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Communication

Nutrient Characterization and Mineral Composition of *Aruana* in a Silvopastoral System with Nitrogen Fertilization

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Abstract: The objective was to characterize the nutrient and mineral composition of *M. maximus* cv *Aruana* grazed by lambs in a silvopastoral system or full sun with added nitrogen. The treatments are: (1) *Aruana* grazed in full sun with nitrogen fertilization, (2) *Aruana* grazed in full sun without nitrogen fertilization, (3) *Aruana* grazed in a silvopastoral system with nitrogen fertilization and (4) *Aruana* grazed in a silvopastoral system without nitrogen fertilization. The nutrient dynamics, urinary excretion of N and the amount returned of the nutrient were determined from four sheep in a Latin square experimental design; the analysis of the mineral composition of the leaves and stalks of the grass was carried out by collecting samples from the plots used, totaling six in each treatment evaluated. The silvopastoral system provided high levels of P and K in forage plants. Urinary N excretion and urine mineral contents were influenced by the systems evaluated and the use of N fertilizer. N did not show differences for leaves and canes.

Keywords: *Megathyrsus maximus*; nitrogen; silvopastoral; urinary excretion

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1. Introduction

Grazing sheep production in subtropical regions has an unexploited potential, despite its favorable characteristics, such as productive forage and precipitation throughout the year, that allow the sustainable production of quality lambs [1].

Tropical forage quality and climatic conditions are limitations for productivity in animal production, affecting animal behavior and grazing [2]. The adoption of integrated systems, such as silvopastoral systems, and management techniques, such as targeted fertilizer application, can increase animal production under these conditions [3].

Megathyrsus maximus cv. *Aruana* is an important, economically and ecologically suitable option for sheep production. It has characteristics such as good response to nitrogen fertilization, adaptability to shading, high dry matter yield and nutritive value [4]. However, low soil nitrogen availability is still a limiting factor in tropical and subtropical forage areas [3]. The introduction of the nutrient through chemical fertilization and cycling processes is commonly observed to overcome this limitation [5]. Practices such as fertilization enable the manipulation of mineral nutrients in forage, which can lead to significant improvements in the quality of the pasture provided to grazing animals [6].

Nutrient cycling in terrestrial ecosystems occurs through the deposition of animal waste, such as urine and feces, into the soil. Herbivores play an important role as transporters of nutrients, such as nitrogen, as they consume plant material at one site and excrete it at other sites [7–9]. Nitrogen cycling is essential to prevent pasture degradation, and its speed and processes of degradation, mineralization and immobilization depend on the carbon:nitrogen ratio in the soil organic matter (SOM) [10].

In situations where fertilizer replacement in pastures is not usual, nutrients contained in animal excreta play an important role for pasture perenniality and sustainability. Of all the forage consumed during grazing, only a small proportion is retained in animal products, and most of it is returned through excretions. N is one of the nutrients with the highest potential for excretion in both feces and urine, raising the dynamics of the system as a whole. The hypothesis tested was that the mineral composition of forage is positively influenced by nitrogen fertilization and the silvopastoral system. The objective was to discover the nutrient characterization and mineral composition of *M. maximus* grazed by lambs in a silvopastoral system or full sun with added N.

2. Materials and Methods

2.1. Localization

The experiment was conducted at the Sheep and Goat Raising Teaching and Research Unit of the Federal University of Technology—Paraná, Dois Vizinhos campus. The institution is located in the Southwest region of Paraná, under latitude 25°42′ S and longitude 53°03′ W, with an approximate altitude of 520 m above sea level. The soil is classified as a red dystrophic latosol of clayey texture [11]. The region is characterized by a humid subtropical mesothermal (Cfa) climate, with an average annual precipitation of 1953 mm and average annual temperatures of 25.2 (maximum) and 14.7 °C (minimum) [12] (Figure 1).

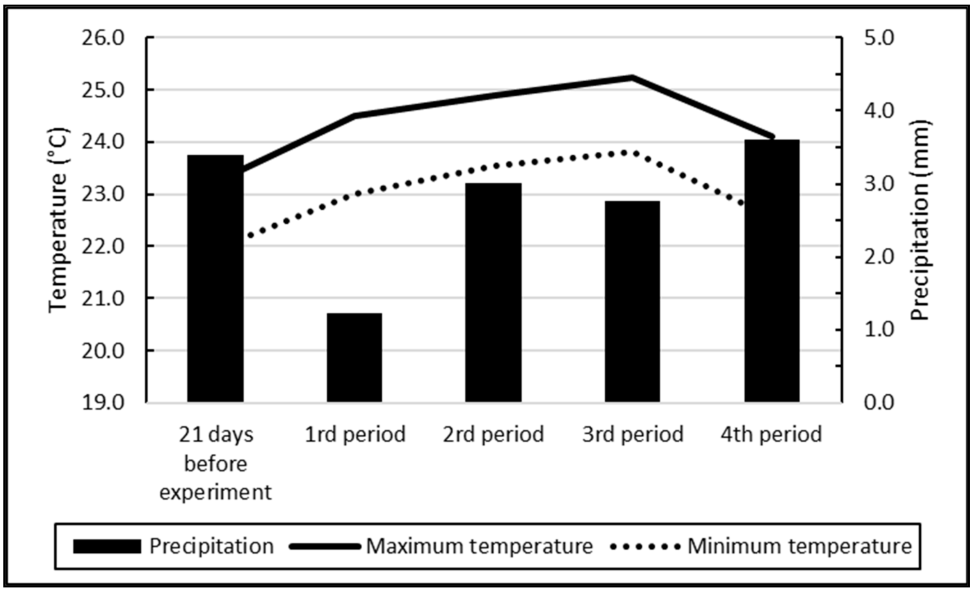

Figure 1. Meteorological data (minimum and maximum temperatures, °C and precipitation, mm) observed during field evaluations (November 2019 to March 2020).

2.2. Animals, Diets and Experimental Design

This study was approved by the Ethics Committee on Animal Use (CEUA-Federal University of Technology–Paraná–protocol no. 2019-34), in accordance with the ethical principles of animal experimentation recommended by the National Council for the Control of Animal Experimentation (CONCEA).

The treatments were: 1—*Aruana* pasture in full sun with nitrogen fertilization, 2—*Aruana* pasture in full sun without nitrogen fertilization, 3—*Aruana* pasture in silvopastoral system with nitrogen fertilization and 4—*Aruana* pasture in silvopastoral system without nitrogen fertilization.

The experiment was set up in a total area containing *Aruana* pasture divided into two systems: 06 pastures in a silvopastoral system (400 m^2 of area) with laurel (*Cordia trichotoma*) and canafistula (*Peltophorum dubium*) and 06 pastures in full sun (400 m^2 of area). The pasture area was established in 2010, and the tree component was implemented in the years 2013/2014 in the silvopastoral system, in the east–west direction with the arrangement of trees in 4 double rows at the ends of each of the paddocks, with an interval of 10 m between them and spacing of 2.00 m (between plants) and 1.50 m (between rows). According to the forest survey conducted in 2019, the *C. trichotoma* specimens had an average crown diameter of 3.05 m, crown height of 3.10 m and average height of 7.90 m. In 2018, the data concerning *P. dubium* were surveyed, being: average crown diameter of 2.20 m, crown height of 2.00 m and height of 3.90 m. The shade area provided by each of the trees averaged 36 m^2 [13]. Both systems were provided with drinking troughs and troughs for the mineral supplementation of the animals.

Prior to the implementation of the experiment, the soil pH of the total area was corrected using dolomitic limestone (PRNT 95.2%) in order to raise the base saturation to 70% (V%: 55.9%). In the plots treated with nitrogen fertilization, this was performed in the form of agricultural urea (46% total N), with a single application at a dose of 200 kg N ha^{-1}, performed after a uniformization cut to improve fertilization efficiency, approximately 15 days before the beginning of the experimental evaluations.

Twenty-four lambs (Dorper $\times$ Santa Ines), uncastrated males with a mean age of 60 $\pm$ 15 days and mean weight of 23.00 $\pm$ 3.50 kg, were used for grazing, randomly distributed in equal numbers among all the paddocks. To determine the nutrient dynamics, four lambs with the same characteristics and mean weight of 17.75 $\pm$ 1.00 kg were used, submitted to the treatments in a 4 $\times$ 4 Latin square (4 treatments $\times$ 4 periods), kept in each paddock for 5 days of adaptation and 10 days of evaluation. Each of the four animals walked on at least one paddock corresponding to the treatment in question during the experimental period.

The animals were weighed every 15 days, or as soon as they were moved from one enclosure to another during an evaluation period. The animal load (AL), in kg BW ha^{-1}, was calculated from the sum of the body weight of each animal and the area of each paddock.

2.3. Measurement and Sampling Techniques

The forage evaluations were performed at the beginning of each experimental period, aiming to provide a constant supply of 10% forage mass (MF) (10 kg DM/100 kg BW animal/day). The available FC was determined using the double sampling method [13], based on a visual estimate of 12 points and three real estimates of each experimental stand, by cutting the forage close to the ground with the aid of a square (area 0.25 m^2) in the real points. The grazing method used was continuous stocking and variable stocking rate, according to the "put and take" technique [14] and according to the available FM. The forage, leaf and stalk samples were collected in order to evaluate the mineral composition.

The soil samples were collected (0–20 cm) using a mug auger at 10 randomly spaced points in each experimental subarea, avoiding areas with the presence of feces. At the end, the sub-samples were concentrated, forming composite samples that were dried in a forced air oven at 55 °C for 72 h, ground in a 1 mm sieve knife mill and later analyzed for organic carbon (OC), organic matter (OCM) and total nitrogen (TN).

Spot urine samples were collected from each animal on day 15 of the experimental period. The four animals were placed in individual hanging pens (0.96 m^2 area) with a drinking trough approximately 4 h after the morning feeding in order to collect urine portions by spontaneous urination. After collection, 10 mL urine samples were diluted in 40 mL of H_2SO_4 (0.036 N) and stored in a freezer (−20 °C).

2.4. Chemical Analysis

The forage and soil samples were dried in an oven with forced air circulation at 55 °C for 72 h and ground in a mill with a 1 mm sieve. Dry matter (DM—method 934.01) was determined according to [15] (2000); P, K, Ca, Mg and S by nitropercloric digestion (P: colorimetry; S: turbidimetry; K: flame photometry; Ca and Mg: atomic absorption spectrophotometry) and N by sulfuric digestion [16]. Organic matter (OM) and organic carbon (OC) contents in the soil samples were determined by the pH method in $CaCl_2$ 0.01 mol L^{-1}; OC(1)/OM(1) colorimetric method [17]. The contents of P and K were extracted with a Mehlich-1 solution [18].

The urinary creatinine concentrations were analyzed using a commercial kit (Labtest® Lagoa Santa, Brazil). The daily urinary volume was calculated using the ratio between daily creatinine excretion and its concentration in spot urine samples using the value 19.82 mg creatinine/kg body weight [19].

From the estimated urine productions (UP), animal stocking (AS) and determined N contents, the total urine excretion (TUE) was calculated [20]:

$$\text{TUE} = [\text{UP}/\text{BW}] \times \text{AS} \tag{1}$$

where BW corresponds to the animal's body weight (kg/ha·day).

The amounts of N returned via urine (NRU) (g/ha·day^{-1}) were determined using the previously calculated excretion and urine N concentration [20]:

$$\text{NRU} = \text{NC} \times \text{TUE} \tag{2}$$

where: NC corresponds to nitrogen concentration (g/L).

2.5. Statistical Analysis

The OM, OC and N contents of the soil samples and the mineral contents of the leaves and canes were analyzed independently by ANOVA, and when they showed significant difference ($p < 0.05$), the means were compared by the Tukey test.

The variables BW, stocking density, total urine excretion, urinary N and the amount of N returned via urine were analyzed by ANOVA, and when they showed significant differences, the means were compared by Tukey's test ($p < 0.05$). The dependent variables were analyzed in the 4×4 split-block Latin square design with fixed effects (period and treatment) and random effect (animal).

The statistical model used was

$$\text{Yijk: } \mu + \text{Ai} + \text{Pj} + \text{Tk} + \text{eijk} \tag{3}$$

where Yijk: observation related to i-th animal, j-th period and k-th treatment; μ: general mean; Ai: effect corresponding to i-th animal (1, 2, 3 and 4); Pj: effect corresponding to j-th period (1, 2, 3 and 4); Tk: effect corresponding to the k-th treatment (1—*Aruana* pasture in full sun with nitrogen fertilization, 2—*Aruana* pasture in full sun without nitrogen fertilization, 3—*Aruana* pasture in a silvopastoral system with nitrogen fertilization and 4—*Aruana* pasture in a silvopastoral system without nitrogen fertilization); eijk: random error associated with i-th animal, j-th period and k-th treatment.

3. Results

The amount of nitrogen returned via urine was not affected ($p = 0.4872$) by the different treatments. The same was observed for the levels of OC ($p = 0.5384$), OM ($p = 0.5371$), P ($p = 0.6114$) and K (K = 0.9158) (Table 1).

Table 1. Effect of nitrogen fertilization and shading on organic matter, organic carbon and total nitrogen contents and standard error of the mean of soil samples collected at the end of the last experimental period in the different treatments.

Variable	Treatments				
	Full Sun		Silvopastoral		
	Fertilization	Without Fertilization	Fertilization	Without Fertilization	Mean [6]
OM (g/dm^3) [1]	43.37 ± 2.92	46.23 ± 1.19	47.00 ± 0.72	45.33 ± 1.45	45.48
OM (kg/ha) [1]	86.733 ± 5.842	92.466 ± 2.384	94.000 ± 1.442	90.666 ± 2.899	90.966
OC (g/dm^3) [2]	25.13 ± 1.71	26.83 ± 0.69	27.23 ± 0.43	26.30 ± 0.84	26.37
OC (kg/ha) [2]	50.266 ± 3.414	53.666 ± 1.377	54.466 ± 851	52.600 ± 1.677	52.750
N (mg/kg) [3]	2356.67 ± 234.21	2380.00 ± 122.52	2338.00 ± 78.00	2090.67 ± 76.93	2291.33
N (kg/ha) [3]	4713.33 ± 488.41	4760.00 ± 424.41	4676.00 ± 156.11	4181.33 ± 153.86	4582.67
P (mg/dm^3) [4]	9.20 ± 2.20	10.25 ± 1.32	9.66 ± 2.42	6.89 ± 1.03	9.00
P (kg/ha) [4]	18.40 ± 4.41	20.51 ± 2.64	19.32 ± 4.85	13.79 ± 2.07	18.00
K (mg/dm^3) [5]	84.72 ± 29.46	112.09 ± 19.20	110.78 ± 34.18	102.96 ± 37.53	102.63
K (kg/ha) [5]	169.43 ± 58.92	224.17 ± 38.40	221.56 ± 68.37	205.93 ± 75.05	205.27

[1] OM—organic matter; [2] OC—organic carbon; [3] N—total nitrogen; [4] P—phosphorus; [5] K—potassium; [6] Mean—standard error of the mean.

The total amount of urine excreted and the amount of nitrogen excreted in the urine did not differ between the different treatments (Table 2).

Table 2. Effect of nitrogen fertilization and shading on body weight (kg), stocking rate (kg/ha), total urine excretion (L/ha × day), urinary nitrogen (g/L) and amount of nitrogen returned via urine (g/ha × day) and standard error of the mean of lambs in shaded and full sun system with and without nitrogen fertilization.

Variable	Treatments					
	Full Sun		Silvopastoral			
	Fertilization	Without Fertilization	Fertilization	Without Fertilization	Mean [1]	*p* Value
Body weight (kg)	21.31 ± 2.08	21.02 ± 1.79	21.19 ± 1.65	20.81 ± 0.65	21.05	0.9966
Livestock density (kg/ha)	23.30 ± 4.79	17.26 ± 2.34	19.67 ± 3.34	17.24 ± 1.93	19.37	0.5410
Total urine excretion (L/ha × day)	0.732 ± 0.14	0.709 ± 0.16	0.695 ± 0.10	0.512 ± 0.07	0.662	0.5949
Urinary nitrogen (g/L)	0.459 ± 0.08	0.473 ± 0.06	0.435 ± 0.08	0.405 ± 0.09	0.443	0.9367
Nitrogen returned via urine (g/ha × day)	0.361 ± 0.12	0.334 ± 0.09	0.282 ± 0.02	0.196 ± 0.05	0.293	0.4887

[1] MEAN—standard error of the mean.

The phosphorus (P) contents were higher in the leaves and stalks of the silvopastoral system, indicating a positive influence of shading on its concentration (Table 3). The magnesium (Mg) concentration in the leaves and stems was higher in the full sun treatment with fertilization, while in stems without fertilization, there was no significant difference (Table 3).

Table 3. Effect of nitrogen fertilization and shading on mineral contents (g/kg) and standard error of the mean of leaf and stem samples of *Megathyrsus maximus* cv. *Aruana* in the silvopastoral system and full sun with and without fertilization.

Variable	Leaves					Stems				
	Treatments				Mean	Treatments				Mean [1]
	Full Sun		Silvopastoral			Full Sun		Silvopastoral		
	Fertilization	Without Fertilization	Fertilization	Without Fertilization		Fertilization	Without Fertilization	Fertilization	Without Fertilization	
P	2.61 ± 0.11 b	3.27 ± 0.07 a	2.57 ± 0.19 b	2.94 ± 0.05 ab	2.85	1.67 ± 0.03 b	2.12 ± 0.05 a	1.55 ± 0.14 b	1.79 ± 0.17 ab	1.78
K	17.95 ± 1.00 b	16.79 ± 1.78 b	26.17 ± 0.52 a	24.25 ± 1.29 a	21.29	18.13 ± 1.85	15.47 ± 2.46	20.99 ± 2.50	16.62 ± 1.25	17.80
Ca	6.47 ± 0.18 a	5.74 ± 0.12 b	6.38 ± 0.18 ab	6.25 ± 0.18 ab	6.21	3.53 ± 0.08	3.44 ± 0.05	3.55 ± 0.05	3.42 ± 0.11	3.48
Mg	6.46 ± 0.20 a	5.17 ± 0.28 b	5.28 ± 0.31 b	5.17 ± 0.15 b	5.52	6.06 ± 0.28 a	5.29 ± 0.32 ab	4.76 ± 0.35 b	4.96 ± 0.14 b	5.27
S	2.34 ± 0.08 ab	2.51 ± 0.05 a	2.19 ± 0.06 b	2.56 ± 0.04 a	2.40	1.94 ± 0.05 b	2.56 ± 0.12 a	1.73 ± 0.05 b	2.57 ± 0.12 a	2.20
N	28.03 ± 2.52	27.03 ± 1.46	25.34 ± 1.56	26.79 ± 0.59	26.80	12.62 ± 1.60	18.96 ± 2.95	14.50 ± 1.24	12.10 ± 2.17	14.54

[1] Mean—standard error of the mean. Averages followed by different letters in the same row comparing the effect of fertilization and shading are different ($p < 0.05$).

4. Discussion

No significant differences were observed in the soil's organic matter (OM), organic carbon (OC), total nitrogen (N), phosphorus (P) and potassium (K) contents among the different treatments. This finding suggests that nitrogen fertilization and shading did not have a substantial impact on the soil's mineral composition, at least during the period of this study. Short-term alterations in soil mineral content may not be easily discernible [21].

The total amount of urine excreted and the amount of nitrogen excreted in urine did not differ significantly between the various treatments. This suggests that neither nitrogen fertilization nor shading had a pronounced effect on nitrogen excretion by grazing animals. This finding contradicts the hypothesis that shading might reduce the stress of the animals and, consequently, influence their nitrogen excretion. Ref. [22] (2018) states that animals that consume higher levels of protein in their diet may present greater excretion of urinary N and greater losses of this nutrient through volatilization; however, this statement was not observed in animals that consumed fertilized pasture, since their nutritional requirements were met according to [23] (2007), expressing the results obtained in this research. Additional long-term studies may be necessary to fully elucidate the relationship between shading and nitrogen cycling in silvopastoral systems. The relationship between shading and nitrogen excretion may be complex and multifaceted [24].

In terms of plant mineral content, the phosphorus (P) levels were significantly higher in the leaves and stalks of the silvopastoral system compared to the full sun treatment, indicating a positive influence of shading on P concentration [25]. While the soil mineral content remained largely unaffected by the treatments, the nutrient composition of the plants showed notable variations [26]. This result may have been influenced by the presence of shading, the type of tree species implanted in the system and the nutritional composition and rate of litter degradation [27,28]. In a study carried out by [29] (2014), comparing the mineral composition of the dry mass of the aerial part (MSPA) of Mombaça grass (*Megathyrsus maximus* Jacq.) in a silvopastoral system with babassu (*Attalea speciosa*) and in a system without shading, a difference was observed in the P composition of forages between systems, corroborating the information obtained in this study. These findings emphasize the need for a comprehensive understanding of the intricate relationships within silvopastoral systems to optimize their productivity and sustainability.

5. Conclusions

Short-term nitrogen fertilization and shading had minimal impact on soil mineral content. Neither treatment significantly altered nitrogen excretion by grazing animals. However, notable differences in plant mineral content were observed: shading increased phosphorus levels, while magnesium concentration was higher in the fully sunlit, fertilized treatment. These findings emphasize the complexity of nutrient dynamics in silvopastoral systems, warranting further research.

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Informed Consent Statement: Not applicable.

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