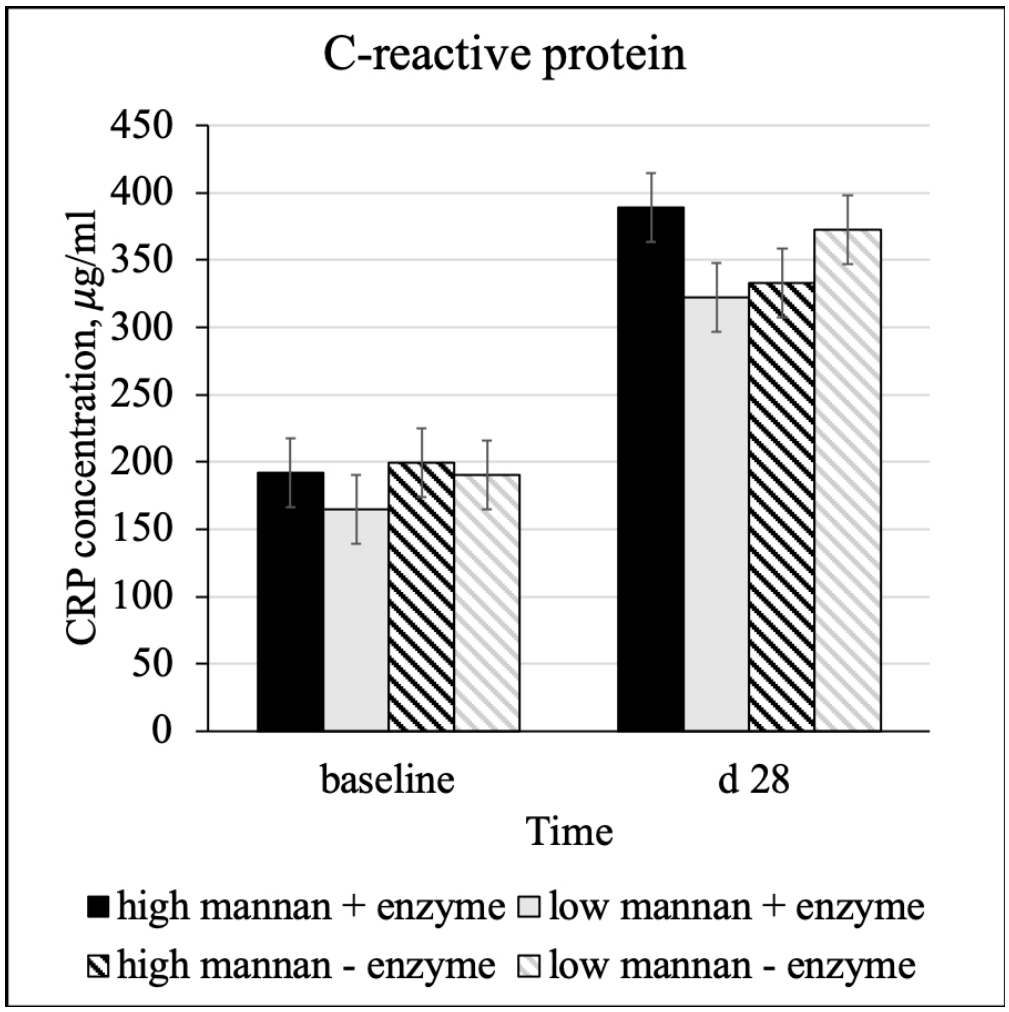
**Figure 1.** Effects of dietary mannan level and β-mannanase supplementation on nursery pig serum haptoglobin concentration ($\mu$g/ml) at baseline (d 0) and after 28 d on the experimental diet. Two levels of dietary mannan were achieved by replacing a proportion of the corn and soybean meal in the low mannan diet (0.4% β-mannan) with copra meal for a high mannan diet (2.8% β-mannan). For the enzyme positive diet, β-mannanase (Hemicell HT 1.5, Elanco Animal Health, Greenfield, IN; endo-1,4-β-D-mannanase from *Bacillus lentus* with not less than 237 million units per kg of product) was included at 0.05%. The main effect of time (baseline or d 28) was significant ($P < 0.0001$) but time did not have any significant interactions with the effects of mannan, enzyme, or the combination of the 2 ($P \geq 0.437$). Dietary mannan level, β-mannanase supplementation, or the interaction of the two effects did not significantly impact serum haptoglobin concentrations ($P \geq 0.160$).

**Figure 2**. Effects of dietary mannan level and β-mannanase supplementation on nursery pig serum C-reactive protein concentration ($\mu$g/ml) at baseline (d 0) and after 28 d on the experimental diet. Two levels of dietary mannan were achieved by replacing a proportion of the corn and soybean meal in the low mannan diet (0.4% β-mannan) with copra meal for a high mannan diet (2.8% β-mannan). For the enzyme positive diet, β-mannanase (Hemicell HT 1.5, Elanco Animal Health, Greenfield, IN; endo-1,4-β-D-mannanase from *Bacillus lentus* with not less than 237 million units per kg of product) was included at 0.05%. The main effect of time (baseline or d 28) was significant ($P < 0.0001$) but time did not have any significant interactions with the effects of mannan, enzyme, or the combination of the two ($P \geq 0.332$). Neither dietary mannan level nor β-mannanase supplementation significantly impacted serum C-reactive protein concentrations ($P \geq$

0.201). The interaction of mannan level and enzyme inclusion tended to affect C-reactive protein concentrations ($P = 0.079$).




152x152mm (150 x 150 DPI)




152x152mm (150 x 150 DPI)