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Original Article

Effect of Genetically Modified Organisms Feed Ingredients (Corn And Soybean) in Diet on Egg Production, Egg Broken Rate and Egg Quality in Layers

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ABSTRACT

This study was conducted to examine the effect of feeding genetically modified organisms (GMO) compared with non - GMO diet on performance of layers in terms of egg production, egg broken rate and egg quality in laying hen. One hundred and ninety-two Hy-line brown laying hens were used in the feeding for four weeks. All the birds were randomly assigned into two dietary treatments groups, each with eight replicates (12 hens/ replicate). Significant difference was not found in egg production in layers fed GMO feed in diet compared with the non - GMO treatment. Whereas in terms of egg quality, a significant increase was observed in yolk color and eggshell thickness of layers fed GMO diet compared with layers fed non-GMO diet at 1st week. Additionally, there was a significant increase in eggshell thickness of layers fed GMO diet compared with layers fed non - GMO diet at 2nd, and 4th week. Layers fed GMO diet showed higher egg yolk value compared to the non-GMO diet in 1st week. However, no significant differences were found in other profiles such as egg quality including egg weight, albumen height, haugh units, shell color, and shell strength from 1st to 4th week since layers fed GMO. In conclusion, layers fed GMO diet showed significant improvement in eggshell thickness and yolk color and no significant difference was found in egg production. Layers fed GMO diets may enhance the egg quality, and further research need to be done.

INTRODUCTION

Since 1996, when large-scale cultivation of genetically modified (GM) crops began, the area cultivated with these crops has kept growing around the world. In 2019, 190.4 million hectares of these crops were grown worldwide (ISAAA, 2020). Genetic modification is the process of changing an organism's genes using advanced biology and technology. It is used to change an organism's rDNA to better serve the needs of farmers and consumers (Van Eenennaam *et al.*, 2014). Genetically modified organisms (GMOs) are plants, animals, and microorganisms that have been altered by recombinant DNA technology. This technology was used to insert foreign genes into the genomes of recipient organisms, which led to organisms with the trait of interest (Flachowsky, *et al.*, 2005). Plants can be genetically modified to be more resistant to herbicides, insects, and other pests, as well as to make their products better (e.g., less antinutritive substances and more valuable ones like vitamins or fatty acids). Corn, soybean, cotton, and canola are the main crops that get the most attention when it comes to genetic engineering in agriculture. About 70% to 90% of the GM plants grown are used as food for farm animals that make food (Flachowsky *et al.*, 2012). Genetic engineering was used to produce a high protein soybean that had a lot more crude protein and essential amino acids than normal soybeans (Swiatkiewicz & Arczewskawłosek, 2011). Soybeans that have been genetically modified to produce traits that increase the nutritional value for poultry, like reduced trypsin inhibitor soybeans and low-lectin soybeans, low-oligosaccharide SBM, and high-protein soybean meal (SBM) (Stein *et al.*, 2008). Many more studies have shown that a lot of GM crops and their non-GM counterparts are very similar in terms of nutrients and animal performance (Aulrich *et al.*, 2001; Mireles *et al.*, 2000; Flachowsky & Aulrich, 2001). However, the public, particularly in Europe, is opposed to crop biotechnology products (Tait, 2000; Atherton, 2002). It's common for people to get emotional when talking about these things. One concern is that transgenes could get into animal feed products. Other concerns include antibiotic resistance genes as unwanted cloning by-products, allergenicity of newly generated proteins, or equivalence of the transgenic plant and original plants. Einspanier *et al.* (2001) found that when a plant is digested, its DNA and proteins are broken down in the same way as normal plant DNA and proteins. Recombinant DNA (recDNA) and recombinant proteins from commercially grown GM plants have not been found in animal organs or animal products. GMO feed materials did not have an adverse impact on the performance, quality, or digestibility of meat, eggs, and milk, or on the health of poultry, pigs, fish, cows, and dairy cows. (Hammond *et al.* 1996; Flachowsky, *et al.*, 2005; Flachowsky *et al.*, 2007).

It is one of the objectives of animal nutrition studies that use plants that have been genetically modified to exhibit agronomic properties to determine whether the changed plants are nutritionally equal to their conventional counterparts. Because of this, the purpose of this study was to investigate the effect of feeding genetically modified organisms (GMO) to layers in comparison to layers fed a non-GMO diet on the performance and quality of their eggs.

MATERIALS AND METHODS

The Animal Care and Use Committee of Dankook University evaluated and approved the experimental procedures, where the management and care of the animals used in the experiments were described (Approval No: Dk-1-2157).

Test of corn and soybean meal

GMO and non-GMO corn and soybean samples were verified for GMO in Kogenebiotech Co., LTD (Seoul, Korea). An in-depth qualitative investigation of GMO in corn was carried out utilizing specific primer pairs for the reference gene (SSIIb), the 35S promoter, the NOS terminator, the DP-098140-6 gene, and the DAS-40278-9 gene, among other genes. Additionally, a qualitative analysis for genetically modified organisms (GMOs) in soybean was carried out using specific primer pairs for the genes Lectin (reference gene), 35S Promoter, MON89788, DP305423-1, NOS Terminator, DO356043-5, MON87701, MON87708, MON87769, CV127, and DAS-68416-4, respectively. The results of our feeding trial revealed that the corn and soybean meal used in our experiment were non-GMO (Table 1 and 2).

Table 1 – GMO qualitative analysis results of corn.

Analysis Item[1]	Non-Corn
SSIIb	Detected
NOS Terminator	Not detected
DP-098140-6	Not detected
35S Promoter	Not detected
DAS40278-9	Not detected

[1]Independent laboratory Kogenebiotech Co. LTD reported.

Table 2 – GMO qualitative analysis results of soybean meal.

Analysis Item1	Non-SBM
Soybean reference gene (lectin)	Detected
35S Promoter	Not detected
NOS Terminator	Not detected
MON89788	Not detected
DP305423-1	Not detected
DO356043-5	Not detected
MON87701	Not detected
MON87705	Not detected
CV127	Not detected
MON87708	Not detected
MON87769	Not detected
DAS-44406-6	Not detected
DAS-68416-4	Not detected

[1]Independent laboratory Kogenebiotech Co.LTD reported.

In this experiment corn and soybean meal were main raw materials. So to make sure about the use of non-GMO diets in this experiment, only corn and soybean meal were included in the formula. Qualitative analysis of GMO is presented in Table 1 and 2. Four genes including NOS Terminator, DP-098140-6, 35S Promoter, DAS40278-9 were not detected non-GMO corn. In addition, twelve genes including 35S Promoter, NOS Terminator, DP305423-1, DO356043-5, MON87701, MON89788, MON87705, MON87708, MON87769, CV127, DAS-44406-6, DAS-68416-4 were not detected in non-GMO soybean meal. Those results proved that the corn and soybean meal used in non-GMO meal were really non-GMOs.

Table 3 – Compositions of corn and soybean meal sample.

Analyses[1]	Corn		Soybean meal	
	Non-GMO	GMO	Non-GMO	GMO
Proximate Analyses, %				
Moisture	14.19	14.55	11.24	12.52
Crude Fat	3.11	3.02	0.67	1.20
Crude Protein	6.82	7.25	47.39	45.42
Crude Fibre	1.82	1.85	6.09	4.22
Crude Ash	1.50	1.54	8.44	6.32
Phosphorus	0.20	0.22	0.54	0.60
Neutral detergent fiber	10.26	10.86		
Acid detergent fiber	2.39	2.48		
KOH-SOL			77.52	77.44

[1]Reported on an as-is basis.

Corn and soybean meal analysis

On samples from each of the four lots of components, approximate analysis of corn and soybean meal was performed on the corn and soybean meal (AOAC, 2000). Daehan Feed Mill provided all of the feed components used in this feeding trial (both non-GMO and GMO corn and soybean), which included both non-GMO and GMO varieties (Daehan Feed Co. Ltd, Incheon, Korea). The amino acid content of the samples was determined using an amino acid analyzer (Biochrom 20, Pharmacia Biotech, Cambridge, England).

Experimental design, diet, animal and housing

A total of 192 Hy-line Brown laying hens (47 weeks old) were randomly divided into two groups and subjected to a four-week feeding trial in which they were fed one of two nutritional treatments. Under each treatment group, there were eight replications (12 hens per replication), with each replicate consisting of twelve adjacent cages (each representing a replicate). Hens were housed in individual cages of 38.1cm x 50cm x 40cm. Each cage was supplied with a nipple drinker, a trough, and an egg collecting plate. All of the hens were housed in a windowless, environmentally controlled room (temperature 26°C) and had a daily lighting schedule of 16 hours of light with 8 hours of darkness. Hens had access of ad libitum feed and free access to water through the nipple of an automatic drinker and a common feeder, respectively. Table 4 describes the composition of the diet. The following were the dietary treatments: 1) GMO feed, GMO corn and soybean meal based basal diet; and 2) Non-GMO feed, non-GMO corn and soybean meal based basal diet. The experimental diets were developed in accordance with the guidelines in the Hy-line brown breeder's manual and to meet the requirements of the National Research Council (NRC, 1994). Corn, soybean meal, and corn gluten meal were combined into a formula to serve as the main raw materials in this experiment, ensure that non-GMO diets were used throughout the process. For the proximate analysis, samples of each of the four lots of ingredients were taken and analyzed separately (AOAC.2000).

Table 4 – Basal diet composition (as-fed basis).

Raw material, %	Non-GMO	GMO
GMO Corn	-	48.96
GMO SBM (CP 45%)	-	13.33
Corn (Non-GMO)	50.19	-
SBM (Non-GMO)	14.04	-
Rice	3	3
Sesame Meal	3	3
Distillers dried grain soluble (Corn)	17.05	19.06
Tallow	1	1
Limestone	10.08	10.08
Mono-di-calcium phosphorus	0.62	0.62
Salt	0.19	0.09
$NaHCO_3$	0.21	0.22
Methionine (99%, L-Form)	0.11	0.12
Lysine (50%)	0.21	0.22
Choline (50%)	0.1	0.1
Vitamin premix[1]	0.1	0.1
Mineral premix[2]	0.1	0.1
Total	100	100
Calculated composition, %		
DM	88.39	88.35
Crude protein	17	17
Crude fat	3.76	3.96
Crude fiber	2.85	3.29
Crude ash	13	13.32
ME (kcal/kg)	2800	2800
Calcium	3.84	3.97
Total phosphorus	0.43	0.39
Methionine + Cysteine	0.73	0.7

[1]Provided per kg of complete diet: vitamin A, 11,025 IU; vitamin D_3, 1,103 IU; vitamin E, 44 IU; vitamin K, 4.4 mg; riboflavin, 8.3 mg; niacin, 50 mg; thiamine, 4 mg; d-pantothenic, 29 mg; choline, 166 mg; and vitamin B_{12}, 33 μg.

[2]Provided per kg of complete diet: Cu (as $CuSO_4$•$5H_2O$), 12 mg; Zn (as $ZnSO_4$), 85 mg; Mn (as MnO_2), 8 mg; I (as KI), 0.28 mg; and Se (as Na_2SeO3•$5H_2O$), 0.15 mg.

Sampling and measurements

Throughout the duration of the research, daily records of egg production were recorded (four weeks). Hen-day production (Hen-day production= total number of eggs divided by the number of days) was used to measure egg production. Besides from that, the egg quality was tested at the 1st, 2nd, 3rd and 4th weeks of the study period. In all, 30 sellable eggs (15 eggs per treatment) were collected randomly at

17:00 h from each cage at the end of the 1st, 2nd, 3rd and 4th (respectively), and the egg quality was assessed at 20:00 h the same day. By using an egg multi-tester, egg weight, yolk color, and Haugh unit (HU) were determined (Touhoku Rhythm Co. Lt., Tokyo, Japan). The breaking strength of the eggshells was calculated by using an eggshell force gauge model II (RobotmSation Co., Ltd., Tokyo, Japan). The thickness of the eggshell was measured with a dial pipe gauge (Ozaki MFG Co., Ltd., Tokyo, Japan) and the results were recorded. The average eggshell thickness was calculated by comparing the thickness of the shell at the rounded end, pointed end, and middle of the egg (excluding the inner membrane).

Statistical analysis

All of the experimental data were analyzed using general linear model (GLM) procedure (SAS Inst. Inc., Cary, North Carolina) with cage was being defined as the experimental unit (SAS Institute, Inc, Cary, NC, USA). Differences among treatments were separated by T-test. The results were expressed as the least squares means and standard error. Statements of statistical significance were based on $p<0.05$.

RESULT AND DISCUSSION

Corn and soybean meal analysis

The results of proximate analysis of corn and soybean meal presented in Table 3 were reported as the percentage by weight on an as-is basis. In corn, the crude protein of GMO corn was 0.43% higher than that of non-GMO corn. On the other hand, 1.97% higher crude protein was observed in non-GMO soybean meal compared to that of GMO soybean meal. Crude fat was lower in GMO corn compared to non-GMO corn but increase in GMO soybean meal than non-GMO soybean meal. The nutrient compositions of GMO grain are better compared to that of non-GMO materials. Probably due to fewer challenging factors such as insect, herbicide or abiotic stress affecting the accumulation of nutrients in the growth process. Previously, World Health Organization (WHO) with Organization for Economic Co-operation and Development, and the United Nations Food and Agriculture Organization have all stated that genetically modified (GM) crops are nutritionally equivalent to conventional wheat, corn, and tomato varieties currently available on the market (Venneria *et al.*, 2008). However, Rayan & Abbott (2015) found increased biochemical components (protein, fat, fiber and fatty acids) in the GMO corn samples compared to the non-GMO control samples. But they noted that the observed increases may be for the synergistic effect of new traits introduced into corn varieties. The development of genetically modified crops, particularly the first generation, has the potential to improve insect or herbicide resistance, as well as abiotic stress tolerance. In this study we considered that the results were unlikely to be biologically significant because they fell well within the range of values reported in the literature.

Egg performance

It can be seen in Table 5 that there are no differences in egg production and egg breaking rate between laying hens fed GMO and non-GMO supplements. During the first until the fourth week of the experiment, layers fed diets supplemented with GMO or non-GMO feed ingredients exhibited no statistically significant difference ($p>0.05$) in egg production, showing that egg production in layers fed non-GMO and GMO feed ingredients was equivalent. In close agreement with our findings, no differences in performance were seen between laying hens fed diets using genetically modified (GM) corn and laying hens fed diets created with non-GM corn (Jacobs *et al.*, 2008). Similiarly, Mejia *et al.* (2010) found that laying hen performance, as measured by egg production and egg quality, was similar between hens fed diets formulated with SBM prepared from transgenic 305423 soybeans, nontransgenic near-isoline SBM, and nontransgenic commercial SBM, regardless of whether the SBM was derived from transgenic 305423 soybeans, nontransgenic near-isoline SBM, or nontransgenic commercial SBM. Previously in a laying hen feeding trials conducted by Ma *et al.* (2013) found that egg production was equivalent between groups of laying hens fed non-GMO and GMO ingredients. Conversely, Scheideler *et al.* (2008) found that dietary GM corn treatment significantly affected egg production parameters for laying hen. The different results could be because of the different types of genetically modified organisms, the concentration of each composite diet formulation, or the way the birds were raised. However, Herman *et al.* (2007) explained that the Bt corn was genetically modified to protect it from pests that damage it and to make it resistant to herbicide. There were no changes made to the nutritional composition of DAS-59122-7 corn, so the nutritional value of the corn should be the same as the first line.

Table 5 – The effects of GMO supplementation on egg production and egg broken rate in laying hens1.

Items	Non-GMO	GMO	SEM[2]	*p-value*
Egg production, %				
1 wk	87.20	88.54	1.52	0.67
2 wk	87.95	90.18	1.20	0.39
3 wk	90.18	90.03	0.77	0.91
4 wk	90.77	91.22	0.63	0.71
Egg broken rate, %				
1 wk	0.00	0.00	-	-
2 wk	0.00	0.00	-	-
3 wk	0.00	0.00	-	-
4 wk	0.00	0.00	-	-

[1]Abbreviation: A, Non-GMO diet; B, GMO diet.

[2]Standard error of means.

Egg quality

In terms of egg quality (Table 6), a significant difference (*p<0.05*) was observed in yolk color in the 1st week and a tendency (*p*<0.10) was found in the 2nd week. GMO group showed higher yolk color value compared to the non-GMO group. Additionally, significant difference (*p<0.05*) was observed in eggshell thickness of layers fed GMO diet compared with layers fed non – GMO diet in the 1st, 2nd, 3rd, and 4th weeks of the experiment. There was a significant increase (*p<0.05*) in eggshell thickness of layers fed GMO diet compared with layers fed non - GMO diet in the 1st, 2nd, and 4th weeks whereas, non-GMO group showed significantly (*p>0.05*) decreased eggshell thickness. However, no significant differences (*p>0.05*) were found in eggshell color and shell strength, as well as other profiles of egg quality including egg weight, albumen height, haugh units. in laying hens fed GMO diet compared to the layer fed non – GMO diet.

Previously Mejia *et al*and expression of the soybean acetolactate synthase protein (GM-HRA. (2010) found in hens fed 305423 soybean meal showed similar egg production and egg quality traits in hen fed diets formulated using near-isoline soybean meal. The egg yolk colour is affected by the feed content of carotenoids, and xanthophyll isomers, lutein and zeaxanthin (Zaheer, 2017). Yellow corn is the major source of these pigment in layer hen. Higher yolk color value represents darker yellow yolk. In the first week of feeding trial, although this value increased significantly (*p*<0.05) in the GMO group compared to the non-GMO group, but in the next week non-GMO group tended to show increased (*p*<0.10) value. It is unclear why the color scores increased in GMO group. Scheideler *et al.* (2008) found significant differences in egg yolk color when GMO corn diet was compared with two types of conventional corn as diet in layer hen. They assume that this is because conventional corn has more xanthophylls than the other corn treatments, which makes the eggs laid by hens fed this diet have a higher Roche color fan score. The color of the egg yolk comes from the absorption and deposition of the pigment present in the hen diet, as hen cannot synthesize these pigments (Breithaupt, 2007).

Table 6 – The effects of GMO supplementation on egg quality in laying hens1.

Items	Non-GMO	GMO	SEM[2]	*p-value*
1wk				
Egg weight, g	62.61	62.62	0.87	0.99
Albumen height, mm	8.47	8.43	0.25	0.92
HU	90.66	89.02	1.45	0.49
Yolk color	7.00	7.77	0.21	0.02
Shell color	9.60	9.27	0.29	0.455
Strength, kg/cm^2	4.66	4.54	0.18	0.65
Eggshell thickness, mm^{-2}	40.66	43.34	0.47	<0.0001
2wk				
Egg weight, g	61.01	61.77	0.82	0.55
Albumen height, mm	8.36	8.52	0.22	0.66
HU	90.42	91.48	1.35	0.62
Yolk color	8.57	8.28	0.11	0.09
Shell color	11.83	12.17	0.34	0.51
Strength, kg/cm^2	4.16	4.35	0.12	0.27
Eggshell thickness, mm^{-2}	42.53	45.59	0.50	0.002
3wk				
Egg weight, g	60.42	61.59	0.86	0.31
Albumen height, mm	8.22	8.41	0.23	0.55
HU	91.76	90.12	1.39	0.51
Yolk color	8.45	8.28	0.10	0.30
Shell color	9.80	10.17	0.35	0.42
Strength, kg/cm^2	4.56	4.49	0.16	0.73
Eggshell thickness, mm^{-2}	38.87	37.81	0.47	0.04
4wk				
Egg weight, g	61.27	61.46	0.72	0.87
Albumen height, mm	7.73	7.42	0.30	0.48
HU	91.62	90.73	1.86	0.74
Yolk color	8.43	8.22	0.08	0.13
Shell color	11.97	11.57	0.44	0.56
Strength, kg/cm^2	4.40	4.48	0.15	0.74
Eggshell thickness, mm^{-2}	41.69	43.88	0.48	0.003

[1]Abbreviation: A, Non-GMO diet; B, GMO diet.

[2]Standard error of means.

In this study we found higher egg shell thickness in GMO supplemented group. For egg shell formulation, laying hen depends on the dietary calcium content (Jiang *et al.*, 2013). In this experiment, diet formulated with GMO grain contain little higher calcium where other calcium source was similar. This higher dietary calcium in GMO group could be the reason for the difference in the eggshell thickness. Previously, Jacobs *et al.* (2008) found no significant difference in shell weight in layers when genetically modified corn (Bt corn) was supplied. They noted that, the purpose of GMO crops is to protect from devastating insect pests. Increased yield, improved plant health, and less dependent on chemical pesticides are also benefits of insect-protected corn, both economically and environmentally.

CONCLUSION

In conclusion, layers GMO corn-soybean basal diet had no adverse effects on egg production characteristic and egg quality. Moreover, layers fed GMO indicated positive effects on egg quality including increased eggshell thickness and yolk color. Further experiments are required to determine the reason for this result.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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