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Research Article
Effect of α-galactosidase Supplementation in Diet on Egg Production, Egg Quality and Dietary Digestibility of Laying Hens

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Abstract

Background and Objective: The carbohydrates in soybean meal consist of approximately 10% oligosaccharides and are linked by an α-1,6 galactosidase bond which cannot be broken down in the small intestine of monogastric animals due to the absence of endogenous α-1,6 galactosidase. This experiment was conducted to determine the effect of α-galactosidase supplementation in the laying hen diet on egg performance, egg quality and dietary digestibility. **Methodology:** A total of 576 Lohmann brown-classic hens (28 weeks old) were divided into 3 groups of 8 replications with 24 hens each. This experiment design was completely randomized design and there were 3 experimental diets, (1) Positive control diet, (2) Positive control diet decreased by 88 kcal kg^{-1} ME and supplemented with α-galactosidase at 0.022 and (3) Positive control decreased by 88 kcal kg^{-1} ME without any supplementation of α-galactosidase (negative control). **Results:** At the end of the 16 weeks of feeding trial, using α-galactosidase significantly improved egg production ($p<0.05$), egg mass ($p<0.05$), feed conversion ratio (FCR, $p<0.01$) and feed cost per egg ($p<0.05$), similar to the positive control. Feeding with α-galactosidase significantly improved the specific gravity ($p<0.01$), shell thickness ($p<0.05$), eggshell weight (<0.05) and tended to improve dietary digestibility ($p<0.10$) and decrease retention time in the gastro-intestinal tract ($p<0.05$). In addition, the apparent metabolizable energy (AME) increased ($p<0.01$) by α-galactosidase when compared to the negative control. However, the dietary treatment did not influence the other parameters of egg quality and feces score. **Conclusion:** Supplementation of α-galactosidase with reducing the metabolizable energy improved egg production performance, egg quality and feed digestibility.

Key words: α-galactosidase, metabolizable energy, egg production, egg quality, dietary digestibility

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Soybean (*Glycine max* L.) meal (SBM) is widely used as a high-quality protein source in most swine and poultry-producing countries and its gross energy (GE) is about 4,100 kcal kg^{-1} and metabolizable energy (ME) is about 2,100 kcal kg^{-1}, thus, about 2,000 kcal kg^{-1} is unusable because it cannot be digested and utilized[1]. The carbohydrates in SBM consist of approximately 10% oligosaccharides, galacto-oligosaccharides (5% sucrose, 4% stachyose and 1% raffinose) and 25% non-starch polysaccharides consisting of 8% cellulose and 17% pectin polysaccharides[2]. Each molecule of sucrose, raffinose, stachyose and verbascose is linked by an α-1,6 galactosidase bond and this bond cannot be broken down in the small intestine of monogastric animals because of the absence of endogenous α-1,6 galactosidase[3,4].

Grieshop *et al.*[5] reported that free sucrose, raffinose, stachyose and verbascose were present in SBM as 65.8, 11.8, 49.8 and 2.2 g kg^{-1} dry meter (DM), respectively. The 10% galacto-oligosaccharide equates to 129.6 g kg^{-1} DM which can be converted into 518.4 kcal kg^{-1} SBM. Indigestible galacto-oligosaccharides may decrease energy utilization, decrease the digestion and absorption of dietary nutrients, increase the digesta passage rate and increase viscosity[6].

In previous research, Scheideler and Webber[7] studied the role of α-galactosidase in improving egg production and the ME of the diet in laying hens without any effect on egg quality. Similarly, Perryman *et al.*[8] showed that ultra-low oligosaccharide SBM improved the FCR and increased the AME in broilers. Ao *et al.*[9] showed that supplementation with α-galactosidase could increase the AME in a corn-soybean diet, which was in agreement with the results of Waldroup *et al.*[10], who reported that the ME of SBM was improved by 10% with α-galactosidase addition because this enzyme can break down the α-galactosidic bond in SBM and the animals were then able to absorb more nutrients into their bodies[11]. Therefore, the objectives of this experiment were to evaluate the effect of alpha-galactosidase supplementation in the diet on egg production, egg quality and dietary digestibility of laying hens.

MATERIALS AND METHODS

Animal management: In total, 576 Lohmann brown-classic hens were used. At age of 27 weeks, the hens were divided into 24 units, with each group consisting of 24 hens. At age of 28 weeks, all 24 units were randomly divided into 3 groups, with each group consisting of 8 replicates. During the 16 weeks of the experiment, an evaporative cooling system was used to control air ventilation and temperature. The hens were housed in wire cages with 4 birds per cage. The lighting program was set of 16 h. Feed was offered twice daily as *ad libitum* and water were provided using water nipples.

Experimental diets: The exogenous enzyme used in this experiment was α-galactosidase (commercial preparations from AGal-Pro 280P). The activity of the enzyme was 176 IU kg^{-1}. The experimental diets were: (1) A positive control diet formulated to meet the nutrient standards for ME and amino acid in leading recommended guidelines (2,750 kcal kg^{-1}), (2) A positive control diet decreased by 88 kcal kg^{-1} ME and supplemented with α-galactosidase (0.022%) and (3) A positive control diet decreased by 88 kcal kg^{-1} ME without any supplemental α-galactosidase (negative control). Inert filler (corn starch) was added as needed to adjust the differences in the quantity of added enzyme. The composition of the diets is shown in Table 1 and 2.

Table 1: Feed ingredients of experimental diets

Ingredients	Control	α-galactosidase	Negative control
Corn	57.20	59.25	59.25
Rice bran oil	2.21	0.53	0.53
Rice solvent bran	4.00	4.00	4.00
Soybean meal (48%)	24.36	23.99	23.99
DL-methionine	0.12	0.12	0.12
Monocalcium phosphate (22%)	1.55	1.54	1.54
Calcium carbonate	8.91	8.92	8.92
Salt	0.25	0.25	0.25
Premix*	0.50	0.50	0.50
Choline chloride (60%)	0.08	0.08	0.08
Corn starch	0.02	-	0.02
α-galactosidase	-	0.02	-
Sodium bicarbonate	0.80	0.80	0.80
Total	100.00	100.00	100.00
Cost kg^{-1} (THB)	14.37	13.89	13.83

*Premix: Consisted of vitamin A: 5.0 MIU, D3: MIU, E: 4,000 IU, K3: 0.6 g, B1: 0.8 g, B6: 1.2 g, B12: 0.0025 g, Nicotinic acid: 5.00 g, Pantothenic acid: 3.76 g, Folic acid: 0.2 g, Biotin: 0.036 g, Mn: 24.00 g, Zn: 20.00 g, Fe: 16.00 g, Cu: 4.00 g, iodine: 0.8 g, Co: 0.08 g, Se: 0.04 g and carrier added to 1.00 kg premix

Table 2: Chemical composition of experimental diets

Ingredients	Control	α-galactosidase	Negative control
ME* for poultry (kcal kg^{-1})	2,750.00	2,750.00**	2,662.00
Crude protein (%)	17.00	17.00	17.00
Crude fat (%)	4.53	2.94	2.94
Crude fiber (%)	3.38	3.41	3.41
Calcium (%)	3.73	3.73	3.73
Available phosphorus (%)	0.38	0.38	0.38
Salt (%)	0.25	0.25	0.25
Lysine (%)	0.87	0.87	0.87
Methionine+cystine (%)	0.68	0.68	0.68
Methionine (%)	0.39	0.39	0.39
Threonine (%)	0.63	0.62	0.62
Tryptophan (%)	0.19	0.19	0.19

*ME: Metabolizable energy, **α-galactosidase at 0.02 g equated to 88 kcal kg^{-1} ME

Parameters recorded

Egg production: At 2 weekly intervals, egg production (%), mean egg weight, feed consumption, egg mass (g/hen/day), feed conversion ratio (FCR) (kg feed kg^{-1} egg mass), feed cost per egg and mortality (%) were recorded.

Egg quality: At the end of the 2 weekly intervals, all eggs from each experimental unit were weighed and 4 eggs from each replication having a weight close to the replications mean were chosen for egg quality analysis of: Specific gravity, shell thickness (mm), albumen (mm), albumen (g), yolk (g), eggshell (g), albumen (%), yolk (%), eggshell (%), albumen:yolk ratio, Haugh unit and yolk color. Measurement procedures are detailed below.

The specific gravity of the whole egg was measured by immersing eggs in salt solutions of different specific gravities to see at what concentration of solution, eggs floated.

Following the steps of Robert and Ball[12], shell weight was measured by breaking an egg, carefully rinsing the pieces of shell, drying them and then measuring the shell weight. The shell weight was then calculated as a proportion of egg weight to give the percentage of shell. Mitutoyo Model 2109-10 Dial Comparator Gauge, mounted on a frame, was used to measure shell thickness.

The albumen was measured from the height of the albumen at 1 cm distance from the edge of the yolk, following cracking the egg on a plate. The albumen height was converted to Haugh units, based on the calculation of Haugh[12]. Yolk and eggshell weight was measured using a suitable balance. The albumen weight was determined as the whole egg weight minus the yolk weight and the egg shell weight.

Feed digestibility: Organic matter digestibility was calculated using the formula of Maynard and Loosli[13], with acid insoluble ash (AIA) as the dietary marker, based on the following equation:

$$\text{Organic matter digestibility} = 100 - \frac{(\%\,\text{marker in diet} \times \%\,\text{organic matter in feces})}{(\%\,\text{marker in feces} \times \%\,\text{organic matter in diet})} \times 100$$

The retention time was measured at the end of the trial period. All birds were fed with 0.5% chromic oxide in their diets as an external marker. When the feces turned green, this was recorded as the beginning time. When the feces turned back to their ordinary color, this was recorded as the finishing time.

Feces scores were divided into 5 grades based on the stability characteristics of the feces. Grade 5 described the highest rated solids, down to grade 1 being the lowest stability. There were 4 contributors scoring at the same time[14].

Apparent metabolizable energy: The apparent metabolizable energy (AME) was determined using the equation of Scott and Boldaji[15], using AIA as the dietary and digestive marker, based on the following equation:

$$\text{Apparent metabolizable energy} = GE_{diet} - \left[GE_{excreta/digesta} \times \left(\frac{\text{marker}_{diet}}{\text{marker}_{excreta/digesta}} \right) \right]$$

Statistical analyses: Data were evaluated using one-way ANOVA in a completely randomized design. Differences of means among treatments were tested for significance using Duncan's multiple range test at the 5% significance level[16].

RESULTS AND DISCUSSION

Egg production: The effect of α-galactosidase supplementation in the experimental diets on egg production shown in Table 3. Feeding the α-galactosidase diet significantly improved the egg production (%) ($p<0.05$), egg mass ($p<0.05$), FCR ($p<0.01$) and feed cost per egg ($p<0.05$) compared to hens fed the negative control diet. There was no significant effect on egg weight, feed intake and mortality. Egg weight was not affected, but egg mass was attached and this improved the percentage egg production and this improvement led to a decrease in the FCR compared with the negative control. The cost saving could be substantial with the addition of α-galactosidase enzyme (480 THB t^{-1} feed), with equal performance to the positive control. The galacto-oligosaccharides in SBM cannot be digested by monogastric animals, but this could provide digestible nutrients and animal energy. This was in agreement with Scheideler and Weber[7], who found that egg production was enhanced ($p<0.05$) by feeding with α-galactosidase. Hens fed the negative control diet had lower egg production compared to the positive control.

The egg weight, feed intake and mortality were not significantly affected by α-galactosidase treatments. Kidd *et al.*[17] reported reduced mortality during heat stress in birds fed an enzyme with α-galactosidase activity.

Egg quality: The effects of α-galactosidase supplementation in the experimental diets on egg quality are shown in Table 4. Supplementation with α-galactosidase diet significantly

Table 3: Effect of α-galactosidase supplementation on egg performance of laying hens

Items	Control	α-galactosidase	Negative control	p-value*	SEM**
Egg production (%)	90.83±1.31[a]	90.57±1.59[a]	88.69±1.96[b]	0.03	0.38
Egg weight (g)	61.27±0.97	61.44±0.71	61.12±0.88	0.76	0.17
Egg mass	55.66±0.93[a]	55.66±1.28[a]	54.21±1.22[b]	0.03	0.27
Feed intake (g)	115.49±3.07	116.99±2.58	116.81±2.05	0.47	0.53
FCR	2.08±0.03[b]	2.10±0.03[b]	2.16±0.03[a]	<0.01	0.01
Feed cost per egg (THB kg^{-1} egg)	29.87±0.44[a]	29.23±0.39[b]	29.86±0.49[a]	0.01	0.10
Egg/dozen	21.95±0.60	21.54±0.40	21.89±0.44	0.22	0.10
Mortality	3.65±4.69	5.73±7.02	2.60±3.10	0.48	1.05

*[a,b]Treatment means with different superscripts in the same row are significantly different (p<0.05), values reported represent the Mean±SD, **Standard error of mean (SEM) represented the spread that the mean of 'a' that was idea of the accuracy of the mean

Table 4: Effect of α-galactosidase supplementation on egg quality of laying hens

Items	Control	α-galactosidase	Negative control	p-value*	SEM**
Specific gravity	1.094±0.00[a]	1.095±0.00[a]	1.093±0.00[b]	0.01	0.00
Shell thickness (mm)	0.411±0.02[a]	0.408±0.01[a]	0.392±0.02[b]	0.05	0.00
Albumen (mm)	8.02±0.31	8.26±0.25	8.28±0.28	0.15	0.06
Albumen (g)	40.24±0.90	40.54±0.74	40.34±0.83	0.76	0.16
Yolk (g)	14.96±0.51	14.80±0.32	14.62±0.46	0.33	0.09
Eggshell (g)	5.95±0.14[b]	6.09±0.11[a]	5.98±0.09[ab]	0.05	0.03
Albumen (%)	65.82±0.69	66.00±0.40	66.20±0.60	0.44	0.12
Yolk (%)	24.45±0.60	24.08±0.35	23.98±0.54	0.17	0.11
Eggshell (%)	9.73±0.25	9.92±0.21	9.82±0.19	0.26	0.05
Albumen:Yolk ratio	2.70±0.09	2.75±0.05	2.77±0.09	0.22	0.02
Haugh unit	89.03±1.74	90.28±1.42	90.48±1.48	0.15	0.33
Yolk color	5.78±0.24	5.93±0.11	5.79±0.19	0.24	0.04

*[a,b]Treatment means with different superscripts in the same row are significantly different (p<0.05), values reported represent the Mean±SD, **Standard error of mean (SEM) represented the spread that the mean of 'a' that was idea of the accuracy of the mean

Table 5: Effect of α-galactosidase supplementation on dietary digestibility of laying hens

Items	Control	α-galactosidase	Negative control	p-value*	SEM**
Digestibility (%)	78.55±7.51[a]	73.43±3.75[ab]	62.72±18.12[b]	0.08	3.01
ME (kcal kg^{-1})	2854.66±72.56[A]	2722.63±60.11[B]	2595.34±178.33[C]	<0.01	27.57
Retention time (min)	171.50±2.74[a]	168.00±1.10[b]	169.67±1.21[ab]	0.02	0.54
Feces score	3.27±0.43	3.39±0.46	3.30±0.39	0.87	0.10

*[a,b]Treatment means with different superscripts in the same row are significantly different (p<0.05) and [A,B,C] was highly significant different (p<0.01), values reported represent the Mean±SD, **Standard error of mean (SEM) represented the spread that the mean of 'a' that was idea of the accuracy of the mean

improved the specific gravity (p<0.01), shell thickness (mm) and eggshell weight (p<0.05) compared to birds fed the negative control diet. There were no significant differences for the other parameters (p>0.05). Shell thickness, specific gravity and eggshell weight have been found to be related to the egg shell strength and eggshell mass quality[12]. The strength of eggshell and the eggshell mass were better and the breakage of egg bubbles was more difficult when α-galactosidase was included in the diet[18].

Feed digestibility: The effects of α-galactosidase supplementation in the experimental diets on feed digestibility are shown in Table 5. Feeding with α-galactosidase was not significantly different (p>0.05) but tended to improve feed digestibility compared to the positive control (p<0.10). The advantage provided by the α-galactosidase enzyme could be the hydrolysis of raffinose to glucose and galactose which are more accessible to digestive enzymes and allow more rapid diffusion of absorbable nutrients to intestinal mucosa[11].

After the decrease of 88 kcal kg^{-1} ME in the diet and supplementation with α-galactosidase, the ME was highly significant (p<0.01) when compared with other treatments and also increased the ME to meet the standards for metabolizable energy of birds. Therefore, α-galactosidase could release energy to birds for maintaining their life and improved the efficiency of animal production. Knudsen[19] reported that there were 63.8 g kg^{-1} of oligosaccharides that could not be digested but supplemental α-galactosidase enabled down these oligosaccharide resulting in an additional 255.2 kcal kg^{-1} ME for birds to maintain their life. Knap *et al.*[20] reported that supplemental α-galactosidase resulted in a verifiable and significant improvement in energy bioavailability of broilers.

Feeding with α-galactosidase significantly decreased the retention time in the GI tract ($p<0.01$). After the enzyme facilitated greater access for substrate digestion, the digesta passage rate would be decreased[11]. Therefore, animals could absorb more nutrients. In addition, Zhang *et al.*[21] found that feeding with α-galactosidase significantly reduced the chyme viscosity of the ileum.

There were no significant differences in the feces scores among treatments ($p>0.05$). The reason for this was not clear, but the solid feces could reduce the problem of ammonia concentration in the evaporation house.

The benefits of using α-galactosidase in the diet were to (1) Increase metabolizable energy that provided more energy for maintaining bird growth and egg production, (2) Decrease retention time, so the birds could absorb more nutrients and to (3) Reduce feed costs.

CONCLUSION

This study supported the hypothesis that supplementation with α-galactosidase improved the egg production, egg quality, dietary digestibility and decreased the retention time for soybean meal. The cost saving could be substantial with the addition of α-galactosidase enzyme, while maintain comparable equal performance to the positive control.

SIGNIFICANCE STATEMENT

This study discovered that α-galactosidase supplementation in the diet can be beneficial for increasing the metabolizable energy and saving on cost. This study will help researchers to uncover critical areas of egg production, egg quality and decreased retention time that to date have not been explored. Thus, a new perspective on the application of dietary α-galactosidase enzyme may result.

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REFERENCES

1. USDA., 2009. USDA National nutrient database for standard reference release 18. United States Department of Agriculture, USA.
2. Choct, M., 1997. Feed non-starch polysaccharides: Chemical structures and nutritional significance. Feed Milling Int., 7: 13-26.
3. Choct, M., Y. Dersjant-Li, J. McLeish and M. Peisker, 2010. Soy oligosaccharides and soluble non-starch polysaccharides: A review of digestion, nutritive and anti-nutritive effects in pigs and poultry. Asian-Aust. J. Anim. Sci., 23: 1386-1398.
4. Gitzelmann, R. and S. Auriccio, 1965. The handling of soya α-galactosides by a normal and a galactosemic child. Pediatrics, 36: 231-235.
5. Grieshop, C.M., C.T. Kadzere, G.M. Clapper, E.A. Flickinger, L.L. Bauer, R.L. Frazier and G.C. Fahey Jr., 2003. Chemical and nutritional characteristics of United States soybeans and soybean meals. Agric. Food Chem., 51: 7684-7691.
6. Coon, C.N., K.L. Leske, O. Akavanichan and T.K. Cheng, 1990. Effect of oligosaccharide-free soybean meal on true metabolizable energy and fiber digestion in adult roosters. Poult. Sci., 69: 787-793.
7. Scheideler, S.E. and T. Weber, 2003. Role of α-galactosidase in corn-soy based layer rations. The Nebraska Poultry Report, pp: 20-22.
8. Perryman, K.R., H. Olanrewaju and W.A. Dozier III, 2013. Growth performance and meat yields of broiler chickens fed diets containing low and ultra-low oligosaccharide soybean meals during a 6-week production period. Poult. Sci., 92: 1292-1304.
9. Ao, T., A.H. Cantor, A.J. Pescatore, M.J. Ford, J.L. Pierce and K.A. Dawson, 2009. Effect of enzyme supplementation and acidification of diets on nutrient digestibility and growth performance of broiler chicks. Poult. Sci., 88: 111-117.
10. Waldroup, P.W., C.A. Keen, F. Yan and K. Zhang, 2006. The effect of levels of α-galactosidase enzyme on performance of Broilers fed diets based on corn and soybean meal. J. Applied Poult. Res., 15: 48-57.
11. Graham, K.K., M.S. Kerley, J.D. Firman and G.L. Allee, 2002. The effect of enzyme treatment of soybean meal on oligosaccharide disappearance and chick growth performance. Poult. Sci., 81: 1014-1019.
12. Roberts, J.R. and W. Ball, 2003. Egg quality guidelines for the Australian egg industry. Australian Egg Corporation Limited Publication 03/19, pp: 32.
13. Maynard, L.A. and J.K. Loosli, 1956. Animal Nutrition. 4th Edn., McGraw Hill, New York, pp: 484.
14. Gerhold, R.W., A.L. Fuller, R.B. Beckstead and L.R. McDougald, 2010. Low-dose immunization of northern bobwhites (*Colinus virginianus*) with *Eimeria lettyae* provides protection against a high-dose challenge. Avian Dis., 54: 1220-1223.

15. Scott, T.A. and F. Boldaji, 1997. Comparison of inert markers [chromic oxide or insoluble ash (Celite)] for determining apparent metabolizable energy of wheat-or barley-based broiler diets with or without enzymes. Poult. Sci., 76: 594-598.
16. Duncan, D.B., 1955. Multiple range and multiple F tests. Biometrics, 11: 1-42.
17. Kidd, M.T., Jr.G.W. Morgan, C.D. Zumwalt, C.J. Price, P.A. Welch, F.L. Brinkhaus and E.A. Fontana, 2001. α-Galactosidase enzyme supplementation to corn and soybean meal broiler diets. J. Applied Poult. Res., 10: 186-193.
18. Sloan, D.R., R.H. Harms, A.G. Abdullah and K.K. Kuchinski, 2000. Variation in egg content density makes egg specific gravity a poor indicator of shell weight. J. Applied Anim. Res., 18: 121-128.
19. Knudsen, K.E.B., 1997. Carbohydrate and lignin contents of plant materials used in animal feeding. Anim. Feed. Sci. Technol., 67: 319-338.
20. Knap, I.H., A. Ohmann and N. Dale, 1996. Improved bioavailability of energy and growth performance from adding α-galactosidase (from *Aspergillus* sp.) to soybean meal-based diets. Proceedings of the Australian Poultry Science Symposium, (APSS'96), Sydney, NSW, Australia, pp: 153-156.
21. Zhang, B., Y. Cao, Y. Chen, Y. Li, S. Qiao and Y. Ma, 2010. Effects of α-galactosidase supplementation on performance and energy metabolism for broilers fed corn-non-dehulled soybean meal diets. Asian-Aust. J. Anim. Sci., 23: 1340-1347.