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Effect of dietary β-mannanase on productive performance, egg quality, and utilization of dietary energy and nutrients in aged laying hens raised under hot climatic conditions

Moon Chan Kim[1], Jong Hyuk Kim[1], Franco Martinez Pitargue[1], Do Yoon Koo[1], Hyeon Seok Choi[1], and Dong Yong Kil[1,*]

* **Corresponding Author:** Dong Yong Kil
Tel: +82-31-670-3028, **Fax:** +82-31-676-2196,
E-mail: dongyong@cau.ac.kr

[1] Department of Animal Science and Technology, Chung-Ang University, Anseong 17546, Korea

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Objective: The objective of this experiment was to investigate the effect of dietary β-mannanase on productive performance, egg quality, and utilization of dietary energy and nutrients in aged laying hens raised under hot climatic conditions.
Methods: A total of 320 84-wk-old Hy-line Brown aged laying hens were allotted to one of four treatments with eight replicates in a completely randomized design. Two dietary treatments with high energy (HE; 2,800 kcal/kg nitrogen-corrected apparent metabolizable energy [AME_n]) and low energy (LE; 2,700 kcal/kg AME_n) were formulated. Two additional diets were prepared by adding 0.04% (MN4) or 0.08% β-mannanase (MN8) to LE treatment diets. The feeding trial was conducted for 28 d, covering a period from July to August in South Korea. The average daily room temperature and relative humidity were 29.2°C and 83%, respectively.
Results: Productive performance, egg quality, and cloacal temperature were not influenced by dietary treatments. The measured AME_n values for MN8 diets were similar to those for HE diets, which were greater ($p<0.05$) than those for LE and MN4 diets. However, the AME_n values for MN8 diets did not differ from those for LE and MN4 diets.
Conclusion: The addition of β-mannanase to low energy diets increases energy values for diets fed to aged laying hens. However, this increase has little positive impacts on performance and egg quality. These results indicate that dietary β-mannanase does not mitigate the heat stress of aged laying hens raised under hot climatic conditions.

Keywords: Aged Laying Hen; β-Mannanase; Egg Quality; Hot Climatic Condition; Performance

INTRODUCTION

High environmental temperature and humidity lead to an adverse effect on livestock production and health, with poultry being the most sensitive of livestock animals to the heat stress owing to low ability to dissipate body heat load [1]. Thus, a variety of strategies related to nutrition and management for mitigating heat stress in poultry has been studied and practiced [1-3]. Identification and reduction of dietary components associated with increasing heat production may be one possible procedure to decrease heat stress in poultry.

Dietary nonstarch polysaccharides (NSPs) are recognized to be the major components showing various anti-nutritional actions such as decreased nutrient digestion and absorption in the small intestine [4]. This decreased utilization of nutrients in the small intestine is also related to increased fermentation in the lower part of the gastrointestinal tract (GIT) of poultry [5]. Choct et al [6] reported that high intake of soluble NSPs greatly increased the small intestinal fermentation of broiler chickens. Thus, it is expected that decreased intake and/or increased utilization of NSPs in the GIT may decrease heat production of chickens because fermentative heat production

is decreased. This expectation is supported by Nian et al [7] who observed a significant decrease in total heat production of chickens fed diets containing NSPs-degrading enzymes compared with those fed diets containing no enzymes. Thus, it may be speculated that dietary NSPs-degrading enzymes have a beneficial effect on poultry production at heat stress conditions because NSP-degrading enzymes decreases fermentative heat production. However, limited poultry experiments have been performed in this regard.

One of the main NSPs in poultry diets is β-mannan, which accounts for 15% to 37% of total contents of NSPs in diets [8]. Dietary β-mannanase, which is an exogenous enzyme that hydrolyzes β-mannan, has been reported to improve poultry productivity by increasing energy and nutrient utilization via decreasing anti-nutritional effects of β-mannan including increased digesta viscosity [9-11]. These positive effects of dietary β-mannanase may also result in decreased heat loads of poultry by lowering fermentative heat production in the GIT, which lead to a decrease in heat stress if the poultry is raised under hot climatic conditions. In addition, it was reported that aged laying hens are likely more susceptible to heat stress than younger laying hens [12]. However, to our knowledge, no experiments have been performed to investigate the efficacy of dietary β-mannanase for aged laying hens raised under hot climate conditions.

The objective of the present experiment, therefore, was to investigate the effect of dietary β-mannanase on productive performance, egg quality, and utilization of dietary energy and nutrients in aged laying hens raised under hot climatic conditions.

MATERIALS AND METHODS

Animals and experimental design

All experimental procedures were reviewed and approved by the Animal Care and the Use Committee at Chung-Ang University. A total of 320 84-wk-old Hy-line Brown aged laying hens were allotted to one of four dietary treatments with eight replicates in a completely randomized design. Each replicate consisted of five consecutive cages and two hens per each cage were placed together in a conventional layer facility. A space allowance of 500 cm^2 per hen was provided. Two dietary treatments with high energy (HE; 2,800 kcal/kg nitrogen-corrected apparent metabolizable energy [AME_n]) and low energy (LE; 2,700 kcal/kg AME_n) were formulated mainly with corn, soybean meal, wheat, and corn distillers dried grains with solubles (Table 1). The differences in energy levels of those diets were achieved primarily by different inclusion levels of tallow. The diets were prepared as a mash form. Two additional diets were prepared by adding 0.04% (MN4) or 0.08% β-mannanase (MN8; CTC bio Inc., Seoul, South Korea) to LE treatment diets at the expense of cornstarch. The declared activity of β-mannanase used in this experiment was 800,000 unit/kg. All diets contained 1.0% celite as an indigestible marker to increase the concentrations of acid-insoluble ash (AIA) for the determination of nutrient and energy utilization in the diets. All nutrients were included to meet or exceed nutrient recommendation of NRC [13] for laying hens. Hens were allowed free access to feed and water throughout the experiment.

Table 1. Composition and nutrient content of experimental diets (as-fed basis)

Items (%, unless noted)	High energy diet (HE)	Low energy diet (LE)
Ingredients (%)		
Corn	53.42	56.46
Soybean meal, 46% CP	19.36	18.52
Wheat	5.00	5.00
Dried distillers grains with solubles	5.00	5.00
Tallow	2.58	0.40
Mono-dicalcium phosphate	1.05	1.04
Limestone	11.56	11.56
DL-methionine	0.13	0.12
Sodium chloride	0.20	0.20
Mineral premix[1)]	0.10	0.10
Vitamin premix[2)]	0.20	0.20
Sodium bicarbonate	0.10	0.10
Cornstarch	0.20	0.20
Celite	1.00	1.00
Ethoxyquin	0.10	0.10
Total	100.00	100.00
Nutrient content[3)]		
AMEn (kcal/kg)	2,800	2,700
CP	14.22	14.90
Lysine	0.75	0.74
Methionine+cysteine	0.67	0.65
Crude fiber	2.85	2.87
NDF	8.51	8.72
Calcium	4.50	4.50
Non-phytate phosphorus	0.34	0.34

CP, crude protein; AME_n, nitrogen-corrected apparent metabolizable energy; NDF, neutral detergent fiber.

[1)] Provided per kilogram of the complete diet: Zn (as ZnO), 60 mg; Mn (as $MnSO_2 \cdot H_2O$), 50 mg; Fe (as $FeSO_4 \cdot 7H2_0$), 50 mg; Cu (as $CuSO_4 \cdot 5H_2O$), 6 mg; Co (as $CoCO_3$), 250 μg; I (as $Ca(IO_3)_2 \cdot H_2O$), 1 mg; Se (as Na_2SeO_3), 150 μg.

[2)] Provided per kilogram of the complete diet: vitamin A (from vitamin A acetate), 12,500 IU; vitamin D_3, 2,500 IU; vitamin E (from DL-α-tocopheryl acetate), 20 IU; vitamin K_3, 2 mg; vitamin B_1, 2 mg; vitamin B_2, 5 mg; vitamin B_6, 3 mg; vitamin B_{12}, 18 μg; calcium pantothenate, 8 mg; folic acid, 1 mg; biotin, 50 μg; niacin, 24 mg.

[3)] Calculated values [13].

The experiment lasted for 28 d (from 84 to 87 wk of age) during the hot seasons, covering a period from the middle of July to the middle of August in South Korea. The layer facility had a mechanical ventilation system to control room temperature and air quality, but the minimal ventilation was performed during the experiment because this experiment was designed to increase room temperature as simulated in commercial layer farms during the hot season. Room temperature and relative humidity were recorded at three times daily using an electronic data recorder. The average daily room temperature was 29.2°C with average highest temperatures of 30.9°C and average lowest temperature of 27.5°C during the experiment. The average relative humidity was 83%. Hens were provided with 16 h of light and 8 h of dark-

ness daily.

Data collection and chemical analysis

Detailed procedures for data collection of productive performance and egg quality have been demonstrated previously [14,15]. In short, laying performance including hen-d egg production, egg weight, egg mass, and soft and broken egg production was recorded daily, whereas feed intake and feed conversion ratio (FCR) were recorded weekly. The data for laying performance were summarized for overall experiment. The body weight (BW) of individual hens was measured at the start and the end of the experiment. The cloacal temperature was determined from four birds per replicate at 2nd wk and 4th wk of the experiment with a digital thermometer, which was inserted 3 cm into the cloaca.

Egg quality was measured with four eggs randomly collected from each replicate at the end of the experiment [15]. Eggshell color was determined using the eggshell color fan (Samyangsa, Kangwon-do, Republic of Korea; 15 = very dark brown and 1 = very light and pale). Egg yolk color was estimated by the Roche color fan (Hoffman-La Roche Ltd., Basel, Switzerland; 15 = dark orange and 1 = light pale). Eggshell strength was determined using a Texture analyzer (model TAHDi 500, Stable Micro System, Godalming, UK) and was displayed as the unit of compression force per unit eggshell surface area. Eggshell thickness was measured at three different regions (top, middle, and bottom) using a dial pipe gauge (model 7360, Mitutoyo Co., Ltd., Kawasaki, Japan). Haugh unit (HU) values were measured using a micrometer (model S-8400, B.C. Ames Co., Waltham, MA, USA), and the HU values were calculated from the egg weight and albumen height as described by Eisen et al [16].

To measure utilization of energy and nutrients in diets, excreta samples from four birds per replicate were collected for 3 d after the completion of the experiment. Collected excreta were pooled and stored at –20°C before analysis. Excreta samples were dried in a force-air drying oven at 60°C for 72 h and finely ground for subsequent analysis.

The diets and excreta samples were analyzed for gross energy using bomb calorimeter (Parr Instrument, Moline, IL, USA), dry matter (DM, method 934.01), ash (method 942.05), and nitrogen (N, method 990.93) using standard procedures of AOAC [17]. The diets and excreta samples were also analyzed for AIA in triplicate according to the method of Vogtmann et al [18]. Apparent total tract retention (ATTR) of nutrients (DM, ash, and N) was calculated using the indicator method described by Kong and Adeola [19]. The AME_n values for all diets also were determined [20].

Statistical analysis

All data were analyzed by an analysis of variance in a completely randomized design using the PROC MIXED procedure (SAS Institute Inc., Cary, NC, USA). The replicate was considered an experimental unit in all data analyses (n = 8). Outlier data were examined using the UNIVARIATE procedure of SAS; however, no outliers were identified. The LSMEANS procedure was used to calculate treatment means and the PDIFF option of SAS was used to separate the means if the difference was significant. Significance for statistical tests was set at $p<0.05$.

RESULTS

Productive performance, including final BW, hen-day egg production, egg weight, egg mass, feed intake, FCR, and soft-broken egg production, of aged laying hens raised under hot climatic conditions was not affected by dietary treatments (Table 2). Egg quality such as egg yolk color, eggshell color, eggshell strength, and HU values also was not influenced by dietary treatments (Table 3). However, eggshell thickness for hens fed MN4 diets was greater ($p<0.05$) than for hens fed other diets. The cloacal temperature of hens measured at 2nd wk and 4th wk of the experiment did not differ among dietary treatments (Table 4).

The ATTR values for DM, ash, and N in diets were not influenced by dietary treatments (Table 5). The measured AME_n

Table 2. Effect of dietary β-mannanase on productive performance of aged laying hens raised under hot climatic conditions[1)]

Items	Dietary treatments[2)]				SEM	p-value
	HE	LE	MN4	MN8		
Initial body weight (kg)	2.30	2.26	2.27	2.30	0.029	0.714
Final body weight (kg)	2.58	2.50	2.50	2.56	0.028	0.100
Body weight gain (kg)	0.28	0.24	0.23	0.26	0.032	0.582
Hen-day egg production (%)	75.9	77.3	78.2	78.4	1.66	0.710
Egg weight (g)	66	66	67	66	0.6	0.094
Egg mass (g)	50.1	50.6	52.7	51.4	1.05	0.358
Feed intake (g/hen/d)	109	108	112	112	1.8	0.222
Feed conversion ratio (g/g)	2.18	2.13	2.14	2.19	0.047	0.782
Soft and broken egg production (%)	1.3	1.8	1.4	1.3	0.04	0.824

SEM, standard error of the mean; AME_n, nitrogen-corrected apparent metabolizable energy.
1) Data are least squares means of eight replicates per treatment.
2) HE, high energy diet (2,800 kcal AME_n/kg); LE, low energy diet (2,700 kcal AME_n/kg); MN4, LE+0.04% β-mannanase; MN8, LE+0.08% β-mannanase.

Table 3. Effect of dietary β-mannanase on egg quality of aged laying hens raised under hot climatic conditions[1)]

Items	Dietary treatments[2)]				SEM	p-value
	HE	LE	MN4	MN8		
Egg yolk color (Roche color fan)	6.7	6.6	6.4	5.8	0.24	0.065
Eggshell color (Color fan)	12.8	12.0	12.8	12.8	0.30	0.174
Eggshell strength (kg/cm^2)	2.59	2.57	2.86	2.63	0.14	0.399
Eggshell thickness (μm)	404.8^b	394.2^b	419.5^a	401.8^b	5.00	0.011
Haugh unit	83.7	80.9	83.6	87.1	2.31	0.315

SEM, standard error of the mean; AME$_n$, nitrogen-corrected apparent metabolizable energy.
[1)] Data are least squares means of eight replicates per treatment. Four eggs per replicate were analyzed.
[2)] HE, high energy diet (2,800 kcal AME$_n$/kg); LE, low energy diet (2,700 kcal AME$_n$/kg); MN4, LE+0.04% β-mannanase; MN8, LE+0.08% β-mannanase.
[a,b] Means in the same row with different superscripts are different ($p < 0.05$).

Table 4. Effect of dietary β-mannanase on cloacal temperature of aged laying hens raised under hot climatic conditions[1)]

Items	Dietary treatments[2)]				SEM	p-value
	HE	LE	MN4	MN8		
Cloacal temperature (°C)						
2 wk	40.8	40.8	40.8	40.7	0.04	0.181
4 wk	40.8	40.7	41.0	40.8	0.13	0.435

SEM, standard error of the mean; AME$_n$, nitrogen-corrected apparent metabolizable energy.
[1)] Data are least squares means of eight replicates per treatment. Four hens per replicate were measured.
[2)] HE, high energy diet (2,800 kcal AME$_n$/kg); LE, low energy diet (2,700 kcal AME$_n$/kg); MN4, LE+0.04% β-mannanase; MN8, LE+0.08% β-mannanase.

Table 5. Effect of dietary β-mannanase on ATTR of nutrients and AMEn of diets fed to aged laying hens raised under hot climatic conditions[1)]

Items	Dietary treatments[2)]				SEM	p-value
	HE	LE	MN4	MN8		
ATTR of nutrients						
DM (%)	73.3	73.8	74.2	75.3	0.70	0.251
Ash (%)	31.2	27.1	24.1	29.9	3.12	0.399
N (%)	55.7	56.6	56.7	59.8	2.05	0.521
AME$_n$ (kcal/kg)	2,874^a	2,779^b	2,798^b	2,822^{ab}	0.09	0.022

ATTR, apparent total tract retention; AME$_n$, nitrogen-corrected apparent metabolizable energy; SEM, standard error of the mean; DM, dry matter; N, nitrogen.
[1)] Data are least squares means of eight replicates per treatment. Four hens per replicate were measured.
[2)] HE, high energy diet (2,800 kcal AME$_n$/kg); LE, low energy diet (2,700 kcal AME$_n$/kg); MN4, LE+0.04% β-mannanase; MN8, LE+0.08% β-mannanase.
[a,b] Means in the same row with different superscripts are different ($p < 0.05$).

values for HE diets were greater ($p<0.05$) than those for LE and MN4 diets. However, the measured AME$_n$ values for MN8 diets did not differ from those for other 3 diets.

DISCUSSION

Previous experiments reported that a variety of dietary NSPs-degrading enzymes increases nutrient digestion and absorption of poultry [21-23]. Similarly, dietary β-mannanase has been known to increase nutrient digestion and utilization in the small intestine of animals, and thus decrease the amounts of fermentable nutrients reaching the hindgut of animals [24-26]. Based on these previous experiments, we hypothesized that dietary β-mannanase may ameliorate heat stress in laying hens raised under hot climatic conditions because decreased fermentation and its heat production in the GIT could decrease heat loads in hens' body. Moreover, it has been postulated that laying hens may be more susceptible to heat stress as they become aged because of increased fermentative capacity (e.g., increased size of hindgut) and increased insulation due to increasing body fat content and heavier feather covers with age [12,27]. Therefore, it was expected that dietary β-mannanase may be effective in decreasing heat production from the fermentation of aged laying hens, which subsequently improves productive performance and egg quality of aged laying hens raised under hot climatic conditions.

In the current experiment, however, cloacal temperature was not affected by dietary β-mannanase, and the measured temperature was in the normal range of body temperature for laying hens, indicating that hindgut fermentation and its heat production of aged laying hens may not be affected by dietary β-mannanase. Subsequently, productive performance and egg quality were not affected by dietary treatments. A possible reason for this observation may be due to the relatively small proportion of heat production from the GIT fermentation in the total heat production of laying hens [5]. Thus, although it was not measured in this experiment, the extent of possible reduction in fermentative heat production as a result of dietary β-mannanase may not be considerable. In addition, the relatively short period (i.e., 4 weeks) of feeding low energy diets containing β-mannanase to hens in this experiment may be another possible reason why no significant changes in productive performance and egg quality were observed. Wu et al [28] reported that feeding low energy diets containing β-mannanase had no effects on productive performance during the first 4 weeks of feeding periods, but improved productive performance during the following 5 to 8 weeks of feeding periods. However, they also found inconsistent results for productive performance during the last 9 to 12 weeks and 0 to 12 weeks of feeding periods. Further research regarding the effect of various feeding period of dietary β-mannanase in laying

hens is required.

Unexpectedly, eggshell thickness was greater for hens fed low energy diets containing 0.04% β-mannanase (MN4) than for hens fed other diets. Likewise, the eggshell strength also was numerically greatest for hens fed MN4 diets among dietary treatments despite no significant differences. The reason for this improvement in eggshell hardness is unclear because a greater addition of β-mannanase (i.e., MN8 treatment diets) to low energy diets did not improve eggshell thickness and strength compared with other diets.

The ATTR values for DM, ash, and N in high energy diets were not different from those observed in low energy diets. In addition, the ATTR values for DM, ash, and N were not affected by addition of 0.4% or 0.8% β-mannanase to low energy diets. Previous experiments have reported that addition of β-mannanase to diets improved energy and nutrient utilization in broiler chickens [9,25,29] and pigs [26]. The potential mode of action for this improvement has been related to decreased anti-nutritional effects of β-mannan as well as prebiotic effects of mannan-oligosaccharides as a degraded form of β-mannan [9,26,30]. Therefore, we expected that feeding diets containing β-mannanase to aged laying hens may also improve nutrient utilization; however, we failed to detect significant improvement although an insignificant increase in the ATTR values for DM and N was observed by adding β-mannanase to diets. There is a lack of previous experiments regarding the effect of dietary β-mannanase on nutrient utilization in adult poultry such as aged laying hens. Therefore, the results are difficult to explain, but we speculate that the efficacy of dietary β-mannanase is likely influenced by the age of poultry. Further research regarding the efficacy of dietary β-mannanase in different ages of poultry is required.

The measured AME_n values for HE and LE diets (2,874 and 2,779 kcal/kg, respectively) were similar to their formulated AME_n values (2,800 and 2,700 kcal/kg, respectively). The difference between AME_n values for HE and LE treatment diets was approximately 95 kcal/kg, which was close to the difference (i.e., 100 kcal/kg) as we intended in the diet formulation. This result confirmed the credibility of our measurements of nutrient utilization and energy values in the current experiment.

Increasing addition of β-mannanase to LE treatment diets increased AME_n values for low energy diets, and the values for diets containing 0.08% β-mannanase were similar to high energy diets, indicating that dietary β-mannanase can increase energy utilization, and thus, show an energy-saving effect. Similar increases in the AME values by dietary β-mannanase have also been reported in broiler chickens [25]. In the current experiment, however, this increase in the AME_n values did not lead to a positive effect on productive performance and egg quality of aged laying hens raised under hot climatic conditions. Similar results were observed by Wu et al [28] who reported that feeding low energy diets containing 0.05% β-mannanase to aged laying hens (98 wk of age) had no positive effects on productive performance during the 12-week of feeding trial. Therefore, the addition of β-mannanase to low energy diets increases energy values for diets fed to aged laying hens; however, this positive effect does not result in significant benefits on productive performance and egg quality of aged laying hens raised under hot climatic conditions. This observation may be due to the fact that the energy requirements of aged laying hens raised under hot climatic conditions were increased, but the increase in the energy supply by dietary β-mannanase did not satisfy the energy requirements.

In conclusion, the addition of β-mannanase to low energy diets increases energy values for diets fed to aged laying hens. However, this increase has little positive impacts on productive performance and egg quality. These results indicate that dietary β-mannanase does not mitigate the heat stress of aged laying hens raised under hot climatic conditions.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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