Evaluation of the nutritional equivalency of soybean meal with the genetically modified trait DP-3Ø5423-1 when fed to laying hens

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ABSTRACT An experiment using 336 Hy-Line W-36 Single Comb White Leghorn hens was conducted to evaluate transgenic soybeans containing the *gm-fad2-1* gene fragment and the *gm-hra* gene. Transcription of the *gm-fad2-1* gene fragment results in an increased level of oleic acid (18:1) in the seed, and expression of the soybean acetolactate synthase protein (GM-HRA) encoded by the modified *gm-hra* gene, is used as a selectable marker during transformation. Pullets (20 wk of age) were placed in cage lots (7 hens/cage, 2 cages/lot) and were randomly assigned to 1 of 4 corn-soybean meal dietary treatments (6 lots/treatment) formulated with the following soybean meals: nontransgenic near-isoline control (control), nontransgenic commercial reference soybean meal A (92M72), nontransgenic commercial reference soybean meal B (93B15), or transgenic soybean meal produced from soybeans containing event DP-3Ø5423-1 (305423). Weeks 20 to 24 were a preconditioning period, and the 4 experimental diets were then fed from 25 to 36 wk of age. Differences between the 305423 and control group means were evaluated, with statistical significance at $P < 0.05$. Body weight, hen-day egg production, egg mass, feed consumption, and feed efficiency for hens fed the 305423 soybean meal were not significantly different from the respective values for hens fed diets formulated with the near-isoline soybean meal. Likewise, egg component weights, Haugh unit measures, and egg weights were similar regardless of the soybean meal source. This research indicates that performance of hens fed diets containing 305423 soybean meal, as measured by egg production and egg quality, was similar to that of hens fed diets formulated with the near-isoline control and commercial soybean meals.

Key words: *gm-fad2-1*, oleic acid, soybean meal, egg production, egg quality

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INTRODUCTION

Soybean meal (**SBM**) is the main protein source used in poultry diets and in most of the livestock industries throughout the world (Stein et al., 2008). The protein quality of SBM is high for poultry, and SBM is a particularly good source of lysine, arginine, and tryptophan, but it is deficient in methionine plus cysteine, threonine, and valine (Baker, 2000). In addition to protein and amino acids, soybean products may contribute approximately 25% of the ME in poultry diets (Dale, 2000).

Research on soybeans with genetic modifications for output traits that increase nutritional value includes SBM with reduced oligosaccharides (Parsons et al., 2000), high-protein soybeans (Edwards et al., 2000), reduced trypsin-inhibitor soybeans (Batal and Parsons, 2003), and, most recently, soybeans with elevated proportions of oleic acid (McNaughton et al., 2008). Soybean plants containing the event DP-3Ø5423-1 (**305423**) were generated by insertion of the *gm-fad2-1* gene fragment and the *gm-hra* gene. Transcription of the *gm-fad2-1* gene fragment suppresses the endogenous *FAD2-1* gene and results in an increased level of oleic acid and decreased levels of linoleic acid, linolenic acid, and, to a lesser extent, palmitic acid in the seed (Buhr et al., 2002; Stoutjesdijk et al., 2002). High-oleic soybean oils used in food applications have improved frying performance resulting from their greater oxidative stability (Mounts et al., 1988; Neff et al., 1994; Warner et al., 1997). The expressed protein encoded by the *gm-hra* gene is a modified version of soybean acetolactate synthase protein (GM-HRA) that is used as a selectable marker during transformation. The objective of this study was to evaluate the nutritional equivalence of SBM prepared from 305423 soybeans by comparing the performance and egg production of laying hens fed diets containing 305423 SBM with those fed diets

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containing nontransgenic control (having a comparable genetic background) or commercial SBM.

MATERIALS AND METHODS

Experimental Design

The Institutional Animal Care and Use Committee and the Institutional Biosafety Committee approved all animal care, housing, and handling procedures. An experiment was conducted using 336 Hy-Line W-36 Single Comb White Leghorns hens (20 wk of age). Hens were fed a preconditioning diet from 20 to 24 wk of age (Table 1). The experiment was designed according to International Life Sciences Institute (2003) guidelines, with an adequate number of replicates to detect a 5% difference between treatments at $P < 0.05$ and a minimum power of 80%; calculations for the number of replicates were performed using the variance observed in a previous trial at this facility. The hens were housed in a caged layer house of commercial design, with water and feed provided ad libitum. Six replicate groups of 14 hens each (2 adjacent cages containing 7 hens/60.9 × 58.4 cm cage) were allotted to 4 dietary treatments in a completely randomized design so that the mean BW was similar for each treatment. The dietary treatments consisted of corn-SBM diets formulated with 1 of the 4 SBM sources: nontransgenic near-isoline control SBM (control), transgenic SBM produced from soybeans containing event DP-3Ø5423-1 (305423), nontransgenic commercial reference SBM A (**92M72**), or nontransgenic commercial reference SBM B (**93B15**).

Pioneer Hi-Bred grew all the soybean sources in a field production trial conducted in 2007 near York, Nebraska. The nontransgenic near-isoline control, transgenic 92M72 and 93B15, and transgenic 305423 test soybeans were processed into meal at Texas A&M University (College Station, TX). Flaked soybean material underwent solvent extraction using commercial hexane, followed by removal of residual hexane, toasting of the material, and particle size reduction of the toasted material to meal. Identity preservation procedures were followed throughout the processing and inventory systems to maintain the identity of each soybean source and the resulting processed meal. Event-specific real-time PCR testing confirmed the presence of event DP-3Ø5423-1 in 305423 SBM and its absence from the control SBM and varieties 92M72 and 93B15. All SBM samples were analyzed in duplicate for selected nutrients at Cumberland Valley Analytical Services (Hagerstown, MD). Dry matter (method 930.15), protein (method 990.03), and calcium and phosphorus (method 985.01) analyses were performed according to AOAC International (2000) methods; fat analysis was performed according to AOAC (2006) method 2003.05; neutral detergent fiber by the method of Van Soest et al. (1991) with modification (Whatman 934-AH glass micro-fiber filters with 1.5-μm particle retention, Whatman International, Maidstone, UK); acid detergent fiber by AOAC International (2000) method 973.18 with modification

Table 1. Nutrient composition of soybean meals used to prepare test diets (as-is basis)[1]

Nutrient, %	Control	305423	92M72	93B15
DM	92.70	92.03	93.00	93.71
Protein	49.6	49.7	50.8	49.1
Crude fat	1.7	1.7	1.6	1.4
Gross energy, cal/g	4,393	4,420	4,413	4,434
Neutral detergent fiber	14.2	9.4	10.7	11.5
Acid detergent fiber	7.7	4.2	5.0	6.2
Calcium	0.34	0.31	0.30	0.39
Phosphorus	0.73	0.81	0.78	0.77
Arginine	3.59	3.47	3.67	3.39
Cysteine	0.72	0.70	0.78	0.72
Histidine	1.41	1.33	1.38	1.23
Isoleucine	2.38	2.17	2.32	2.12
Leucine	4.12	3.70	3.94	3.61
Lysine	3.07	2.94	3.08	2.96
Methionine	0.70	0.66	0.74	0.66
Phenylalanine	2.52	2.36	2.48	2.30
Threonine	1.96	1.79	1.96	1.75
Tryptophan	0.74	0.68	0.75	0.71
Tyrosine	1.84	1.74	1.83	1.73
Valine	2.50	2.34	2.45	2.21
Alanine	2.19	2.03	2.18	1.98
Aspartic acid	5.90	5.31	5.81	5.27
Glutamic acid	8.88	8.37	9.07	8.17
Glycine	2.18	1.97	2.13	1.96
Proline	2.37	2.23	2.52	2.21
Serine	2.31	2.00	2.26	1.96

[1]All soybean meal samples were analyzed in duplicate. Diets: control = nontransgenic near-isoline control soybean meal; 305423 = transgenic soybean meal produced from soybeans containing event DP-3Ø5423-1; 92M72 = nontransgenic commercial reference soybean meal A; 93B15 = nontransgenic commercial reference soybean meal B (all from Pioneer Hi-Bred, Johnston, IA).

Table 2. Ingredient composition of test diets (%)[1]

Item	Preconditioning diet[2]	Control	305423	92M72	93B15
Ingredient					
Ground corn	63.90	63.77	63.83	64.41	63.49
Soybean meal	23.46	23.59	23.53	22.95	23.87
Soybean oil	1.20	1.20	1.20	1.20	1.20
Limestone	9.32	9.32	9.32	9.32	9.32
Dicalcium phosphate	1.27	1.27	1.27	1.27	1.27
Salt	0.30	0.30	0.30	0.30	0.30
Poultry VTM 88[3]	0.50	0.50	0.50	0.50	0.50
DL-Methionine	0.05	0.05	0.05	0.05	0.05
Analysis[4]					
Protein	15.99	16.17	15.95	16.02	15.87
TME_n, kcal/kg	2,906	2,905	2,905	2,911	2,902
Calcium	3.90	4.04	3.96	3.99	3.92
Sodium	0.15	0.15	0.15	0.15	0.15
Total phosphorus		0.63	0.61	0.62	0.64
Available phosphorus	0.35	0.35	0.35	0.35	0.35
Lysine		0.89	0.91	0.91	0.90
Methionine + cysteine		0.55	0.56	0.58	0.55

[1]Diets: control = nontransgenic near-isoline control soybean meal; 305423 = transgenic soybean meal produced from soybeans containing event DP-3Ø5423-1; 92M72 = nontransgenic commercial reference soybean meal A; 93B15 = nontransgenic commercial reference soybean meal B (all from Pioneer Hi-Bred, Johnston, IA).

[2]Fed from 20 to 24 wk.

[3]Poultry VTM 88 vitamin and trace mineral premix (Archer Daniels Midland, Quincy, IL); guaranteed analysis (min): selenium, 40 mg/kg; vitamin A, 272,400 IU/kg; vitamin D_3, 90,800 ICU/kg; vitamin E, 590 IU/kg; menadione, 36 mg/kg; vitamin B_{12}, 0.39 mg/kg; biotin, 1.4 mg/kg; choline, 14,755 mg/kg; folic acid, 45 mg/kg; niacin, 1,362 mg/kg; pantothenic acid, 363 mg/kg; pyridoxine, 36 mg/kg; riboflavin, 182 mg/kg; and thiamine, 45 mg/kg.

[4]All values for the preconditioning diet are calculated values (NRC, 1994). Values for TME_n, sodium, and available phosphorus are calculated; all other values are analyzed values (n = 3).

(Whatman 934-AH glass micro-fiber filters with 1.5-µm particle retention); and crude fat by an AOAC (2006) method. Soybean meal samples were also analyzed for gross energy content with a bomb calorimeter (Parr Instruments Model 1271, Parr Instruments, Moline, IL) at Pioneer Hi-Bred (Urbandale, IA), and amino acid analysis was conducted by University of Missouri Agricultural Experiment Station Chemical Laboratories (Columbia, MO) in accordance with AOAC International (2000) methods (988.15, 982.30, and 994.12). The analyzed nutrient compositions (Table 1) of the test SBM were used in diet formulations. All diets were produced at the Pioneer Livestock Nutrition Center (Polk City, IA). Diets were mixed in the order of 1) control, 2) 92M72, 3) 93B15, and 4) 305423, and the mixing and bagging system was cleaned between treatments by flushing with nontransgenic soybean hulls. All diets contained a similar amount of SBM and were balanced based on actual nutrient composition data to be isonitrogenous and isocaloric (based on gross energy), and were then balanced for analyzed TSAA and lysine, threonine, tryptophan, and arginine. Dietary energy concentration was changed by altering the level of corn inclusion. The 4 experimental diets were formulated to contain 3.90% calcium and at least 0.35% nonphytate phosphorus when using NRC (1994) table values (Table 2). Samples of each diet were collected at the time of diet preparation for nutrient analysis, as described previously.

Hens were exposed to a 15-h daily photoperiod from 20 to 25 wk of age, which was then increased 0.5 h weekly to provide a 17-h photoperiod by 29 wk of age; this was maintained throughout the remainder of the experiment. The experiment was divided into three 4-week phases: phase 1 (25 to 28 wk of age), phase 2 (29 to 32 wk of age), and phase 3 (33 to 36 wk of age). Body weights were taken at the beginning of the study and at the end of each phase, and BW gains were calculated for each phase. Weekly feed intakes (g/hen per day) were measured. Feed efficiency ratios were calculated every 2 wk and averaged for each phase. Feed efficiency was calculated as [egg weight (g) × (egg production/100)]/feed intake (g) per hen per day.

Eggs were collected daily, and egg production and egg mass (grams of egg produced per day) were determined weekly for each phase. Egg weight, number of cracked eggs, and egg grade measures were determined for eggs collected on 2 d of egg production during the last week of each phase. Egg components (albumen, yolk, and wet shell weights) and Haugh units were determined on 4 eggs/cage lot during the last week of each phase.

Statistical Analysis

Performance and egg quality data were summarized for each phase and for the entire experiment. For all data, the cage lot (2 adjacent cages) was considered the experimental unit. Data were analyzed using the PROC MIXED procedure of SAS (SAS Institute, 1990); the model included treatment, phase, and the treatment × phase interaction as fixed effects, and cage lot (treatment) was designated as a random effect. The true comparison effect of interest in this study was that of the 305423 transgenic treatment vs. the control treatment. Therefore, estimate statements were used to generate the treatment comparisons between control and 305423 transgenic SBM groups for each performance and egg quality trait. Differences between means were considered significant at $P < 0.05$. False discovery rate, as described by Benjamini and Hochberg (1995), was applied across all treatments to control the false positive rate; this process takes into account multiple comparisons and aids in determining meaningful differences by controlling the experiment-wise error rate. The false discovery rate-adjusted P-value was reviewed if statistically significant differences ($P < 0.05$) generated from the estimate comparison statement were observed for a trait. Data generated from the commercial SBM varieties (92M72 and 93B15) were used in estimations of experimental variability; least squares means were generated for each reference treatment, but statistical comparisons between 92M72 and 93B15 and the control or 305423 treatment groups were not generated from estimate statements. Instead, these data were used to construct a 95% tolerance interval containing 99% of the observed trait values from birds fed commercial SBM diets, as described by Graybill (1976). The tolerance intervals were a supplement to the statistical comparison between the control and 305423 groups, with their purpose being to estimate the expected response range of laying hens obtained from the same supplier and exposed to the same conditions as laying hens fed the control and 305423 diets. Data from the control and 305423 groups were evaluated to determine if the observed values were contained within the tolerance interval. If an observed value for a treatment was contained within the tolerance interval, that value was considered to be consistent with how hens would perform when fed diets containing a similar amount of commercial SBM.

RESULTS AND DISCUSSION

Body weights, BW gain, and feed intakes were not affected ($P > 0.05$) by dietary treatment (Table 3). A significant ($P < 0.05$) treatment × phase interaction for initial hen BW was observed in the overall analysis [control initial BW (IBW) = 1,381.4 g; 305423 IBW = 1,391.3 g; 92M72 IBW = 1,404.5 g; 93B15 IBW = 1,388.7; SEM = 16.0). However, the statistical contrast between the control and 305423 groups demonstrated that the treatment × phase interaction was not significant for those 2 groups. Phase results were as expected for the typical production cycle of a laying hen. Hen-day egg production, egg weight, egg mass, and feed efficiency were also similar between hens fed the near-isoline control diet and the transgenic 305423 diet. Hen-day and egg mass values that were below the lower tolerance interval boundary (81.60% and 46.5 g of egg/d, respectively) were observed in the 305423 diet group with 1 cage lot (74.23% and 42.1 g of egg/d) that experienced a production decline in the second and third phases; these hens did not exhibit any overt signs of molting. The production decline was most likely an aberration caused by 1 or more hens going out of production for that time and not because of a treatment effect, especially because the other 5 cage lots maintained their egg production throughout all 3 periods at levels similar to all other treatments. Observed egg mass and feed efficiency values of the control and 305423 groups fell within the tolerance intervals calculated from hens fed the 92M72 and 93B15 diets. Flighty, hysteria-type behavior was observed across all treatment groups during the 4 wk of the preconditioning period. This behavior resulted in extending the preconditioning diet period by 1 wk, for a total of 5 wk. Because the behavior was observed across all treatment groups, the decision was made to continue with the study with feed intakes measured every 2 wk, as opposed to a weekly basis, to reduce the stress associated with measuring intake. Overall production data from this flock were, in fact, comparable with previous production results with Hy-Line W36 hens at this facility (Jacobs et al., 2008). Mortalities in this study were limited to 1 hen each from the control, 305423, and 92M72 treatment groups. The necropsy examinations showed the cause of death as heart hemorrhage, which was not treatment related but likely related to the flighty, hysteria-type behavior of this particular flock.

Egg components (albumen, yolk, and wet shell weight) were not different ($P > 0.05$) between hens fed the control and 305423 diets (Table 4); all observed values of the control and 305423 groups fell within the individual component tolerance intervals. No differences were observed for egg weight class distributions (jumbo, extra large, large, medium, and small) between the hens fed the control and 305423 diets. The highest observed estimated egg weight value (73.0 g) occurred with hens fed the control diet; this value was just outside the tolerance interval (72.4 g) and was due to a jumbo egg produced in the third phase. The occurrence of jumbo eggs is not uncommon and is not considered treatment related, as evidenced by their occurrence in the 93M15 treatment group; more important, the class percentage means were not statistically different between the control and 305423 groups. Haugh unit values did not differ ($P > 0.05$) between hens fed the control and 305423 diets (Table 4), and all values fell within the US grade AA category (72+ Haugh units).

The results of this study indicate 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, or nontransgenic commercial SBM. The 305423 soybeans were genetically modified for an increased level of oleic acid and decreased levels of linoleic, linolenic, and, to a lesser extent, palmitic acid in the seed. No significant differences were found in the amino acid composition of the 305423 and control soybeans (Pavely, 2007). Previous research by McNaughton et al. (2008) when feeding broilers 305423 SBM revealed no significant differences in growth performance, liver and kidney yield, or

Table 3. Performance, egg performance, and production efficiency of hens fed diets containing the control or 305423 diet[1]

				Control vs. 305423			Reference soybean meal group[5]	
Response[2]	Control	305423	SEM[3]	FDR P-value[6]	Raw P-value[7]	Tolerance interval[4]	92M72	93B15
Performance								
BW gain, g	42.4	43.6	3.7	0.97	0.83	−47.6 to 148.9	54.9	46.4
Feed intake, g/hen per d	93.4	93.3	0.9	0.97	0.95	78.4 to 109.5	93.5	94.3
Egg production								
Egg production, hen-day, %	91.98	89.73	1.31	0.95	0.24	81.60 to 103.71	92.87	92.45
Egg weight, g	57.1	57.3	0.2	0.95	0.62	48.2 to 66.9	57.8	57.2
Egg mass,[8] g of egg produced/d	52.5	51.4	0.8	0.95	0.32	46.5 to 60.0	53.7	52.8
Feed efficiency,[9] g of egg/g of feed	0.562	0.550	0.006	0.95	0.16	0.474 to 0.661	0.575	0.560

[1]Values are means for 6 replicate groups of 14 hens each. Diets: control = nontransgenic near-isoline control soybean meal; 305423 = transgenic soybean meal produced from soybeans containing event DP-3Ø5423-1; 92M72 = nontransgenic commercial reference soybean meal A; 93B15 = nontransgenic commercial reference soybean meal B (all from Pioneer Hi-Bred, Johnston, IA).

[2]Means for the control and 305423 soybeans were not significantly different ($P > 0.05$) for any of the phases or the overall period (25 to 36 wk of age).

[3]SEM = pooled SEM.

[4]Lower and upper limits of a 95% tolerance interval on 99% of the observed performance trait values from hens fed the 92M72 and 93B15 diets.

[5]Commercial reference soybean meal least squares means included for reference purposes only. The true comparison of interest was the control vs. 305423 soybean meal.

[6]P-value adjusted using the false discovery rate.

[7]Nonadjusted P-value generated from the estimate statement.

[8]Egg mass calculated as egg weight (g) × (egg production/100).

[9]Feed efficiency calculated as [egg weight (g) × (egg production/100)]/feed intake (g) per hen per day.

carcass yield when compared with near-isoline control SBM. Other studies in broilers (Brake and Vlachos, 1998; Brake et al., 2003, 2005; Taylor et al., 2004, 2007; McNaughton et al., 2007) have shown that growth performance is not influenced by soybeans that have been genetically modified. Genetically modified maize lines were also evaluated in laying hens (Aeschbacher et al., 2005; Halle et al., 2006; Jacobs et al., 2008), and re-

Table 4. Component weights and Haugh unit measures of eggs produced from hens fed the control or 305423 diet[1]

				Control vs. 305423			Reference soybean meal group[5]	
Quality trait[2]	Control	305423	SEM[3]	FDR P-value[6]	Raw P-value[7]	Tolerance interval[4]	92M72	93B15
Component weight								
Albumen weight, g	35.6	35.8	0.3	0.95	0.70	31.0 to 39.7	35.7	35.1
Yolk weight, g	15.1	15.2	0.1	0.95	0.70	10.5 to 19.8	15.2	15.1
Wet shell weight, g	8.0	8.0	0.1	0.95	0.61	5.9 to 10.0	7.9	8.0
Weight class distribution								
Estimated egg weight,[8] g	56.8	57.1	0.3	0.95	0.51	42.3 to 72.4	57.9	56.9
Jumbo	0.28	0.00	0.18	0.95	0.28		0.00	0.22
Extra large	6.79	6.67	1.89	0.97	0.97		10.97	8.05
Large	46.64	48.80	2.92	0.95	0.61		52.67	43.20
Medium	42.38	42.69	2.38	0.97	0.93		33.08	46.59
Small	3.48	1.35	0.96	0.95	0.13		2.41	1.49
Haugh units	100.3	100.0	0.9	0.97	0.82	78.8 to 122.5	99.8	101.4

[1]Values are means for 6 replicate groups of 14 hens each. Diets: control = nontransgenic near-isoline control soybean meal; 305423 = transgenic soybean meal produced from soybeans containing event DP-3Ø5423-1; 92M72 = nontransgenic commercial reference soybean meal A; 93B15 = nontransgenic commercial reference soybean meal B (all from Pioneer Hi-Bred, Johnston, IA).

[2]Means for the control and 305423 soybean meals were not significantly different ($P > 0.05$) for any of the phases or the overall period. Phase 1 = 25 to 28 wk, phase 2 = 29 to 32 wk, and phase 3 = 33 to 36 wk.

[3]SEM = pooled SEM.

[4]Lower and upper limits of a 95% tolerance interval on 99% of the observed performance trait values from hens fed the 92M72 and 93B15 diets.

[5]Commercial reference soybean meal least squares means included for reference purposes only. The true comparison of interest was the control vs. 305423 soybean meal.

[6]P-value adjusted using the false discovery rate.

[7]Nonadjusted P-value generated from estimate statement.

[8]Estimated egg weights calculated by assigning the centroid of the difference between class weight breaks plus the class weight break value to each egg in the respective weight class. Class weight breaks: jumbo eggs = 70 g/egg; extra large eggs = 64 g/egg; large eggs = 56 g/egg; medium eggs = 49 g/egg; small eggs = 42 g/egg. These values were used to generate the tolerance interval for the set of eggs that was graded.

sults showed that performance was similar for hens fed control and genetically modified maize lines. The use of soybean oil with an increased level of oleic acid may have potential benefits for human health. According to Lichtenstein et al. (2006), increased intake of oils high in monounsaturated fatty acids, such as oleic acid, may decrease the risk of cardiovascular disease.

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