Appendix 20

Proximate and Fibre Composition of Forage from Imidazolinone-Tolerant Soybean BPS-CV127-9 Produced in Brazil in 2007/2008 and Comparison with that from Isogenic control and Commercial Soybean Varieties

The Chemical Company
Plant Science LLC

Proximate and Fiber Composition of Forage from Imidazolinone-Tolerant Soybean BPS-CV127-9 Produced in Brazil in 2007/2008 and Comparison with that from Isoline Control and Conventional Soybean Varieties

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No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA §10(d) (1) (A), (B), or (C).

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STATEMENT OF COMPLIANCE

This study was not conducted in compliance with the requirements of 40 CFR Part 160.

The data generated by ITAL on behalf of BASF Plant Science in support of product safety comply with generally accepted scientific procedures. ITAL is an ISO 9001 compliant laboratory. Record keeping is consistent with procedures used throughout the research community. This report accurately presents the raw data developed during the study.

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ABBREVIATIONS AND DEFINITIONS

ADF	Acid detergent fiber
AHAS	Acetohydroxyacid synthase
AOAC	American Organization of Analytical Chemists
AOCS	American Oil Chemists Society
CF	Crude fiber
DW	Dry weight
FW	Fresh weight
NDF	Neutral detergent fiber

Proximate and Fiber Composition of Forage from Imidazolinone-Tolerant Soybean BPS-CV127-9 Produced in Brazil in 2007/2008 and Comparison with that from Isoline Control and Conventional Soybean Varieties

SUMMARY

Soybean (*Glycine max* L.) plants have been developed by BASF Plant Science, L.L.C (BPS) and EMBRAPA (Empresa Brasileira de Pesquisa Agropecuaria) that are tolerant to the imidazolinone class of agricultural herbicides. The herbicide-tolerant soybean plants BPS-CV127-9 (hereafter referred to as BPS-CV127-9) were produced by introduction of an imidazolinone-tolerant acetohydroxyacid synthase large subunit (*ahasl*) from *Arabidopsis thaliana* into the soybean plant genome. The herbicide tolerance in BPS-CV127-9 will allow growers to treat the soybean crop with imidazolinone herbicides without causing injury to the plant at normal field application rates. Therefore, introduction of BPS-CV127-9 offers soybean growers an additional tool for controlling weeds. An important component of the safety assessment of BPS-CV127-9 is to demonstrate that the nutrient composition of any consumed component is similar to that of the isoline control variety as well as other conventional commercial soybean varieties, thereby confirming that BPS-CV127-9 is appropriate for use as conventional soybean food and feed products. Therefore, proximates, and fiber (crude, acid and neutral detergent fibers) were quantitated in BPS-CV127-9 forage, and compared with that found in the isoline, nontransgenic, conventional soybean control and two other commercial conventional varieties. The forage was produced from plants grown in replicated field trials at six locations in Brazil during the summer of 2007/2008. The field trials were conducted at sites located near Santo Antonio de Posse, Uberaba, Londrina, Brasilia, Santo Antonio de Goias, and Sete Lagoas. Analysis of the proximates and fiber found in forage showed that BPS-CV127-9 is compositionally equivalent to the isoline control, and comparable to the other conventional soybean varieties included in the study.

In summary, these compositional analyses demonstrate that the introduction of the *ahas* gene from *Arabidopsis thaliana* into the soybean genome, together with treatment by imidazolinone herbicide on BPS-CV127-9 does not impact the nutritional composition of BPS-CV127-9 forage. Results of these analyses demonstrate that forage from BPS-CV127-9 is compositionally equivalent to, and as nutritious as, forage from the isoline control as well as other conventional soybean varieties.

INTRODUCTION

Soybean (*Glycine max* L.) plants have been developed that are tolerant to the imidazolinone class of agricultural herbicides. The herbicide-tolerant soybean plants, referred to as BPS-CV127-9, were produced by introduction of an imidazolinone-tolerant acetohydroxyacid synthase large subunit (*ahasl*) gene from *Arabidopsis thaliana* into the soybean plant genome via biolistics. Acetohydroxyacid synthase (AHAS) is a key enzyme in plants, bacteria, and fungi that is required for the biosynthesis of the branched-chain amino acids valine, leucine, and isoleucine. Herbicides of the imidazolinone class, function by binding near the active site of the catalytic AHAS large subunit, thereby preventing normal functioning of the enzyme (Pang *et al*., 2002). Several *ahas* genes encoding AHAS enzymes that are tolerant to imidazolinone herbicides have been discovered in plants through mutagenesis and selection and have been used to create imidazolinone-tolerant maize (*Zea mays* L.), rice (*Oryza sativa* L.), wheat (*Triticum aestivum* L.), oilseed rape (*Brassica napus* and *B. juncea* L.), and sunflower (*Helianthus annuus* L.). These crops were developed through mutagenesis, selection, and conventional breeding technologies and have been commercialized under the Clearfield® brand name since 1992. There are five single point mutations in *ahas* genes that have been found to result in tolerance to imidazolinones in plants (Tan *et al*., 2005). One of these, a mutation that results in a substitution of a serine residue with an asparagine at position 653 (relative to the AHAS enzyme from *Arabidopsis thaliana*), is known to result in tolerance to imidazolinone herbicides with no cross-tolerance to other AHAS inhibitors (Lee *et al*., 1999). The imidazolinone-tolerant AHAS large subunit *csr1-2* gene (Sathasivan *et al*., 1990) from *Arabidopsis thaliana* that has the S653N mutation was transformed into soybean (*Glycine max* L.) plants with the native *A. thaliana* promoter, to produce soybean plants that are tolerant to imidazolinone herbicides. This has led to the development of BPS-CV127-9 by BASF and Embrapa.

Soybean has many uses in animal and human nutrition. The main soybean product fed to animals is the defatted/toasted soybean meal (OECD, 2001). However, other components of the soybean plant, including the forage, are also fed to a limited extent to animals, primarily to cattle. Soybean forage is typically harvested between the time the plants reach the sixth node stage to the beginning of pod formation. Therefore, the purpose of the current study was to demonstrate that the forage of BPS-CV127-9, treated with an imidazolinone herbicide, is substantially equivalent in composition to forage from the isoline and other conventional soybean varieties, and that BPS-CV127-9 forage is appropriate for use in animal feed. The forage was produced from plants grown in replicated field trials at six locations in Brazil during the summer of 2007/2008. The components analyzed included: proximates (moisture, fat, ash, protein, carbohydrates and calories) and fiber (crude, acid and neutral detergent fibers). The results of analyses were subjected to statistical analysis and values for BPS-CV127-9 were compared to the isoline control and to two commercial conventional soybean varieties.

MATERIALS AND METHODS

Forage source. Imidazolinone treated BPS-CV127-9 plants (abbreviated as CV127+imi in the data Tables) together with the isoline control variety, and two other conventional soybean varieties [Monsoy 8001 (Std 1) and Coodetec 217 (Std 2), respectively] were grown at six locations in Brazil during the 2007/2008 growing season. The field trials were conducted at sites located near Santo Antonio de Posse, Uberaba, Londrina, Brasilia, Santo Antonio de Goias, and Sete Lagoas. The plants were grown under standard agronomic practices in a complete randomized block design with three replicate blocks per location. With the exception of the BPS-CV127-9 plants treated with the imidazolinone herbicide at 70 g/ai/ha, all other entries in the study were treated with Bentazon + Acifluorfen-sodium (commercial name Volt) at the rate of 1.0 liters/ha. Three plants at the full-flowering stage of development in each replicate plot were harvested by cutting the plants at the base of the stem near ground level. The three plants from each plot or treatment replicate were pooled together in a plastic bag and shipped on wet ice to the Instituto de Technologia de Alimentos (ITAL) in Campinas, Brazil, for compositional analysis. At ITAL, the three plants from each replicate plot were chopped and mixed to produce a composite sample. After partial drying and weighing the samples were reduced to a fine powder using a Waring blender. Samples were stored in air-tight glass bottles until analyzed. Results were recorded on a fresh weight (FW) basis and adjusted for moisture content to calculate the corresponding dry weight (DW) value. Statistical analysis was conducted using the dry weight data.

Analytical methods. *Ash.* The method used was based on AOAC International (2005) method 942.05. The sample was placed in an electric furnace at 550°C and ignited to drive off all volatile organic matter. The nonvolatile matter remaining was quantitated gravimetrically and calculated to determine percent ash. The limit of quantitation for this study was 0.1% FW.

Carbohydrates. The method used was based on the USDA Agriculture Handbook No. 8 (1963) method. The total carbohydrate level was calculated by difference using the fresh weight-derived data and the following equation: % carbohydrates = 100% - (% protein + % fat + % moisture + % ash). The limit of quantitation for this study was 0.1% FW.

Fat. The method used was ISO 1443. The sample was weighed into a beaker and 4M HCL was added. The extract was then evaporated, dried, and weighed. The fat was extracted from the dried preparation using petroleum ether. The limit of quantitation for this study was 0.1% FW.

Moisture. The method used was based on AOAC International (2005) methods Bc 2-49. The sample was dried in a vacuum oven at 95 - 100°C to a constant weight. The moisture weight loss was determined and converted to percent moisture. The limit of quantitation for this study was 0.1% FW.

Protein. The method used was based on AOAC International (2005) method 954.01. Nitrogenous compounds in the sample were reduced in the presence of boiling sulfuric acid and a $CuSO_4$ + K_2SO_4 + Se mixture to form ammonia. The acid digest was made alkaline. The ammonia was distilled and then titrated with a standard acid. The percent nitrogen was calculated and converted to protein using the factor 6.25. The limit of quantitation for this study was 0.100% FW.

Crude Fiber (CF). Crude fiber was measured as the loss on ignition of dried residue remaining after digestion of the sample with 1.25% solutions of sulfuric acid and sodium hydroxide according to the method of Beythien and Diemair (1963). The limit of quantitation for this study was 0.1 g/g FW sample.

Acid Detergent Fiber (ADF). The method was based on AOAC International (1975) method 7.057. The sample was placed in a fritted vessel and washed with an acidic boiling detergent solution that dissolved the protein, carbohydrate, and ash. After an acetone wash to remove the fats and pigments; the lignocellulose fraction was collected on the frit and quantitated gravimetrically. The limit of quantitation for this study was 0.1% FW.

Neutral Detergent Fiber (NDF). The method used for sample preparation was the AOAC International (1995) method 920.85. Analysis for NDF was performed on the samples using the method of Van Soest *et al*. (1991). Samples were placed in a fritted vessel and washed with a neutral boiling detergent solution that dissolved the protein, carbohydrate, enzyme, and ash. After an acetone wash to remove the fats and pigments, the hemicellulose, cellulose, and lignin fractions were collected on the frit and quantitated gravimetrically. The limit of quantitation for this study was 0.1% FW.

Statistical analysis. Analysis of variance was carried out on the data using SAS Version 9.1 (SAS Institute Inc., Cary, NC) following two procedures, the General Linear Model and the Mixed Model. With the exception of moisture content, all data were expressed on a dry weight basis for statistical analyses. Differences were assessed across location and by location. The model for across location:

y = variety + location + variety x location + block(location) + e
Random effects: location, variety x location, block(location).
Where y is the response variable (any analyte measured)

Contrasts were carried out to compare each of the conventional varieties with the BPS-CV127-9 + imi treatment. Differences were considered statistically significant at the 0.05 confidence level.

RESULTS AND DISCUSSION

Proximate composition. Forage samples were analyzed for moisture, crude fat, protein, and ash. The carbohydrate composition and calorie values were calculated. The results on a dry weight basis (except for moisture) are presented for each field location in Table 1, and data were also analyzed across locations and results presented in Table 3. The data show that there were no statistically significant differences in the moisture, content between forage from BPS-CV127-9 plants treated with imidazolinone (CV127+imi) and the isoline control or either conventional standard soybean variety at any location. Furthermore, there were no statistically significant differences in ash, protein, carbohydrates and calorie content between the CV127+imi treatment and the isoline control at any of the field test sites. Only in the case of forage fat content were statistically significant differences observed between the CV127+imi treatment and the isoline control. These differences were only observed at two field sites (Uberaba and Sete Lagoas), and in these cases the differences were inconsistent (CV127+imi treatment had a higher forage fat content than the isoline at Uberaba and a lower value at the Sete Lagoas site). When forage proximate composition of the CV127+imi treatment was compared to composition of the conventional standard soybean varieties, there were only scattered instances of statistically significant differences. For example, there were no statistically significant differences in the ash level of CV127+imi forage compared to levels in the conventional standard varieties at four of the locations, and only significant differences were observed at the Santo Antoinio de Goias and Uberaba field sites. Similarly, statistically significant differences in forage fat level were only observed between the CV127+imi treatment and the conventional standard soybean varieties at the Uberaba and Brasilia field sites, and these differences were not consistent between the two field sites. For three locations, the protein level in one of the conventional standard soybean varieties was statistically significantly different from the CV127+imi treatment at the specific location. For two locations the carbohydrate levels were statistically significantly lower in one or both of the conventional standard soybean variety forage than the CV127+imi forage, and the calorie levels were different in one or both of the conventional standard varieties at three locations, but not consistently higher or lower.

In summary, no forage proximate component of the isoline control or the conventional standard soybean varieties was consistently statistically significantly different at all locations from the CV127+imi forage. The forage proximate composition of the CV127+imi treatment was equivalent to that of the isoline control and comparable to the composition of the conventional standard soybean varieties. In the few instances where differences were observed between the CV127+imi treatment and the conventional standard soybean varieties, these were most likely due to germplasm differences. When the proximate levels were evaluated across locations, the forage proximate composition of CV127+imi treatment was never statistically significantly different from that of the isoline control and only occasionally from either of the two conventional standard soybean varieties (Table 3). Finally, forage proximate levels of the CV127+imi treatment were comparable to published values for soybean forage (OECD, 2001) which are presented in Table 4. Specific differences are most likely due to characteristics of the BPS-CV127-9 variety, adapted for Brazilian growing conditions, compared to the adaptation characteristics of the soybean varieties included in the OECD report. These results indicate that forage prepared from BPS-CV127-9 does not differ significantly in its proximate composition from its isoline control, and is comparable to other conventional soybean varieties.

Fiber composition. Forage samples were analyzed for crude fiber (CF), acid detergent fiber (ADF) and neutral detergent fiber (NDF). Results are shown on a dry weight basis and are presented for each field location in Table 2, and data were also analyzed across locations and results presented in Table 3. The data show that there were no statistically significant differences in levels of any of the fiber fractions between forage from BPS-CV127-9 plants treated with imidazolinone (CV127+imi) and the isoline control at any field location (Table 2). When forage fiber composition of the CV127+imi treatment was compared to that of the conventional standard soybean varieties, there were only scattered instances of statistically significant differences. For example, there were no statistically significant differences in forage crude fiber content between the CV127+imi treatment and the conventional standard 2 soybean variety at any of the field locations. Statistically significant differences were only observed between CV127+imi and conventional standard 1 soybean variety for this analyte at two field locations. Similarly, for both forage ADF and NDF, there were no statistically significant differences between CV127+imi and the conventional standard soybean varieties at five of the six field locations, and significant differences were only observed at the Brasilia field site. When the fiber levels were evaluated across locations, forage fiber in the CV127+imi treatment was never statistically significantly different from that of the isoline control and only in the case of crude fiber was there a significant difference from the conventional standard 1 soybean variety (Table 3). Finally, forage ADF and NDF levels of the CV127+imi treatment were comparable to published values for soybean forage (OECD, 2001) which are presented in Table 4. Specific differences are most likely due to characteristics of the BPS-CV127-9 variety, adapted for Brazilian growing conditions, compared to the soybean varieties included in the OECD report. In summary, the forage fiber content of CV127+imi was never statistically significantly different from its isoline control and in most cases, with only the few exceptions described above, were the values statistically significantly different from any of the conventional standard soybean varieties. These results show that forage fiber produced by BPS-CV127-9, treated with imidazolinone herbicide, is equivalent to the isoline control and in the same range as the fiber of forage from conventional soybean varieties with a history of safe food and feed uses as well as safety to the environment.

CONCLUSION

Soybean has many uses in animal and human nutrition. The grain is typically processed into two commodity products, oil and meal. The defatted toasted meal is commonly used in livestock feed. The various soybean protein fractions derived from processing nontoasted defatted soybean meal is used in different human foods. Also, the soybean oil is used in different food products including cooking oil and salad dressings. The forage is occasionally used as animal feed. An important component of the safety assessment of BPS-CV127-9 is to demonstrate that the nutrient composition of the forage used in animal feed is equivalent to that of the isoline control variety as well as other conventional commercial soybean varieties, thereby confirming that BPS-CV127-9 grain is appropriate for use in soybean feed products including forage. Therefore, proximates and fiber (crude, acid and neutral detergent fibers) were quantitated in BPS-CV127-9 forage, and compared with that found in the isoline soybean control and two other commercial conventional soybean varieties adapted for commercial production in Brazil. The analytes measured are important nutrient components of soybean forage. Results of these compositional analyses showed that BPS-CV127-9 forage is compositionally equivalent to the isoline control and in general comparable to the other two conventional soybean varieties examined in this study, as well as to other commercial conventional soybean varieties.

In summary, these compositional analyses demonstrate that the introduction of the *ahas* gene from *Arabidopsis thaliana* into the soybean genome, together with treatment by imidazolinone herbicide on BPS-CV127-9 does not impact the nutritional composition of forage produced by BPS-CV127-9. Results of these analyses demonstrate that grain from BPS-CV127-9 is compositionally equivalent to, and as nutritious as, forage from the isoline control as well as other conventional soybean varieties.

RECORDS RETENTION: Raw data, the original copy of this report, and other relevant records are archived at BASF, 26 Davis Drive, Research Triangle Park, NC, USA 27709.

STUDY PERSONNEL: Statistical analysis work reported herein conducted by [redacted], Ph.D., BASF Plant Science, LLC, Research Triangle Park, NC 27705.

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Table 1. Proximate Composition of Forage of Soybean BPS-CV 127-9 (CV127 + Imi), the Isoline Control and Two Conventional Soybean Varieties (Std 1 and Std 2), Grown at Six Locations in Brazil in the 2007/2008 Season

Location	Line	N	Moisture	Ash	Fat	Protein	Carbohydrates	Calories
			g/100g FW	g/100g DW				kcal/100g DW
			mean ± standard deviation (range)					
Santo Antonio de Goias	Isoline	3	82.0 ± 1.0 (81.2 – 83.1)	9.1 ± 0.9 (8.2 – 9.9)	2.3 ± 0.6 (1.7 – 2.7)	17.9 ± 0.4 (17.6 – 18.3)	70.8 ± 1.0 (69.8 – 71.8)	375 ± 8 (367 – 383
	CV127 + imi	3	81.8 ± 0.8 (81.1 – 82.7)	9.3 ± 1.1 (8.3 – 10.4)	3.0 ± 0.2 (2.7 – 3.1)	17.9 ± 1.3 (16.5 – 19.0)	69.7 ± 0.9 (68.8 – 70.5)	380 ± 4 (376 – 383)
	Std 1	3	82.9 ± 0.7 (82.2 – 83.5)	10.9 ± 0.3* (10.6 – 11.2)	2.7 ± 0.3 (2.5 – 3.1)	21.3 ± 3.2* (17.6 – 23.1)	65.1 ± 3.6* (62.4 – 69.2)	370 ± 2* (368 – 371)
	Std 2	3	82.0 ± 1.3 (80.9 – 83.5)	10.2 ± 0.4 (9.7 – 10.5)	2.6 ± 0.4 (2.3 – 3.0)	19.3 ± 1.4 (17.9 – 20.7)	68.2 ± 2.1 (66.1 – 70.2)	375 ± 3 (372 – 377)
Uberaba	Isoline	3	79.8 ± 0.8 (79.0 – 80.6)	7.9 ± 0.7 (7.1 – 8.5)	2.5 ± 0.3* (2.1 – 2.7)	18.4 ± 0.2 (18.3 – 18.6)	71.3 ± 0.6 (70.8 – 71.9)	381 ± 5 (376 – 386)
	CV127 + imi	3	79.1 ± 0.3 (78.8 – 79.3)	7.3 ± 0.3 (7.0 – 7.5)	3.1 ± 0.3 (2.9 – 3.4)	17.5 ± 1.1 (16.6 – 18.7)	72.0 ± 1.4 (70.3 – 72.9)	385 ± 2 (383 – 387)
	Std 1	3	80.0 ± 1.7 (78.4 – 81.8)	8.4 ± 0.9* (7.6 – 9.3)	2.5 ± 0.2* (2.3 – 2.7)	18.6 ± 0.3 (18.3 – 18.9)	70.5 ± 0.6 (69.8 – 70.9)	378 ± 2 (376 – 380)
	Std 2	3	80.1 ± 0.7 (79.3 – 80.5)	8.4 ± 0.1 (8.3 – 8.5)	2.3 ± 0.2* (2.1 – 2.5)	18.8 ± 0.5* (18.4 – 19.3)	70.6 ± 0.5 (70.0 – 70.9)	376 ± 5* (372 – 382)
Sete Lagoas	Isoline	3	80.8 ± 0.7 (80.2 – 81.6)	8.1 ± 0.4 (7.6 – 8.3)	2.4 ± 0.3* (2.1 – 2.7)	17.9 ± 0.5 (17.5 – 18.5)	71.9 ± 0.8 (71.2 – 72.7)	380 ± 1 (379 – 381)
	CV127 + imi	3	79.4 ± 1.1 (78.4 – 80.5)	8.6 ± 0.2 (8.4 – 8.8)	1.9 ± 0.1 (1.9 – 2.0)	17.3 ± 0.4 (16.9 – 17.7)	72.2 ± 0.4 (71.8 – 72.6)	376 ± 2 (375 – 379)
	Std 1	3	81.7 ± 2.3 (79.2 – 83.7)	8.5 ± 0.4 (8.0 – 8.7)	2.2 ± 0.3 (1.9 – 2.4)	19.3 ± 0.6 (18.6 – 19.7)	70.0 ± 0.7 (69.3 – 70.7)	378 ± 4 (373 – 380)
	Std 2	3	77.9 ± 4.6 (73.1 – 82.2)	8.3 ± 1.3 (6.8 – 9.1)	2.2 ± 0.2 (1.9 – 2.3)	19.4 ± 2.1 (17.0 – 20.7)	70.1 ± 2.9 (68.4 – 73.5)	379 ± 6 (375 – 386)

Table 1. Continued

Location	Line	N	Moisture	Ash	Fat	Protein	Carbohydrates	Calories
			g/100g FW	g/100g DW				kcal/100g DW
			mean ± standard deviation (range)					
Londrina	Isoline	3	80.7 ± 0.7 (80.1 – 81.4)	8.2 ± 0.5 (7.8 – 8.8)	2.0 ± 0.3 (1.7 – 2.2)	16.2 ± 0.9 (15.1 – 16.9)	71.3 ± 1.6 (70.1 – 73.1)	374 ± 3 (371 – 376)
	CV127 + imi	3	81.2 ± 1.1 (80.3 – 82.4)	8.8 ± 1.1 (7.6 – 9.8)	2.3 ± 0.5 (1.9 – 2.8)	17.0 ± 2.2 (15.3 – 19.5)	73.4 ± 2.7 (70.3 – 75.6)	377 ± 8 (371 – 386)
	Std 1	3	81.8 ± 0.6 (81.3 – 82.5)	8.4 ± 1.8 (6.4 – 9.7)	2.2 ± 0.2 (2.0 – 2.4)	17.5 ± 1.4 (15.9 – 18.7)	72.5 ± 3.5 (68.4 – 74.9)	377 ± 5 (372 – 381)
	Std 2	3	82.4 ± 1.1 (81.7 – 83.7)	9.1 ± 0.2 (8.9 – 9.3)	2.1 ± 0.1 (2.0 – 2.2)	17.8 ± 0.5 (17.3 – 18.3)	71.5 ± 0.5 (71.0 – 71.8)	377 ± 3 (374 – 380)
Brasilia	Isoline	3	81.9 ± 1.1 (80.9 – 83.1)	9.2 ± 1.1 (8.1 – 10.3)	3.4 ± 0.2 (3.2 – 3.5)	18.8 ± 1.0 (17.6 – 19.5)	68.5 ± 2.2 (66.9 – 71.0)	381 ± 2 (379 – 383)
	CV127 + imi	3	81.4 ± 1.1 (80.7 – 82.6)	9.3 ± 1.2 (8.6 – 10.7)	3.3 ± 0.1 (3.2 – 3.4)	17.4 ± 1.4 (16.6 – 19.0)	70.0 ± 2.9 (66.7 – 71.9)	377 ± 3 (374 – 380)
	Std 1	3	82.1 ± 0.3 (81.8 – 82.3)	8.7 ± 0.6 (8.1 – 9.3)	3.4 ± 0.1 (3.4 – 3.5)	19.3 ± 1.8 (17.6 – 21.1)	68.6 ± 2.4 (65.9 – 70.6)	383 ± 3* (380 – 385)
	Std 2	3	81.9 ± 0.5 (81.4 – 82.4)	9.0 ± 0.3 (8.7 – 9.2)	3.8 ± 0.3* (3.6 – 4.1)	20.5 ± 1.6* (19.5 – 22.4)	66.8 ± 1.8 (64.8 – 68.3)	387 ± 1* (386 – 387)
Santo Antonio de Posse	Isoline	3	83.3 ± 0.9 (82.7 – 84.3)	8.6 ± 0.4 (8.2 – 8.9)	2.2 ± 0.1 (2.2 – 2.3)	16.9 ± 0.4 (16.5 – 17.3)	72.4 ± 0.5 (71.8 – 72.8)	378 ± 3 (376 – 382)
	CV127 + imi	3	83.6 ± 1.5 (82.5 – 85.3)	8.6 ± 0.3 (8.4 – 8.9)	2.1 ± 0.4 (1.6 – 2.4)	16.7 ± 1.7 (15.3 – 18.6)	72.5 ± 1.6 (70.9 – 74.1)	377 ± 4 (374 – 381)
	Std 1	3	85.0 ± 0.3 (84.8 – 85.3)	9.2 ± 0.3 (8.9 – 9.4)	2.3 ± 0.2 (2.2 – 2.5)	18.1 ± 0.3 (17.8 – 18.3)	70.4 ± 0.3* (70.1 – 70.7)	374 ± 1 (373 – 375)
	Std 2	3	84.0 ± 0.3 (83.7 – 84.2)	8.9 ± 0.2 (8.8 – 9.1)	1.9 ± 0.3 (1.7 – 2.2)	18.6 ± 0.8 (17.7 – 19.1)	70.4 ± 0.7* (69.8 – 71.2)	375 ± 2 (373 – 377)

*Statistically significantly different from CV127 + imi at $p < 0.05$.

Table 2. Fiber Composition of Forage of Soybean BPS-CV127-9 (CV127 + imi), the Isoline Control and Two Conventional Soybean Varieties (Std 1 and Std 2), Grown at Six Locations in Brazil in the 2007/2008 Season

Location	Line	N	Crude Fiber	ADF	NDF
			mean ± standard deviation (range) g/100 g DW		
Santo Antonio de Goias	Isoline	3	29.0 ± 0.9 (28.4 – 30.0)	34.87 ± 0.81 (34.04 – 35.65)	44.36 ± 3.25 (40.61 – 46.31)
	CV127 + imi	3	29.7 ± 0.6 (29.1 – 30.3)	33.66 ± 0.27 (33.39 – 33.92)	41.32 ± 1.27 (40.07 – 42.60)
	Std 1	3^	26.9 ± 2.6* (25.1 – 29.9)	32.33 ± 1.92 (30.97 – 33.69)	42.56 ± 3.79 (39.88 – 45.24)
	Std 2	3^	28.8 ± 1.3 (27.9 – 30.3)	33.45 ± 0.28 (33.25 – 33.65)	44.67 ± 0.92 (44.02 – 45.32)
Uberaba	Isoline	3	30.4 ± 1.4 (30.3 – 31.9)	36.93 ± 1.42 (35.56 – 38.39)	46.40 ± 1.89 (44.88 – 48.52)
	CV127 + imi	3	29.6 ± 1.1 (28.6 – 30.7)	35.44 ± 1.68 (33.50 – 36.47)	46.30 ± 1.93 (44.19 – 47.96)
	Std 1	3	30.1 ± 1.1 (29.4 – 31.4)	36.72 ± 1.23 (35.51 – 37.97)	44.66 ± 1.70 (42.94 – 46.33)
	Std 2	3	30.5 ± 0.6 (29.9 – 31.0)	36.05 ± 1.31 (34.65 – 37.25)	44.88 ± 0.23 (44.67 – 45.13)
Sete Lagoas	Isoline	3	28.7 ± 1.1 (27.5 – 29.7)	35.40 ± 1.15 (34.33 – 36.62)	44.77 ± 1.84 (42.69 – 46.20)
	CV127 + imi	3	28.7 ± 0.7 (27.9 – 29.1)	35.03 ± 1.24 (34.13 – 36.45)	44.56 ± 1.75 (43.11 – 46.50)
	Std 1	3	27.0 ± 0.2* (26.8 – 27.2)	35.76 ± 1.28 (34.29 – 36.66)	43.74 ± 1.71 (42.11 – 45.52)
	Std 2	3	28.8 ± 0.6 (28.3 – 29.5)	35.34 ± 0.73 (34.91 – 36.18)	43.84 ± 1.73 (42.13 – 45.58)
Londrina	Isoline	3	29.4 ± 1.6 (27.6 – 30.4)	35.91 ± 2.70 (34.34 – 39.03)	44.70 ± 3.37 (42.21 – 48.54)
	CV127 + imi	3	30.2 ± 0.9 (29.2 – 30.8)	37.26 ± 1.09 (36.08 – 38.23)	45.31 ± 0.40 (44.84 – 45.56)
	Std 1	3	29.1 ± 2.5 (26.3 – 30.6)	36.71 ± 3.93 (32.31 – 39.88)	44.78 ± 4.85 (39.33 – 48.61)
	Std 2	3	29.7 ± 1.0 (28.6 – 30.5)	36.83 ± 2.03 (35.48 – 39.17)	45.44 ± 1.79 (43.79 – 47.34)
Brasilia	Isoline	3	28.3 ± 0.4 (27.9 – 28.6)	35.08 ± 2.85 (31.79 – 36.82)	42.49 ± 2.77 (39.71 – 45.25)
	CV127 + imi	3	28.8 ± 0.6 (28.2 – 29.3)	35.97 ± 1.13 (34.75 – 36.97)	43.97 ± 0.53 (43.38 – 44.40)
	Std 1	3	27.6 ± 0.6 (26.9 – 28.1)	31.00 ± 2.81* (28.94 – 34.20)	41.68 ± 1.80 (39.68 – 43.18)
	Std 2	3	27.8 ± 1.2 (27.0 – 29.1)	32.17 ± 1.97 (30.43 – 34.30)	40.28 ± 1.21* (38.93 – 41.28)
Santo Antonio de Posse	Isoline	3	32.6 ± 0.7 (31.9 – 33.2)	41.23 ± 1.50 (39.83 – 42.81)	48.99 ± 1.96 (46.99 – 50.90)
	CV127 + imi	3	31.9 ± 1.2 (30.6 – 32.7)	41.07 ± 1.31 (39.62 – 42.16)	50.69 ± 2.18 (48.49 – 52.85)
	Std 1	3	31.8 ± 0.4 (31.6 – 32.3)	40.45 ± 2.25 (37.86 – 41.82)	48.66 ± 2.29 (46.16 – 50.67)
	Std 2	3	32.2 ± 1.3 (30.8 – 33.1)	41.33 ± 3.00 (38.67 – 44.58)	48.95 ± 2.88 (47.26 – 52.28)

*Statistically significantly different from CV 127 + imi at $p < 0.05$. ^ N= 2 for ADF and NDF.

Table 3. Proximate and Fiber Composition of Soybean Forage, comparing CV127 + imi to the Isoline Control and Two Conventional Soybean Varieties (Std 1 and Std 2) Across Six Locations in Brazil in the 2007/2008 Season

Analyte/Unit	Isoline	CV127 + imi	Std 1	Std 2
	N=18**			
Proximates	mean (range)			
Moisture g/100g FW	81.4ab (79.0 – 84.3)	81.1b (78.4 – 85.3)	82.3a (78.4 – 85.3)	81.4a (73.1 – 84.2)
Ash g/100g DW	8.5c (7.1 – 10.3)	8.6 bc (7.0 – 10.7)	9.0a (6.4 – 11.2)	9.0ab (6.8 – 10.5)
Fat g/100g DW	2.5a (1.7 – 3.5)	2.6 a (1.6 – 3.4)	2.6 a (1.9 – 3.5)	2.5 a (1.7 – 4.1)
Protein g/100g DW	17.7b (15.1 – 19.5)	17.3b (15.3 – 19.5)	19.0a (15.9 – 23.1)	19.1a (17.0 – 22.4)
Carbohydrates g/100g DW	71.0a (66.9 – 73.1)	71.6a (66.7 – 75.6)	69.5b (62.4 – 74.9)	69.6b (64.8 – 73.5)
Calories kcal/100gDW	378a (367 – 386)	379a (371 – 387)	377a (368 – 385)	378a (372 – 387)
Fiber				
Crude Fiber g/100g DW	29.8a (27.5 – 33.2)	29.8a (27.9 – 32.7)	28.8b (25.1 – 32.3)	29.6a (27.0 – 33.1)
ADF g/100g DW	36.57a (31.79 – 42.81)	36.41a (33.39 – 42.16)	35.68a (28.94 – 41.82)	36.00a (30.43 – 44.58)
NDF g/100g DW	45.28a (39.71 – 50.90)	45.36a (40.07 – 52.85)	44.45a (39.33 – 50.67)	44.68a (38.93 – 52.28)

*Numbers followed by the same letter are not statistically significantly different at $p < 0.05$.
**N=17 for Std 1 and Std 2 for ADF and NDF

Table 4. Proximate and Fiber Content of Soybean Forage (% of Dry Matter) as Reported in OECD (2001).

Nutrient	Soybean Forage
Moisture	74 - 79
Protein	11.2 - 17.3
Fat	3.1 - 5.1
Ash	8.8 - 10.5
NDF	34 - 40
ADF	32 - 38