

β -Mannanase, more than just a digestive enzyme for corn/soy diets

Dr. Hung-Yu Hsiao and Dr. Frank L. Jin
ChemGen Corp.

Introduction:

The use of feed enzymes such as xylanase, β -glucanase and phytase to improve the digestion and absorption of dietary nutrients has become very common for diets containing appreciable levels of xylan (from wheat), β -glucan (from barley) and phytic acids, respectively. However, corn and soy, the main feed ingredients used in Asia do not have much soluble xylan and β -glucan. Recently, Hemicell[®], a β -mannanase based feed enzyme has been shown to improve growth performance in swine and poultry fed with corn/soy based diets (1-4). Experiments described here, along with other trial results, suggest that β -mannanase is more than a simple digestive enzyme in its function to improve the performance of pigs and poultry production.

Impacts on Digestibility and Performance:

Following traditional ilea digestibility test with β -mannanase, Radcliffe and Kornegay (5) were able to show a significant improvement in digestibility of dry matter and numerical improvement in protein, lysine and other amino acids digestibility by using pigs (45-75 kg) fed with a typical corn/soy/fat diet at 16% CP or 12% CP and at 3500 kcal/kg ME (Table 1). The net benefit from β -mannanase in improving digestibility of nutrients is much larger in feeding 16% CP diet than the benefit from feeding 12% CP diet.

Results from two feeding trials on weaning pigs are also described here. Petty, *et al.* at Oklahoma State University (2) randomly allocated 117 pigs with average ages of 3-wk post-weaning to three dietary treatments. The major ingredients in the basal diet are corn, SBM and soy oil. The diets for Control and β -mannanase groups had the same nutrient density. The diet in Soy oil group had ME of 100kcal/kg higher than other two diets. The experiment included 10 pen replicates per treatment. Results are shown in Table 2. For the entire 21 days feeding period, the addition of β -mannanase to the diet increased feed conversion about 4.75% ($P < 0.01$) compared with that of pigs fed with Control diets, and it also gave a numerically better performance than pigs fed with the diet having 100 kcal/kg higher metabolic energy. These results implied that addition of β -mannanase had an energy benefit of more than 100kcal/kg. The same research group reported similar results when testing on grower and finishing pigs (22 kg -110 kg).

Dr. Jowaman Khajareern and her colleague at Khon Kaen University in Thailand also tested the same β -mannanase on the weaning pigs. A total of 96 crossbred piglets (Durox [Yorkshire x Landrac]) weaning at 21 days of age with balanced sex and genetic background were divided into two treatments with 4 replications of 12 piglets per replicate (2 replications for males and 2 replications for females). All piglets were housed in an environmentally regulated nursery pen throughout the study (21-56 days of age). A typical local piglet diet was used with ingredients such as broken rice (39.8%), rice bran (6%), fish meal (9%), SBM (16.8%), skim milk replacer (5.00%), whey powder (20%) and rice bran oil (1.5%). Test results are shown in Table 3. β -Mannanase addition significantly improved feed conversion up to 8.9%, weight gain about 4.15% and uniformity about 21%. An economic benefit of 23.3 baht per piglet was calculated.

Impacts on growth performance under antibiotics free or environmental stressful conditions:

The feeds used for the three trials described above were all supplemented with various kinds of antibiotics as growth promoters. Dr. Zhao Kebin and his master student Ms. Ma Yanfeng at Chinese Academy of Agricultural Science studied the impact of replacing antibiotics with β -mannanase fortified with xylanase on early-weaned pigs. In their trial, piglets weaned at 28 days were pre-fed for 7 days and, then, randomly allocated into three treatment groups. Each group has 4 repeats (2 for males and 2 for females). The major ingredients used in piglet diet are corn (60%), SBM (16%), extruded full fat soy (13.0%), fish meal (4%) and soybean oil (0.7%). Test results are shown in Table 4. Supplementation with β -mannanase and xylanase significantly improved feed conversion up to 10.8% over the Control group fed with the antibiotics free diet. β -mannanase and xylanase also supported a growth performance similar to feeds supplemented with antibiotics.

Dr. In-Ho Kim, et al at Dankook University in Korea tested β -mannanase on growing pigs fed with an antibiotics free diet in the Summer of 2002 (6). Ninety six pigs [D \times Y \times L] were randomly assigned by weight to four dietary treatments. The experimental design was a randomized complete block design with six pen replicates per treatment. Pigs were penned with four pigs per pen. Dietary treatments were a factorial arrangement of diet complexity (2,250 vs. 2100 kcal/kg NE for a 150 kcal/kg NE difference) and two levels of Hemicell[®] preparation (0 vs. 0.05%). All diets were corn-soybean meal based. The Results of the 6 week trial is shown in Table 5. Feed conversion responded very well to the energy and enzyme variations. The Feed:Gain improved 4.6 and 5.5% in the high and low NE diets, respectively, in response to enzyme addition. The high-energy diet without β -mannanase yielded virtually identical performance to the low energy diet with the enzyme. It clearly implies a benefit of 150 kcal/kg of NE due to supplementation of β -mannanase under the test conditions.

Beside the pen trials described above, O'Quinn *et al.*, conducted a commercial grow-finish swine trial using a total of 5350 pigs in the Summer of 2001 (7). A commercial farm with 6 barns was used and each barn fitted with two feed lines to allow the control and β -mannanase treatments to grow in the same house. Sex and weight were balanced as much as practically possible. Pigs of Dekalb genetics started at approximately 18.8 kg and ended at approximately 113 kg in about 20 weeks. BMD at 30 g/ton and Paylean at 4.5 g/ton were used according to commercial feeding practice. The results are shown in Table 6. Supplementation with β -mannanase tended to reduce mortality ($P=0.09$) and the reduction was 23.7%. Similarly, cull and lightweight pigs were reduced by 59.6% ($P=0.0007$). Feeds containing β -mannanase reduced losses (cull and mortality) by 4.63% of the herd ($P=0.002$). The net result was an extra 126 pigs sent to the market. Pigs fed β -mannanase had also increased ADG (3.2%, $P=0.04$) and ADFI (4.9%, $P=0.004$). An improved feed intake is considered to be very positive during the hot summer in North Carolina. Feed conversion adjusted for mortality and cull was statistically similar ($P=0.22$). Dressing percentage tended to be increased by feeding β -mannanase ($P=0.08$).

Conclusion and Discussion:

A much larger relative performance improvement by feeding β -mannanase to the pigs under stressful conditions than in normal conditions has been demonstrated both in pen and field trials as shown here. The data also suggests that β -Mannanase is highly effective in swine diets in the absence of antibiotics and can be considered as a good substitute when antibiotics are not used.

β -Galactomannans are a group of soluble hemicelluloses found in feed mainly associated with plant proteins. β -Galactomannan content in soybean meal is about 1.2 (48% SBM) to 1.6% (44% SBM). A typical corn/soy diet at 16% CP for grower pigs contains about 20-22% SBM resulting in 0.25-0.32% β -galactomannan. Therefore, it is very difficult to explain the energy impact of 150 kcal/kg NE (Table 4) from feeding β -mannanase solely based on digestibility improvement. ChemGen Corp. has performed quite a few studies to probe the mechanism for the consistently observed response. Most of these trials used broilers challenged with *Eimeria sp.* and *Clostridium perfringenes* (1). β -Mannanase has been shown to significantly improve performance up to 10-15% and reduce mortality in disease challenged birds.

It is well documented that water-soluble β -galactomannans or its derivatives isolated from mushrooms or extracts of the plant *Aloe vera* were able to stimulate immune response when contacting cells such as macrophages (8-9). β -Galactomannan in feed, if allowed to cross the intestinal mucosa, can behave as stimulators of the innate immune system resulting in increased proliferation of macrophages and monocytes and resultant cytokine production. Immune response can reduce feed intake and nutrient utilization. Animals under stressful environments tend to have compromised intestinal integrity, for example due to protozoan parasite infections like *Eimeria*. We believe this results in easier

access of β -mannan from the intestinal lumen into mucosa and causes additional immune stress over an already heavily burden immune system. The data is consistent with a mechanism for the β -mannanase effect on stressed animals that is simply the reduction of high molecular weight β -galactomannan content in feed entering the intestinal tract. This reduction consequently reduces the unnecessary and non-productive immune stress. Mannans and its derivatives are very unique in this respect among all the fibers found in commonly used feed ingredients. This may reflect an evolutionary process that resulted in an innate defence system in vertebrates that is very reactive against high molecular weight mannans. The reason for this is that many pathogens that contain various kinds of mannan in their structure and cell surface. A few of examples include fungi, *Clostridium perfringenes*, *Mycobacterium* etc., (10-12). Therefore, β -mannanase is clearly a different kind of feed enzyme not merely improving nutrient digestion and absorption.

Table 1. Ileal digestibility of finishing pigs fed diets with and without β -mannanase

Items	Diets ¹				SEM	Probability ²
	12	12	16	16		
Crude protein, %						
β -mannanase*	-	+	-	+		
Dry Matter	74.13	75.32	75.05	77.04	.37	0.001
Ca	51.52	53.47	56.61	59.14	1.31	0.12
N	70.71	71.07	77.48	78.87	.58	0.14

1: Each mean represents 24 observations.

2: w vs. w/o β -mannanase

* Applied at 110 million units / ton of feed, ChemGen Corp.

Table 2. U.S. Trial: Effects of β -mannanase and soybean oil on growth performance of weanling pigs

Item	Treatment			SE
	Control	Soybean Oil	β -mannanase*	
ME, kcal/kg	3,297	3,396	3,295	
CP/Lysine, %	21.5/1.20	21.41/1.20	21.50/1.20	
Initial weight, kg	13.63	13.66	13.54	.07
Final weight, kg	24.77	24.99	25.00	.22
ADG, g/day	543	553	558	9.7
ADFI, g/day	955	941	938	12.3
Feed : Gain	1.761 ^b	1.701 ^a	1.681 ^a	.02

* Applied at 110 million units / ton of feed, ChemGen Corp.

^{a&b} Different superscripts mean statistically significant differences (P<0.05)

Table 3. Trial in Thailand (21-56 days): The effect of addition of β -mannanase to diet on the performance of weaning piglets.

Item	Control	β -mannanase*	Pooled SEM
ME, kcal/kg	3,250	3,250	
CP, %	20.30%	20.18%	
Initial weight, kg	6.90	6.90	
Final weight, kg	22.54	23.19	2.090
Body weight gain, kg	15.64	16.29	2.090
ADG, kg/day	0.412	0.429	0.055
ADFI, kg/day	0.583	0.587	0.018
Feed : Gain	1.47 ^a	1.34 ^b	0.010
Uniformity of body weight, %	83.45	85.24	1.573
Feed cost/kg BWG, Baht	25.374	23.485	1.734
¹ Net profit/head, Baht	563.04	586.35	75.251
Economic benefit return/head, Baht		+23.31	

¹: Average sale price is assumed to be 36 Baht/kg.

* Applied at 110 million units / ton of feed, ChemGen Corp.

^{a&b} Different superscripts mean statistically significant differences (P<0.05)

Table 4. China Trial (35 -56 days): Comparing impact of antibiotics and enzyme supplementation on weaning pigs

Items	Control	Antibiotics	Enzyme
DE, kcal/kg	3,345		
CP/Lysine, %	20.4/1.25		
β-Mannanase/xylanase*	-	-	+
Arsanilic acid	-	7 ppm	-
Colistin sulphate	-	20 ppm	-
Initial weight, kg	8.54 ± 0.04	8.95 ± 0.07	8.78 ± 0.11
Final weight, kg	17.3 ± 0.07	18.6 ± 0.14	18.0 ± 0.04
ADG, g/day	417.1 ± 11.3	459.5 ± 10.2	439.3 ± 11.2
ADFI, g/day	699.2 ± 36	675.2 ± 39	657.2 ± 41
Feed : Gain	1.67 ± 0.07 ^a	1.46 ± 0.01 ^b	1.49 ± 0.09 ^b

* Applied at 110 million units of β-Mannanase and 200 million units of xylanase / ton of feed, ChemGen Corp.

^{a&b} Different superscripts mean statistically significant differences (P<0.05)

Table 5. Korea trial (6 weeks): Effects of β-mannanase on growth performance in growing pigs¹

	High NE		Low NE			Contrast ²		
	2,250		2,100					
NE, kcal/kg	2,250		2,100					
CP/Digestible Lysine, %	16.57/0.85		16.20/0.85					
β-Mannanase	+	-	+	-	SE ³	1	2	3
ADG (g)	506	494	491	473	16	0.201	0.964	0.853
ADFI (g)	1,593	1,621	1,604	1,633	29	0.813	0.134	0.553
Feed: Gain	3.145 ^c	3.289 ^b	3.268 ^b	3.448 ^a	0.007	0.027	0.030	0.951

¹ Ninety six pigs with an average initial body weight of 34.43kg.

² Probability for contrast: 1) high NE vs. low NE; 2) w vs. w/o β-mannanase; 3) interaction.

³ Pooled standard error.

^{a, b&c} Different superscripts mean statistically significant differences (P<0.05)

Table 6: U.S. Field Trial (20 weeks): Effects of β-mannanase on grower-finishing pigs

Treatment	Control	β-mannanase*	Probability
Total pigs	2,633	2,717	
Initial weight, kg	19.18 ± 3.06	18.53 ± 1.37	0.32
Final weight, kg	111.26 ± 2.66	113.82 ± 2.43	0.06
Body weight gain, kg	92.08	95.29	
ADG, kg/day	0.694 ± 0.0136	0.717 ± 0.0227	0.04
ADFI, kg/day	1.766 ± 0.0409	1.852 ± 0.0499	0.004
Feed : Gain	2.544	2.588	0.22
% Culls	6.44 ± 1.52	2.60 ± 1.50	0.0007
% Mortality	3.34 ± 1.05	2.55 ± 0.89	0.09
% Mortality and Cull	9.78 ± 2.11	5.15 ± 2.08	0.002
Carcass wt. kg	88.298 ± 8.467	88.480 ± 8.204	0.25
% Carcass Yield	76.74 ± 0.62	77.16 ± 0.22	0.08

* Applied at 110 million units / ton of feed, ChemGen Corp.

References:

1. Jackson, M. E., Anderson, D.M., Hsiao, H.Y, Mathis, G.E., and Fodge, D.W., 2003. Beneficial Effect of β -Mannanase Feed Enzyme on Performance of Chicks Challenged with *Eimeria* sp. and *Clostridium perfringens*. *Avian Diseases* 47:759-763.
2. Petty, L.A., Carter, S.D., Senne, B.W., and Shriver, J. A., 2002. Effects of β -mannanase addition to corn-soybean meal diets on growth performance, carcass traits, and nutrient digestibility of weaning and growing-finishing pig. *Journal of Animal Science* 80:1012-1019.
3. Odetallah, N., Ferket, P.R., Grimes, J.L., and McNaughton, J.L., 2002. Effect of mannan-endo-1,4- β -mannosidase on the growth performance of turkeys fed diets containing 44 and 48% crude protein soybean meal. *Poultry Science* 81:1322-1331.
4. Jackson, M. E., D. W. Fodge, and H. Y. Hsiao., 1999. Effects of β -Mannanase in corn-soybean meal diets on laying hen performance. *Poultry Science* 78:1737-1741.
5. Robbins, B.C., Rice, J.P., Radcliffe, J.S., Pleasant, R.S., and Kornegay, E.T., 1999. The effects of Hemicell[®] on digestibilities of minerals, energy, and amino acids in pigs fitted with steered ileo-cecal valve cannulas and fed a low and high protein corn-soybean meal diet. *Journal of Animal Science* 77:197-198; Suppl. 1
6. Kim, I.H., Kim, J.H., Hong, J.W., Kwon, O.S., Min, B.J., Lee, W.B., Shon, K.S., Jackson, M.E., and Jin, F.L., 2003. Effects of β - Mannanase enzyme addition on swine performance fed low and high energy diets without antibiotics. 9th International Symposium on Digestive Physiology in Pigs. May 14-17, 2003. Banff, Alberta, Canada.
7. O'Quinn, P.R., Funderburke, D.W., Funderburke, C.L., and James, R.L., 2002. Influence of dietary supplementation with β -mannanase on performance of finishing pigs in a commercial system. *Journal of Animal Science* 80:65; Suppl. 2
8. Zhang, L., and I. R. Tizzard, 1996. Activation of a mouse macrophage cell line by acemannan: the major carbohydrate fraction from *Aloe vera* gel. *Immunopharmacology* 35: 119-128
9. Ross, S. A., C. J. G. Duncan, D. S. Pasco, and N. Pugh, 2002. Isolation of a galactomannan that enhances macrophage activation from the edible fungus *Morchella esculenta*. *J. of Agric. and Food Chem.* 50:5683-5685.

10. Jones, G. H. and C. E. Ballou, 1968. Studies on the structure of Yeast mannan. *J. Biol. Chem.* 244:043-1051

11. Cherniak, R., K. I. Dayalu, and R. G. Jones, 1983. Analysis of the common polysaccharide antigens from the cell envelope of *Clostridium perfringens* Type A. *Carbohydr. Res.* 119:171-90

12. Ludwiczak, P., T. Brando, B. Monsarrat and G. Puzo, 2001, Structural Characterization of *Mycobacterium tuberculosis* Lipoarabinomannans by the combination of capillary electrophoresis and matrix-assisted laser desorption/ionization time of flight mass spectrometry. *Anal. Chem.* 73:2323-2330