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Biofertilizer in the nutritional quality of alfalfa (*Medicago sativa* L.)[1]

Biofertilizante na qualidade nutricional da alfafa (*Medicago sativa* L.)

Rodrigo Luis Lemes[2]; Cecílio Viega Soares Filho[3*]; Manoel Garcia Neto[3];
Reges Heinrichs[4]

Abstract

The objective of this study was to evaluate the response of alfalfa (*Medicago sativa* L.) in the nutritional composition to the application of biofertilizers. The experiment was conducted with increasing doses of biofertilizers in a greenhouse at the Faculty of Veterinary Medicine / UNESP, Araçatuba - Sao Paulo, Brazil, from April to October 2010. The experimental design was completely randomized with six biofertilizer doses from cattle manure (0, 25, 50, 100, 200, and 400 m^3 ha^{-1}) and five replications. Cuts were performed, on average, every 27 days, 10 cm above the ground when 10% of the plants were flowering. Biofertilization had a positive significant impact on foliar nitrogen, potassium, calcium, magnesium, sulfur, and shoot iron concentrations. The values of crude protein, acid detergent fiber, and neutral detergent fiber did not differ between doses of biofertilizers. Biofertilization is a viable alternative for nutrition of this species, showing positive results in the nutritional composition of alfalfa. However, but long-term studies are necessary to assess the environmental impact of these fertilizers.
Key words: Biofertilizer. Composition. Nitrogen. Crude protein. Cattle manure.

Resumo

O objetivo deste trabalho foi avaliar a resposta da alfafa (*Medicago sativa* L.) na composição nutricional à aplicação de biofertilizante. O experimento foi realizado em casa de vegetação na Faculdade de Medicina Veterinária/UNESP, Campus de Araçatuba, São Paulo, Brasil, de abril a outubro de 2010 com doses crescentes de biofertilizante. O delineamento experimental foi inteiramente ao acaso, sendo seis doses de biofertilizante oriundo de esterco bovino (0, 25, 50, 100, 200 e 400 m^3 ha^{-1}) e cinco repetições. Os cortes foram realizados, em média, a cada 27 dias, quando as plantas estavam com 10% de florescimento a 10 cm de altura do solo. Os teores foliares nitrogênio, potássio, cálcio, magnésio, enxofre e ferro da parte aérea tiveram melhora significativa. Os valores de proteína bruta, fibra em detergente ácido e fibra em detergente neutro não diferiram entre as doses de biofertilizante. O biofertilizante é uma alternativa viável para ser utilizado na nutrição desse vegetal, mostrando resultado positivo na composição nutricional da alfafa, mas estudos de longo prazo devem ser realizados para avaliar melhor o impacto ambiental.
Palavras-chave: Biofertilizante. Composição. Nitrogênio. Proteína bruta. Esterco bovino.

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[2] Zootecnista, Mestre em Ciência Animal, Faculdade de Medicina Veterinária, Universidade Estadual Paulista “Julio de Mesquita Filho”, UNESP, Campus de Araçatuba, Araçatuba, SP, Brasil. E-mail: rllemes@gmail.com
[3] Profs. Adjunto, Departamento. Apoio, Produção e Saúde Animal, Faculdade de Medicina Veterinária, UNESP, Campus de Araçatuba, Araçatuba, SP, Brasil. E-mail: cecilio@fmva.unesp.br; mgarcia@fmva.unesp.br
[4] Prof. Adjunto, Faculdade de Ciências Agrárias e Tecnológicas, UNESP, Campus de Dracena, Dracena, SP, Brasil. E-mail: reges@dracena.unesp.br
[*] Author for correspondence

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Introduction

Brazil is a major producer of dairy and meat, especially at low production costs since most properties are currently under pastures, which contributes to making the country internationally competitive (SILVA JÚNIOR et al., 2008).

Some producers use supplement concentrated in animal feed for maximizing production, however, this increases the production costs. For this reason, legumes are being used as forage as a more economical alternative to animal supplementation. Alfalfa (*Medicago sativa* L.) has been used for its nutritional quality, high productivity, ability to fix atmospheric nitrogen (N_2), and high palatability (MOREIRA et al., 2007; RODRIGUES et al., 2008); it is also popular due to. Its low seasonality in forage production.

A concentrated production of animal products is associated with the accumulation of waste, called organic fertilizers or biofertilizers, which can become a serious problem if not properly managed. To solve this problem efficiently, producers use animal waste as an input into agricultural production. The main organic fertilizers available for use in agriculture are poultry litter, pig manure, and manure of animals in general, vinasse, and green manure (SILVA et al., 2006). This practice reduces pollution and production costs and is regarded as a sustainable management practice (RTS, 2009).

Bovine biofertilizers originating from anaerobic digesters have the characteristic of containing nitrogen in large quantities. Soares Filho et al. (2015) applied bovine biofertilizer in *Cynodon dactylon* cv. Tierra Verde and found that it positively influenced the soil chemical properties and foliar growth.

However, Brazil still does not have a national waste policy (ABREU JUNIOR et al., 2005), and there is no consensus in plant nutrition (FONTES et al., 1992), especially regarding the impact of nitrogen on the production and composition of alfalfa. Thus, the aim of this study was to evaluate the nutritional characteristics of the aerial parts of alfalfa with increasing doses of bovine biofertilizers.

Material and Methods

The experiment was conducted at an altitude of 415 m from April to October 2010 in a greenhouse with a transparent plastic cover, in the Service Department of Animal Production and Health, Faculty of Veterinary Medicine of Araçatuba, Sao Paulo State University (UNESP), Brazil.

Soil was collected in a pasture area at a depth of 0-20 cm and classified as Ultisol. Prior to analysis, the samples were mixed and sieved through a 4 mm mesh. A soil sample was used for analysis, showing the following chemical composition: P = 3 mg dm^{-3}; MO = 17 g dm^{-3}; pH em $CaCl_2$ = 4.6; K = 1.6 $mmol_c$ dm^{-3}; Ca = 13 $mmol_c$ dm^{-3}; Mg = 6 $mmol_c$ dm^{-3}; H+Al = 34 $mmol_c$ dm^{-3}; sum of bases = 20.6 $mmol_c$ dm^{-3}, cation exchange capacity = 54.6 $mmol_c$ dm^{-3}, and base saturation = 37.7%.

Base saturation was increased to 80% (RAIJ et al., 1997) by the application of $CaCO_3$ and $MgCO_3$ in a ratio of 3:1. The soil samples were then incubated for 30 days in a pot under natural conditions, maintaining a humidity of 80% of field capacity.

After incubation, the soil was dried under natural conditions for seven days. The soil from the pots was placed individually in plastic trays and the following nutrients were added: $Ca(H_2PO_4)_2$, 200 mg dm^{-3} of P; K_2SO_4, 150 mg dm^{-3} of K and 61.53 mg dm^{-3} S; H_3BO_3, 0.5 mg dm^{-3} B; $CuSO_4$ 1.0 mg dm^{-3} Cu; H_2MoO_4, 0.1 mg dm^{-3} Mo; $MnSO_4$, 3 mg dm^{-3} Mn, and $ZnSO_4$, 2.0 mg dm^{-3} of Zn.

The biofertilizer used in this experiment originated from cow manure after being fermented in biodigester. The chemical characteristics were: 0.300 g N L^{-1}; 0.057 g P L^{-1}; 0.188 g K L^{-1}; 0.105 g Ca L^{-1}; 0.057 g Mg L^{-1}, 1 mg Mn L^{-1}; 1 mg Fe L^{-1}, and 1 mg Zn L^{-1}.

The experimental design was completely randomized, with six doses of biofertilizer (0, 25, 50, 100, 200, and 400 m^3 ha^{-1}) and five replications (cuts), totaling 30 experimental units. The proposed application was fractionated into three parts to avoid soaking of the soil samples with high doses of fertilizers. The first dose was applied during sowing, the second 30 days after sowing and third nine days after application of the second dose and 20 days before the first harvest.

In May 2010, alfalfa seeds were sown and thinning was carried out after 15 days, leaving only five uniform plants per 5 l pot. For harvesting, we made five cuts were 10 cm above the ground when 10% of the plants were flowering, precisely on the following dates 07/19/2010 (59 days after sowing), 08/09/2010 (21 days of age), 09/02/2010 (24 days of age), 09/24/2010 (22 days of age) and 10/21/2010 (27 days of age).

The severed shoots were wrapped in paper bags and dried at 65° C until constant weight, according to the methodology described in Silva and Queiroz (2002). The concentration of N, P, K, Ca, Mg, S, B, Cu, Fe, Mn, and Zn in the aerial part of the plant was analyzed in the first, second, and fifth cut. Determination of these elements followed the method described by Malavolta et al. (1997). Concentrations of neutral detergent fiber and fiber shoot acid detergent were analyzed in all cuts and determined according to the methodology described by Campos et al. (2004). Protein content was determined using Micro-Kjeldahl and multiplying the percentage of nitrogen by 6.25 (AOAC, 1990).

Absorbed nutrient values in the shoots were tested for normality of errors and homogeneity of variances. Statistical analyses were performed using SAS (Statistical Analysis System, version 9.1). We used ANOVA and Duncan´s test for multiple comparisons of averages at a significance level of 5% significance level. Regression analyses were performed according to the biofertilizers doses (PIMENTEL-GOMES; GARCIA, 2002).

Results and Discussion

According to the ANOVA results, the nutrients N, K, Ca, Mg, and Fe absorbed by alfalfa shoots showed significant differences ($P < 0.05$) between doses of biofertilizers and linearly responded to a dose of 400 m^3 ha^{-1} (Figures 1, 2, and 3). We found a lower N content in the control treatment (24.46 g kg^{-1}), and higher N values 400 m^3 ha^{-1} (29.77 g kg^{-1}), representing a 22% higher concentration. This finding is probably a result of the amount of nitrogen provided using biofertilizer (120 kg ha^{-1}) and might also be due to small, but insignificant increases in MO concentrations. These values are closer to those obtained by Moreira et al. (2002) who evaluated the effect of different doses and sources of P in alfalfa; however, the authors did not find significant differences between P sources.

We observed lower N values than Oliveira et al. (2004) who evaluated N in alfalfa cultivars in green house experiments and found higher N concentrations in plants treated with the highest dose (16 mmol N L^{-1}); however, they did not detect differences between cultivars. Another factor that could have caused such low N concentrations in our experiment soil pH index which was slightly lower at the end of the experiment than the value of 6.8 recommended by Honda and Honda (1990); low pH values can damage N_2 fixation efficiency in *Sinorhizobium meliloti*.

Figure 1. Absorbed nitrogen, potassium, and calcium values according to the doses of cattle manure biofertilizer in alfalfa.

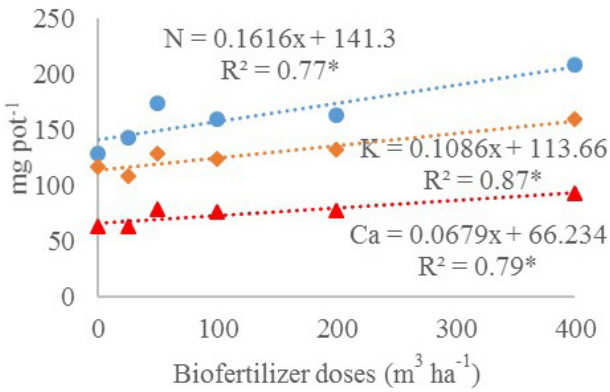


Figure 2. Absorbed magnesium and sulfur concentrations according to the doses of cattle manure biofertilizer in alfalfa.

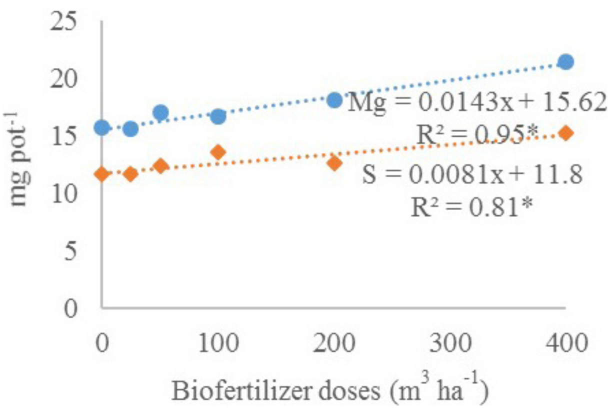
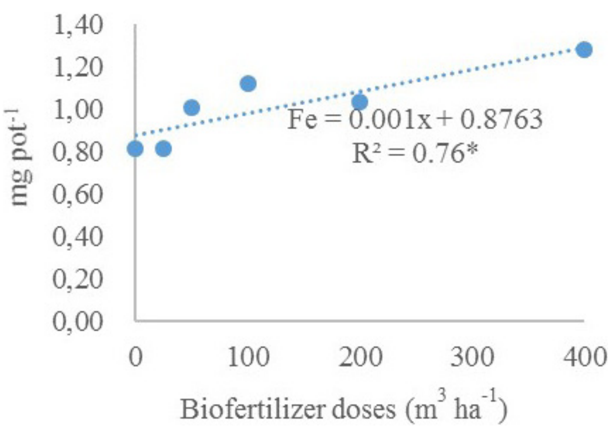


Figure 3. Number of absorbed iron according to the doses of cattle manure biofertilizer in fertilizer alfalfa.



Vasileva and Kostov (2015) also found a positive effect of the application of cattle manure at doses of 70, 140, and 210 kg N ha^{-1} in alfalfa. The increase in the N content in the biomass responded significantly to the dose of 140 kg N ha^{-1}. Yolcu (2015) found a linear increase in N concentration evaluating the effect of cattle manure of Hungarian vetch and barley grown in intercropping mixtures in response to three rates (0, 10 and 20 Mg N ha^{-1}). However, Rashid et al. (2014) evaluated the application of solid cattle manure at the dose of 200 kg N ha^{-1} and found the cumulative increase of absorbed N was 26% higher compared to a treatment with non-fertilized perennial ryegrass.

The amount of absorbed N increases linearly with the application of biofertilizers but is proportional to biomass increases in the aerial part of alfalfa (LEMES et al., 2013), showing that absorbed N was diluted in the dry shoot mass without significant increases in concentrations, featuring the dilution effect (JARREL; BEVERLY, 1981). Similar results were found by Araujo et al. (2011) who assessed the biomass of four forage species (*Desmanthus virgatus, Macroptillium martii, M. lathyroides*, and *Cenchrus ciliaris*) grown with and without organic manure (20 Mg ha^{-1}); N concentrations did not differ significantly between all four species.

A biofertilizer dose of 400 m^3 ha^{-1} resulted in highest K absorptions (Figure 1), and the concentration was within the range established by Moreira et al. (1997) and Raij et al. (1997). One of the reasons for using an increased amount of K with a further application of biofertilizer is the low nutrient availability in the soil; according to Raij et al. (1997), soil K values at the end of the experiment were relatively low due to a high K uptake by alfalfa. Low K values could also be an effect of competitive inhibition of K, Ca, and Mg, verified by Moreira et al. (1999) in an experiment with various Ca: Mg ratios and different K levels in alfalfa. Araújo et al. (2011) found slight increases in K concentrations through manure application in four species. Since K is an element retained in plant tissues, even in those already digested, manure treatment has a considerable influence on the final concentration and availability of K. Yolcu (2015) also found an increased K concentration when up to 20 Mg ha^{-1} cattle manure were applied to the consortium grazing vetch with barley.

The largest amount of absorbed Ca per pot was achieved with a treatment of 400 m^3 ha^{-1}, which represented a 49% absorption greater than the control (Figure 1). In all treatments, the concentration of this nutrient was within the range found by Moreira et al. (1997) and Raij et al. (1997). This increase is consistent with the concentration of Ca in the soil which increased with increasing doses of biofertilizer. Similar results were also obtained by Carneiro et al. (2009) who worked on soils with mycorrhizal fungi and obtained an increase of the Ca amount by pot according to elevated levels of P. Yolcu (2015) also observed increased Ca concentrations when up to 20 Mg ha^{-1} cattle manure was applied to cattle grazing vetch intercropped with barley. However, Lloveras et al. (2004) found a decrease in Ca concentration with higher applications of pig manure.

Leaf Mg concentration was higher at a biofertilizer dose of 400 m^3 ha^{-1} (Figure 2). Retention was 37.51% higher than at a dose of 25 m^3 ha^{-1} which had the lowest absorption. These data reflect Mg concentrations in the soil after the last cut. The values resulting from the treatments 200 and 400 m^3 ha^{-1} (3.07 and 3.09 g kg^{-1}, respectively) were higher than those found by Lloveras et al. (2004) who applied swine manure and mineral fertilizer for two years; however, they found no significant differences between treatments. Salmeron et al. (2010), also in an experiment using pig manure, found significant differences between treatments in the second year of the experiment, but the concentrations were lower (2.2 to 2.4 g kg^{-1}). However, Yolcu (2015) reported lower leaf Mg concentration values (1.78, 2.65, and 2.08 g kg^{-1}) at doses of 0, 10, and 20 Mg ha^{-1} cattle manure applied to grassland vetch intercropped with barley.

The highest absorption rate of S was found in the treatment 400 m^3 ha^{-1} (Figure 2), which was 30.53% greater than in the control treatment. This concentration was similar to the levels described by Raij et al. (1997) and Yolcu (2015) and higher than levels observed by Moreira et al. (1997) who reported significant differences in S concentration in dry mass on the basis of gypsum rates. However, Lloveras et al. (2004) found no significant difference in S absorption when applying different doses of pig manure.

Nitrogen and sulfur are important elements in plant nutrition because they are constituents of amino acids such as cysteine, cystine, and methionine and participate in metabolic processes of photosynthesis; an adequate nitrogen to sulfur ratio (N: S) indicates a good nutritional status of the plant Malavolta et al. (1997). As the concentrations of N and S responded similarly, we found an N: S ratio of about 11: 1 in the control treatment, 13.7: 1 for a dose of 400 m^3 ha^{-1} and 14: 1 for a dose of 50 m^3 ha^{-1}; these values are close to those indicated as suitable for grasses (MOREIRA et al., 2007).

Reijneveld et al. (2014) studied the relationship between soil fertility, forage quality and the composition of manure applied in dairy farms pastures and found a positive linear correlation between the nutrients S, P, K, and Mg in the soil and grass shoot.

The amount of P leaf per pot had no difference according to the F test; the average number was 19.27 mg pot^{-1} (Table 1). The P content in dry mass was above the critical value of 2.4 g kg^{-1} established by Andrew and Robins (1969). A similar increase in P concentration was observed by Lloveras et al. (2004) with applications of 25 and 50 m^3 ha^{-1} per year of pig manure to alfalfa. However, Salmeron et al. (2010) found no differences in P concentrations when they applied different doses of swine manure in the Ebro Valley, Spain; similar observations were made by Yolcu (2015) with an application of cattle manure. On the other hand, Araujo et al. (2011) found small differences in P concentrations with the application of cattle manure (20 Mg ha^{-1}), however, the accumulation of P in shoots was significant, indicating the potential P supply by cattle manure.

Table 1. Shoot nutrients in alfalfa according to the cuts by pot.

Treatment	Phosphor		Boron		Copper		Manganese		Zinc	
Mean BD	19.27 (± 2.278)		0.31 (± 0.037)		0.04 (± 0.004)		0.35 (± 0.037)		0.17 (± 0.014)	
	----- mg pot^{-1} -----									
1° Cut	19.33	B	0.406	A	0.046	A	0.311	B	0.200	A
2° Cut	15.37	C	0.259	B	0.034	B	0.268	B	0.139	B
5° Cut	23.45	A	0.282	B	0.045	A	0.473	A	0.159	B
CV (%)	16.20		11.51		16.81		20.49		13.67	

Means followed by different capital letters differ significantly by Duncan´s test at 5% probability. BD = Biofertilizer doses. CV= Coefficient of variation.

We found no significant differences in boron amounts (Table 1). The concentration did not reach the levels achieved by Moreira et al. (2000), but was close to the maximum values described by Raij et al. (1997). Lloveras et al. (2004) conducted a similar study in Spain and did not observe statistically significant differences between treatments; however, they found values lower than in our study. Yolcu (2015) found a linear response of B to the highest dose of cattle manure applied to the consortium grazing vetch with barley.

The treatments 200 and 400 m^3 ha^{-1} differed from 0 to 25 m^3 ha^{-1}, and 400 m^3 ha^{-1} recovered 39.27% less Fe compared to the control (Figure 3). However, all concentrations were in agreement with the literature (MOREIRA et al., 2002; RAIJ et al., 1997). Moreira et al. (2002) also found differences in the concentrations of this mineral according to the dose and source of the applied P.

The levels of the micronutrients Cu, Mn, and Zn showed no significant differences between treatments according to the F test (Table 1). Cu and Zn concentrations were below those found in the literature (MOREIRA et al., 2000; RAIJ et al., 1997), but Mn concentration was within the range defined by Raij et al. (1997).

Divergent results were found by Salmerón et al. (2010), who observed a significant increase in Cu concentrations in the first year of the trial and in Zn values in the second year with increasing pig manure applications to alfalfa. Lloveras et al. (2004) also found different results in the concentration of Cu with various pig manure application levels.

With regard to cuts, all the nutrients evaluated in this experiment showed significant differences, however, the cuts cannot be considered as a treatment, but rather as a condition of the experiment. The greater absorptions of Mn, P, N, and Mg (Table 1) occurred in the fifth cut. The amount of K was decreasing with the cuts because this nutrient was available only at low levels in the soil at the end of the experiment, not meeting the needs of the plant.

Smaller amounts of the elements Cu, Ca, S, and Fe (Table 1) per pot were found in the second cut. Moreira et al. (2000) also found a higher level of B of the first cut of alfalfa and higher levels of Cu and Mn in the fifth section in an experiment to test the Ca: Mg ratio. However, unlike in our study, (Table

1) they found higher amounts of Zn in the sixth cutting.

Xavier et al. (2005) tested the efficiency of inoculants and found a higher concentration of N in the first cut, in contrast to our observations. Moreira et al. (2002) worked with different doses of P and found the highest concentration in the third, fourth, and fifth cutting of alfalfa. The contents of Cu, Fe, Mn, and Zn in our study were similar to those found by Moreira et al. (2000).

The concentration of crude protein did not reach statistical difference (P> 0.05) between the biofertilizer doses according to the F test, with an average of 17.37% (Table 2). These values are close to those found by Pompeu et al. (2003) who evaluated the alfalfa cultivate Crioula, in an experiment with the application of nitrogen fertilizer. However, Benke et al. (2013) reported increases in the concentration of crude protein in feed barley with application rates of 0, 60, 120, and 180 Mg ha^{-1} of cattle manure.

Thus, the concentration of crude protein in our study was close to that recommended by the EMBRAPA (2002) to supply concentrate for dairy cows. Lloveras et al. (2000) also found no significant difference in the concentration of crude protein in an alfalfa experiment with an N application of 30 kg ha^{-1} yr of nitrogen; however, the protein concentration was higher (20.04 to 23.58%).

Oliveira et al. (2004) found significantly higher N concentrations in an experiment using nitrogen rate eight times higher than the control in the greenhouse; however, they found no significant difference in a field experiment with applications of 450 kg nitrogen ha^{-1} yr^{-1}.

Table 2. Chemical characteristics of the aerial part of alfalfa according to the cuts by pot.

Treatment	CP		NDF		ADF	
Mean BD	17.37 (± 1.33)		37.93 (± 0.97)		24.27 (± 0.87)	
			%			
1° Cut	15.79	B	37.91	A	25.19	A
2° Cut	17.77	AB	35.55	B	22.56	B
5° Cut	18.58	A	38.71	A	22.64	B
CV (%)	13.39		8.53		9.12	

Means followed by different capital letters differ significantly by Duncan´s test at 5% probability. BD = Biofertilizer doses. CV= Coefficient of variation.

The protein content was significantly higher for the fifth cut (Table 2). This concentration increase may be due to better root formation and an improved ability to absorb nutrients or due to the climatic variation how light and temperature. Kawas et al. (1990), the protein content changes according to alfalfa maturity stage.

We found statistically significant differences among neutral detergent fiber contents the F test between biofertilizer doses studied, with an average of 37.93% (Table 2). Fontes et al. (1992) provided the same amount of nitrogen as provided by the biofertilizer using urea; they also found no significant difference between treatments and fiber values of neutral detergent. Similarly, Pompeu et al. (2003) used nitrogen fertilizer and found no significant difference between treatments. However, the fiber content in the neutral detergent was higher, probably because of the more advanced age of the plants, as the first cut was made 107 days after sowing.

Similarly, the fiber content in the acid detergent was not significantly different between biofertilizer doses according to the F test, with an average content of 24.27% (Table 2). Similar values were found by Cherney et al. (1994), in an experiment testing ammonium nitrate doses in alfalfa collected 71 days after sowing; they also found no significant difference between treatments.

The fiber content in neutral and acid detergent was significantly different between cuts (Table 2). These values may have varied probably by a small difference in the plant maturity stage, because the cuts were made after a visual assessment of maturation, which might entail slight errors. According to Kawas et al. (1990), acid detergent fiber and neutral detergent fiber vary with the growth stage of the plant. The fiber values of neutral detergent were within the range established by Kawas et al. (1990), as pre-bloom and early bloom. The fiber content of acid detergent was similar to values defined as pre-flowering by Kawas et al. (1990). Yoottasanong et al. (2015) worked with cattle manure doses (0, 8, 16 and 24 Mg ha^{-1}) in *Panicum maximum* cv. TD58 and *Stylosanthes hamata* cv. Verano and found a positive effect on NDF up to a dose of 8 Mg ha^{-1}; however, FDA content had no significant effect.

Conclusion

The nutrients N, K, Ca, Mg, S, and Fe in alfalfa shoots responded linearly to biofertilizer doses of up to 400 m^3 ha^{-1}. The nutritional value was not influenced by the application of biofertilizers. Cattle manure is a viable alternative for alfalfa nutrition, but long-term studies are needed to the associated environmental impact.

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References

ABREU JUNIOR, C. H.; BOARETTO, A. E.; MURAOKA, T.; KIEHL, J. C. Uso agrícola de resíduos orgânicos: propriedades químicas do solo e produção vegetal. *Tópicos Especiais em Ciência do Solo*, Viçosa, MG, v. 4, n. 1, p. 391-470, 2005.

ANDREW, C. S.; ROBINS, M. F. The effect of phosphorus on the growth and chemical composition of some tropical pasture legumes. I. Growth and critical percentages of phosphorus. *Australian Journal of Agricultural Research*, Melbourne, v. 20, n. 2, p. 665-674, 1969.

ARAUJO, E. R.; SILVA, T. O. da; MENEZES, R. S. C.; FRAGA, V. da S.; SAMPAIO, E. V. de S. B. Biomassa e nutrição mineral de forrageiras cultivadas em solos do semiárido adubados com esterco. *Revista Brasileira de Engenharia Agrícola e Ambiental,* Campina Grande, v. 15, n. 9, p. 890-895, 2011.

ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS - AOAC. Official methods of analysis. 15th ed. Washington: AOAC, 1990. 1117 p.

BENKE, M. B.; INDRARATNE, S. P.; HAO, X. Long-term manure applications impact on irrigated barley forage mineral concentrations. *Agronomy Journal*, Madison, v. 105, n. 5, p. 1441-1450, 2013.

CAMPOS, F. P.; NUSSIO, C. M. B.; NUSSIO, L. G. *Métodos de análise de alimentos*. Piracicaba: FEALQ, 2004. 135 p.

CARNEIRO, R. F. V.; EVANGELISTA, A. R.; FERREIRA, A. S. Crescimento vegetativo e aquisição de nutrientes pela alfafa em resposta à micorriza e doses de fósforo. *Revista Brasileira de Ciências Agrárias,* Recife, v. 4, n. 3, p. 267-273, 2009. Disponível em: <http://redalyc.uaemex.mx/src/inicio/ArtPdfRed.jsp?iCve=1190 12585006>. Acesso em: 10 jul. 2011.

CHERNEY, D. J. R.; CHERNEY, J. H.; PELL, A. N. Inorganic nitrogen supply effects on alfalfa forage quality. *Journal of Dairy Science*, Champaign, v. 77, n. 12, p. 230-236, 1994.

EMPRESA BRASILEIRA DE PESQUISA AGROPECUÁRIA - EMBRAPA. Produção de leite no sudeste do Brasil. São Carlos: EMBRAPA, 2002. Disponível em: <http://sistemasdeproducao.cnptia.embrapa.br/FontesHTML/Leite/LeiteSudeste/introducao.html>. Acesso em: 3 jun. 2011.

FONTES, P. C. R.; CÓZER, A. C.; MARTINS, C. E.; VILELA, D. Resposta da cultura da alfafa *(Medicago sativa* L.) à adubação nitrogenada. *Revista da Sociedade Brasileira de Zootecnia*, Viçosa, MG, v. 21, n. 6, p. 996-1001, 1992.

HONDA, C. S.; HONDA, A. M. *Cultura da alfafa*. Cambará: IARA Artes Gráficas, 1990. 245 p.

JARREL, W. M.; BEVERLY, R. B. The dilution effect in plant nutrition studies. *Advances in Agronomy*, San Diego, v. 34, n. 1, p. 197-224, 1981.

KAWAS, J. R.; JORGENSEN, N. A.; LU, C. D. Influence of alfalfa maturity on feed intake and site of nutrient digestion in sheep. *Journal of Animal Science*, Champaign, v. 68, n. 12, p. 4376-4386, 1990.

LEMES, R. L.; SOARES FILHO, C. V.; GARCIA NETO, M.; HEINRICHS, R. The chemical properties of soil for alfalfa production after biofertilizer application. *Semina: Ciências Agrárias*, Londrina, v. 34, n. 5, p. 2211-2218, 2013.

LLOVERAS, J.; ARA'N, M.; VILLAR, P.; BALLESTA, A.; ARCAYA, A.; VILANOVA, X.; DELGADO, I.; MUNOZ, F. Efecto del abono nitrogenado invernal en la producción y calidad de la alfalfa en los regadíos del Urgell. In: REUNIÓN IBÉRICA DE PASTOS Y FORRAJES, 3., 2000, Bragança-La Coruña. *Anales*... Bragança-La Coruña: Sociedad Española para el Estudio de los Pastos, 2000. p. 255-261.

______. Effect of swine slurry on alfalfa production and on tissue and soil nutrient concentration. *Agronomy Journal*, Madison, v. 96, n. 4, p. 986-991, 2004.

MALAVOLTA, E.; VITTI, G. C.; OLIVEIRA, S. A. *Avaliação do estado nutricional das plantas*: princípios e aplicações. Piracicaba: Potafos, 1997. 319 p.

MOREIRA, A.; BERNARDI, A. C. C.; RASSINI, J. B.; FERREIRA, R. de P.; OLIVEIRA, P. P. A. *Fertilidade do solo e estado nutricional da alfafa cultivada nos trópicos*. São Carlos: Embrapa Pecuária Sudeste, 2007. 40 p. (Embrapa Pecuária Sudeste. Documentos, 67).

MOREIRA, A.; CARVALHO, J. G.; EVANGELISTA, A. R. Efeito de doses de enxofre na produção e composição mineral da alfafa. *Pesquisa Agropecuária Brasileira*, Brasília, v. 32, n. 5, p. 533-538, 1997.

______. Influência da relação cálcio:magnésio do corretivo na nodulação, produção e composição mineral da alfafa. *Pesquisa Agropecuária Brasileira*, Brasília, v. 34, n. 2, p. 249-255, 1999.

MOREIRA, A.; CARVALHO, J. G.; MORAES, L. A. C.; SALVADOR, J. O. Efeito da relação cálcio e magnésio do corretivo sobre micronutrientes na alfafa. *Pesquisa Agropecuária Brasileira*, Brasília, v. 35, n. 10, p. 2051-2060, 2000.

MOREIRA, A.; MALAVOLTA, E.; MORAES, L. A. C.; HEINRICHS, R. Fontes e doses de fósforo na concentração de nitrogênio e micronutrientes na alfafa e na centrosema. *Boletim de Indústria Animal*, Nova Odessa, v. 59, n. 2, p. 157-165, 2002.

OLIVEIRA, W. S.; OLIVEIRA, P. P. A.; DUARTE, F. R. S.; TSAI, S. M. Alfalfa yield and quality as function of nitrogen fertilization and symbiosis with *Sinorhizobium meliloti*. *Scientia Agricola*, Piracicaba, v. 61, n. 4, p. 433-438, 2004.

PIMENTEL-GOMES, F.; GARCIA, C. H. *Estatística aplicada a experimentos agronômicos e florestais*: exposição com exemplos e orientações para uso de aplicativos. Piracicaba: FEALQ, 2002. 309 p.

POMPEU, R. C. F. F.; UCHOA, F. C.; NEIVA, J. N. M.; OLIVEIRA FILHO, G. S. de; PAULA NETO, F. L. de; SILVA, E. S. da; LOBO, R. N. B.; BOTREL, M. A. Produção de matéria seca e qualidade de quatorze cultivares de alfafa (*Medicago sativa* L.) sob irrigação no Estado do Ceará. *Revista Ciência Agronômica*, Fortaleza, v. 34, n. 2, p. 153-160, 2003.

RAIJ, B. Van; CANTARELLA, H.; QUAGGIO, J. A.; FURLANI, A. M. C. (Ed.). *Recomendação de adubação e calagem para o Estado de São Paulo*. Campinas: Instituto Agronômico de Campinas, 1997. 285 p. (Boletim técnico, 100).

RASHID, M.; GOEDE, R. G. M.; NUNEZ, G. A.; BRUSSAARD, L.; LANTINGA, E. A. Soil pH and earthworms affect herbage nitrogen recovery from solid cattle manure in production grassland. *Soil Biology and Biochemistry*, Elmsford, v. 68, n. 1, p. 1-8, 2014.

REDE DE TECNOLOGIA SUSTENTÁVEL - RTS. Gestão de dejetos animais passa por expansão. Brasília: IBICT, 2009. Disponível em: <http://rts.ibict.br/noticias/destaque-2/imagens/gestao-de-dejetos-animais-passa-por-expansao>. Acesso em: 3 jun. 2015.

REIJNEVELD, J. A.; ABBINK, G. W.; TERMORSHUIZEN, A. J.; OENEMA, O. Relationship between soil fertility herbage quality and manure composition on grassland-based dairy farms. *European Journal of Agronomy*, Elmsford, v. 56, n. 5, p. 9-18, 2014.

RODRIGUES, A. A; CAMERÓN, E. A.; VILELA, D. Utilização da Alfafa em pastejo para alimentação de vacas leiteiras. In: FERREIRA, R. P. (Ed.). *Cultivo e utilização da alfafa nos trópicos*. Brasília: EMBRAPA, 2008. p. 361-392.

SALMERÓN, M.; CAVERO, J.; DELGADO, I.; ISLA, R. Yield and environmental effects of summer pig slurry applications to irrigated alfalfa under mediterranean conditions. *Agronomy Journal,* Madison, v. 102, n. 2, p. 559-567, 2010.

SILVA, A. A.; COSTA, A. M.; XAVIER, C. A. N.; MORALES, M. M.; LANA, R. M. Q. Efeito da aplicação de dejetos liquidos de suínos e fertilizantes minerais na absorção de nutrientes em pastagem *Brachiaria decumbens*. In: ENCONTRO LATINO AMERICANO DE INICIAÇÃO CIENTÍFICA, 10.; ENCONTRO LATINO AMERICANO DE PÓS-GRADUAÇÃO - UNIVERSIDADE DO VALE DO PARAÍBA, 6., São José dos Campos. *Anais*... São José dos Campos: Univap, 2006. v. 1, p. 2887-2890.

SILVA, D. J.; QUEIROZ, A. C. *Análise de alimentos*: métodos químicos e biológicos. 3. ed. Viçosa, MG: UFV, 2002. 160 p.

SILVA JÚNIOR, M. C.; PINTO, F. A. C.; FONSECA, D. M.; QUEIROZ, D. M.; MACIEL, B. F Detecção do efeito da adubação nitrogenada em *Brachiaria decumbens* Stapf. utilizando um sistema de sensoriamento remoto**.** *Revista Brasileira de Zootecnia*, Viçosa, MG, v. 37, n. 3, p. 411-419, 2008.

SOARES FILHO, C. V.; HEINRICHS, R.; PERRI, S. H. V.; CORREIA, A. de C. Atributos químicos no solo e produção de *Cynodon dactylon* cv. Tierra Verde sob doses de biofertilizante orgânico. *Revista Brasileira de Saúde e Produção Animal,* Salvador, v. 16, n. 1, p. 23-35, 2015.

VASILEVA, V.; KOSTOV, O. Effect of mineral and organic fertilization on alfalfa forage and soil fertility. *Emirates Journal of Food and Agriculture***,** Maqam, v. 27, n. 9, p. 678-686, 2015.

XAVIER, D. F.; GOMES, F. T.; LEDO, F. J. da S.; PEREIRA, A. V. Eficiência de inoculantes de rizóbio na nodulação de alfafa em solo de cerrado. *Revista Brasileira de Zootecnia*, Viçosa, MG, v. 34, n. 3, p. 781-785, 2005.

YOLCU, H. Effect of applications of organic solid cattle manure on Hungarian vetch and barley intercropping mixtures grown on soils of different depths. *Grass and Forage Science*, Cheshire, v. 70, n. 3, p. 428-438, 2015.

YOOTTASANONG, C.; PHOLSEN, S.; HIGGS, D. E. B. Dry matter yields and forage quality of grass alone and grass plus legume mixture in relation to cattle manure rates and production methods. *Pakistan Journal of Biological Sciences,* Faisalabad, v. 18, n. 7, p. 324-332, 2015.