

1 **RUNNING TITLE: Effect of  $\beta$ -Mannanase**

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4 TITLE: Effects of  $\beta$ -Mannanase in Corn-Soy Diets on Commercial Leghorns in  
5 Second Cycle Hens

6 **ABSTRACT**  $\beta$ -Mannanase (Hemicell<sup>®</sup>) is a unique enzyme-based feed ingredient, which  
7 can hydrolyze  $\beta$ -mannan, an anti-nutritional fiber in feed. Because soybean meal contains  
8  $\beta$ -mannan and its derivatives, addition of  $\beta$ -mannanase may improve soybean-meal  
9 utilization. The purpose of this study was to evaluate the effect of  $\beta$ -mannanase on  
10 performance of commercial Leghorns fed corn-soybean meal based diets. In this  
11 experiment, three diets were formulated. The metabolizable energy content for diet 1  
12 (high-energy diet) was 2951 kcal/kg, which was 120 kcal/kg higher than diet 2 (low-  
13 energy diet supplemented with  $\beta$ -mannanase) and diet 3 (low-energy diet without  $\beta$ -  
14 mannanase). Hy-Line W-36 hens (n = 720, 98 wk old) were randomly divided into three  
15 dietary treatments (16 replicates of 15 hens per treatment). The trial lasted for 12 weeks.  
16 Overall average feed conversion of hens fed the low-energy diet supplemented with  $\beta$ -  
17 mannanase was similar to that of hens fed the high-energy diet, and both were  
18 significantly lower than that of hens fed the low-energy diet without  $\beta$ -mannanase. There  
19 were no significant differences in overall average egg production and egg mass among  
20 three dietary treatments for the 12-wk period. However, the addition of  $\beta$ -mannanase  
21 significantly increased average egg production and egg mass of hens fed the low-energy  
22 diet from week 5 to 8. There were no significant differences in feed intake, egg specific  
23 gravity, egg weight, mortality, body weight, and body weight variability among three  
24 dietary treatments.  $\beta$ -mannanase supplementation improved energy utilization of corn-  
25 soybean layer diets, and has the potential to reduce the cost of practical laying hen diets

26 containing  $\beta$ -mannan.

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28 *Key words:* energy,  $\beta$ -mannanase, hens, energy utilization

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## INTRODUCTION

30           The addition of feed enzymes to improve dietary nutrient utilization has become  
31 popular during the last ten years. Phytase, which can improve availability of phosphorus  
32 in feed, is commonly used in commercial poultry diets today. There are growing interests  
33 in the potential of other enzyme products to improve performance of poultry provided  
34 with corn-soybean meal based diets.

35           Hemicell<sup>®</sup> is a fermentation product of *Bacillus lentus*. Its active ingredient is  $\beta$ -  
36 mannanase, which can hydrolyze  $\beta$ -mannan in feed.  $\beta$ -Mannan in ingredients such as  
37 guar, soybean meal, and sesame meal has been known as a powerful anti-nutritional  
38 factor.  $\beta$ -Mannans are linear polysaccharides composed of repeating D-mannose units  
39 with  $\beta$ -1, 4 bonds and D-galactose units. Daskiran et al. (2004) and Ray et al. (1982)  
40 reported that  $\beta$ -mannan significantly reduced growth, and increased feed:gain ratio in  
41 broilers. Some studies have been conducted to evaluate the effect of  $\beta$ -mannanase on  
42 nutrient utilization in several monogastric species. Daskiran et al. (2004) demonstrated  
43 that  $\beta$ -mannanase improved feed: gain ratio and reduced water: feed ratio and dry fecal  
44 output of broilers by degrading the  $\beta$ -mannans. McNaughton et al. (1998) reported that  $\beta$ -  
45 mannanase improved metabolizable energy, growth, and feed conversion in broilers.  
46 Petty et al. (1999) and Odetallah et al. (2002) indicated that  $\beta$ -mannanase also improved  
47 feed efficiency of swine and turkey respectively.

48           Corn-soybean meal based diets are the most popular for laying hens in US.  
49 Because soybean meal contains  $\beta$ -mannan and its derivatives such as  $\beta$ -galacto-mannan  
50 and  $\beta$ -gluco-mannan, addition of  $\beta$ -mannanase may improve soybean-meal utilization.

51 Patel and McGinnis (1985) found that  $\beta$ -mannan significantly decreased egg production,  
52 egg weight and feed intake in laying hens.

53 Little research has been conducted to investigate the effect of  $\beta$ -mannanase on  
54 performance of laying hens fed corn-soy diet (Jackson et al., 1999). The goal of this study  
55 was to evaluate the effect of  $\beta$ -mannanase on egg production, feed intake, feed  
56 conversion, egg weight, egg specific gravity, mortality, body weight and body weight  
57 variability in commercial Leghorns.

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## MATERIAL AND METHODS

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60 Three diets were formulated for Hy-Line W-36 hens (Table 1). The metabolizable  
61 energy content of Diet 1 (high-energy diet) was 120 kcal/kg higher than that of Diet 2  
62 (low-energy diet supplemented with  $\beta$ -mannanase) and Diet 3 (low-energy diet without  
63  $\beta$ -mannanase) (Table 1).

64 In this experiment, 720 Hy-Line W-36 hens (molted at 66 wks) in the second  
65 cycle (98 wks old) were randomly divided into three treatments (16 replicates of 15 hens  
66 per treatment). Three hens were housed in a 40.6  $\times$  45.7 cm cage and five adjoining cages  
67 consisted of a group. Replicates were equally distributed into upper and lower cage level  
68 to minimize cage level effect. All hens were housed in an environmentally controlled  
69 house with temperature maintained at approximately 25.6°C (21.1°C during the night and  
70 28.9°C during the day). The house has controlled ventilation and lighting (16 hr/day), but  
71 no control on relative humidity. All hens were supplied with feed and water *ad libitum*.  
72 Feed consumption was recorded weekly, egg production was recorded daily, and egg  
73 weight and egg specific gravity were recorded bi-weekly. Egg weight and egg specific  
74 gravity were measured using all eggs produced during two consecutive days. Egg specific  
75 gravity was determined using 11 gradient saline solutions varying in specific gravity from  
76 1.060 to 1.100 with 0.005-unit increments (Holder and Bradford, 1979). Mortality was  
77 determined daily and egg production and feed consumption were adjusted to a hen-day  
78 basis. Body weight was obtained by weighing three hens per replicate at the end of the  
79 experiment. Body weight was not determined at the beginning of the experiment in an  
80 effort to minimize stress on the hens prior to the test. Live weight uniformity was  
81 measured by body weight variability (CV), which was calculated as (sample standard

82 deviation/mean)  $\times$  100. Egg mass (g egg/hen/d) and feed conversion (g feed/g egg) were  
83 calculated from egg production, egg weight and feed consumption. Feed samples were  
84 sent for  $\beta$ -mannanase enzyme activity analysis<sup>1</sup>.

85 Data were subjected to one-way ANOVA by using general linear model  
86 procedure (PROC GLM) in SAS/STAT (2000). If differences in treatment means were  
87 detected by ANOVA, Duncan's Multiple Range Test was applied to separate means.  
88 Statements of statistical significance are based on a probability of ( $P \leq 0.05$ ).

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<sup>1</sup>ChemGen Corp., Gaithersburg, MD.

## RESULTS AND DISCUSSION

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91           There were no significant differences in overall average egg production and egg  
92 mass among three dietary treatments for the 12-wk period (Table 2 and Table 3).  
93 However, average egg production and egg mass from week 5 to 8 of hens fed the low-  
94 energy diet supplemented with  $\beta$ -mannanase were similar to those of hens fed the high-  
95 energy diet, and both were significantly higher than those of hens fed the low-energy diet  
96 without  $\beta$ -mannanase (Table 2 and Table 3). These results are in agreement with those of  
97 Jackson et al. (1999) who observed a significant increase in egg production by adding  $\beta$ -  
98 mannanase to layer diets.

99           There were significant differences in overall average feed conversion (g feed/g  
100 egg) among three dietary treatments (Table 4). Overall average feed conversion of hens  
101 fed the low-energy diet supplemented with  $\beta$ -mannanase was similar to that of hens fed  
102 the high-energy diet, and both were significantly lower than that of hens fed the low-  
103 energy diet without  $\beta$ -mannanase. The addition of  $\beta$ -mannanase improved feed  
104 conversion of hens fed the low-energy diet approximate 4.2 %. This observation is  
105 consistent with those of Daskiran et al. (2004) and Jackson et al. (2004) who reported that  
106  $\beta$ -mannanase improved feed/gain ratios in broilers. No significant differences in feed  
107 intake, egg weight, egg specific gravity, mortality, body weight, and body weight  
108 variability were observed among three dietary treatments (Tables 2-5). Mean values of  
109 mortality, body weight, and body weight variability for this experiment were 1.95%, 1.69  
110 kg, and 15.05% respectively.

111           Based on the metabolizable energy content of diets, egg mass and feed intake,  
112 hens fed the low-energy diet supplemented with  $\beta$ -mannanase consumed 5.84 kcal ME to

113 produce one gram egg daily, compared to 5.93 kcal ME and 6.07 kcal ME in the high and  
114 low energy diets respectively. Hens fed the low-energy diet supplemented with  $\beta$ -  
115 mannanase had better efficiency in utilizing dietary energy. This study demonstrated that  
116 degradation of  $\beta$ -mannans resulted in more energy available for production purposes in  
117 laying hens. Similarly, Radcliffe et al. (1999) observed increased apparent energy  
118 digestibility in swine with  $\beta$ -mannanase addition to corn-soybean meal based diets.

119         Significant effects of  $\beta$ -mannanase on performance of laying hens may not be  
120 explained by simply making  $\beta$ -mannan available as an energy source. The mode of action  
121 of  $\beta$ -mannanase is complex. An improvement in energy metabolism may be anticipated  
122 with an increase in  $\beta$ -mannan digestion. Experiments have demonstrated reductions in  
123 insulin secretion associated with  $\beta$ -mannan intake in swine (Leeds et al., 1980; Sambrook  
124 and Rainbird, 1985) and in humans (Morgan et al., 1985) and reduced glucose absorption  
125 in swine (Rainbird et al., 1984).

126          $\beta$ -mannans are highly viscous and may have adverse effects on the digestive  
127 systems. Viscosity reduction has been suggested as a primary reason for improved  
128 performance with certain endolytic enzymes used in association with barley based high  
129 viscous cereals (Rotter et al., 1989, 1990). This may be a contributing factor in  
130 performance enhancement.

131         A third but important mode of action is a reduction in innate immune stimulation  
132 associated with a reduction in the  $\beta$ -mannan content of substrate entering the intestinal  
133 tract.  $\beta$ -mannans crossing the intestinal mucosa are potent stimulators of the innate  
134 immune system resulting in increased proliferation of macrophages and monocytes and  
135 resultant cytokine production. These result in exacerbated disease symptoms and reduced



136 nutrient utilization. This has been observed using galactomannans derived from fungi  
137 (Ross et al., 2002). Improved performance of laying hens with enzyme addition to diets is  
138 likely a result of a combination of the three proposed modes of action.

139         The economic benefit of  $\beta$ -mannanase is dependent on enzyme price, feed  
140 ingredient prices, and the price of eggs. For example, if poultry oil price is high,  $\beta$ -  
141 mannanase may be a good alternative for poultry oil to reduce the cost of layer diets. This  
142 research demonstrated that the addition of  $\beta$ -mannanase improved energy utilization in  
143 typical corn-soy poultry diet. More research needs to be conducted to determine the  
144 optimum  $\beta$ -mannanase level in feed for the best performance and maximum profits of  
145 laying hens.

146         In conclusion, the addition of  $\beta$ -mannanase significantly improved overall average  
147 feed conversion of hens fed the low-energy diet. There were no significant differences in  
148 overall average egg production and egg mass among three dietary treatments for the 12-  
149 wk period. However,  $\beta$ -mannanase supplementation significantly increased average egg  
150 production and egg mass of hens fed the low-energy diet from week 5 to 8.  $\beta$ -Mannanase  
151 supplementation improved energy utilization of corn-soybean layer diets, and has the  
152 potential to reduce the cost of layer diets containing  $\beta$ -mannan.

## REFERENCES

153

154 Daskiran, M., R. G. Teeter, D. W. Fodge, and H. Y. Hsiao. 2004. An evaluation of endo-  
155  $\beta$ -D-mannanase (Hemicell) effects on broiler performance and energy use in diets  
156 varying in  $\beta$ -mannan content. *Poult. Sci.* 83:662-668.

157 Jackson, M. E., D. W. Fodge, and H. Y. Hsiao. 1999. Effects of  $\beta$ -mannanase in corn-  
158 soybean meal diets on laying hen performance. *Poult. Sci.* 78:1737-1741.

159 Jackson, M.E., K. Geronian, A. Knox, J. McNab, and E. McCartney. 2004. A dose-  
160 response study with the feed enzyme  $\beta$ -mannanase in broilers provided with corn-  
161 soybean meal based diets in the absence of antibiotic growth promoters. *Poult. Sci.*  
162 83:1992-1996.

163 Holder, D. P., and M. V. Bradford. 1979. Relationship of specific gravity of chicken eggs  
164 to number of cracked eggs and percent shell. *Poult. Sci.* 58:250-251.

165 Leeds, A. R., S. S. Kang,, A. G. Low, and I. E. Sambrook. 1980. The pig as a model for  
166 studies on the mode of action of guar gum in normal and diabetic man. *Proc. Nutr.*  
167 *Soc.* 39:44.

168 McNaughton, J. L., H. Hsiao, D. Anderson, and D. W. Fodge. 1998. Corn/soy/fat diets  
169 for broilers,  $\beta$ -mannanase and improved feed conversion. *Poult. Sci.* 77(Suppl.  
170 1):153. (Abstr.)

171 Morgan, L. M., J. A. Tredger, A. Madden, P. Kwasowski, and V. Marks. 1985. The  
172 effect of guar gum on carbohydrate, fat and protein stimulated gut hormone secretion:  
173 Modification of postprandial gastric inhibitory polypeptide and gastrin responses. *Br.*  
174 *J. Nutr.* 53:467-475.

175 Odetallah, H. N., P. R. Ferket, J. L. Grimes, and J. L. McNaughton. 2002. Effect of

176 mannan-endo-1, 4-  $\beta$ -mannosidase on the growth performance of turkeys fed diets  
177 containing 44 and 48% crude protein soybean meal. *Poult. Sci.* 81:1322-1331.

178 Patel, M. B., and J. McGinnis. 1985. The effect of autoclaving and enzyme  
179 supplementation of guar meal on the performance of chicks and laying hens. *Poult.*  
180 *Sci.* 64:1148-1156.

181 Petty, L. A., S. D. Carter, B. W. Senne, and J. A. Shriver. 1999. Effects of Hemicell<sup>®</sup>  
182 addition to nursery diets on growth performance of weanling pigs. *J. Anim. Sci.*  
183 77(Suppl.):195. (Abstr.)

184 Radcliffe, J. S., B. C. Robbins, J. P. Rice, R. S. Pleasant, and E. T. Kornegay. 1999. The  
185 effects of Hemicell<sup>®</sup> on digestibilities of minerals, energy, and amino acids in pigs  
186 fitted with steered ileo-cecal cannulas and fed a low and high protein corn-soybean  
187 meal diet. *J. Anim. Sci.* 77(Suppl. 1.):197. (Abstr.)

188 Rainbird, A. L., A. G. Low, and T. Zebrowska. 1984. Effect of guar gum on glucose and  
189 water absorption from isolated loops of jejunum in conscious growing pigs. *Br. J.*  
190 *Nutr.* 52:489-498.

191 Ray, S., M. H. Pubols, and J. McGinnis. 1982. The effect of a purified guar degrading  
192 enzyme on chick growth. *Poult. Sci.* 61:488-494.

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

196 Rotter, B. A., R. R. Marquardt, W. Guenter, and G. H. Crow. 1990. Evaluation of three  
197 enzymic methods as predictors of in vivo response to enzyme supplementation of  
198 barley-based diets when fed to young chicks. *J. Sci. Food Agric.* 50: 19-27.

199 Rotter, B. A., M. Neskar, R. R. Marquardt, and W. Guenter. 1989. Effect of different  
200 enzyme preparations on the nutritional value of barley in chicken diets. *Nutr. Rep.*  
201 *Int.* 39:107-120.

202 Sambrook, I. E, and A. L. Rainbird. 1985. The effect of guar gum and level and source of  
203 dietary fat on glucose tolerance in growing pigs. *Br. J. Nutr.* 54:27-35.

204 SAS institute. 2000. *SAS/STAT User's Guide*. SAS Institute Inc., Cary, NC.

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**TABLE 1. Ingredient and nutrient content of the experimental diets**

Ingredient (%)	Experimental diets		
	1	2	3
Corn	64.43	67.82	67.87
Soybean meal (48% protein)	21.18	20.40	20.40
CaCO <sub>3</sub>	7.36	7.37	7.37
Hardshell <sup>1</sup>	2.00	2.00	2.00
Dicalcium phosphate	1.09	1.08	1.08
Poultry oil	3.00	0.35	0.35
NaCl	0.39	0.39	0.39
Vitamin Premix <sup>2</sup>	0.25	0.25	0.25
Mineral Premix <sup>3</sup>	0.25	0.25	0.25
DL-methionine	0.05	0.04	0.04
Hemicell <sup>®4</sup>	0.00	0.05	0.00
Calculated analysis			
Crude protein (%)	15.35	15.24	15.25
ME (Kcal/kg)	2951	2831	2831
Calcium (%)	4.00	4.00	4.00
aP <sup>5</sup> (%)	0.30	0.30	0.30
Methionine (%)	0.32	0.31	0.31
Methionine + cystine (%)	0.59	0.58	0.58
Lysine (%)	0.79	0.77	0.77
Analyzed value (MU <sup>6</sup> /ton of feed)			
<u><math>\beta</math>-Mannanase activity</u>	16.08	125.04	15.62

207 <sup>1</sup>Hardshell = large particle (passing US mesh #4 and retained by US mesh #6) CaCO<sub>3</sub> supplied by  
 208 Franklin Industrial Minerals, Lowell, Florida.

209 <sup>2</sup>Provided per kilogram of diet: vitamin A (as retinyl acetate), 8,000 IU; cholecalciferol, 2,200  
 210 ICU; vitamin E (as DL- $\alpha$ -tocopheryl acetate), 8 IU; vitamin B<sub>12</sub>, 0.02 mg; riboflavin, 5.5 mg; D-  
 211 calcium pantothenic acid, 13 mg; niacin, 36 mg; choline, 500 mg; folic acid, 0.5 mg; vitamin B<sub>1</sub>  
 212 (thiamin mononitrate), 1 mg; pyridoxine, 2.2 mg; biotin, 0.05 mg; vitamin K (menadione sodium  
 213 bisulfate complex), 2 mg.

214 <sup>3</sup>Provided per kilogram of diet: manganese, 65 mg; iodine, 1 mg; ferrous carbonate, 55 mg;  
 215 copper oxide, 6 mg; zinc oxide, 55 mg; sodium selenium, 0.3 mg.

216 <sup>4</sup>ChemGen Corp., Gaithersburg, MD 20877, recommended addition rate = 0.05%.

217 <sup>5</sup>aP = available phosphorus.

218 <sup>6</sup>MU = 10<sup>6</sup>  $\beta$ -mannanase enzyme activity unit determined by ChemGen Corp.

219 **TABLE 2. Effect of  $\beta$ -mannanase on egg production and feed intake**

Dietary treatment		Egg production (%)				Feed intake (g feed/hen/d)			
ME level	Enzyme	1-4 wk	5-8 wk	9-12 wk	Overall	1-4 wk	5-8 wk	9-12 wk	Overall
High	-	73.68	72.31 <sup>a</sup>	70.45	72.14	100.19	97.76	92.86	96.94
Low	+	74.05	72.11 <sup>a</sup>	68.78	71.65	100.02	98.64	94.35	97.63
Low	-	73.23	68.36 <sup>b</sup>	66.73	69.44	101.22	99.55	95.57	98.78
	SEM	1.22	1.01	1.28	1.06	0.76	0.83	0.96	0.78
	Probability	0.8936	0.0197	0.1301	0.1728	0.6986	0.5662	0.3847	0.5028

220 <sup>a-b</sup> Values of the same column with no common superscripts are significantly different ( $P \leq 0.05$ ).

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222 **TABLE 3. Effect of  $\beta$ -mannanase on egg weight and egg mass**

Dietary treatment		Egg weight (g)				Egg mass (g egg/hen/d)			
ME level	Enzyme	1-4 wk	5-8 wk	9-12 wk	Overall	1-4 wk	5-8 wk	9-12 wk	Overall
High	-	66.97	66.76	66.90	66.88	49.35	48.29 <sup>a</sup>	47.14	48.26
Low	+	66.26	66.09	65.85	66.07	48.94	47.66 <sup>a</sup>	45.31	47.30
Low	-	66.71	66.32	66.31	66.45	48.82	45.32 <sup>b</sup>	44.20	46.11
	SEM	0.34	0.32	0.31	0.30	0.88	0.76	0.85	0.74
	Probability	0.3468	0.3265	0.0700	0.1736	0.9049	0.0198	0.0566	0.1313

223 <sup>a-b</sup> Values of the same column with no common superscripts are significantly different ( $P \leq 0.05$ ).

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225 **TABLE 4. Effect of  $\beta$ -mannanase on feed conversion and egg specific gravity**

Dietary treatment		Feed conversion (g feed/g egg)				Egg specific gravity (unit)			
ME level	Enzyme	1-4 wk	5-8 wk	9-12 wk	Overall	1-4 wk	5-8 wk	9-12 wk	Overall
High	-	2.04	2.03 <sup>a</sup>	1.98 <sup>a</sup>	2.01 <sup>a</sup>	1.0718 <sup>a</sup>	1.0702	1.0751	1.0724
Low	+	2.03	2.07 <sup>a</sup>	2.08 <sup>b</sup>	2.06 <sup>a</sup>	1.0699 <sup>b</sup>	1.0702	1.0750	1.0717
Low	-	2.08	2.20 <sup>b</sup>	2.17 <sup>b</sup>	2.15 <sup>b</sup>	1.0704 <sup>b</sup>	1.0694	1.0745	1.0715
SEM		0.020	0.016	0.022	0.016	0.0001	0.0001	0.0001	0.0001
Probability		0.4413	0.0001	0.0013	0.0015	0.0001	0.2007	0.5929	0.0868

226 <sup>a-b</sup> Values of the same column with no common superscripts are significantly different ( $P \leq 0.05$ ).

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