# SEASONAL CHANGES IN LEVELS OF CHLOROPHYLL, NITRATE AND TOTAL SOLUBLE CARBOHYDRATES OF BRACHIARIA DECUMBENS IN A LONG-TERM SILVOPASTORAL SYSTEM

**Marina Aparecida LIMA [1]; Domingos Sávio Campos PACIULLO [2]; Adriano NUNES-NESI [3]; Wagner Luiz ARAÚJO [4]; Mirton José Frota MORENZ [5]; Daiana Lopes LELIS [6]; Auxiliadora Oliveira MARTINS [7]; Carlos Augusto de Miranda GOMIDE [8]; Renato de Aragão Ribeiro RODRIGUES [9]; Fernanda Helena Martins CHIZZOTTI [10]**

[1] Animal Scientist. Manager. Brazilian Institute of Development and Sustainability; [2] Agronomist. Researcher. Embrapa Dairy Cattle; [3] Agronomist. Professor. Federal University of Viçosa; [4] Agronomist. Professor. Federal University of Viçosa; [5] Animal Scientist. Researcher. Embrapa Dairy Cattle; [6] Animal Scientist. PhD Student. Federal University of Viçosa; [7] Agronomist. PostDoc Student. Federal University of Viçosa; [8] Agronomist. Researcher. Embrapa Dairy Cattle; [9] Biologist. Researcher. Embrapa Soils; [10] Animal Scientist. Professor. Federal University of Viçosa

**ABSTRACT**
Our objective was to quantify the levels of chlorophyll (Chl), nitrate and total soluble carbohydrates in leaf blades of *Brachiaria decumbens* grown in the understory of a silvopastoral system (SPS) under intense shade and compare them to those grown in an open pasture (OP), with *B. decumbens* under full sunlight. Two treatments, corresponding to the specific production system (SPS and OP) were evaluated over four seasons: spring 2016, summer 2017, autumn 2017, and winter 2017. In the SPS, we observed an increase in the levels of chlorophyll and nitrate, and a reduction in total soluble carbohydrates relative to those in OP. This pattern of response was also influenced by the seasons, with higher levels of chlorophyll and nitrate during seasons with greater availability of resources. In contrast, total soluble carbohydrates were lower in spring and summer and higher in autumn and winter. Our results demonstrated that intense light restriction (70% shading) in an SPS reduces the levels of total soluble carbohydrates and increases the levels of chlorophyll and nitrate, which are directly related to the productivity, longevity, and nutritional value of the forage.
**Key words:** nitrate; shading; silvopastoral system

## INTRODUCTION

The silvopastoral system (SPS) has been considered a promising alternative for sustainable management of land use (PACIULLO et al., 2017). Despite recent advances in our understanding of the morphophysiological responses of shaded forages, little is currently known concerning the responses of metabolites related to carbon and nitrogen in forages grown in SPS. Accordingly, it remains unclear how photosynthetically active radiation (PAR) reductions modifies the levels of these metabolites in the forage and how these metabolites may vary over the seasons due to changes in climatic conditions. This information would likely provide a better understanding of how these changes are related to forage productivity and the performance of animals that consume that forage. Based on that, we hypothesized that intense shade provided by trees in the SPS alters the levels of chlorophyll, nitrate, and total soluble carbohydrates in *B. decumbens*. We therefore quantified levels of levels of chlorophyll, nitrate, and total soluble carbohydrates in leaf blades of *B. decumbens* growing under the understory of an SPS under intense shading and compare it to those found in leaf blades grown in an OP (*B. decumbens* under full sunlight) over four seasons (spring 2016, summer 2017, autumn 2017, and winter 2017) in southeastern Brazil.

## MATERIAL AND METHODS

The experiment was conducted at Embrapa Dairy Cattle research farm, in Coronel Pacheco, Minas Gerais, Brazil (21°33'S and 43°06'W; 410 m a.s.l.). The experiment began on September 22, 2016, and ended 22 September, 2017, and was composed of an SPS and OP, established in November 1997 in a mountainous area. Evaluations were performed in the grove (intra-rows) of SPS and the OP. The forage was composed of *B. decumbens*. The grove in the SPS was composed of trees of the species *Eucalyptus grandis* and tree legumes *Acacia mangium*, which were planted perpendicular to the incline of the slope in a north-south direction to prevent surface erosion of soils. Trees were arranged in groves comprising four parallel rows with an intra-row spacing of 3.0 m and an inter-row spacing of 3.0 m, totaling 81 trees/ha. The trees were planted alternately (mixed) in each of the four rows. The percentage of shading in the experimental area in the SPS was measured using the AccuPAR LP-80 ceptometer (Decagon Devices, Pullman, WA, USA), where, ten through non-destructive measures, the understory PAR was evaluated. Measurements were made during clear skies at 9:00 am, 12:00 pm, and 3:00 pm, one meter above ground. From these data, we calculated a reduction of the incidence of PAR by approximately 70% relative to OP, thus characterizing an intensely shaded environment. The experimental area of 500 $m^2$ was subdivided into 16 plots (8 plots for each system) of 2 $m^2$ distributed into two blocks. Before the experiment began, animals were excluded from the experimental area for one year, so that there was no residual effect of excreta previously deposited on the soil. Throughout the experimental period, the plots were periodically harvested to maintain an herbage height of 35 cm ± 10 cm, and the plant cuttings were removed from the plots, simulating continuous grazing. Two treatments, corresponding to a specific production system (SPS and OP), were evaluated over four seasons (spring 2016, summer 2017, autumn 2017, and winter 2017). We used a randomized block design (two blocks) with four replicates (plots) within a block ($n$ = 8), and the season was considered a repeated measure. In each plot, three tillers were collected in a usable area of 1 $m^2$, in plants located in the central part of the plots, leaving a 0.5 m border at the ends. From each tiller the last completely expanded leaf blade (recognized by +1 leaf blade) was collected, every 28 days, between 08:00–10:00 h. The central vein was removed from each leaf blade, and the three leaf blades collected from each plot were combined to form a single replicate. After harvesting, the leaf samples were snap frozen in liquid nitrogen and stored at −80°C until further analyses. The snap frozen samples were lyophilized (L 120, Liotop brand) for 76 h at −50°C. The levels of metabolites were quantified according to procedures in the following references: total chlorophyll contents ($a$ + $b$) (PORRA et al., 1989); nitrate (FRITZ et al., 2006). The quantification of total soluble carbohydrates was performed by the phenol-sulfuric method (DUBOIS et al., 1956; MASUKO et al., 2005). All metabolites were determined in an OptiMax Tunable Microplate Reader (ELISA) microplate reader. Repeated measures analyses were conducted with the PROC MIXED procedure in SAS® (SAS Institute Inc., Cary, NC, USA). The means of the two systems were compared using the F-test, and the season means were compared using the Tukey–Kramer test of the LSMEANS command. For all analyses, $\alpha = 0.05$ was defined as the critical level of probability for type I error.

## RESULTS AND DISCUSSIONS

The levels of total chlorophyll and nitrate in leaf blades of *B. decumbens* were significantly different between the production systems and seasons (Figure 1). There was an interaction between production systems and seasons for the variables total Chl ($p = 0.0002$) and nitrate ($p < 0.0001$). When comparing the systems, higher levels of total Chl were found in the SPS in all seasons (Figure 1a). In both systems, higher levels of total Chl were observed in the autumn. Chlorophylls are the most abundant photosynthetic pigments in plants and are responsible for absorbing light energy and converting it to chemical energy. Several studies of tropical forages have observed that, under shading environments, the amount of Chl increases in a direct relationship with to monoculture cultivation (DIAS-FILHO, 2002; OLIVEIRA et al., 2013). These higher levels, also observed in our study, are associated with the increases in the photosynthetic efficiency in response to light restriction (DO NASCIMENTO et al., 2019). As plants become more efficient at absorbing light energy, their growth increases. The higher levels of Chls observed in the SPS relative to OP can also be correlated with the greater availability of nitrogen in the SPS. Higher levels of Chls were observed during autumn in both systems and can be explained, at least partially, by the greater yet atypical precipitation during this season, even with reduced light intensity. Therefore, the increased amount of chlorophyll enhanced light capture efficiency even with a reduction in light intensity and temperature in autumn. The lower chlorophyll levels observed during winter may be, however, associated with a reduction in light intensity, temperatures, and water in the soil, resulting in a decrease in photosynthetic rates and growth. Higher levels of nitrate were observed in SPS than OP in spring, summer, and winter, with no difference between systems in autumn (Figure 1b). Moreover, the SPS was characterized by the highest levels in spring, followed by summer and the lowest levels in autumn and winter. Nitrate levels in the winter were reduced by 92% (0.26 g/kg) relative to spring (3.18 g/kg), a period in which less nitrogen absorption may occur as it is less mobilized in the soil due to the reduction of water content. Open pasture, on the other hand, maintained similar nitrate levels throughout the experimental period. The higher levels of nitrate observed in SPS relative to OP can be explained by the lower amount of PAR that reaches the understory, affecting the internal assimilation rates of nitrogen due to the lower availability of carbon skeletons via photosynthesis. The low light availability is also a stress. Neel and Belesky (2017) also observed higher levels of nitrate in $C_3$ forage in SPS relative to OP. Nitrate can accumulate in vacuoles without causing any harmful effects to the plant, unlike nitrite and ammonium ions, which must be reduced and assimilated immediately. It is important to mention, however, that it high levels of nitrate can affect the ingestive behavior of animals that consume this forage and consequently influence animal production. Accordingly, it has been observed that animals prefer to graze in sunnier areas over shaded areas (NEEL et al., 2008). Notably, the nitrate concentrations found in the present study were below the limits considered toxic to animals. Nitrate values between 6.6 to 13.3 g/kg of DM can reduce the daily intake of DM by 50%, and values between 13.3 to 19.9 g/kg of DM can reduce the DM consumption by up to 75% (ESSIG et al., 1988). The levels of total soluble carbohydrates were influenced ($p = 0.0001$) by the interaction between the production system and season (Figure 2). During the spring and summer, there was no difference between the systems. In autumn and winter, SPS showed lower levels of total soluble carbohydrates relative to those of OP. Higher levels of total soluble carbohydrates in the SPS were observed in winter, followed by autumn, which did not differ from spring, and a lower level was observed in summer, which was similar to spring. In OP, higher levels of total soluble carbohydrates were observed in winter, followed by autumn, spring, and lower levels were observed in summer. The similarity in the levels of total soluble carbohydrates between systems during spring and summer can be explained, at least partially, by the higher synthesis and use of carbohydrates for biomass production during seasons with more favorable climatic conditions. During autumn and winter, when usually plant growth is reduced the trend is that these compounds are stored and also transported to drain organs (stem and roots). However, in these seasons, the levels of total soluble carbohydrates were lower in SPS than in OP. In general, under shade, forage was characterized by lower levels of carbohydrates. This reduction may be associated with a thinner leaf mesophyll under shaded environments to increase photosynthetic efficiency (GEREMIA, 2016). According to Ciavarella et al. (2000), under shaded environments, grasses prioritize the allocation of photoassimilates to maintain and increase leaf area and stems that ultimately also increase light uptake. Plant growth and development are dependent on the interaction between the metabolism of carbon and nitrogen (NUNES-NESI et al., 2010). Our, results indicate that changes in forage growth are also associated with metabolic changes. Our results indicate that metabolites related to carbon and nitrogen are altered with shading and that magnitude of these effects may vary with the season.

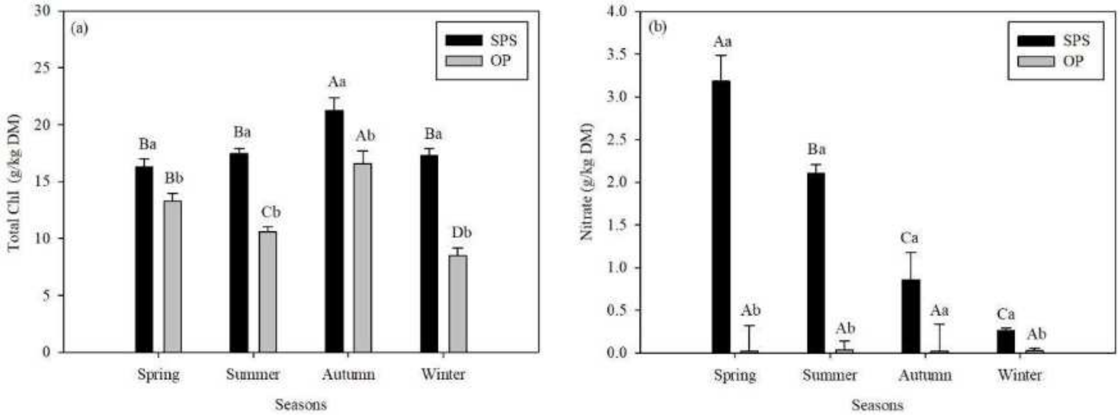


Figure 1. Total chlorophyll (Chl) and Nitrate (d) yield (g/kg; 100 % dry-matter basis) in leaf blades of Brachiaria decumbens in a silvopastoral system (SPS) and open pasture (OP) by season (spring, summer, autumn, and winter). Means followed by different letters, lowercase letters comparing the systems in each season, and uppercase letters comparing each system in the seasons available, are significantly different ($p < 0.05$). Vertical bars indicate the standard errors of the means.

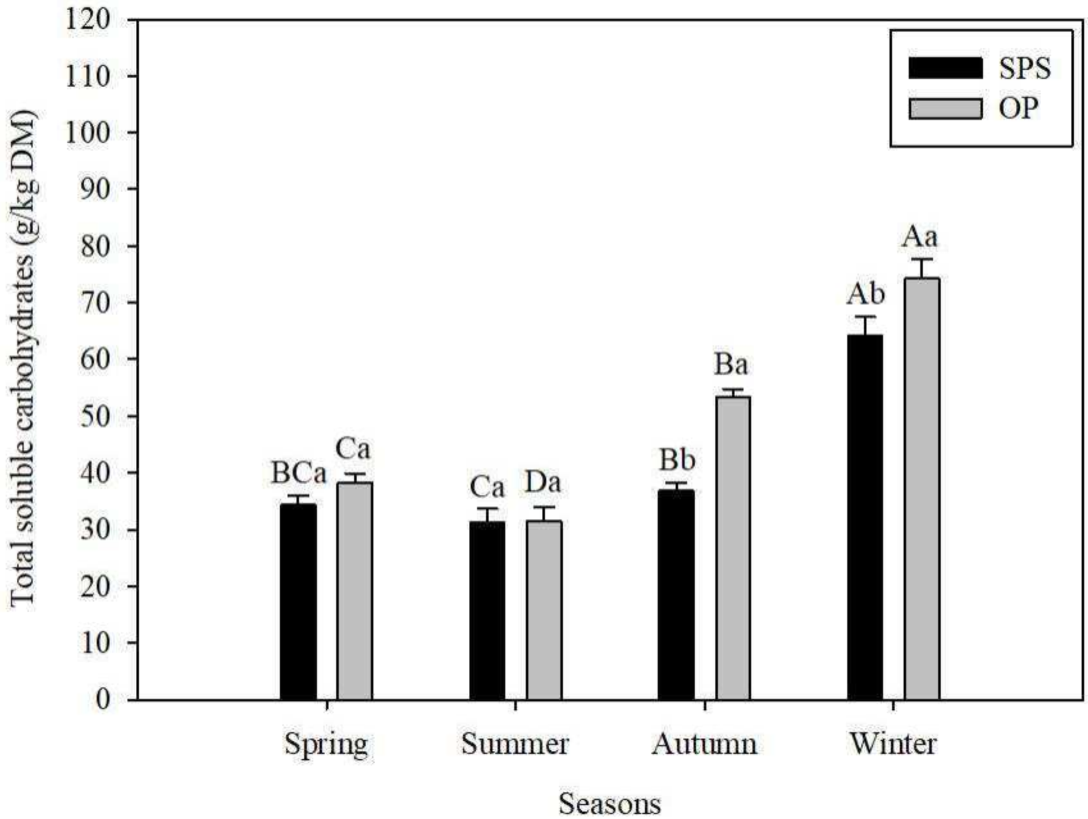


Figure 2. Total soluble carbohydrates (g/kg; 100% dry-matter basis) in leaf blades of Brachiaria decumbens in a silvopastoral system (SPS) and open pasture (OP) by season (spring, summer, autumn, and winter). Means followed by different letters, lowercase letters comparing the systems in each season, and uppercase letters comparing each system in the seasons available, are significantly different ($p < 0.05$). Vertical bars indicate the standard errors of the means.

## CONCLUSIONS

Our results demonstrated that intense light restriction (70% shading) in a SPS reduces the levels of total soluble carbohydrates, while it increases the levels of chlorophyll and nitrate; higher fluctuations in these metabolites are observed in the seasons of higher growth (spring and summer). Notably, these metabolites are directly related to the productivity, persistence, and nutritional value of the forage. That being said, for a comprehensive understanding of carbon/nitrogen interactions in forage in response to shade, much higher resolution at the whole-plant, cellular, and sub-cellular level is likely required. In summary, our results revealed that intense shading should be avoided and therefore silvicultural interventions, such as thinning or pruning of trees in the SPS, are highly recommended, thereby focusing on animal production that stabilizes the production and quality of the forage in the long-term.

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