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Original Article

# *Effects of β-mannanase on Egg Production Performance, Egg Quality, Intestinal Microbiota, Viscosity, and Ammonia Concentration in Laying Hens*

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## ABSTRACT

Two experiments were conducted to evaluate the effects of β-mannanase on egg production performance, egg quality, intestinal microbiota, viscosity, and ammonia concentration in laying hens. In Exp. 1, two hundred and seventy 30-wk-old Hy-Line Brown laying hens were assigned to 6 diets arranged in a 3 × 2 factorial of three levels of MEn and CP [(a corn-soybean meal based diet (HEHP), a diet containing 50 kcal of MEn/kg and 1.0% less energy and CP than the HEHP (MEMP), and a diet containing 80 kcal of MEn/kg and 1.5% less energy and CP than HEHP (LELP)], and β-mannanase supplementation (0 or 0.04%). In Exp. 2, A total of two hundred and sixteen62-wk-old Hy-Line Brown laying hens were assigned to 6 dietary treatments in a 3 × 2 factorial arrangement. In Exp. 1, β-mannanase supplementation increased egg production rate in hens fed LELP diet, but not in those fed HEHP or MEMP diet (interaction, $p<0.01$), and the interaction was significant ($p<0.01$) for egg mass. β-mannanase supplementation decreased ($p<0.05$) ammonia concentration. In Exp. 2, the supplementation of β-mannanase increased egg production rate and egg mass in hens fed LELP diet, whereas no differences were found in those fed HEHP or MELP diet (interaction, $p<0.01$). The supplementation of β-mannanase in a lower energy and protein diet resulted in similar production performance when compared to high-energy and high-protein diet during early and late stages of egg production.

## INTRODUCTION

In monogastric animal diets, exogenous enzymes have been used to reduce anti-nutritive factors such as non-starch polysaccharides (NSP), the polysaccharide present in the common corn-soybean meal (SBM) diets. β-mannans, one of the NSP, are mainly found in the hull and fiber fractions of SBM and make up to 1.3 or 1.6% in the dehulled or nondehulled SBM, respectively (Hsiao *et al.*, 2006). The water-soluble structure of NSP increases digesta viscosity (Lee *et al.*, 2003), which may limit the access of digestive enzymes to their substrates (Dale, 1997) and therefore decreases nutrients digestibility (Smits *et al.*, 1997). Additionally, highly viscous NSP present in feed may negatively alter microbiota profiles by providing substrates for the fermentation of potentially pathogenic bacteria in the hindgut, such as *Escherichia coli* and *Clostridium spp.* (Hopwood *et al.*, 2002). The use of NSP enzymes can minimize the quantity of undigested NSP that reaches the hindgut and limit proliferation of potential pathogenic microbes (O'Neill *et al.*, 2014).

β-mannanase supplementation has shown to increase AMEn and has beneficial effects on growth performance when added into poultry diets (Kong *et al.*, 2011; Kim *et al.*, 2017). In laying hens, egg

production of hens fed a reduced energy diet was similar ompared to an energy sufficient diet when β-mannanase was added into the diet (Jackson *et al*., 1999). Latham *et al*. (2016), studying the efficacy of β-mannanase in broiler diet, found that the addition of this enzyme in diets with reduced metabolizable energy and crude protein promoted similar growth performance to those fed a diet with adequate energy and nutrient levels. However, there is limited information on how β-mannanase would affect intestinal microbiota and viscosity in laying hens fed reducedenergy and crude protein diets. Therefore, the objective of this study was to evaluate the effects of β-mannanase on egg production performance, intestinal microbiota, and viscosity of laying hens when added into corn-SBM based diets with different levels of energy and crude protein during early or late egg production period.

## MATERIALS AND METHODS

The experimental protocol was approved by the Institutional Animal Care and Use Committee at Konkuk University (KU13188). β-mannanase (CTCZYME®, 800,000 U/kg) were provided by CTCBIO Inc. (Seoul, Republic of Korea).

### Experiment 1

#### *Animals and experimental design*

A total of two hundred and seventy30-wk-old Hy-Line Brown laying hens were used in this study. Five replicates of 9 hens each (3 adjacent cages containing 3 hens/ 45 × 62 × 66 cm cage) were randomly allotted to 6 dietary treatments based on 3 × 2 factorial arrangement. The three diets containing different levels of energy and CP were made as follows: HEHP, a corn-soybean meal based diet that met or exceeded NRC (1994) nutrient requirements containing 2,810 kcal of MEn and 18.0% CP per kg feed; MEMP, a diet that contained lower energy and CP levels than HEHP with 2,760 kcal of MEn and 17.0% CP per kg feed; LELP, a diet that contained lower energy and CP levels than MEMP with 2,730 kcal of MEn and 16.5% CP per kg feed. Each of the three diets was supplemented with either 0 or 0.04% β-mannanase to make 6 dietary treatments (Table 1). The experiment lasted 7 wks. A room temperature of 25 ± 3 °C and a photoperiod of 16 L: 8 D were maintained throughout the experimental period. Hens were given *ad libitum* access to water and diets.

**Table 1 –** Ingredients and chemical composition of the experimental diets (Exp. 1)[1].

| | Treatments | | |
|---|---|---|---|
| | HEHP | MEMP | LELP |
| Ingredients, % | | | |
| Yellow corn, ground | 55.47 | 55.95 | 57.01 |
| Soybean meal (44%) | 22.21 | 20.21 | 19.50 |
| Wheat bran | 3.64 | 6.52 | 7.24 |
| Corn gluten meal | 5.00 | 4.23 | 3.65 |
| Tallow | 2.08 | 1.50 | 1.00 |
| DL-Met | 0.07 | 0.05 | 0.06 |
| Choline chloride (50%) | 0.13 | 0.13 | 0.13 |
| Dicalcium phosphate | 1.37 | 1.33 | 1.31 |
| Limestone | 9.31 | 9.35 | 9.36 |
| Salt | 0.33 | 0.33 | 0.33 |
| Vitamin mixture[2] | 0.18 | 0.18 | 0.18 |
| Mineral mixture[3] | 0.22 | 0.22 | 0.22 |
| Total | 100.00 | 100.00 | 100.00 |
| Calculated values | | | |
| CP, % | 18.00 | 17.00 | 16.50 |
| MEn, kcal/kg | 2,810 | 2,760 | 2,730 |
| Met + Cys, % | 0.72 | 0.67 | 0.65 |
| Lys, % | 0.85 | 0.80 | 0.78 |
| Ca, % | 3.90 | 3.90 | 3.90 |
| Avail. P, % | 0.35 | 0.35 | 0.35 |

[1]HEHP, a diet that met NRC (1994) nutrient requirements for laying hens over 18 wks old containing 2,810 kcal of MEn and 18.00% CP per kg; MEMP, a diet that contained lower energy and CP levels than HEHP with 2,760 kcal of MEn and 17.00% CP per kg; LELP, a diet that contained lower energy and CP levels than HEHP and MEMP with 2,730 kcal of MEn and 16.50% CP per kg.

[2]Vitamin mixture provided the following nutrients per kg: vitamin A, 40,000,000 IU; vitamin D3, 8,000,000 IU; vitamin E, 10,000 IU; vitamin K3, 4,000 mg; vitamin $B_1$, 4,000 mg; vitamin $B_2$, 12,000 mg; vitamin $B_6$, 6,000 mg; vitamin $B_{12}$, 20,000μg; pantothenic acid, 20,000 mg; folic acid, 2,000 mg; nicotinic acid, 60,000 mg.

[3]Mineral mixture provided the following nutrients per kg: Fe, 30,000 mg; Zn, 25,000 mg; Mn, 20,000 mg; Co, 150 mg; Cu, 5,000 mg; Ca, 250 mg; Se, 100 mg.

#### *Sample collection*

Feed intake was recorded weekly by replicate. Eggs were collected daily, and egg production and egg mass were determined weekly. The mean egg weight was measured on a weekly basis and the quality of the eggs were measured biweekly according to the method described by Zheng *et al.* (2012).

At the end of the experiment, 10 birds per treatment were slaughtered using carbon dioxide gas. Ammonia concentration of the cecal digesta was measured according to Lee *et al*. (2010) using an ammonia assay kit (Product codeAA0100, Sigma).

### Experiment 2

#### *Animals and experimental design*

A total of two hundred and sixteen 62-wk-old Hy-Line Brown laying hens were used in this study. Four replicates of 9 hens each (3 adjacent cages containing 3 hens/ 45 × 62 × 66 cm cage) were randomly allotted

to 6 dietary treatments based on 3 × 2 factorial arrangement. The three diets containing different levels of energy and CP were made as follows: HEHP, a corn-soybean meal based diet that met or exceeded NRC (1994) nutrient requirements containing 2,770 kcal of MEn and 16.0% CP per kg feed; MELP, a diet that contained lower energy and CP levels than HEHP with 2,700 kcal of MEn and 15.0% CP per kg feed; LELP, a diet that contained lower energy than MELP with 2,650 kcal of MEn and 15.0% CP per kg feed. Each of the three diets was supplemented with either 0 or 0.04% β-mannanase to make 6 dietary treatments (Table 5). The experiment lasted 7 wks. A room temperature of 25 ± 3 °C and a photoperiod of 16 L: 8 D were maintained throughout the experimental period. Hens were given *ad libitum* access to water and diets.

### *Sample collection*

Feed intake was recorded weekly by replicate. Egg production rate and egg mass were recorded on a daily basis and determined on a replicate basis throughout the experiment. The mean egg weight and egg qualities were measured with the same method as shown in Exp. 1.

At the end of the experiment, 10 birds per treatment were slaughtered using carbon dioxide gas. Cecal microbiota was measured according to the method described by Zheng *et al*. (2012).

Ileal digesta were collected to measure viscosity according to the method described by Lee *et al.* (2010). Ammonia concentration of the cecal digesta was measured according to Lee *et al.* (2010) using an ammonia assay kit (Product code AA0100, Sigma).

## Statistical analysis

Data were analyzed as a complete randomized design by two-way ANOVA using the GLM procedure of SAS (SAS Inc., 2002) to determine effects of energy and protein levels, and enzyme, and interactions. The cage lot (3 adjacent cages) was considered as an experimental unit. Differences were declared significant when $p<0.05$ or highly significant at $p<0.01$, and trends were noted when $0.05 \leq p<0.10$. When significant interactions occurred, means among treatments were compared in a pairwise manner using the probability of differences (PDIFF) option of SAS.

# RESULTS

## Experiment 1

### *Egg production*

During the whole experimental period, no interactions between β-mannanase and energy and protein levels were observed for feed intake; however, there was an increase ($p<0.05$) in feed intake with β-mannanase supplementation (Table 2). An interaction between β-mannanase and energy and protein levels was observed for egg production rate ($p<0.01$). Supplementation of β-mannanase increased ($p<0.05$) egg production rate in laying hens when added into LELP diet, but did not affect that when supplemented into HEHP or MEMP diet. The interaction between β-mannanase and energy and protein levels tended to be significant ($p=0.09$) for egg weight. β-mannanase and the diet effects were shown in egg weight, respectively ($p<0.01$; $p<0.01$). An interaction between the enzyme and the energy and protein levels was found for egg mass ($p<0.01$). Supplementation of β-mannanase increased ($p<0.01$) egg mass in hens fed HEHP or LELP diet but did not affect that when fed MEMP diet. The egg mass of hens fed HEHP and MEMP diets were higher ($p<0.01$) than those fed LELP diet when fed without β-mannanase.

### *Egg quality*

No differences or interactions were observed for egg quality including eggshell color, eggshell thickness, eggshell strength, egg yolk color, or Haugh Unit (Table 3).

### *Ammonia concentration*

No interactions between β-mannanase and energy and protein levels were observed for ammonia concentration; however, there was a decrease ($p<0.05$) in ammonia concentration with β-mannanase supplementation (Table 4).

**Table 2 –** Egg production performance of laying hens fed diets with three levels of energy and crude protein and β-mannanase from 32 to 39 weeks of age (Exp.1)[1].

| Item | Diet: | HEHP | | MEMP | | LELP | | | *p*-value | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | β-mannanase: | - | + | - | + | - | + | SEM | Diet | Enzyme | Interaction |
| Feed intake, g/d/bird | | 120.9 | 123.7 | 120.0 | 123.2 | 121.2 | 126.3 | 2.79 | 0.80 | 0.03 | 0.62 |
| Egg production, % | | 95.55$^{a}$ | 93.53$^{ab}$ | 91.67$^{b}$ | 92.93$^{ab}$ | 90.60$^{bc}$ | 95.04$^{a}$ | 0.42 | 0.21 | < 0.01 | < 0.01 |
| Egg weight, g/egg | | 64.08 | 65.07 | 64.33 | 64.09 | 63.28 | 63.90 | 0.28 | < 0.01 | < 0.01 | 0.09 |
| Egg mass, g/d/bird | | 59.35$^{b}$ | 60.83$^{a}$ | 58.99$^{b}$ | 59.58$^{b}$ | 57.35$^{c}$ | 60.74$^{a}$ | 0.25 | < 0.01 | < 0.01 | < 0.01 |

[1]Values are presented as the least square of means of 5 replicates (each replicate was composed of 3 cages, 3 hens/cage).

**Table 3 –** Egg quality of laying hens fed diets with three levels of energy and crude protein and β-mannanase from 32 to 39 weeks of age (Exp.1) [1].

| Item | Diet: | HEHP | | MEMP | | LELP | | | *p*-value | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | β-mannanase: | - | + | - | + | - | + | SEM | Diet | Enzyme | Interaction |
| Eggshell color, unit | | 25.14 | 25.08 | 25.64 | 26.59 | 25.01 | 26.15 | 0.81 | 0.47 | 0.31 | 0.73 |
| Eggshell thickness, 0.01mm | | 35.80 | 35.27 | 34.96 | 35.32 | 35.26 | 35.24 | 0.30 | 0.42 | 0.81 | 0.35 |
| Eggshell strength, kg/cm$^2$ | | 3.36 | 3.50 | 3.54 | 3.34 | 3.38 | 3.30 | 0.08 | 0.40 | 0.45 | 0.12 |
| Egg yolk color, Roche yolk color fan | | 7.38 | 7.33 | 7.38 | 7.29 | 7.26 | 7.26 | 0.09 | 0.57 | 0.52 | 0.88 |
| Haugh Unit | | 94.21 | 96.11 | 95.50 | 93.63 | 95.25 | 95.68 | 1.17 | 0.74 | 0.87 | 0.29 |

[1]Values are presented as the least square of means of 5 replicates (each replicate was composed of 3 cages, 3 hens/cage).

**Table 4 –** Cecalmicrobiota of laying hens fed diets with three levels of energy and crude protein and β-mannanase from 32 to 39 weeks of age (Exp.1)[1].

| Item | Diet: | HEHP | | MEMP | | LELP | | | *p*-value | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | β-mannanase: | - | + | - | + | - | + | SEM | Diet | Enzyme | Interaction |
| Total microbes, log10 cfu/g$^2$ | | 5.08 | 5.05 | 5.06 | 4.98 | 5.33 | 5.11 | 0.17 | 0.48 | 0.44 | 0.86 |
| Lactic acid bacteria, log10 cfu/g | | 7.16$^{ab}$ | 7.15$^{ab}$ | 7.17$^{ab}$ | 7.23$^{a}$ | 7.37$^{a}$ | 6.89$^{b}$ | 0.08 | 0.05 | < 0.01 | < 0.01 |
| Coliformbacteria, log10 cfu/g | | 5.86 | 5.65 | 5.72 | 5.35 | 5.77 | 5.04 | 0.12 | 0.02 | < 0.01 | 0.09 |
| Ammonia concentration, ug/mL | | 1.49 | 1.16 | 1.38 | 1.33 | 1.56 | 1.08 | 0.10 | 0.93 | < 0.01 | 0.12 |

[1]Values are presented as the least square of means of 5 replicates (each replicate was composed of 3 cages, 3 hens/cage).

**Table 5 –** Ingredients and chemical composition of the experimental diets (Exp. 2)[1].

| | Treatment | | |
|---|---|---|---|
| | HEHP | MELP | LELP |
| Ingredients, % | | | |
| Yellow corn, ground | 59.14 | 57.58 | 55.17 |
| Soybean meal (44%) | 18.93 | 17.15 | 16.68 |
| Wheat bran | 6.32 | 10.00 | 10.00 |
| Rice bran | 0.00 | 0.95 | 4.22 |
| Corn gluten meal | 3.19 | 1.98 | 1.90 |
| Tallow | 1.00 | 0.80 | 0.50 |
| DL-Met | 0.04 | 0.04 | 0.04 |
| Choline chloride (50%) | 0.09 | 0.09 | 0.09 |
| Dicalcium phosphate | 1.06 | 0.98 | 0.95 |
| Limestone | 9.53 | 9.73 | 9.75 |
| Salt | 0.30 | 0.30 | 0.30 |
| Vitamin mixture[2] | 0.18 | 0.18 | 0.18 |
| Mineral mixture[3] | 0.22 | 0.22 | 0.22 |
| Total | 100.00 | 100.00 | 100.00 |
| Calculated values | | | |
| CP, % | 16.00 | 15.00 | 15.00 |
| MEn, kcal/kg | 2,770 | 2,700 | 2,650 |
| Met + Cys, % | 0.62 | 0.59 | 0.59 |
| Lys, % | 0.76 | 0.72 | 0.72 |
| Ca, % | 3.90 | 3.95 | 3.95 |
| Avail. P, % | 0.30 | 0.30 | 0.30 |

[1]HEHP, a diet that met or exceeded NRC (1994) nutrient requirements containing 2,770 kcal of MEn and 16.00% CP per kg; MELP, a diet that contained lower energy and CP levels than HEHP with 2,700 kcal of MEn and 15.00% CP per kg; LELP, a diet that contained lower energy than MELP with 2,650 kcal of MEn and 15.00% CP per kg.

[2]Vitamin mixture provided the following nutrients per kg: vitamin A, 40,000,000 IU; vitamin D3, 8,000,000 IU; vitamin E, 10,000 IU; vitamin K3, 4,000 mg; vitamin $B_1$, 4,000 mg; vitamin $B_2$, 12,000 mg; vitamin $B_6$, 6,000 mg; vitamin $B_{12}$, 20,000μg; pantothenic acid, 20,000 mg; folic acid, 2,000 mg; nicotinic acid, 60,000 mg.

[3]Mineral mixture provided the following nutrients per kg: Fe, 30,000 mg; Zn, 25,000 mg; Mn, 20,000 mg; Co, 150 mg; Cu, 5,000 mg; Ca, 250 mg; Se, 100 mg.

## Experiment 2

### *Egg production*

No differences or interactions were observed for feed intake (Table 6). An interaction between β-mannanase and energy and protein levels was observed for egg production rate ($p$<0.01). Supplementation of β-mannanase increased ($p$<0.01) egg production rate in laying hens when added into LELP diet, whereas no differences were found when supplemented into HEHP or MELP diet. An interaction between β-mannanase and energy and protein levels was observed for egg weight ($p$<0.01) and egg mass ($p$<0.01), respectively. Supplementation of β-mannanase increased ($p$<0.01) egg mass of laying hens when added into LELP diet but did not affect that when added into HEHP or MELP diet. β-mannanase supplementation increased ($p$<0.01) egg mass.

### *Egg quality*

No differences or interactions were observed for eggshell color, eggshell thickness, egg yolk color, or Haugh Unit (Table 7); however, there was a trend for decreased ($p$=0.09) eggshell strength by feeding LELP diet compared to feeding HEHP or MELP diets. The Haugh Unit was lower ($p$<0.05) when feeding HEHP or LELP diet than feeding MELP diet.

### *Cecal microbiota, ammonia concentration, and viscosity of ileal digesta*

The interaction between β-mannanase and energy and protein levels tended to be significant ($p$=0.07)

**Table 6 –** Egg production performance of laying hens fed diets with three levels of energy and crude protein and β-mannanase from 62 to 69 weeks of age (Exp. 2)[1].

| Item | Diet: | HEHP | | MELP | | LELP | | | *p*-value | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | β-mannanase: | - | + | - | + | - | + | SEM | Diet | Enzyme | Interaction |
| Feed intake, g/d/bird | | 126.96 | 125.43 | 131.70 | 123.59 | 122.69 | 121.51 | 4.30 | 0.43 | 0.32 | 0.67 |
| Egg production, % | | 83.62$^{ab}$ | 81.95$^{ab}$ | 80.11$^{b}$ | 82.29$^{ab}$ | 73.56$^{c}$ | 86.39$^{a}$ | 1.39 | 0.16 | < 0.01 | < 0.01 |
| Egg weight, g/egg | | 68.82$^{ab}$ | 68.24$^{b}$ | 68.20$^{b}$ | 68.80$^{ab}$ | 68.37$^{ab}$ | 69.08$^{a}$ | 0.18 | 0.39 | 0.10 | < 0.01 |
| Egg mass, g/d/bird | | 57.56$^{ab}$ | 55.92$^{ab}$ | 54.63$^{b}$ | 56.62$^{ab}$ | 50.31$^{c}$ | 59.68$^{a}$ | 0.96 | 0.21 | < 0.01 | < 0.01 |

[1]Values are presented as the least square of means of 4 replicates (each replicate was composed of 3 cages, 3 hens/cage).

**Table 7 –** Egg quality of laying hens fed diets with three levels of energy and crude protein and β-mannanase from 62 to 69 weeks of age (Exp. 2)[1].

| Item | Diet: | HEHP | | MELP | | LELP | | | p-value | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | β-mannanase: | - | + | - | + | - | + | SEM | Diet | Enzyme | Interaction |
| Eggshell color, unit | | 24.21 | 24.33 | 24.85 | 24.90 | 24.63 | 24.05 | 0.41 | 0.97 | 0.56 | 0.17 |
| Eggshell thickness, 0.01mm | | 35.81 | 35.15 | 34.84 | 35.61 | 35.07 | 34.28 | 0.41 | 0.16 | 0.50 | 0.14 |
| Eggshell strength, kg/cm$^2$ | | 2.80 | 2.85 | 2.99 | 2.98 | 2.84 | 2.78 | 0.08 | 0.09 | 0.95 | 0.82 |
| Egg yolk color, Roche yolk color fan | | 6.85 | 6.73 | 6.77 | 6.94 | 7.09 | 6.81 | 0.15 | 0.58 | 0.55 | 0.35 |
| Haugh Unit | | 83.69 | 86.40 | 88.96 | 90.90 | 88.89 | 87.88 | 1.46 | 0.01 | 0.32 | 0.42 |

[1]Values are presented as the least square of means of 4 replicates (each replicate was composed of 3 cages, 3 hens/cage).

for cecal lactic acid bacteria (Table 8). No differences or interactions were observed for total microbes or coliform bacteria. In addition no differences or interactions were observed for ammonia concentration and viscosity in the ileal digesta.

# DISCUSSION

## Egg production

Results of the current study demonstrate the beneficial effects of the use of β-mannanase on egg production and egg mass in the early and late egg production. It is interesting to note that the egg production and egg mass of hens fed LELP diets supplemented with β-mannanase were similar to those of hens fed HEHP diets without β-mannanase. Similar results were found in a previous study, egg production of hens fed diets with a 99 kcal lower energy levels that supplemented β-mannanase was similar with those fed diets containing sufficient energy levels (Jackson *et al*., 1999). The study showed that β-mannanase can increase egg production, whereas no interactions between the enzyme and energy levels for egg production were found. In a study with broilers, where the birds were fed diets containing 97 kcal lower energy levels than a control diet,with the supplementation of β-mannanase, the growth performance was similar to those fed a control diet (Latham *et al*., 2016). β-mannanase (endo-1,4-β-mannanase) used in the present study cleaves randomly within the β-D-1,4 mannopyranoside linkages, and is speculated to produce mannan-oligosaccharides and a small amount of mannose when added into diets (McCleary, 1988; Dhawan & Kaur, 2007).In the previous studies evaluating the effects of β-mannanase on energy utilization showed it can increase energy values of the diets when fed to laying hens(Kim *et al*., 2017)and broiler chickens(Kong *et al*., 2011). The release of the mannose in the small intestine by the addition of β-mannanase would lead to the increase of the ME of the diet which then can result in decreases in feed intake. However, in the present study,

**Table 8 –** Cecalmicrobiota, ammonia concentration and viscosity of ileal diesta in laying hens fed diets with three levels of energy and crude protein and β-mannanase from 62 to 69 weeks of age (Exp. 2)[1].

| Item | Diet: | HEHP | | MELP | | LELP | | | p-value | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | β-mannanase: | - | + | - | + | - | + | SEM | Diet | Enzyme | Interaction |
| Total microbes, log10 cfu/g | | 6.88 | 6.48 | 5.61 | 6.72 | 6.16 | 6.29 | 0.47 | 0.51 | 0.48 | 0.29 |
| Lactic acid bacteria, log10 cfu/g | | 7.72 | 7.43 | 7.38 | 7.36 | 6.95 | 7.69 | 0.21 | 0.46 | 0.41 | 0.07 |
| Coliformbacteria, log10 cfu/g | | 3.92 | 4.26 | 2.92 | 3.54 | 4.29 | 3.28 | 0.40 | 0.12 | 0.96 | 0.12 |
| Ammonia concentration, ug/mL | | 3.52 | 4.10 | 3.83 | 3.23 | 3.11 | 3.14 | 0.54 | 0.46 | 0.99 | 0.56 |
| Viscosity, mPas | | 4.14 | 3.64 | 3.86 | 3.59 | 3.57 | 3.50 | 0.33 | 0.81 | 0.17 | 0.95 |

[1]Values are presented as the least square of means of 4 replicates (each replicate was composed of 3 cages, 3 hens/cage).

β-mannanase supplementation showed to increase feed intake in the early phase of egg production. It can be speculated that the improved energy utilization by the β-mannanase supplementation might partially improve the production performance; however, it cannot fully explain the beneficial effects shown in the present study. The addition of β-mannanase has shown to lead to the improvement in the digestibility of nutrients such as crude fiber (Li *et al*., 2010), digestibility of amino acids (Mussini *et al*., 2011), and phosphorus digestibility (Lv *et al*., 2013). The beneficial effects showed on egg production might be related to the positive effects on the nutrient absorption, but further research is needed to elucidate it.

### Cecal microbiota, ammonia concentration, and viscosity of ileal digesta

Excessive NSPs presented in the diet may lead to the growth of potential harmful intestinal microbiota (Choct *et al*., 2010). Mannan-oligosaccharides have been shown to improve growth performance by reducing potential pathogen populations in the large intestine (Mourão et al., 2006). They reduce the colonization of pathogens (Spring *et al*., 2000), maintain epithelial integrity (Baurhoo *et al*., 2007), and enhance immune function (Davis *et al*., 2004). Additionally, the reduction of the intestinal viscosity is the main purpose of the use of exogenous enzymes and it showed to be linked with the enhanced intestinal ecosystem (Adeola & Cowieson, 2011). The intestinal microbiota and viscosity were measured to establish a possible mechanism of promoting egg production. These may partially explain the positive effects on egg production shown in the present study. In the present study, supplementation of β-mannanase tended to increase cecal lactic acid bacteria in hens fed LELP, but not in those fed HEHP or MELP diet. The alteration in the composition of gut microbiota in the large intestine might have reduced the pathogenic bacterial attachment to the enterocytes (Spring *et al*., 2000) which can enhance mucosal immune system and production performance. No differences were observed in the viscosity of ileal digesta in the present study. The inconsistent results may be related to the diet composition and age of birds. Furthermore, ammonia concentration decreased with β-mannanase supplementation in the ceca in the Exp. 1.β-mannanase supplementation reduced excreted nitrogen and increased amino acid digestibility when fed to broiler chickens (Ferreira *et al*., 2016). The reduction in ammonia concentration shown in the present study might be related to improvement in crude protein digestibility.

In conclusion, supplementation of β-mannanase in a low-energy and low-protein diet, laying hens were able to produce similar production performance when compared to a high-energy and high-protein diet during early and late egg production. Supplementation of β-mannanase can increase lactic acid bacteria population, but further research is needed to elucidate it.

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