INTRODUCTION OF FORAGE LEGUMES INTO ARUANA GUINEAGRASS PASTURE

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Abstract
The experiment was developed at the Instituto de Zootecnia in Nova Odessa-SP. It was designed to evaluate forage mass, forage legumes ratio in the available forage mass and the transfer potential of the nitrogen fixed by the forage legumes to Aruana Guineagrass pasture grazed by lambs under intermittent grazing. The treatments consisted of the introduction of four accessions of forage legumes in the pasture of Megathyrsus maximum cv. Aruana and a treatment of Aruana Guineagrass pasture (without legumes), totalling five treatments. The Aruana Guineagrass pasture was established five years ago. The forage legumes treatments were: Neonotonia wightii NO 253 (Perennial soy NO 253), Neonotonia wightii NO 2348 (Perennial soy NO 2348), Macrotyloma axillare NO 279 (Macrotyloma legume) and Calopogonium mucunoides NO 1194 (Calapo legume). The experimental design was made up of random blocks with three replicates. The Calapo legume showed the lowest mean forage mass in the three seasons (400 kg/ha). The mean forage mass of the Perennial soy NO 253, Perennial soy NO 2348 and Macrotyloma legume did not show significant differences in the three seasons. Furthermore, the Calapo legume ratio in the pasture was the lowest in the total forage mass (13%) among the intercropped forage legumes (31.9% at 43.2%). Perennial soy NO 253, Perennial soy NO 2348 and Macrotyloma legume showed suitable ratio in the forage mass when intercropped with Aruana Guineagrass. These forage legumes also promoted the highest crude protein content in the Aruana Guineagrass. Thus, it is possible to advise the introduction of Perennial soy NO253, Perennial soy NO2548 or Macrotyloma legume in established Aruana grass pasture.

Keyword
Calapo legume, Macrotyloma legume, Crude protein, Perennial soy

INTRODUÇÃO DE LEGUMINOSAS EM PASTAGEM DE CAPIM-ARUANA PASTEJADA POR OVINOS SOB LOTAÇÃO INTERMITENTE

Resumo
O experimento foi desenvolvido no Instituto de Zootecnia, em Nova Odessa-SP. Foi projetado para avaliar a massa de forragem, a proporção de leguminosas forrageiras na massa de forragem disponível e o potencial de transferência do nitrogênio fixado pelas leguminosas forrageiras para o pasto de capim Aruana, pastejados por cordeiros sob pastejo intermitente. Os tratamentos consistiram na introdução de quatro acessos de leguminosas forrageiras no pasto de Megathyrsus maximum cv. Aruanã e um tratamento de pasto de capim-aruana (sem leguminosas), totalizando cinco tratamentos. O pasto de capim Aruana foi estabelecido há cinco anos. Os tratamentos com leguminosas forrageiras foram: Neonotonia wightii NO 253 (Soja perene NO 253), Neonotonia wightii NO 2348 (Soja perene NO 2348), Macrotyloma axillare NO 279 (Macrotyloma legume) e Calopogonium mucunoides NO 1194 (Calopogônia). O delineamento experimental foi o de blocos casualizados com três repetições. A leguminosa Calopogônia apresentou a menor média de forragem nas três estações (400 kg / ha). A massa média de forragem da soja perene NO 253, soja perene NO 2348 e leguminosa Macrotyloma não apresentou diferenças significativas nas três estações do ano (Tabela 1). Além disso, a proporção de Calopogônia no pasto foi a mais baixa na massa total de forragem (13%) entre as leguminosas forrageiras consorciadas (31,9% a 43,2%). A soja perene NO 253, a soja perene NO 2348 e a leguminosa Macrotyloma apresentaram relação adequada na massa de forragem quando consorciadas com capim Aruana. Essas leguminosas forrageiras também promoveram o maior teor de proteína bruta no capim Aruana. Assim, é possível recomendar a introdução da soja perene NO253, soja perene NO2548 ou leguminosa Macrotyloma em pastagens estabelecidas de capim Aruana.

Palavras–chave
Calopogônia, Macrotyloma, proteína bruta, soja perene

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INTRODUCTION

*Urochloa* and *Megathisus* grass are the predominant genera in the monospecific grazing systems in the Brazilian tropical regions. However, the necessity to increase the productivity and efficiency of the resources used in the sustainable intensification process raises new challenges for agricultural systems. There have been many attempts and some success in introducing forage legumes to pasture monocultures in the last years, because grasses x forage legumes intercropping systems permit to explore different areas and aspects of the soil – plant – animal system.

Studies of intercropping systems have shown that intensification of the livestock system is possible using forage legumes (SOUZA et al., 2016; FIORELI et al., 2018; LIMA et al., 2018; TERRA et al., 2019). Researchers agree that the development of grasses x forage legumes intercropping systems is an important pillar to make livestock systems more sustainable and competitive (LUSCHER et al., 2014). Low soil nitrogen availability is a major limitation in tropical and subtropical pasture areas. Reduced application rates of nitrogen fertilizers in these areas is mainly due to the unfavourable economic returns. However, the lack of nitrogen represents one of the main causes of pasture degradation (DUBEAUX and SOLLENBERGER, 2020).

The suitable maintenance of pasture systems requires the replacement of nutrients. Due to its determining role in the grass production, nitrogen is the main nutrient that needs to be supplied in the pasture system. In the conventional practice of pasture management, nitrogen is available to plants in the form of chemical fertilizer and/or is recycled. A sustainable alternative nitrogen supply is the introduction of forage legumes in pasture. This alternative reduced the need for synthetic nitrogen in pasture systems and increases the nutritive value of the diet of the animals grazing the pasture. Forage legumes increase nitrogen in pasture systems through biological nitrogen fixation (BNF) by bacterial symbiosis. In general, *Rhizobium* and *Bradyrizobium* are genera which act in the symbiosis (SOUSSANA and LEMAIRE, 2014; GIMENES et al., 2017). These genera have an enzyme (Nitrogenase) that is able to reduce dinitrogen (N$_2$) to ammonia (NH$_3$) which can be protonated to ammonium (NH$_4$), a form of nitrogen that is taken up by plants. Therefore, intercropping pasture systems (legumes x grasses) can be more productive than pastures fertilized with mineral nitrogen. In addition intercropping pasture systems can be an important strategy for the mitigation of the contribution to climatic change from livestock systems (WERNER, 1986; SOUSSANA and LEMAIRE, 2014).

The greatest challenge to livestock systems using forage legumes x grasses
intercropping is to maintain the ideal ratio of forage legumes in the pasture (30-40%). In the intercropping system, according to the concept of relative acceptability the grass must be the forage preferentially consumed by the animal during most of the year (water period) allowing the good development of the forage legumes and thus ensuring their primary function, which is to supply nitrogen to the system, improving the production and the nutritional value of the intercropped grass. Furthermore, the consumption of legumes must be more intense in the autumn / winter, when the availability and nutritional value of the grasses decrease (WERNER, 1986; CADISCH et al., 1994; ELGERSMA and SOEGAARD, 2018). The consumption of forage legumes is normally relatively lower than grasses, because they have astringent substances in their composition such as tannins and other secondary compounds of the plant metabolism that can limit their consumption.

The objective of this study was to evaluate the forage mass, the forage legumes ratio in the available forage mass and the transfer potential of the nitrogen fixed by the forage legumes to Aruana grass pasture grazed by lambs under intermittent grazing.

MATERIAL AND METHODS

The experiment was developed at the Instituto de Zootecnia in Nova Odessa, SP, Brazil (22° 42’S, 47° 18’W), between January, 2008 and January, 2009. The climate is classified as humid, subtropical, dry winter Cwa type, with mean temperatures below 18°C in the coldest month and above 22°C in the hottest period. Figure 1 shows the climatic dates of the experimental site. The soil of the experimental site is classified as Red Yellow Argisol (Ultisol) (UNITED STATES, 2014).

Figure 1. Precipitation (mm) and mean temperature (°C) from January, 2008 to January, 2009.
The treatments consisted of the introduction of four accessions of forage legumes in the pasture of *Megathyrsus maximum* cv. Aruana and a treatment of Aruana Guineagrass pasture (without legumes), totalling five treatments. The Aruana Guineagrass pasture was established five years ago. The forage legume treatments were: 1- *Neonotonia wightii* NO 253 (Perennial soy NO 253), 2- *Neonotonia wightii* NO 2348 (Perennial soy NO 2348), 3- *Macrotyloma axillare* NO 279 (Macrotyloma legume) and 4- *Calopogonium mucunoides* NO 1194 (Calapo legume). The experimental design was random blocks with three replicates, totalling fifteen experimental plots.

The experimental plots were 6 x 6 m (36 m²) and they were separated by corridors 3 m wide, without fences. The forage legumes were sown after grazing of the grass by lambs to a sward height of 0.13 m (01/08/2008). Before sowing the forage legumes, fertilizers were applied to the soil surface (500 kg/ha of single superphosphate, 100 kg/ha of potassium chloride and 40 kg/ha of FTE-Br 16 (Fritted trace elements – Brazil 16) (WERNER, 1986).

Germination tests were performed on the seeds of the selected forage legumes to determine their sowing density. The sowing densities were: 12 kg/ha of Perennial soy seeds (NO 253), 12 kg/ha of Perennial soy seeds (NO 2348), 10 kg/ha of Macrotyloma legume seeds (NO 279) and 10 kg/ha of Calapo legume seed (NO 1194).

Lambs with mean bodyweight of 36 kg (± 1.6 kg) were kept in the paddocks under intermittent grazing management during the establishment of the forage legumes (from 01/11 to 04/11/2008). Lambs entered the paddocks when the pastures had a sward height of 0.30 - 0.35 m and these animals grazed the paddocks until the sward height reached 0.10 - 0.15 m. The sward height was measured using a graduated stick (sward stick) (BARTHRAM, 1985). The paddocks were rested from May to October for the flowering of the forage legumes and natural reseeding. After this period, the lambs returned to the paddocks under intermittent grazing management according to the sward height established previously.

The plants (forage legumes and grass) were harvested over three sampling seasons (10/21/08; 12/16/08 and 01/27/09). In these sampling seasons the persistence and ratio of the forage legumes were measured in the pasture before the lambs grazed the paddocks (pre-grazing). The evaluations were made with a square (0.5 m²) which was randomly thrown twice in each plot (1 m² per plot). All forage mass at the soil surface were harvested.

After the harvest, the forage mass was divided into the components of forage legume, Aruana Guineagrass and dead material. Then, the components were weighed and a subsample was placed for drying in a forced circulation oven at 65°C, until a constant weight was reached, to determine its dry matter content and forage mass per hectare.

The contributions of each forage legume treatment to the protein content of the Aruana Guineagrass were evaluated by converting the nitrogen content to crude protein using
the conversion factor of 6.25. The nitrogen content was determined by the Kjeldahl semi-micro analytical method.

The data were submitted for variance analysis according to the MIXED procedure of the Statistical Analysis System (SAS) program (SAS, 2009). The means were compared by Tukey test (P ≤ 0.10) according to the LSMEANS procedure of the Statistical Analysis System (SAS) program (SAS, 2009).

RESULTS AND DISCUSSION

There was no significant season x forage legumes treatments interaction. According to Gerdes et al. (2005), the forage mass results were adequate for forage legume x grass intercropping. The total forage mass showed significant difference in only one season (Table 1). The Aruana Guineagrass x Perennial soy NO 253 intercropping showed the largest total forage mass. Zanini et al. (2012) working with Aruana Guineagrass pasture monoculture observed that the more efficient use of this grass was achieved with 0.15 m sward height after-grazing. Thus, the sward height in this study was suitable because the forage consumption by lambs is maximized in dense pastures with many leaves which have approximately 2,000 kg/ha of dry mass in the total forage mass in pre-grazing. According to Hodgson (1990), forage mass less than 2,000 kg/ha can promote a restriction in animal consumption, due to pasture structure characteristics. The Calapo legume showed the lowest mean forage mass in the three seasons (400 kg/ha). The mean forage mass of the Perennial soy NO 253, Perennial soy NO 2348 and Macrotyloma legume did not show significant differences in the three seasons (Table 1). Furthermore, the ratio of Calapo legume in the total forage mass of the pasture was lower (13%) than that of the other intercropped forage legumes (31.9% to 43.2%).

Table 1. Mean forage mass, total forage mass and forage mass of the botanical components, forage legume ratio in the total mass and crude protein content of Aruana Guineagrass (A) in the intercropping treatments (mean of the three sampling seasons) and three sampling seasons (means five treatments).

| Treatments                  | Grass   | Forage legumes | Dead material | Total       | Forage legumes (% total) | Crude protein content (% grass) |
|-----------------------------|---------|----------------|---------------|-------------|-------------------------|---------------------------------|
| Aruana Guineagrass (A)      | 2390    | -              | 1105 a        | 3495 b      | 9.3 c                   |                                 |
| A + Calapo legume           | 2011    | 400 b          | 896 b         | 3306 b      | 13.3 c                  | 9.9 c                           |
| A + Macrotyloma legume      | 1048    | 1364 a         | 542 c         | 2955 b      | 43.2 a                  | 11.9 a                          |
| A+ Perennial soy NO 253     | 1968    | 1383 a         | 911 a         | 4262 a      | 31.9 b                  | 11.5 ab                         |
| A+ Perennial soy NO 2348    | 1325    | 1542 a         | 627 bc        | 3493 b      | 42.2 ab                 | 10.9 b                          |
| Sampling seasons            |         |                |               |             |                         |                                 |
| 10/21/2008                  | 1644    | 1093 a         | 1184 a        | 3921 a      | 28.4 a                  | 10.1 c                          |
| 12/16/2008                  | 1286    | 561 b          | 645 b         | 2493 b      | 21.9 a                  | 11.5 a                          |
| 01/27/2009                  | 2314    | 1159 a         | 649 b         | 4092 a      | 28.1 a                  | 10.6 b                          |

Means followed by different superscript letters in the columns differ from each other by F test (P>0.10).
The results of forage legume mass and total percentage of forage legumes in the pasture showed the lowest persistence of Calapo legume in the intercropping system and these results also reduced the crude protein content from the Aruana Guineagrass. There were no significant differences in the crude protein content between the forage mass of the Aruana Guineagrass pasture monoculture (9.3%) and Calapo legume x Aruana Guineagrass intercropping system (9.9%). Thus, the Calapo legume did not improve the nutritional status of Aruana Guineagrass when used in the intercropping pasture system.

Perennial soy NO 253, Perennial soy NO 2348 and Macrotyloma legume showed suitable ratios in the forage mass when intercropped with Aruana grass. These forage legumes also promoted the highest crude protein content in the Aruana Guineagrass (Table 1). The mean increase in the crude protein content was 2%. These results corroborated those of Aguirre et al. (2016) who observed increases in the crude protein content of 2.6% in grasses intercropped with forage legumes which have a hibernal cycle.

Some studies have reported an increase in the productivity of intercropped pastures (CADISCH et al., 1994; CECATO et al., 2011; GAMA et al., 2013; STURLUDÓTTIR et al., 2013; LUSCHER et al., 2014; MUIR et al., 2014; SOUZA et al., 2016; ARF et al., 2018) due to improvements in the crude protein content as a result of the transfer of nitrogen fixed by the forage legumes to the grasses. Martuscello et al. (2011) verified that the use of Stylosanthes guianensis intercropped with Signal grass (Urochloa decumbens, Syn. Brachiaria decumbens Stapf.) promoted similar results to nitrogen fertilizer. This result according to Martuscello et al. (2011) demonstrated that the use of this forage legume can be a substitute for industrial fertilizer in the establishment and maintenance of pasture longevity. Sturludóttir et al. (2013) observed an increase in the dry mass yield in the grass and forage legumes mixture in relation to the grass monoculture. However, according to Sturludóttir et al. (2013), there was no decrease in the crude protein content of this mixture when compared to the grass monoculture.

Table 1 shows that the lowest forage mass from Aruana Guineagrass occurred in the treatments where Aruana grass was intercropped with Macrotyloma legume or Perennial soy NO 2348. These treatments also showed the largest forage legume ratio in the total forage mass. These results are possibly due to the effect of competition for space and growth factors (light, energy, water and nutrients). The grass component revealed the biggest forage mass in the Aruana Guineagrass pasture monoculture and this can be due to less competition for space, light, water and nutrients. However, this treatment also had the greatest dead material mass and the lowest crude protein content as a result of the nitrogen limitation to maintain its growth.

Among the sampling seasons (Table 1), the sampling from October, 2008 and January,
2009 revealed the greatest amount of forage legume component in the total forage mass. These sampling seasons also showed the biggest total forage mass. However, the sampling from October, 2008 showed the largest amount of dead material mass and the lowest crude protein content in the Aruana Guineagrass component in relation to the other sampling seasons. This observation may be due to the long time that the paddocks were rested (May to October, 2008) for the flowering and natural reseeding of the forage legumes. In addition this sampling coincided with the drought season that promotes the aging and death of plant tissues.

The sampling from December (2008) showed the lowest total forage mass. However, the forage legume component showed the biggest crude protein content in Aruana grass, probably because this season coincided with a short period of growth (21/10 to 16/12/2008), well-distributed rainfall, and thus less dilution effect. However, sampling from December (2008) showed moderate amounts of total forage mass and of the forage legumes component compared to the growth season from January, 2009.

CONCLUSIONS

The crude protein content of the Aruana grass was directly related to the intercropped legume forage ratio. Thus, it is possible to advise the introduction of Perennial soy NO 253, Perennial soy NO 2548 or Macrotyloma legume in established Aruana Guineagrass pasture.

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