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Genotypic difference in physico-chemical grain composition of three species of grain amaranth selected for the Brazilian Savannah

Diferença genotípica na composição físico-química de grãos de três espécies de amaranto de grãos selecionadas para o Cerrado Brasileiro

Diferencia genotípica en la composición físico-química del grano de tres especies de amaranto en grano seleccionadas para la Sabana Brasileña

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Andréia Alves Rosa
Master's Student in Agribusiness
Institution: Universidade de Brasília (UNB)
Address: Campus Darcy Ribeiro, Brasília – DF, CEP: 70910-900
E-mail: arrosalves@gmail.com

Juliana Evangelista da Silva Rocha
Doctor in Agronomy, Phytotechnology
Institution: Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA)
Address: Parque Estação Biológica, s/n, Asa Norte, Brasília – DF,
CEP: 71825-300
E-mail: juliana.evangelista@embrapa.br

Artur Guerra Rosa
Master's Student in Agribusiness
Institution: Universidade de Brasília (UNB)
Address: Campus Darcy Ribeiro, Brasília – DF, CEP: 70910-900
E-mail: arturguerra921@hotmail.com

Carlos Roberto Spehar
Doctor in Genetic Improvement, Plant Nutrition
Institution: Universidade de Brasília (UNB)
Address: Campus Darcy Ribeiro, Brasília – DF, CEP: 70910-900
E-mail: spehar@unb.br

ABSTRACT

Seventeen genotypes, representing three species of grain amaranth, were analyzed for physico-chemical components. These genotypes are considered outstanding in terms of agronomical performance, necessitating an understanding of protein, lipids, fiber, and carbohydrate content. It has been demonstrated that *A. cruentus, A. hypochondriacus*, and *A. caudatus* differ in the content and properties of these compounds. Based on field experiments conducted in the Brazilian Savannahs, these genotypes were initially tested for germination and vigor after 12 years of storage under low moisture conditions at 10°C in air-tight plastic bags. Assuming that the physico-chemical characteristics were maintained, as indicated by an average 80% germination rate, samples of amaranth seeds were milled and used for analysis. The results indicate protein content below expectations, with the highest values above 14g per 100g, while lipids, fiber, and carbohydrates are within the expected range. The possible factors to explain these results are related to the cultivation environment, although genotypic differences are also significant. Additional testing with broad-based selected lines is necessary to improve selection for both agronomic and physico-chemical composition. The present results suggest that efforts should be concentrated on *A. cruentus* and *A. hypochondriacus*.

Keywords: agronomic performance, selection, protein, lipid, fiber, carbohydrates.

RESUMO

Foram analisados dezessete genótipos pertencentes a três espécies de amaranto graneleiro quanto aos seus componentes físico-químicos. Esses genótipos representam os melhores em termos de características agronômicas, para as quais é necessário conhecer o conteúdo de proteínas, lipídios, fibras e carboidratos. Foi demonstrado que *A. cruentus*, *A. hypochondriacus* e *A. caudatus* diferem no conteúdo e nas propriedades desses compostos. Com base em experimentos de campo realizados nas savanas brasileiras, esses genótipos foram inicialmente testados quanto à germinação e vigor após 12 anos de armazenamento em baixa umidade e temperatura de 10º C em bolsas de plástico herméticas. Pressupondo que as características físico-químicas originais foram mantidas, expressas por uma taxa média de germinação de 80%, amostras de sementes assim mantidas foram moídas e utilizadas na análise. Os resultados indicam um teor de proteína abaixo do esperado, com valores mais altos ultrapassando os 14g por 100g, enquanto os lipídios, fibras e carboidratos estão dentro da faixa esperada. Os possíveis fatores que explicam esses resultados estão relacionados ao ambiente de cultivo, embora as diferenças genotípicas também sejam significativas. Testes adicionais com linhagens selecionadas de ampla base genética são necessários para avançar na seleção, visando melhorar o desempenho agronômico associado a características físico-químicas desejáveis. Os resultados sugerem que os esforços devem ser concentrados em *A. cruentus* e *A. hypochondriacus*.

Palavras-chave: desempenho agronômico, seleção, proteínas, lipídios, fibras, carboidratos.

RESUMEN

Se analizaron los componentes fisicoquímicos de diecisiete genotipos que representan tres especies de amaranto en grano. Estos genotipos se consideran sobresalientes en términos de rendimiento agronómico, por lo que es necesario conocer su contenido de proteínas, lípidos, fibra y carbohidratos. Se ha demostrado que A. cruentus, A. hypochondriacus y A. caudatus difieren en el contenido y las propiedades de estos compuestos. Basándose en experimentos de campo realizados en las sabanas brasileñas, estos genotipos se sometieron inicialmente a pruebas de germinación y vigor tras 12 años de almacenamiento en condiciones de baja humedad a 10°C en bolsas de plástico herméticas. Suponiendo que se mantuvieran las características fisicoquímicas, como indica un índice medio de germinación del 80%, se molieron muestras de semillas de amaranto y se utilizaron para el análisis. Los resultados indican un contenido de proteínas por debajo de lo esperado, con los valores más altos por encima de 14 g por 100 g, mientras que los lípidos, la fibra y los hidratos de carbono están dentro de lo esperado. Los posibles factores que explican estos resultados están relacionados con el medio de cultivo, aunque las diferencias genotípicas también son significativas. Es necesario realizar pruebas adicionales con líneas seleccionadas de base amplia para mejorar la selección tanto para la composición agronómica como para la fisicoquímica. Los presentes resultados sugieren que los esfuerzos deberían concentrarse en A. cruentus y A. hypochondriacus.

Palabras clave: rendimiento agronómico, selección, proteína, lípidos, fibra, carbohidratos.

1 INTRODUCTION

Grain amaranth has been cultivated in the Americas for the past 6,000 years (Spehar *et al*., 2007a). While its integration into agricultural systems is a recent development, growth and consumption in Brazil have been limited, despite the nutritional properties of both the grain and the whole plant (Almeida & Sá, 2009; Bressiani *et al*., 1992). The potential adaptation of this crop for commercial production in the Brazilian savannah is expected to contribute to increased supply and regulation of amaranth product prices, potentially popularizing them worldwide (Spehar, 2009).

The organic-mineral composition of amaranth, especially the biological value of its protein, positions it as an alternative for both food and animal feed, offering advantages over cereals and leguminous species (Bressiani *et al*., 1992; Spehar *et al*., 2007b). The grains are nutritious, featuring naturally balanced proteins, high-quality lipids, starch with suitable properties for use in processed foods, and providing fiber, minerals, and vitamins. This creates demand and opportunities for research, development, and production technology (Gorinstein *et al*., 2001; Martirosyan *et al*., 2007; Santos & Costa, 2007). Apart from the grains, the leaves can also be utilized in human diets, with the former being more common – traded as grain or processed into flour, flakes, cereal bars (Coelho, 2006), and biscuits (Marcílio *et al*., 2005).

The protein in amaranth grains exhibits a high proportion of essential amino acids, comparable to milk casein. Due to its gluten-free nature, grain amaranth is essential for individuals with celiac disease (Santos & Costa, 2007) and those seeking alternative diets to reduce cholesterol (Spehar *et al*., 2003).

Amaranth's lipid quality is considered high, especially when compared to other fat sources like soybeans. The grain contains tocotrienols and squalene compounds that affect cholesterol biosynthesis. Experiments with hypercholesterolemic patients using defatted amaranth have illustrated the role of proteins in ameliorating this condition (Chávez-Jáuregui *et al*., 2010). There is a need to better understand the functional activity of lipids and proteins in amaranth, which are present in relatively higher proportions than in maize (Spehar *et al*., 2007b).

It is worthwhile to compare Amaranthus species for the presence of non-glutinous and glutinous starches. In A. hypochondriacus, the granules consist of typical amylopectin, although amylase is also present (Becker *et al*., 1981), whereas in A. caudatus, starch was reported to be non-glutinous in comparison with glutinous A. cruentus. Compared to corn starch, A. cruentus and A. hypochondriacus starches had higher swelling power, lower solubility, greater water uptake, lower susceptibility to α-amylase, higher amylograph viscosity, and much lower amylose content (Stone & Lorenz, 1984). These properties allow for their utilization in processed frozen food as a stabilizing agent (Baker & Rayas-Duarte, 1998). Regarding fibers, grain amaranth is rich in digestible ones and contains considerably higher amounts of iron, calcium, and magnesium than cereals (Spehar *et al*., 2007b).

The most important grain amaranth species discussed here are A. cruentus, A. caudatus, and A. hypochondriacus, and research is ongoing in agronomy, nutrition, and processing in America, Asia, Africa, and Europe (Amaya-Farfan *et al*., 2005; Gimplinger *et al*., 2007). Experimentation across different environments indicates variation in the content of major organic compounds such as proteins, lipids, and carbohydrates, justifying the need for understanding these variations (Spehar *et al*., 2007b).

In Brazil, a partnership between Embrapa and the University of Brasília has resulted in the selection and recommendation of BRS Alegria, the first cultivar of A. cruentus for the savannahs (Spehar *et al*., 2003). It is currently the only option available for commercial production, limiting cultivation and processing. However, there are high-yield breeding lines under study, for which physical and organic compositions are not yet known.

This work aims to evaluate the differences in outstanding Brazilian-Savannah selected genotypes of the three grain amaranth species to support crop improvement and provide alternatives to producers and industries as sources of organic-mineral compounds.

2 MATERIAL AND METHODS

Experiments to evaluate agronomic genotypic performance of A. cruentus, A. caudatus and A. hypochondriacus were conducted in the Brazilian Savannah highlands, located at 15 – 16º S and 47 – 48º W, at altitudes of 900-1,000 m.a.s.l., in the winter, 1998 (Teixeira *et al*., 2003). At physiological maturity (when fruits in the panicle start shedding seeds), plants were harvested, the seeds cleaned and dried down to 12 g 100 g-1, being stored in a cold room at 8-10º C, in sealed plastic bags.

A sample of 100 g seed was collected from seventeen amaranth genotypes, of outstanding agronomic performance, being five of *A. hypochondriacus* (9, 11, 15, 16 and 17), three of *A. caudatus* (1, 2 and 6) and nine of *A. cruentus* (3, 4, 5, 7, 8, 10, 12, 13 and 14). These seeds had been kept in the cold room for 12 years, requiring assessment of biological quality, which was achieved by conducting a pilot test with seeds of all genotypes. These samples were employed in the physico-chemical analysis at the Food Analysis Laboratory of the Faculdade de Agronomia e Medicina Veterinária, Universidade de Brasília, Brasília, Brazil.

Initially the samples were ground in a TECNAL® mill, model TE 631 until reduced into flour, to homogenize and facilitate digestion and chemical reactions in the tests. The preparation for the analyses, followed the procedure provided by Becker *et al*., 1981. The following compounds were analyzed: moisture, dry matter, ash, protein, lipids, carbohydrates (Brasil, 2005) and fibers. Raw fiber was analyzed according to the international standard procedure (AOAC, 1980). Three replicates were used for each sample in the analysis.

Moisture in the samples was determined by the use of a Marconi® ventilated oven, model MA 037, and set at 105 º C, for 24 h, until reaching constant weight. Dry matter was calculated by the ratio of oven-dried sample to the one before drying, while ash was obtained by taking ± 1,0000g of seeds which were placed into crucibles inside a Linn Elektron Therm® furnace muffle, model KK 260, set to 600°C, for 4 h, until full combustion of organic matter.

In protein determination, a Kjeldahl and Tecnal® N-digesting and steel block, model TE 036/1, was used to assess the proportion of N. To calculate crude protein, N value was multiplied by 5.85, according to recommended by Becker *et al*., 1981.

Raw fiber was determined by Marconi® equipment, model MA 444-CI. The rate was calculated by digesting ± 1,0000g of milled sample in 1.25% w/v H2SO4 for 30 minutes, followed by addition of 1.25% w/v NaOH for additional 30 minutes.

The rates of lipids were obtained by n-hexane extraction, at 98ºC in a Tecnal®, model TE-044, Soxhlet extractor for 5 hours.

Carbohydrates were determined by deducting all other components from the total (100 %), i.e., lipid (%L), ash (%As), humidity (% H), fiber (%F) e protein (%P); thus, (CHO) = 100 – (%L + %As + %H + %F + %P).

The data for all genotypes, across and within species, were subject to analysis of variance and comparisons for treatment means were conducted by the Tukey test (p=0.5).

3 RESULTS AND DISCUSSION

The low moisture content in all samples (Tables 1-5) indicates that the seeds were kept in their original composition, despite the field experiment that originated them having been conducted 12 years earlier. To confirm this, pilot germination tests showed no effect of storage on seeds, as measured by germination and vigor (above 80%), providing information on long-term viability for grain amaranth. It is assumed that the association of low moisture and temperature maintained the amaranth seeds without deterioration that could be caused by pests and pathogens (Gimplinger *et al*., 2007; Spehar *et al*., 2007c). Given these considerations, the genotypic comparisons within and among species for seed composition were assumed to be valid.

Initially, all treatments for the three species are compared (Table 1). Then, comparisons can be made for genotypes within *Amaranthus cruentus* (Table 2), *Amaranthus caudatus* (Table 3), and *Amaranthus hypochondriacus* (Table 4); finally, comparisons of the mean value for each species allow for comparing the species for physico-chemical characteristics (Table 5).

The reported protein content in grain amaranth varies according to the species, in the range of 12% to 19% (Spehar *et al*., 2007b). *Amaranthus cruentus* had an average of 15.7%, followed by *Amaranthus hypochondriacus* and *Amaranthus caudatus*, with respective values of 15.5% and 13.5% (Bressani *et al*., 1992). In the present study, comparing all genotypes from the three species (Table 1), indicates 4 and 17 as the ones with values above 14%, the first belonging to *A. cruentus* while the second is a selection within *A. hypochondriacus*. There are several accessions with higher levels than BRS Alegria, the first cultivar for the savannahs. When the means over species are compared (Table 5), the superiority of *A. cruentus* and *A. hypochondriacus* is confirmed, being statistically different from *A. caudatus*.

The relatively lower expected values than the ones found elsewhere (Bressani *et al*., 1992; Gimplinger *et al*., 2007; Spehar *et al*., 2007a) might be related to winter (dry season) cultivation in the savannah. In a study with quinoa, recently re-classified as belonging to the Amaranthaceae family, differences in performance between summer and winter cultivation were found (Rocha *et al*., 2010), thus illustrating the environmental influence on the physico-chemical composition of these pseudocereals. Moreover, protein content in amaranth can also vary according to nitrogen availability and harvest time, diminishing when it is delayed, after plants reach physiological maturity (Spehar *et al*., 2003). Considering the field experiment was nitrogen-fertilized according to recommendations, and plants were harvested at physiological maturity, another factor to be considered is the genotype; the present sample is limited, especially for *A. hypochondriacus* and *A. caudatus*. It must be added that, depending on the conversion factor, the values might be over or underestimated, as in this case (Becker *et al*., 1981; Ascheri *et al*., 2004).

A similar line of thought applies to lipid and fiber content. For the three species, the average was within the range found in experiments of temperate climates, with some values being equivalent (Gimplinger *et al*., 2007). The main fraction of carbohydrates is made up of starch (Spehar *et al*., 2007b), and these results are superior to the 71.8% reported elsewhere (Nieto, 1990). This can be interpreted as the calculation procedure where the value is obtained by difference. Or, by default, smaller values for protein and lipids can be the cause of higher values for carbohydrates.

Overall, it can be said that selecting grain amaranth genotypes for full adaptation to the Brazilian Savannah must be complemented by physico-chemical evaluation (Spehar, 2007b). By analyzing a larger number of accessions, it is possible to identify the ones with desirable content. Considering that each species varies in compounds, emphasis must be placed on *A. cruentus* and *A. hypochondriacus*, based on agronomic performance and composition.

4 CONCLUSIONS

In selecting grain amaranth for cultivation in the savannahs, it is possible to achieve high protein content. However, it is noteworthy that BRS Alegria, the current cultivar for commercial production in the savannahs, may be replaced by other genotypes with superior agronomic performance and higher protein content. Additionally, it's essential to acknowledge that lipid and carbohydrate content can be influenced by selecting genotypes within the species where these components are more abundant. Among these, *A. cruentus* and *A. hypochondriacus* show promising adaptability to commercial production.

Furthermore, reflecting on how these findings can benefit society and academia, the research offers insights into improving crop selection and cultivation methods, potentially leading to higher nutritional yields and agricultural sustainability.

In terms of limitations, this research focused primarily on protein, lipid, and carbohydrate content, but further investigation could delve into other nutritional aspects or agronomic traits. Additionally, field trials and long-term studies are necessary to validate the adaptability and performance of the identified genotypes. Recommendations for future work include expanding the scope of research to encompass broader aspects of grain amaranth cultivation and continuing to explore new genotypes for enhanced productivity and nutritional value.

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APPENDIX 1

Table 1. Physico-chemical characteristics (g 100 g^{-1}) of 17 amaranth genotypes cultivated in the Brazilian Savannah highlands.

Genotype	Species	Moisture	Dry Matter	Ash	Protein	Lipíd	Fiber	Carbohydrate
1	*A. caudatus*	8,59 ^{B}	91,40 ^{G}	2,20 ^{EFG}	11,22 ^{EF}	6,28 ^{ABC}	3,69 ^{CD}	79,51 ^{AB}
2	*A. caudatus*	9,03 ^{B}	90,96 ^{G}	2,57 ^{DEF}	11,72 ^{DE}	6,28 ^{ABC}	5,56 ABC	78,61 ^{ABCD}
3	*A. cruentus*	9,06 ^{B}	90,94 ^{G}	0,27 ^{H}	12,98 ^{B}	5,26 ABCDE	6,88 ^{A}	80,58 ^{A}
4	*A. cruentus*	10,88 ^{A}	89,11 ^{H}	2,47 ^{DEF}	14,19 ^{A}	5,40 ABCDE	2,51 ^{D}	76,96 ^{DE}
BRS Alegria	*A. cruentus*	5,81 ^{EFGH}	94,19 ^{ABCD}	1,82 ^{FG}	12,57 ^{BCD}	4,49 ^{CDE}	7,52 ^{A}	80,24 ^{AB}
6	*A. caudatus*	5,28 ^{H}	94,71 ^{A}	2,80 ^{CDE}	11,97 ^{CDE}	4,58 ^{BCDE}	5,47 ABC	79,81 ^{AB}
7	*A. cruentus*	5,57 ^{FGH}	94,43 ^{ABC}	2,18 ^{EFG}	12,13 BCDE	6,67 ^{A}	4,43 BCD	78,18 ^{BCD}
8	*A. cruentus*	5,89 ^{DEFG}	94,10 ^{BCDE}	1,59 ^{G}	10,55 ^{F}	6,36 ^{AB}	5,20 ABC	80,76 ^{A}
9	*A. hypochondriacus*	5,90 ^{DEFG}	94,09 ^{BCDE}	3,14 ^{BCD}	11,81 ^{CDE}	4,95 ABCDE	3,98 ^{CD}	79,27 ^{AB}
10	*A. cruentus*	5,97 ^{DEF}	94,02 ^{CDE}	1,85 ^{FG}	11,92 ^{CDE}	6,06 ^{ABCD}	6,44 ^{AB}	79,33 ^{AB}
11	*A. hypochondriacus*	5,41 ^{FGH}	94,58 ^{ABC}	3,46 ^{BC}	11,68 ^{DE}	4,87 ABCDE	5,45 ABC	79,17 ^{ABC}
12	*A. cruentus*	5,37 ^{GH}	94,62 ^{AB}	3,51 ^{BC}	13,05 ^{B}	5,62 ABCDE	4,17 BCD	76,91 ^{DE}
13	*A. cruentus*	6.42 ^{CD}	93,58 ^{EF}	2,66 ^{CDEF}	12,65 ^{BCD}	5,18 ABCDE	4,16 BCD	78,62 ^{ABCD}
14	*A. cruentus*	6.55 ^{C}	93,45 ^{F}	3,24 ^{BCD}	12,74 ^{BC}	6,07 ^{ABCD}	7,53 ^{A}	77,05 ^{CDE}
15	*A. hypochondriacus*	5.97 ^{DEF}	94,02 ^{CDE}	5,9 ^{A}	12,55 ^{BCD}	4,04 ^{E}	4,11 BCD	76,64 ^{DE}
16	*A. hypochondriacus*	6.37 ^{CD}	93,62 ^{EF}	5,55 ^{A}	12,25 ^{BCD}	4,34 ^{DE}	7,38 ^{A}	77,01 ^{CDE}
17	*A. hypochondriacus*	6.28 ^{CDE}	93,71 ^{DEF}	3,79 ^{B}	14,59 ^{A}	4,72 ^{BCDE}	5,89 ABC	75,89 ^{E}

Source: Authors. Numbers followed by the same letter in each column do not show statistical difference (Tukey, p=0.05).

APPENDIX 2

Table 2. Physico-chemical characteristics (g 100 g^{-1}) of *Amaranthus cruentus* genotypes.

Genotype	Moisture	Dry Matter	Ash	Protein	Lipid	Fiber	Carbohydrate
3	9,06^{B}	90,94^{E}	0,27^{E}	12,98^{B}	5,26 ^{BC}	6,88^{AB}	80,58^{B}
4	10,88^{A}	89,11^{F}	2,47^{BCD}	14,19^{A}	5,40^{BC}	2,51^{D}	76,96^{H}
BRS Alegria	5,81^{EF}	94,19^{AB}	1,82^{CD}	12,57^{BCD}	4,49^{C}	7,52^{A}	80,24^{C}

Alegria							
7	5,57^{EF}	94,43^{AB}	2,18^{CD}	12,13^{CD}	6,67^{A}	4,43^{BCD}	78,18^{F}
8	5,89^{DEF}	94,10^{ABC}	1,59^{D}	10,55^{E}	6,36^{AB}	5,20^{ABC}	80,76^{A}
10	5,97^{CDE}	94,02^{CD}	1,85^{CD}	11,92^{D}	6,06^{AB}	6,44^{ABC}	79,33^{D}
12	5,37^{F}	94,62^{A}	3,51^{A}	13,05^{B}	5,62^{ABC}	4,17^{CD}	76,91^{I}
13	6.42C^{D}	93,58^{CD}	2,66^{ABC}	12,65^{BCD}	5,18^{BC}	4,16^{CD}	78,62^{E}
14	6.55^{C}	93,45^{D}	3,24^{AB}	12,74^{BC}	6,07^{AB}	7,53^{A}	77,05^{G}

Source: Authors. Numbers followed by the same letter in each column do not show statistical difference (Tukey, p=0.05).

APPENDIX 3

Table 3. Physico-chemical characteristics (g 100 g^{-1}) of *Amaranthus caudatus* genotypes.

Genotype	Moisture	Dry Matter	Ash	Protein	Lipid	Fiber	Carbohydrate
1	8,59^{A}	91,40^{B}	2,20^{A}	11,22^{A}	6,28^{A}	3,69^{A}	79,51^{B}
2	9,03^{A}	90,96^{B}	2,57^{AB}	11,72^{A}	6,28^{A}	5,56^{A}	78,61^{C}
6	5,28^{B}	94,71^{A}	2,80^{B}	11,97^{A}	4,58^{A}	5,47^{A}	79,81^{A}

Source: Authors. Numbers followed by the same letter in each column do not show statistical difference (Tukey, p=0.05).

APPENDIX 4

Table 4. Physico-chemical characteristics (g 100 g^{-1}) of *Amaranthus hypochondriacus* genotypes.

Genotype	Moisture	Dry Matter	Ash	Protein	Lipid	Fiber	Carbohydrate
9	5,90^{B}	94,09^{B}	3,14^{C}	11,81^{BC}	4,95^{A}	3,98^{C}	79,27^{A}
11	5,41^{C}	94,58^{A}	3,46^{BC}	11,68^{C}	4,87^{A}	5,45^{B}	79,17^{B}
15	5.97^{B}	94,02^{B}	5,9^{A}	12,55^{B}	4,04^{A}	4,11^{C}	76,64^{D}
16	6.37^{A}	93,62^{C}	5,55^{A}	12,25^{BC}	4,34^{A}	7,38^{A}	77,01^{C}
17	6.28^{A}	93,71^{C}	3,79^{B}	14,59^{A}	4,72^{A}	5,89^{B}	75,89^{E}

Source: Authors. Numbers followed by the same letter in each column do not show statistical difference (Tukey, p=0.05).

APPENDIX 5

Table 5. Physico-chemical characteristics (g 100 g^{-1}) for the three species of grain amaranth.

Species	Moisture	Dry Matter	Ash	Protein	Lipid	Fiber	Carbohydrate
A. caudatus	7,63^{A}	92,36^{B}	2,52^{B}	12,44^{B}	5,71^{A}	4,91^{A}	66,76^{A}
A. cruentus	6,83^{AB}	93,16^{AB}	2,17^{B}	13,39^{A}	5,68^{A}	5,42^{A}	66,47^{A}
A. hypochondriacus	5,99^{B}	94,00^{A}	4,37^{A}	13,44^{A}	4,58^{B}	5,36^{A}	66,24^{A}

Source: Authors. Numbers followed by the same letter in each column do not show statistical difference (Tukey, p=0.05).