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Suitability of Soybean Meal from Insect-Resistant Soybeans for Broiler Chickens

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ABSTRACT: Benning^{M} and Benning^{MGH} are near-isogenic lines (NILs) of the soybean cultivar Benning, which contain insect-resistance quantitative trait loci (QTLs) from the soybean accession PI 229358. Benning^{M} contains QTL-M, which confers antibiosis and antixenosis. In addition to QTL-M, Benning^{MGH} contains QTL-G, which confers antibiosis, and QTL-H, which confers antixenosis. Soybean meal was produced from Benning and the NILs. Nutritional composition, digestible amino acid content, and nitrogen-corrected true metabolizable energy (TME_N) were equivalent among soybean meals. A 21-day broiler feeding trial was carried out to determine if the QTLs affect soybean meal quality. Weight gain and feed-to-gain ratio were evaluated. No biologically significant differences were detected for broilers fed Benning, Benning^{M}, and Benning^{MGH}. This demonstrates that soybean meal produced from the insect-resistant NILs is equivalent to soybean meal produced from their non-insect-resistant parent cultivar for broiler weight gain.

KEYWORDS: *soybean, insect-resistance QTLs, near-isogenic lines, soybean meal, broiler performance*

INTRODUCTION

Broiler feeding trials have become a standard test to assess the nutritional suitability of genetically modified crops.[1] Some jurisdictions even consider them as providing a screen to guard against the unintentional presence of harmful side effects from the modification.[2,3] In contrast, similar traits obtained via conventional breeding are seldom tested for safety.[4] Insect resistance in soybean [*Glycine max* (L.) Merr.] is an example of a trait that can be obtained either transgenically[5] or conventionally.[6]

Soybean seeds are a major protein source for animal feed.[7] Worldwide, 11% of the crop is lost to animal pests, including insects,[8] of which leaf-chewing insects are economically important in the southern United States.[9] Although soybeans can withstand moderate leaf damage, high levels of defoliation greatly reduce seed yield and quality.[10] Therefore, plant resistance to leaf-chewing insects is essential for preventive pest management; it promotes efficient use of insecticides, diminishing crop production, and environmental concerns. In soybeans, nontransgenic resistance to a broad range of leaf-chewing insects[11−26] is found in the Japanese soybean landrace 'Sodendaizu' PI 229358,[27] from which it has been bred into several modern cultivars.

PI 229358's resistance is conferred via antibiosis and antixenosis.[28,29] In antibiosis, the plant has detrimental effects on insect growth, development, and/or reproduction.[30] In antixenosis, the plant affects insect behavior by discouraging oviposition, colonization, and/or feeding.[30,31] Three quantitative trait loci (QTLs) confer PI 229358's resistance. QTL-M, on chromosome 7, provides both antibiosis and antixenosis. QTL-H, on chromosome 12, conditions antixenosis, whereas QTL-G, on chromosome 18, conditions antibiosis.[28,29] QTL-M is required for the expression of QTL-H and QTL-G.[32] The chemical nature of the resistance conferred by these QTLs remains largely unknown.

Inasmuch as the products from PI 229358 QTLs are detrimental to insect growth and behavior, there is a concern that meal derived from such insect-resistant soybean seed could also have detrimental effects on animals when used for feed. Although rare, a few past efforts to develop disease-resistant cultivars through conventional breeding led to unacceptable levels of undesirable metabolites. The potato cultivar Lenape accumulated high levels of glycoalkaloids,[33] and disease-resistant celery containing high levels of furanocoumarins was associated with dermatitis among grocery store personnel.[34,35] Therefore, it is prudent to ensure that soybean meal produced from plants carrying QTL-M, QTL-G, and QTL-H is as safe and wholesome as soybean meal produced from seed without these QTLs.

To determine if the addition of insect-resistance QTLs has negative effects on the feed quality of soybean meal, soybean meals were produced from soybean NILs containing the QTLs described earlier. Digestible amino acid content and nitrogen-corrected true metabolizable energy (TME_N) were measured for each soybean meal, and diets containing each soybean meal were evaluated in a 21-day broiler feeding trial.

MATERIALS AND METHODS

Soybean Meal Production. Benning[36] and its insect-resistant NILs[6] were used in this study. Benning^{M} contains QTL-M, whereas Benning^{MGH} contains QTL-M, QTL-G, and QTL-H. Figure 1 shows Benning, Benning^{M}, and Benning^{MGH} plants in the field, exposed to

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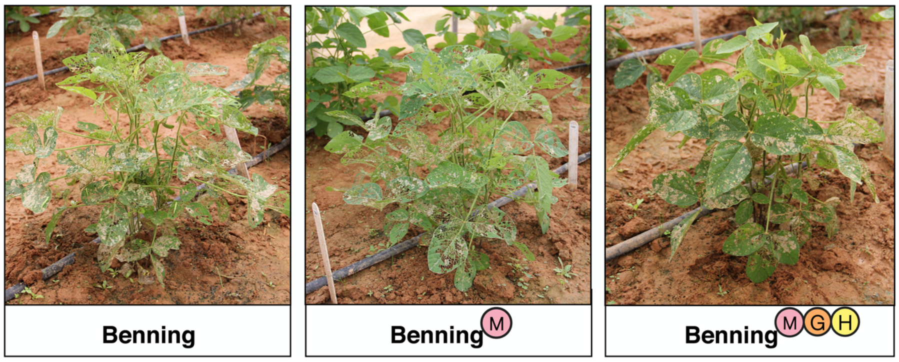

Figure 1. Benning, Benning^M, and Benning^{MGH} soybeans exposed to soybean looper caterpillars in a field-cage assay.

soybean looper caterpillars. Benning is highly defoliated, whereas Benning^M is moderately defoliated, and Benning^{MGH} is the least defoliated. The NILs are similar to Benning for most agronomic characteristics, including seed quality score and protein and oil contents.[6] Benning, Benning^M, and Benning^{MGH} were grown in 2011 at the University of Georgia Plant Sciences Farm. To avoid agronomic differences due to the environment and ensure that differences among soybean meals were due to genotype, the lines were planted on the same date and in the same field prepared the same way. A total of 250 kg of seeds was harvested from each line. The soybean seeds were processed into meal at the Food Protein R&D Center, Texas A&M University (College Station, TX, USA) using industry-standard procedures.[37]

Soybean Meal Composition. Proximate composition and amino acid content were determined for each soybean meal. Amino acid, dry matter, and crude protein analyses were performed according to AOAC methods 994.12, 930.15, and 990.03, respectively.[38] Digestible amino acid content and TME_N were determined according to the methods of Sibbald[39] and Dale and Fuller.[40] To determine the digestible amino acid content of each soybean meal, the digestive tracts of eight cecectomized 60-week-old White Leghorn roosters (*Gallus gallus domesticus* L.) were cleared by a 30-h feed withdrawal. Each rooster was precision-fed 35 g of soybean meal; 8 unfed roosters served as controls. Roosters were distributed to each treatment in a completely randomized design. For each individual rooster, excreta were collected for 48 h after feeding; the samples were dried and analyzed for amino acid content.[38] The amino acid digestibility protocol was modified to determine TME_N, in that noncecectomized roosters were used in the assay. Excreta were analyzed as previously described for TME_N.[39,40] Animal procedures were approved by the Institutional Animal Care and Use Committee of the University of Georgia.

Broiler Assay. The procedure was adapted from that of Davis.[41] One hundred and eighty 1-day-old Cobb × Cobb male broiler chicks were selected from a larger population for uniform body weights. The chicks were maintained in electrically heated brooder batteries (24 pens per battery); each pen housed five chicks. The chicks were given constant illumination and free access to water. Diets were formulated on a digestible amino acid basis (Table 1). The dietary treatments, Benning, Benning^M, and Benning^{MGH}, were assigned in a completely randomized design to each pen. The experiment included 12 replicate pens per treatment. The experimental diets were fed until the chickens were 21 days of age. Weight gain and feed consumption were recorded for each pen at 7, 14, and 21 days of age. Animal procedures were approved by the Institutional Animal Care and Use Committee of the University of Georgia.

Statistical Analysis. Data were analyzed using JMP statistical software version 10.0 (SAS Institute, Inc., Cary, NC, USA). Each data set first was tested for normality using the Shapiro−Wilk test ($P > 0.05$).[42] A one-way ANOVA test ($P > 0.01$) was used to detect differences among soybean genotypes, and a post hoc Tukey−Kramer multiple-comparison test ($P > 0.01$)[43−45] was used to determine significant differences between soybean genotypes.

Table 1. Nutritional Composition of Experimental Diets Containing Soybean Meal from Benning and Its Insect-Resistant Isolines, Benning^M and Benning^{MGH}

	diet[a]		
	Benning	Benning^M	Benning^{MGH}
ingredient %			
corn	52.980	52.174	50.644
soybean meal	40.387	41.156	42.289
soybean oil[b]	2.697	2.742	3.142
limestone	1.297	1.298	1.299
dicalcium phosphate	1.189	1.182	1.174
salt	0.266	0.270	0.266
sodium carbonate	0.245	0.243	0.248
L-lysine, HCl 78.8%	0.151	0.136	0.155
DL-methionine 99%	0.378	0.380	0.370
L-threonine, 98%	0.068	0.075	0.071
choline chloride 60%	0.020	0.020	0.020
Quantum Phytase XT 2,500	0.020	0.020	0.020
vitamin mix[c]	0.227	0.227	0.227
mineral mix[d]	0.075	0.075	0.075
calculated analysis			
AME (kcal/kg)	3031	3031	3031
crude protein (%)	23.467	22.836	22.927
calcium (%)	0.950	0.950	0.950
available phosphorus (%)	0.475	0.475	0.475
digestible total sulfur (%)	0.950	0.950	0.950
digestible lysine (%)	1.250	1.250	1.250
digestible threonine (%)	0.812	0.812	0.812

[a]Starter diet was fed from day 1 to 21 days of age. [b]Restaurant's Pride Advantage Soybean Oil (F.A.B, Inc., Alpharetta, GA, USA). [c]Vitamin mix (DSM Nutritional Products Ltd., Pendergrass, GA, USA) provided the following per 100 g of diet: vitamin A, 551 IU; vitamin D_3, 110 IU; vitamin E, 1.1 IU; vitamin B_{12}, 0.001 mg; riboflavin, 0.44 mg; niacin, 4.41 mg; D-pantothenic acid, 1.12 mg; choline, 19.13 mg; menadione sodium bisulfate, 0.33 mg; folic acid, 0.55 mg; pyridoxine HCl, 0.47 mg; thiamin, 0.22 mg; D-biotin, 0.011 mg; and ethoxyquin, 12.5 mg. [d]Mineral mix (Southeastern Minerals Inc., Bainbridge, GA, USA) provided the following in mg per 100 g of diet: Mn, 6.0; Zn, 5.0; Fe, 3.0; I, 0.15; Cu, 0.05; and Se, 0.05.

RESULTS AND DISCUSSION

Soybean Meal Composition. The overall nutrient profiles of soybean meal from Benning, Benning^M, and Benning^{MGH} were comparable (Table 2), despite minor differences that were detected in amino acid digestibility among soybean meals (Table 2). These variations could be the result of slight differences in seed composition or differences in the small-batch processing of the meals. The TME_N values were similar

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Table 2. Crude Protein, Amino Acid Content, and Amino Acid Digestibility of Benning, Benning^{M}, and Benning^{MGH} Soybean Meals[a]

	Benning	Benning^{M}	Benning^{MGH}
total content %			
dry matter	89.44	92.10	90.27
amino acid			
alanine	2.11	2.00	2.01
arginine	3.37	3.21	3.11
aspartic acid	5.21	4.94	4.89
cysteine	0.71	0.67	0.70
glutamic acid	8.11	7.70	7.45
glycine	2.01	1.91	1.90
histidine	1.25	1.19	1.18
isoleucine	2.11	2.01	1.98
leucine	3.48	3.32	3.25
lysine	2.91	2.78	2.74
methionine	0.59	0.56	0.56
phenylalanine	2.46	2.38	2.31
proline	2.26	2.28	2.23
serine	2.41	2.29	2.19
threonine	1.90	1.81	1.80
tryptophan	0.62	0.61	0.59
tyrosine	1.20	1.12	1.08
valine	2.27	2.17	2.14
digestibility			
alanine	83.10 ± 0.27b	84.95 ± 0.14a	85.22 ± 0.21a
arginine	84.25 ± 0.13c	91.53 ± 010a	88.06 ± 0.09b
aspartic acid	85.17 ± 0.14c	87.75 ± 0.16a	86.26 ± 0.01b
cysteine	72.02 ± 0.05b	76.06 ± 0.15a	74.46 ± 1.14ab
glutamic acid	86.96 ± 0.18b	89.54 ± 0.15a	89.08 ± 0.05a
glycine	71.00 ± 0.17	71.00 ± 0.00	70.35 ± 0.43
histidine	85.88 ± 0.09c	88.97 ± 0.03a	86.61 ± 0.11b
isoleucine	86.97 ± 0.05c	89.71 ± 0.05a	88.66 ± 0.07b
leucine	87.86 ± 0.02c	90.66 ± 0.07a	89.83 ± 0.12b
lysine	87.30 ± 0.04c	90.81 ± 0.15a	88.67 ± 0.07b
methionine	90.57 ± 0.00c	92.32 ± 0.06a	91.43 ± 0.06b
phenylalanine	89.22 ± 0.16c	91.61 ± 0.09a	90.82 ± 0.09b
proline	84.23 ± 0.05c	88.16 ± 0.09a	87.11 ± 0.03b
serine	86.31 ± 0.23b	88.57 ± 0.12a	86.81 ± 0.18b
threonine	82.09 ± 0.17b	83.86 ± 0.19a	83.05 ± 0.17ab
tryptophan	89.20 ± 0.11	89.22 ± 0.06	89.50 ± 0.28
tyrosine	91.24 ± 0.06c	93.44 ± 0.09a	92.38 ± 0.09b
valine	86.28 ± 0.02b	88.84 ± 0.19a	88.18 ± 0.09a
total AA	38.53	37.96	36.72
total EAA	18.15	18.01	17.38
total NEAA	20.38	19.95	19.34

[a]Values are the mean ± SEM. Means within a row with a different letters differ, $P < 0.05$. Amino acid and dry mater analysis were performed according to AOAC methods 994.12 and 930.15, respectively.[38] Amino acid digestibility was determined according to the method of Sibbald.[39] AA, amino acid; EAA, essential amino acid; NEAA, nonessential amino acid.

for the three soybean meals, 2560, 2569, and 2544 kcal kg^{-1} for Benning, Benning^{M}, and Benning^{MGH}, respectively (Table 3). The protein content in the prepared diets was not affected, as each diet was supplemented to adjust for the differences in meal composition (Table 1).

Broiler Assay. The performances of the Cobb × Cobb male broilers were equivalent for the Benning, Benning^{M}, and Benning^{MGH} diets when measured at 7, 14, and 21 days of age (Figure 2). No statistically significant differences were found

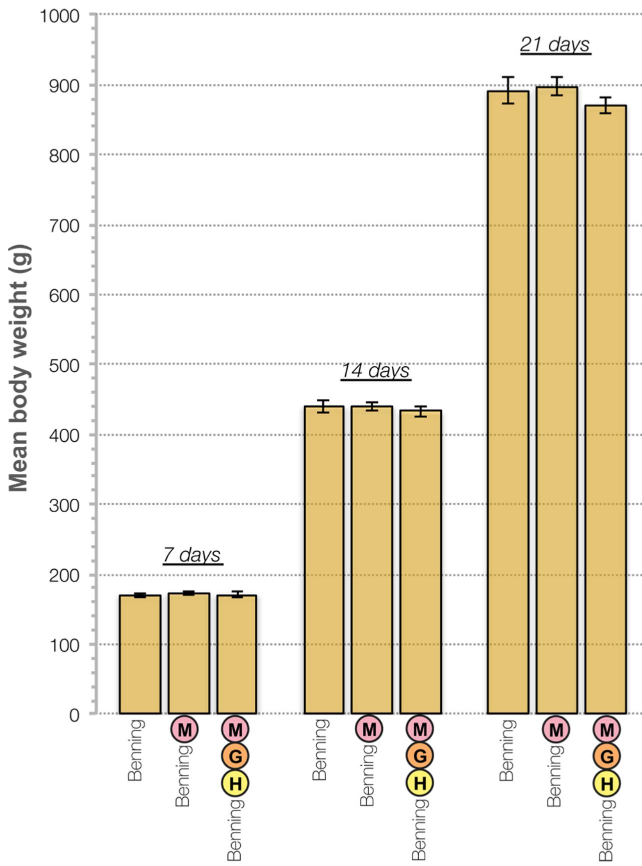

Figure 2. Weight of Cobb × Cobb male broiler chickens feeding on Benning, Benning^{M}, and Benning^{MGH} soybean meals at 7, 14, and 21 days of age.

among diets for weight per chick, weight gain per chick, and feed to gain ratio at 7, 14, and 21 days of age (Table 4). The Cobb−Vantress guidelines for ideal broiler weight at 7, 14, and 21 days of age are 170, 449, and 885 g, respectively.[46] The mean weights of Benning-, Benning^{M}-, and Benning^{MGH}-fed broilers were very close to the ideal weights.

Animal feeding trials are routinely conducted to determine the nutritive value of transgenic crops. In feeding assays with broiler chicks, Kan and Hartnell[47] demonstrated that insect-

Table 3. Chemical Composition and Nitrogen-Corrected True Metabolizable Energy (TME_N) Content of Benning, Benning^{M}, and Benning^{MGH} Soybean Meals

soybean line	TME_N,[a] kcal/kg as is	TME_N,[a] kcal/kg dry	protein, %	fat, %	moisture, %	ash, %
Benning	2560 ± 21	2789 ± 23	49.30	0.87	8.20	6.09
Benning^{M}	2569 ± 23	2880 ± 26	46.39	0.89	10.80	5.90
Benning^{MGH}	2544 ± 23	2832 ± 26	46.15	1.17	10.14	6.03

[a]TME_N was determined using the methodology described by Dale and Fuller.[40]

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Table 4. Growth Performance of Cobb × Cobb Male Broilers Fed Benning, Benning^{M}, and Benning^{MGH} Diets from 1 to 21 Days of Age[a]

	body weight gain (g/bird)	feed to gain
1−7 days of age		
Benning	126 ± 3	1.11 ± 0.01
Benning^{M}	129 ± 2	1.14 ± 0.01
Benning^{MGH}	127 ± 4	1.11 ± 0.02
1−14 days of age		
Benning	395 ± 10	1.20 ± 0.02
Benning^{M}	396 ± 7	1.22 ± 0.02
Benning^{MGH}	389 ± 8	1.19 ± 0.01
1−21 days of age		
Benning	848 ± 20	1.35 ± 0.03
Benning^{M}	853 ± 13	1.38 ± 0.02
Benning^{MGH}	826 ± 11	1.36 ± 0.02

[a]The values are means ± SEM.

resistant soybean meal is nutritionally equivalent to non-transgenic cultivars, and McNaughton et al.[48] determined that high-oleic soybean seeds were comparable to nontransgenic controls. Although the insect-resistant lines Benning^{M} and Benning^{MGH} were developed through conventional breeding and their agronomic characteristics are similar to those of Benning, the rationale for this study was to ensure that the insect-resistance QTLs derived from PI 229358 do not alter the nutritional value and safety of soybean meal. Because PI 229358 has been used in soybean breeding programs worldwide as a source of genetic resistance to leaf-chewing insects, the results of this study are highly relevant. Overall, the nutritional compositions of Benning^{M} and Benning^{MGH} soybean meals are equivalent to that of Benning soybean meal. No biologically significant differences were detected among broiler chicks fed Benning, Benning^{M}, and Benning^{MGH} for weight, weight gain, and feed-to-gain ratio; therefore, there is no indication that meal produced from soybean seed carrying QTL-M, QTL-G, and QTL-H would not be as safe as the insect-susceptible Benning soybean cultivar when used for animal feed.

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Author Contributions

M.A.O. drafted the manuscript. A.J.D. performed the chicken feeding assays. H.R.B. conceived the study and coordinated the production of soybean seed and meals. W.A.P. conceived the study and drafted the manuscript.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

NIL, near-isogenic line; QTL, quantitative trait loci; TME_N, nitrogen-corrected true metabolizable energy

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