Journal of Animal & Plant Sciences, 2018. Vol.38, Issue 1: 6138-6151
Publication date 31/10/2018, http://www.m.elewa.org/JAPS; ISSN 2071-7024

Integrated production factors and chemical-bromatological quality of *Brachiaria brizantha* cv. Marandu intercropped with *Stylosanthes* spp. under organic management

Luiz Henrique X. Silva[3], Eliane S. M. Okada[3], Euclides R. Oliveira[1], Andrea M. A. Gabriel[1], João Paulo G. Soares[2], Juaci V. Malaquias[2], Jefferson R. Gandra[1] , Elaine B. Muniz[4]

[1]*Universidade Federal da Grande Dourados- Faculdade de Ciências Agrarias. Dourados MS. Brazil*
[2]*EMBRAPA. Empresa Brasileira de Pesquisa Agropecuária. Embrapa Cerrados, Planaltina DF. Brazil*
[3]*Universidade Federal de Goiás. Escola de Medicina Veterinária e Zootecnia. Goiânia GO. Brazil*
[4]*Universidade Estadual do Oeste do Paraná Toledo- PR. Brazil*
Corresponding author: jeffersongandra@ufgd.edu.br

Keywords: tropical forages, multivariate analysis, organic farming, and plant variables.

1 ABSTRACT

This study aimed to evaluate by principal component analysis the relationship among chemical-bromatological composition, fractionation of carbohydrate and protein content, *in vitro* dry matter digestibility (IVDMD), and dry matter (DM) production of *Brachiaria brizantha* cv. Marandu intercropped with *Stylosanthes* spp. under organic and conventional fertilization. Forage chemical quality patterns were assessed for each fertilization management by multivariate analyses. The discriminatory capacity was higher for variables resulting from *B. brizantha* cv. Marandu analyses than from *Stylosanthes* spp. However, the expressiveness of these variables had a high similarity with multivariate analyses, mainly when comparing carbohydrate fractionation and IVDMD. In terms of chemical quality and production variables, a principal component analysis proved to be effective for evaluations of different fertilization management types, being more expressive for samples of *B. brizantha* cv. Marandu.

2 INTRODUCTION

Sustainable and bioeconomic livestock technologies are necessary/demanded for organic animal production systems and the forage growth response of tropical species to alternative fertilizers legally accepted for organic systems still is unknown in the Biome Cerrado (Figueiredo e Soares, 2012, Soares *et al*., 2016). These Technologies offers alternatives for organic meat and milk producers to maintain their system since no conventional input for pasture fertilization can be used in Brazil (Soares *et al*., 2014; Brasil, 2011). Forage farming systems must be characterized in terms of fodder quality and production. Therefore, based on plant productive and qualitative responses, the best system to be used can be chosen (Castro Neto *et al*. 2010). Even though there are several ways of assessing forages under different fertilization management types, a set of consistent and coherent factors must be properly adopted. Furthermore, an ideal data analysis method, capable of discriminating qualitative and productive characteristics, is also required for scientific evaluations. The complex correlation among the agricultural parameters of forages (nutritional value and dry matter production) can be understood through a multivariate procedure known as Principal Component Analysis (PCA). The use of multivariate analyses are required when assessing a high number of variables, through the single purpose is determine productive and qualitative forage responses. Overall, univariate analyses are able to evaluate each variable independently, while multivariate ones are able to assess a set of variables. In multivariate statistics, one of the methods used to interpret the structure of a data set from variance and covariance matrices or correlation between certain parameters is the PCA method. This study aimed to analyze the behavior of some variables related to chemical-bromatological composition, fractionation of carbohydrate and protein content, *in vitro* dry matter digestibility, and dry matter (DM) production of *Brachiaria brizantha* cv. Marandu intercropped with *Stylosanthes spp*. under organic and conventional fertilization using the PCA.

3 MATERIAL AND METHODS

Two pasture areas were implanted in an experimental field of Embrapa Cerrados, at Planaltina - DF, Brazil. In these areas, *Brachiaria brizantha* cv. Marandu plants were intercropped with a mix of *Stylosanthes* spp. cultivars (Mineirão, Campo Grande, and Bela), intercalated with rows of pigeon pea (*Cajanus cajan* cv. Mandarim) in different plots. This experiment lasted three years, and evaluation cuts were performed in the rainy and dry seasons of 2013 to 2015, and the plots were standardized after each cut. The soil of the experimental area was a Dystrophic Latosol with low fertility, showing the following chemical characteristics (0-20 cm depth range): pH = 5.8, Al = 0.04 cmol dm^{-3}, Ca + Mg = 1.54 cmolc dm^{-3}, P = 3.61, mg dm^{-3}, and K = 47 mg dm^{-3}. Three months before planting, the soil was corrected for acidity with 2 t ha^{-1} dolomitic limestone, 1 t ha^{-1} agricultural gypsum, and fertilization at planting according to previous analysis. Then, in a green manure area, *Crotalaria juncea* plants were cut and incorporated into the soil by leveling disk harrow, as a source of organic matter and nitrogen, quantifying production, bromatological composition, and incorporated nutrients. Before sowing, the areas under organic management were fertilized with poultry litter (1.5% N), thermopotash (6% K_2O), and thermophosphate (12% P_2O_5), as N, K, and P sources, applying 6.67, 2.00, and 1.00 t ha^{-1}, respectively. Yet, the areas under conventional system were fertilized, prior to sowing, applying potassium chloride (60% K_2O), triple superphosphate (46% P_2O_5), and urea (46% N) at the rates of 200, 260, and 217 kg ha^{-1}, respectively. No source of fertilizer was used for control treatment. Except for *Stylosanthes* spp., all legume and grass seeds were inoculated with diazotrophic bacteria and mycorrhizal fungi, respectively. Forages were cut once in the dry and another in the rainy seasons of each year (Table 1), cuts were made at 10 cm above the soil level using a 1 m^{-2} square frame at three different points inside if the plots. Then, these samples were weighed in the field and subjected to drying in a forced ventilation oven at 65 ºC for 72 hours. Afterwards, they were processed in a Wiley mill and conditioned in bottles for analysis. After this, the pre-dry matter contents were analyzed.

3.1 Soil chemical analyses: Seventy-two soil composite samples were evaluated for chemical properties in both forage cutting periods. These samples were compounded by 20 simple samples per plot, being one replication per plot and collected from 0-20 cm depth range. Chemical characterization followed the method proposed by Embrapa, (1979). The evaluated properties were pH and contents of organic matter (OM), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), aluminum (Al), potential acidity (H+Al), also calculating the values of cation exchange capacity (CEC) and base saturation (BS).

3.2 Chemical and bromatological analyses: Chemical analyses were performed on samples collected randomly using a 1m^2 square frame in two periods of the year; these samples were weighed in the field and then subjected to drying. The samples were dried in a forced-air drying oven (60 ºC for 72 hours) and processed in a Wiley mill, being then conditioned in bottles for the analysis of contents of dry matter (DM), organic matter (OM), crude protein (CP), and ether extract (EE) as described by the (AOAC, 1995). Neutral detergent fiber (NDF), acid detergent fiber (ADF), and lignin (LIG) were assessed as methods described by (Van Soest *et al.* 1991).

***3.3 In vitro* dry matter digestibility:** *In vitro* dry matter digestibility (IVDMD) was determined using rumen inoculum and buffer solution. The ruminal inoculum was obtained from two adult castrated rumen-cannulated Holstein cows (with 380 kg average weight). These animals were maintained in paddocks grown with *B. brizantha* cv. Marandu pasture and received mineral supplementation. Rumen-fluid samples were taken in the morning via ruminal cannula. Then, it was kept in a water bath at 39 °C and purging CO_2 before and after sampling. A total of two liters of rumen-liquid were collected, one per animal, including the solid fraction. The collected material was transferred to a preheated Thermos flask, previously purged with CO_2, and hermetically sealed. Afterwards, this material was filtered through four layers of cotton cloth (gauze) to be used in incubations (McDougal, 1949). Solutions A and B were mixed at a ratio 1: 5, reaching a pH of 6.8 at a constant temperature of 39 ºC, to which were added 40 mL buffer solution, as proposed by McDougal[14] and 10 mL rumen-liquid. Animal diets were evaluated for IVDMD following the method described by Tilley & Terry (1966) and modified by Goering and Van Soest, using an artificial rumen (Daisy-II Fermenter®, Ankom). Samples of 0.5 g were weighed in TNT bags (100 g/m), which were cut and sealed (5.0 x 5.0 cm), as in (Casali *et al.*, 2008). Two bags with no rumen samples were used (blank samples) in each jar for data collection. The sample bags were placed in the jars, evenly distributed. Then, buffer solution and ruminal inoculum were added, and CO_2 was applied to maintain anaerobic conditions. After this procedure, the jars remained in the artificial rumen [Daisy-II Fermenter® (Ankom)] at 39 ºC for 48 hours, with continuous stirring as proposed by Tilley & Terry (1966). Incubation was interrupted after 48 hours, and pepsin and hydrochloric acid were added to stop fermentation and microbial enzymatic activity. This step was taken to simulate the second part of digestibility for another 48 hours. After the end of the second stage, the sample bags were washed with tap water until total removal of digestibility residue, being then transferred to an oven at 105 °C for 12 hours. IVDMD was then determined by weight difference.

3.4 Carbohydrate fractioning: Total and non-fibrous carbohydrates (TC and NFC) were estimated according to Sniffen *et al.* (1992), by the formulas: TC = 100 - (%CP + %EE + %MM), and NFC = 100 - (%CP + %EE + %NDFap + MM), wherein NDFap is the neutral detergent fiber corrected for ashes and proteins and MM is the mineral matter. The fraction B2 was calculated by the difference of NDFap – Fraction C (Sniffen *et al.*, 1992) and the C by lignin percentage multiplied by 2.4, as in Russel *et al.* (1992). **3.5 Fractionation of nitrogen compounds:** The fractions of nitrogen compounds were determined by the following procedures: fraction A was obtained by treating the samples (0.5 g) in 50 mL distilled water for 30 minutes and subsequent addition of 10 mL 10% trichloroacetic acid (TCA) for another 30 minutes. Afterwards, it was filtered through filter paper (Whatman 54) for residual nitrogen determination. By the difference between total and residual nitrogen, the fraction A was obtained (Goering and Van Soest, 1970).
Total soluble nitrogen was determined by incubating the samples (0.5 g) with 50 mL borate-phosphate buffer solution ($NaH_2PO_4.H_2O$ at 12.2 g/L + $Na_2B_4O_7.10H_2O$

at 8.91 g/L + 100 mL/L tertiary butyl alcohol) and 1 mL sodium azide solution (at 10%). After 3 hours of incubation, the residual sample was filtered through filter paper, and then insoluble residual nitrogen analysis was performed in borate-phosphate buffer solution (BPB). Total soluble nitrogen (NNP + soluble protein + peptides) was obtained by the difference between total nitrogen and insoluble nitrogen in BPB. Soluble protein fraction (B1) was determined by the difference between total soluble nitrogen and NNP fraction determined by TCA. Fraction B3 was calculated by the difference between neutral detergent insoluble nitrogen (NDIN) and acid detergent insoluble nitrogen (ADIN) (Sniffen *et al*., 1992). Fraction C was determined by insoluble nitrogen in acid detergent. Fraction B2 was taken as the difference between insoluble fraction in the borate-phosphate buffer and the NDIN fraction. Neutral detergent insoluble protein (NDIP) and in acid detergent insoluble protein (ADIP) was determined by multiplying NDIN and ADIN by 6.25 (Krishnamoorth, 1992). All chemical and digestibility analyses were performed in the laboratory for Animal Nutrition located in the facilities of the Faculty of Agricultural Sciences, Federal University of Grande Dourados, in Dourados – MS, Brazil.

3.6 Statistics: Multivariate analyses were performed on bromatological, fractionation, digestibility, and production data, to verify chemical quality differentiation patterns in forages under different fertilization management. For this purpose, the data were standardized using PROC STANDARD procedure and then, subjected to a discriminant analysis by the PROC DISCRIM procedure. The PRINCOMP procedure (SAS Institute Inc., Cary, NC) was then used to perform the PCA of the values of chemical-bromatological quality for each fertilization management, based on a correlation matrix (Fine and Pousse, 1992). The correlation between fertilization managements and canonical variables was ascertained using the CANDISC procedure (SAS Institute Inc., Cary, NC).

4 RESULTS AND DISCUSSION

4.1 Chemical quality of B. brizantha cv. Marandu in green manure systems: The PCA showed different patterns for each forage property (chemical-bromatological quality, carbohydrate and protein fractionation, IVDMD, and DM production) under the different fertilization systems used (conventional, organic, and control). Figure 1 shows the PCA distribution of the above-mentioned variables with a cumulative variance of 43.67% for both principal components (PC 1 and PC 2). The influence of each variable on these axes is observed by its relationship with both of them. For PC 1 (abscissa), the variables that stand out are B2-protein fraction contents, NDIN, and NDF, while for PC 2 (ordinate) the significant properties were B2-carbohydrate fraction, MM, OM, C-protein fraction, and TC (in the two evaluated layers). The length of the arrows highlights the significance of each variable. Among them, B2 fractions of both carbohydrates and proteins showed moderate availability. In addition, the contents of non-fibrous carbohydrate and A+B1 carbohydrate fraction were also highlighted and interfered directly with *In vitro* DM digestibility, which also presented expressive values. When considering the period between forage cuts, this result was certainly influenced by the high concentrations of high- and medium-availability carbohydrates. In contrast to the method used here, Zanine *et al*. (2008) applied the criterion of minimum eigenvalue equals one to explain the behavior of variables when assessing Tanzania grass (*Panicum maximum*), for being more coherent to the results, and selecting six principal components, while in this study only two were considered.

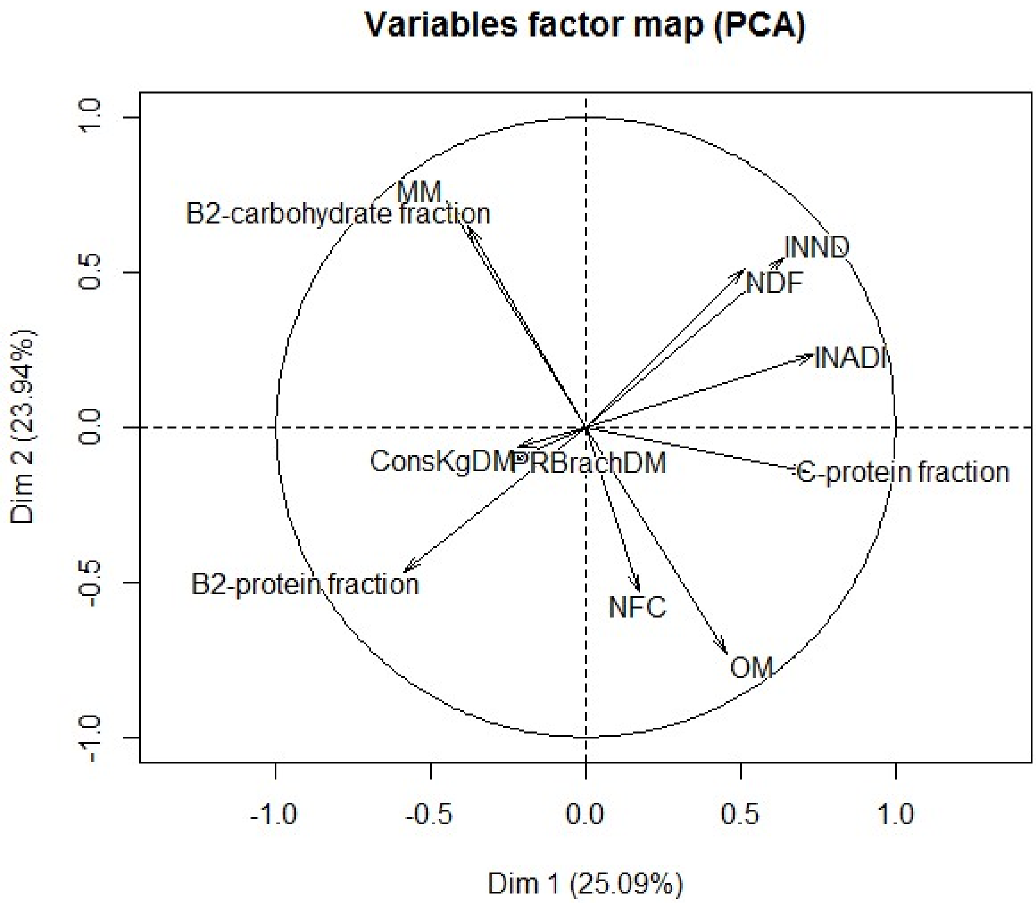

Figure 1: Plot of the distribution of the variables studied by the principal component analysis (PCA) of *Brachiaria brizantha* cv. Marandu under conventional, organic management and control in a system with green manur.

According to Melém Jr. *et al.* (2011), the angle between the arrows shows the degree of inter-correlation between variables, whereas the length of the arrow indicates the importance. Therefore, the degree to which a variable correlates with the axis is determined by the angle between a given arrow and the ordinate axis. When compared to conventional fertilizer and control group, organic fertilization resulted in more similar samples of *Brachiaria* both, chemical-bromatologically and productively, as red dots were closer to the axis (Figure 2). Thus, the fertilization management type promoted non-random patterns of differentiation among values of chemical-bromatological quality for *Brachiaria* forage samples. The most typical pattern (greater discriminatory power) was related to organic fertilizer. Such a result was already expected since organic fertilizers have great potential to change the soil characteristics, hence improving the nutritional quality of forages. In addition, when under this type of fertilization management, the variables showed a higher discriminatory power of the variance, thus promoting a non-random pattern of organization. In general, variations in chemical quality of samples had no random pattern among the different types of fertilization (Figure 2).

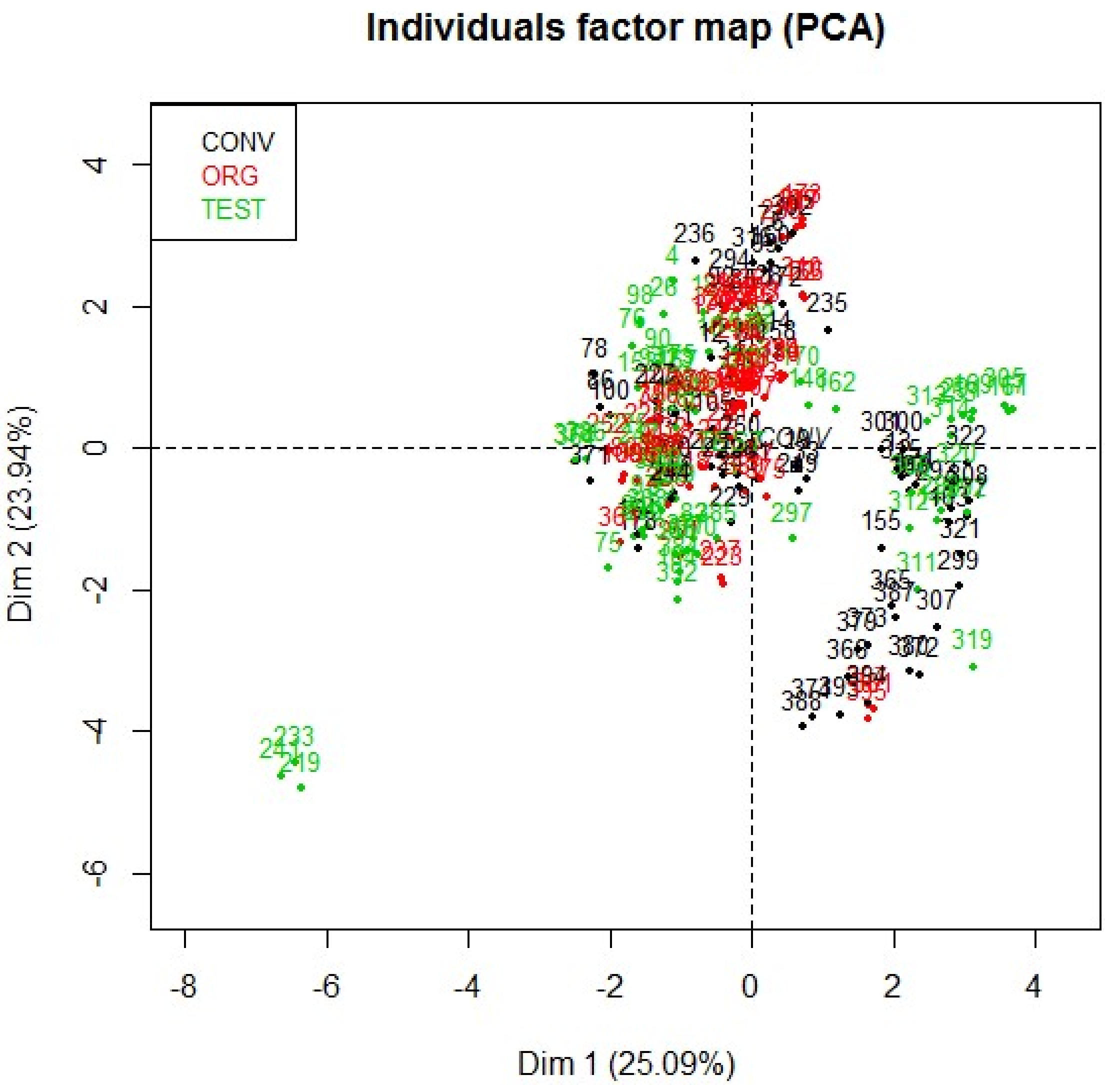

Figure 2: Dispersion of the PCA factors 1 and 2 scores of the samples under conventional fertilization (CONV), organic (ORG) and control (TEST) management, in a system with green manuring.

4.2 Chemical quality of B. brizantha cv. Marandu in conventional fertilization systems: Analyzing the variables jointly (multivariate) via PCA (Figure 3), both components were responsible for 45.19% of the total variation of all studied parameters of Marandu grass under the different fertilization managements (conventional, organic, and control group). Of these, PC 1 was responsible for 26.74% and PC 2 for 18.45% of the total data variation. Among the most expressive variables of PC 1 are DM content, B2-protein fraction, IVDMD, CP, and B1-protein fraction. For PC 2, the variables with greater discriminatory capacity were B2-carbohydrate fraction, NDIN, NFC, A+B1-carbohydrate fraction, MM, and OM. In view of these results, we may infer that only two principal components are enough to explain the variance in samples of B. brizantha cv. Marandu under these management conditions. Chemical-bromatological quality and DM production had an expressively greater capacity to discriminate variance in forage grown under organic and conventional management (Figure 4). Conversely, the plants belonging to the control group had an inexpressive discriminatory capability for the analyzed variables, which might have occurred due to a lack of nutrient sources to the soil (fertilization management).

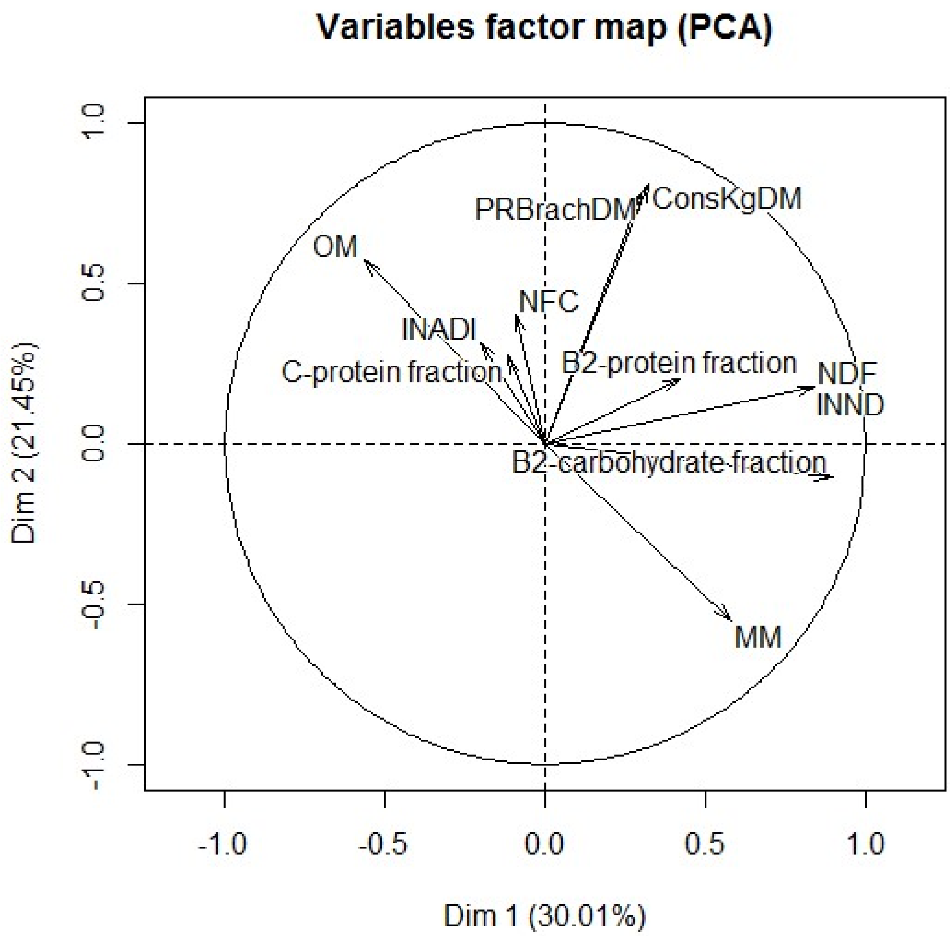

Figure 3: Plot of the distribution of the variables studied by the principal component analysis (PCA) of *Brachiaria brizantha* cv. Marandu under conventional, organic and control management, in a system without green manuring.

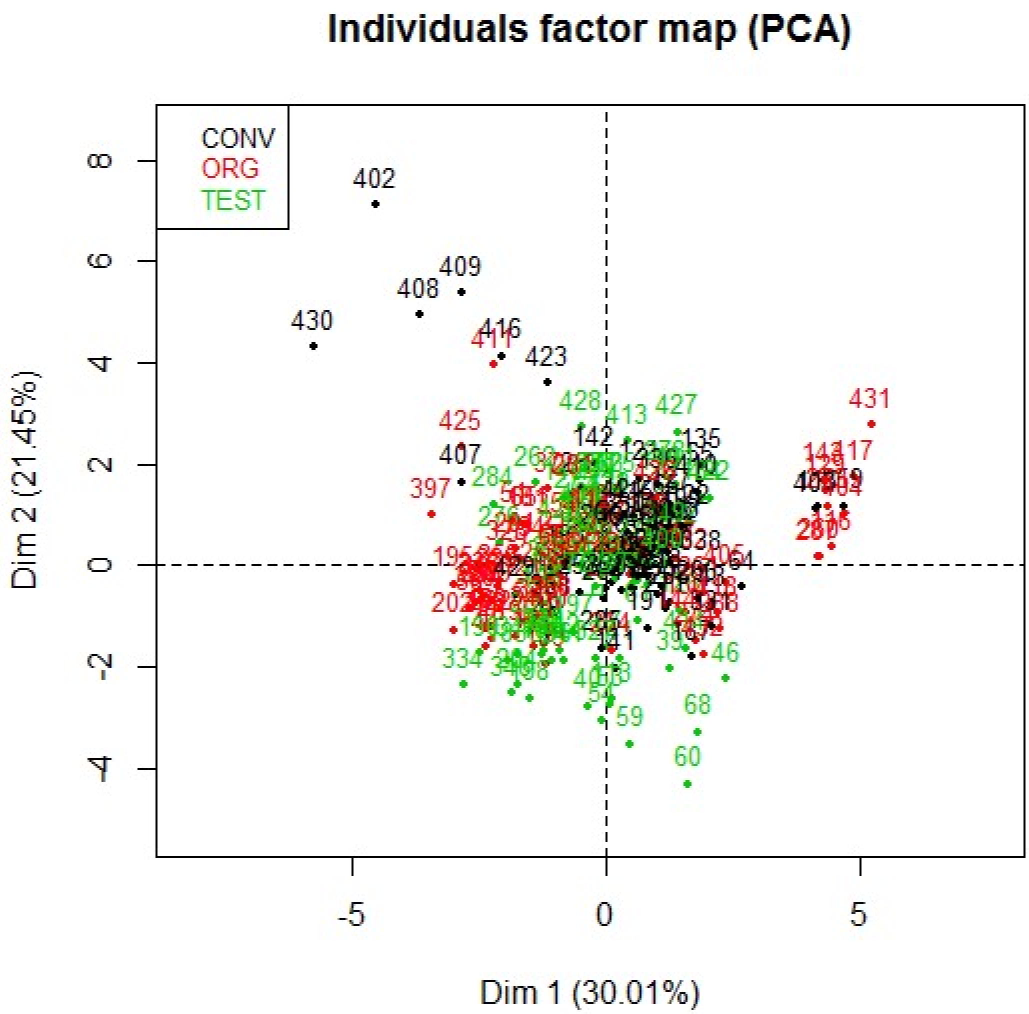

Figure 4: Dispersion of the PCA factors 1 and 2 scores of the samples under conventional fertilization (CONV), organic (ORG) and control (TEST) management, in a system without green manuring.

4.3 Chemical quality of Stylosanthes spp. in green manure systems: The first two components were responsible for 60.59% of the total variation for the studied variables in samples of *Stylosanthes* grown under different fertilization systems (conventional, organic, and control) (Figure 5). Of this variation, PC 1 was responsible for 42.99% and PC 2 for 17.60% of the total data variation. By means of PCA, Alves *et al*. (year) evaluated the relationship between chemical quality and N release in semiarid plants used as green manure. As in our study, these authors also used the first two principal components and found that 81% of the total variance was explained. All nitrogen compound fractions, IVDMD, DM production, and EE were the variables closer to the PC 1, which has the highest percentage of variance between both components. Such importance of these variables can be noted by the length of the arrows. On the other side, the variables approaching PC 2 axis were carbohydrate fractioning, NDF, ADF, OM, MM, INND, and INAD. An inter-correlation can be evidenced between OM and MM. The same was observed between NFC and A+B1 fraction (prompt availability) with B2 and C fractions, NDF, and ADF. Figure 5 shows that the variable IVDMD had the longer arrows, what denotes the importance of this variable for *Stylosanthes* spp. grown under a green manure system; therefore, such results were already expected as this forage species has a superior quality. Additionally, both chemical-bromatological quality and DM production showed an improved ability to discriminate variance only in organic and control groups (Figure 6). This can be explained by the clustering of points near the axis of the dispersion plot. Probably, the more chemical fertilizer is available, mainly N, the less the legume will fix N and, consequently, showing less development. However, a higher clustering capacity was observed in both organic and control groups, where little or no N is released, respectively. This outcome strengthens the theory of legume superiority to develop in low fertility soils.

4.4 Chemical quality of Stylosanthes spp. without green manure systems: The first two components explained 61.58% of the total variation (Figure 7). The first and second principal components accounted for 51.93 and 9.65% of the total variance, respectively. The variables close to the PC 1 were B1- and A-nitrogen compound fractions, OM, TC, LIG, C-carbohydrate fraction, MM, and production for both *Stylosanthes* and *Brachiaria* intercropped with *Stylosanthes*. In the plot, the variables with the greatest importance were TC, OM, B1, LIG, and DM production for *Stylosanthes*. For PC 2, there was an overlap of the variables ADIN, FC, NDFap, ADF, NDF, NDIN, B3 and B2 fractions, which were relevant to explain the total variation, as well as A+B1 carbohydrate fraction and NFC. An inter-correlation between DM production of *Stylosanthes* (PRStyDM) and TC was observed. Given the long period between cuts, this result may justify increases in structural carbohydrate deposition and reductions in moisture content. A second similarity observation between the different ways of evaluating the data is the non-fibrous carbohydrate (NFC) concentration and the average availability (A+B1 fraction) with a remarkable intercorrelation of this with the contents of NDF and FDA, since higher content of these reduces high and medium availability carbohydrates (A+B1 fraction and NFC). Again, among the variables of major importance in the system with no green fertilization were NFC and A+B1 fraction, but IVDMD had no such prominence. This result from the higher concentrations of fraction C and lignin, which interfered directly with digestibility expressivity. Moreover, DM yield also stood out, which can explain the high concentrations of carbohydrates with low or no availability. Figure 8 shows that both chemical-bromatological quality and DM production of *Stylosanthes* spp. samples inserted into the system with no green manure had an expressive capacity of discriminating the variance in organic and control groups. Conversely, the conventional system presented no significant discriminatory capacity for the analyzed variables. However, few consistent groups were formed in relation to the variables for all types of fertilization (conventional, organic, and control). This somewhat random behavior was already expected since a mix of three legume species was used here, which might have hindered a non-random expression of variables when submitted to different fertilization management.

Journal of Animal & Plant Sciences, 2018. Vol.38, Issue 1: 6138-6151
Publication date 31/10/2018, http://www.m.elewa.org/JAPS; ISSN 2071-7024

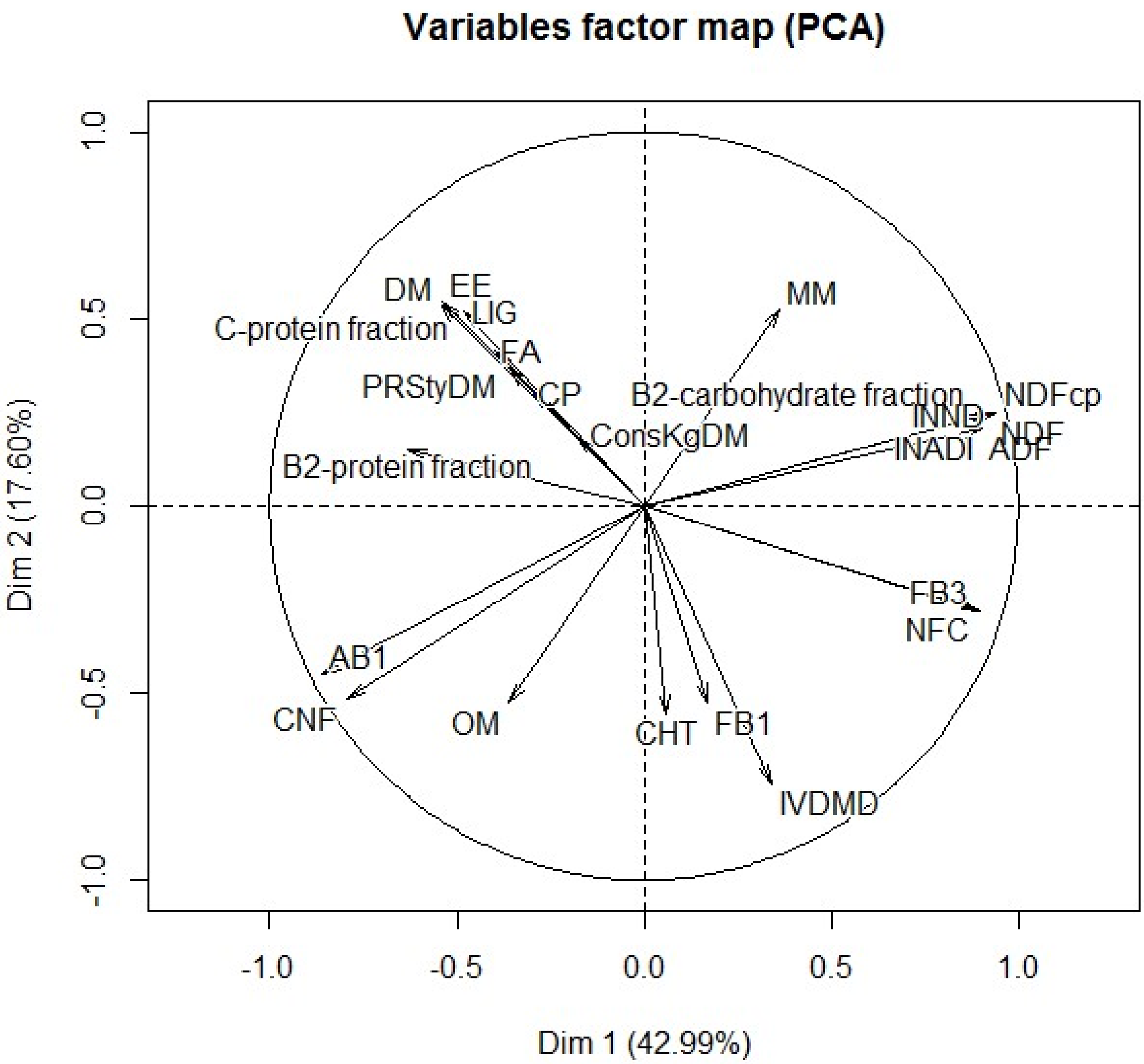

Figure 5: Plot of the distribution of the variables studied by principal component analysis (PCA) of *Stylosanthes spp*. under conventional, organic and control management, in a system with green manuring.

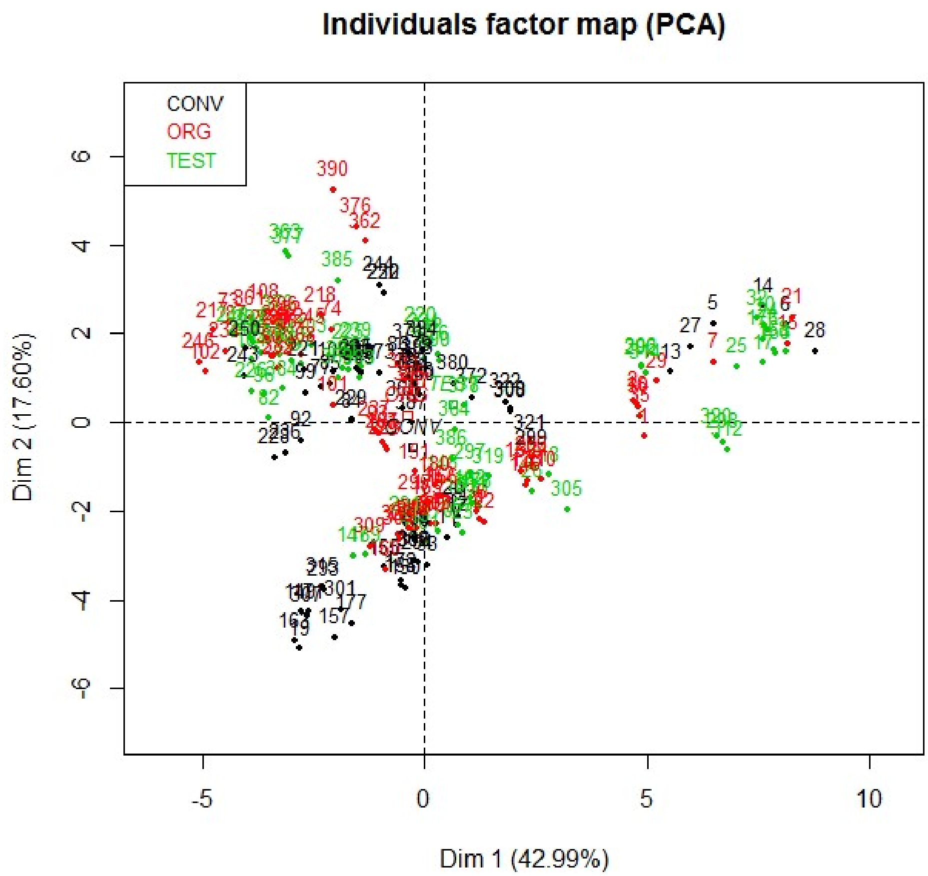

Figure 6: Dispersion of the PCA factors 1 and 2 scores of the samples under conventional fertilization (CONV), organic (ORG) and control (TEST) management, in a system with green manuring.

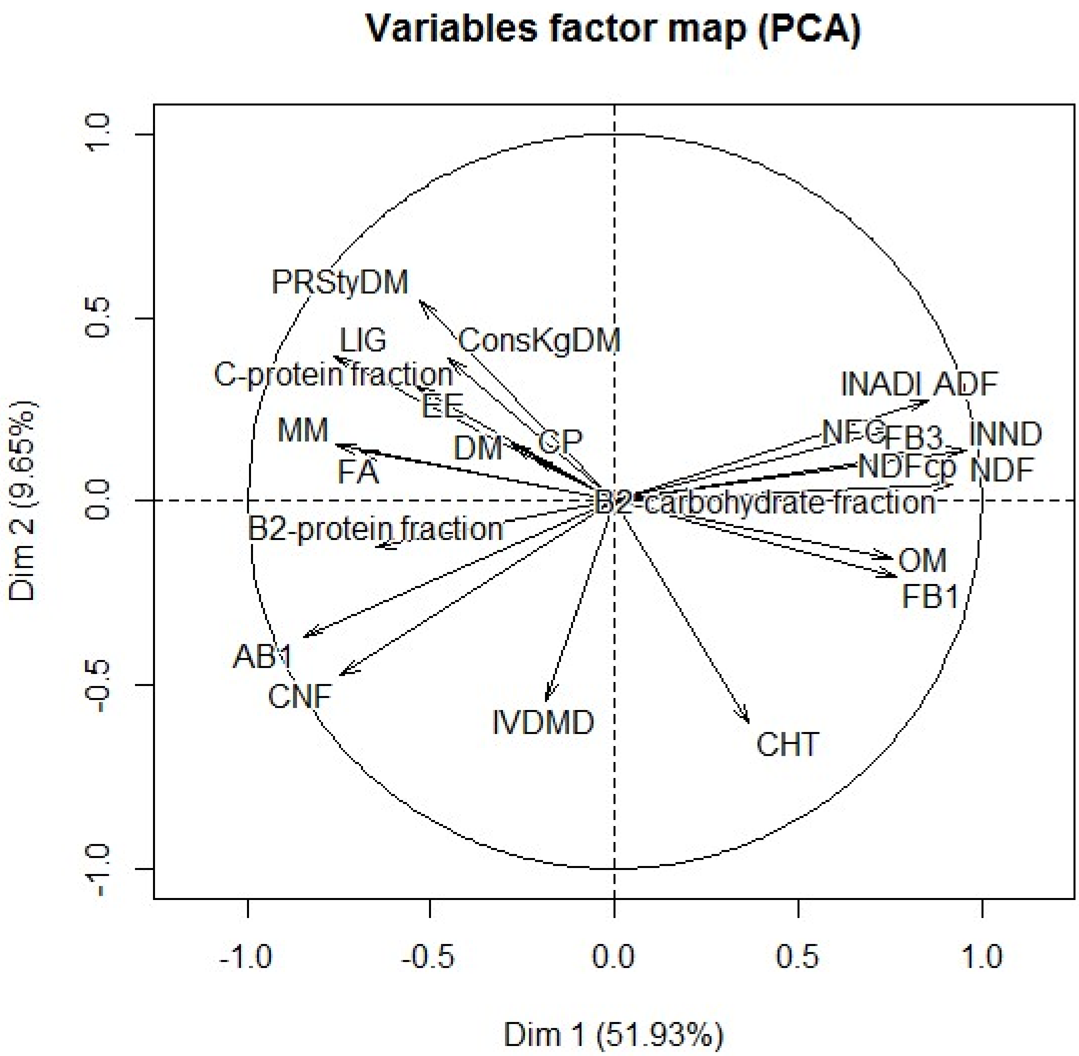

Figure 7: Plot of the distribution of the variables studied by principal component analysis (PCA) of *Stylosanthes spp*. under conventional, organic and control management, in a system without green manuring.

Journal of Animal & Plant Sciences, 2018. Vol.38, Issue 1: 6138-6151
Publication date 31/10/2018, http://www.m.elewa.org/JAPS; ISSN 2071-7024

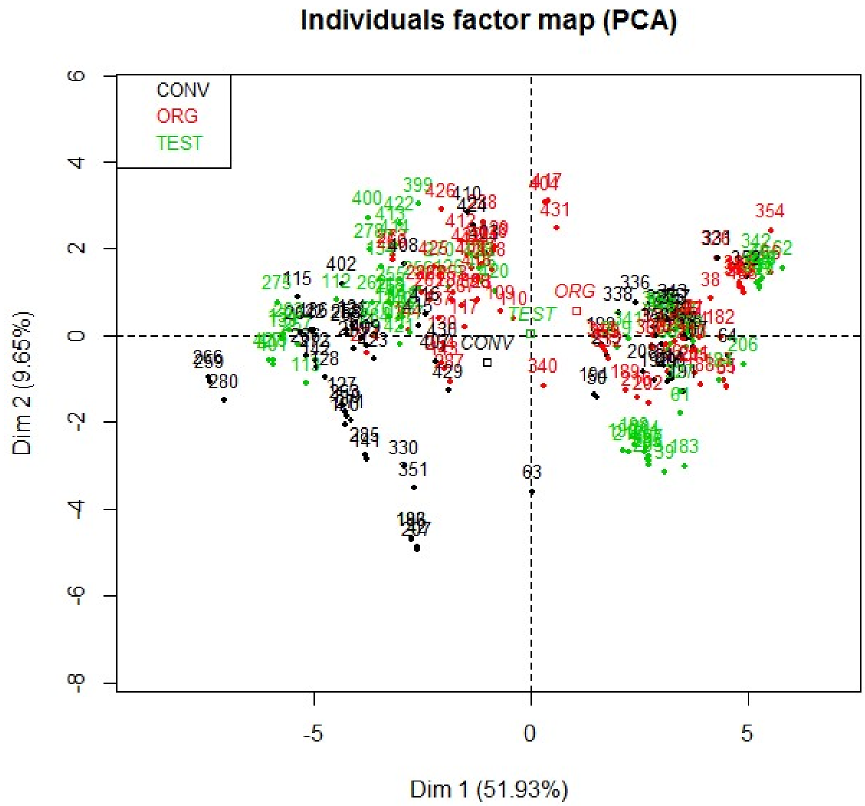

Figure 8: Dispersion of the PCA factors 1 and 2 scores of the samples under conventional fertilization (CONV), organic (ORG) and control (TEST) management, in a system without green manuring.

5 CONCLUSION

The technique of multivariate analysis using the principal components analysis method was more efficient in the expressive determination of *Brachiaria brizantha* cv. Marandu variables, with non-random behavior of the groups.

6 REFERENCES

Association of Official Analytical Chemistry – AOAC: 1995 Official methods of analyses. 16.ed. Arlington: AOAC International, 1025p.

Bernardi JVE, Lacerda LD, Dórea JG, Landim PMB, Gomes JPO, Almeida R, Manzatto AG, Bastos WR: 2009. Aplicação da análise das componentes principais na ordenação dos parâmetros físico-químicos no alto rio madeira e afluentes, Amazônia Ocidental. Geochimica Brasiliensis. 23:79-90.

Casali AO, Detmann E. and Valadares Filho SC: 2008. Influence of incubation time and particle size on the contents of indigestible compounds in food and bovine faeces obtained by in situ procedures. Brazilian Journal of Animal Science. 37: 335-342.

Castro Neto N, Denuzi VSS, Rinaldi RN. and Staduto JAR: 2010. Produção orgânica: uma potencialidade estratégica para a agricultura familiar. Revista Percurso-NEMO 2 : 73-95.

BRAZILIAN COMPANY OF RESEARCH AND AGRICULTURE - EMBRAPA. 1979. National Soil Survey and Conservation Service - SNLCS, Rio de

Janeiro, RJ. Manual of soil analysis methods. Rio de Janeiro: EMBRAPASNLCS. 271p.

Figueiredo EAP, Soares JPG .2012. Organic animal production systems: technical and economic dimensions. In: 49º Annual Meeting of The Brazilian Society of Zootecnia.

Fine J and Pousse A: 1992. Asympotic study of the multivariate functional model. Application to the metric choice in principal component analysis. Statistics 23: 63-83

Goering HK and Van Soest PJ: 1970. Forage fiber analysis (Apparatus, reagents, procedures and some applications). Washington, DC: USDA, (Agricultural Handbook, 379).

Krishnamoorth A: 1982. Mass rearing technique for an indigenous predatory mite, Amblyseius (*Typhlodromus)* tetranychivorus (Gupta) (*Acarina: Phytoseiidae*) in the laboratory. Entomon. 8: 229-234.

McDougal EI: 1949 Studies on ruminal saliva. The composition and output of sheep's saliva. Biochemical Journal.43: 99-109.

Melém Júnior NJ, Fonseca ICB, Brito OR, Decaëns T, Carneiro MM, Matos MFA, Guedes MC, Queiroz JAL, Barroso KO: 2008. Principal components analysis for the evaluation of analytical results of soil fertility in Amapá. Semina: Ciências Agrárias. 29: 499- 506.

Russel JB, O´Connor JD, Fox DG: 1992 A net carbohydrate and protein system for evaluating cattle diets: I. Rumen fermentation. Journal of Animal Science. 70: 3551-3561.

SAS - versão 9.1.3, SAS Institute, Cary, NC 2004.

Sniffen CJ, O'Connor DJ, Van Soest PJ, Fox DG, Russel JB: 1992 A net carbohydrate and protein system for evaluating cattle diets: carbohydrate and protein availability. Journal of Animal Science. 70: 3562-3577.

Sores JPG, Ramos AKB, Braga GJ, Santos JR, Fernandes FD, Martins ES, Reis BR, Malaquias JV. 2016. Organic management of Brachiaria brizantha cv. Marandu pasture mixed with Stylosanthes spp. in the Cerrado. In: International Meeting of Advances in Animal Science, 2016, Jaboticabal. 1 st International Meeting of Advances in Animal Science. Jaboticabal-SP: Unesp/FCAV.

Soares JPG, Neves, DL, Carvalho JM. 2014. Organic beef production: challenges and technologies for an expanding market. In: Oliveira RL, BARBOSA MAAF. (Org.). Bovinocultura de corte: desafios e tecnologias. 2thed.. Salvador: EDUFBA, 1 :701-725.

Tilley JMA and Terry RA: 1963. A two-stage technique for digestion of forage crops. Journal of the British Grassland Society. 18: 104-111.

Zanine AM, Dias, PF, Souto SM, Ferreira DJ, Santos EM, Pinto LFB: 2008. Evaluation of Tanzania grass (Panicum maximum) by means of multivariate analysis methods. Brazilian Journal of Animal Health and Production. 9: 56-64.