Scientific Paper

Effect of nitrogen fertilization on the growth of five perennial pastures in Ecuador

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Abstract

Objective: To evaluate the effect of nitrogen fertilization on the growth and crude protein content of five perennial pastures of Ecuador.

Materials and Methods: To evaluate the effect of nitrogen fertilization on the growth and crude protein content of the species *Lolium perenne* L. var. Alto, *Lolium perenne* x *Lolium multiflorum* var. Shogun, *Festuca arundinacea* Schreb var. Alta Barolex, *Dactylis glomerata* L. var. Quick Draw and *Festuca arundinacea* x *Lolium multiflorum* var. Premium, six progressive doses of nitrogen were applied (0, 70, 140, 210, 280 and 350 kg N ha^{-1} year^{-1}). A split-plot design was used, where the species was placed in the large plot, and the nitrogen doses, in the small ones, with three repetitions. The variables biomass production, crude protein and normalized differential vegetation index, were evaluated. The data were analyzed through the statistical program INFOSTAT®.

Results: The biomass production indicated statistically significant differences ($p < 0,05$) for the studied factors species and nitrogen doses, but not for the interaction of such factors. *D. glomerata* and *F. arundinacea* showed the lowest biomass production values (2 457 and 2 490 kg DM ha^{-1}), compared with *L. perenne*, which had a moderate yield. *F. arundinacea* x *L. multiflorum* and *L. perenne* x *L. multiflorum* reached the highest biomass values (3 407 and 3 364 kg DM ha^{-1}). The crude protein content also increased with the nitrogen doses. In turn, the normalized differential vegetation index increased, also according to a quadratic model, with a different response among species.

Conclusions: All the species responded positively to the nitrogen application doses. It was determined that there is increase in the biomass production, crude protein content and normalized differential vegetation index.

Keywords: production, animal husbandry, vegetation indexes

Introduction

Animal husbandry represents 40 % of the agricultural gross domestic product (GDP) worldwide. This activity generates jobs for three thousand million people and is the main subsistence means for one thousand million throughout the world. Animal husbandry products provide one third of the world protein consumption (Steinfeld *et al*., 2009).

According to FAO (2019), population growth, increase of richness and urbanization have raised the demand for animal husbandry products, particularly in developing countries. It is foreseen that, to feed a population that is estimated to reach 9 600 million people in 2050, the world demand will increase by 70 %.

In the world, the animal husbandry sector uses the largest surface of agricultural lands in grazing and forage crops. This sector also plays an important role in climate change, land, water and biodiversity management. Currently, pressure is exerted for animal production systems to develop technologies that allow them to be sustainable. In order to reach this objective, such aspects as animal health and welfare, responsible utilization of animal genetic resources, sustainable animal nutrition and feeding, must be considered (FAO, 2018).

The efficient use of natural resources in animal husbandry should be focused on three areas: efficiency practices, pastureland management and manure management (Gerber *et al*., 2013). One management strategy in pastures is the use of fertilizers to enhance their production, and in it efficiency practices should be implemented. According to García (2017), at world level, from 30 to 50 % of the crop yield is ascribed to the nutrients provided by fertilizers, nitrogen (N) being the most widely used, followed by phosphorus (P) and potassium (K).

N is the element that limits yields the most in any productive system, and its consideration becomes more important, as the production is more intensive (Alesandri & Alesandri, 2009). The contributions of N to the crops should be made looking

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for economically and environmentally sustainable production (IDEA, 2007).

According to Santillano-Cázares *et al.* (2013), one of the reasons that can affect the cost effectiveness of agricultural productions lies on the fact that farmers could be using more nitrogen fertilizers than necessary.

The use of N and P as fertilizers is a widely spread practice, but their inadequate use can cause contamination of water bodies, and enhance algae growth in detriment of fish and native wild fauna (Alfaro & Salazar, 2005).

The excessive use of N in pastures can cause intoxications in the animals that consume this feed source, because it increases the concentration of nitrates, extremely toxic metabolite that reacts at blood level (Fulkerson *et al.,* 2010).

From these antecedents, the objective of this research was to evaluate the effect of nitrogen fertilization on the growth and crude protein content of perennial pastures in Ecuador.

Materials and Methods

Location. The study was conducted at the Experimental Teaching Academic Center La Tola (CADET, for its initials in Spanish) of the School of Agricultural Sciences (FCAg) of the Central University of Ecuador (UCE). The campus is in the Tumbaco parish, Quito canton, Pichincha province. This facility is located at 2 465 m.a.s.l., latitude 00° 14’ 46”S, longitude 78° 22’ 00” W

Climate characteristics. The study site has average rainfall of 952 mm, and temperature of 16,4 ºC (Date, 2019).

Soil conditions. In the soil analysis of the research area it was determined that N is found in low levels; while P and K appear in high levels. The soil is Molisol, of limey-clayey texture.

Experimental design and treatment. A split-plot design was used, with three blocks and 30 treatments, for a total of 90 experimental units. The large plot was the grass species, and the small one, the N dose. Five types of perennial grass species used as pastures were selected: diploid ryegrass (*Lolium perenne* L. var. Alto), tetraploid ryegrass (*Lolium perenne* x *Lolium multiflorum* var. Shogum), tall fescue (*Festuca arundinacea* Schreb var. Alta Barolex), cock’s foot (*Dactylis glomerata* L. var. Quick Draw) and festulolium (*Festuca arundinacea* x *Lolium multiflorum* var. Premium) and six nitrogen doses were evaluated.

Experimental procedure. In the experiment six N doses were analyzed, and complementary quantities of P, K, magnesium (Mg) and sulfur (S) were supplied (table 1). The utilized sources were ammonium nitrate, triple superphosphate, muriate of potash, ammonium sulfate and magnesium oxide. The N doses were fragmented into eight equal applications, which were performed seven days after each cutting. The other nutrients were applied at planting.

As nutrient sources, ammonium nitrate, triple superphosphate, muriate of potash, ammonium sulfate and magnesium oxide, were used.

The species were established in 7,5-m^2 plots (3 x 2,5 m) in 50 days. Thirty-three kilograms of seed were sown per hectare, in rows separated at 10 cm. At 15 days a germination control was done and re-sowing was carried out.

Four cuttings were evaluated, with interval of 28 days between cuttings. Physiologically, and under the agroclimatic conditions of this experiment, it is in this time that the pasture reaches its maximum growth point, without starting the senescence of its leaves. The plots were managed at field capacity, with a spraying irrigation system. The research

Table 1. N doses and complementary quantities of P, K, Mg and S used in the experiment.

N	P_2O_5	K_2O	MgO	SO_4
	kg ha^{-1} año^{-1}			
0	100	100	60	60
70	100	100	60	60
140	100	100	60	60
210	100	100	60	60
280	100	100	60	60
350	100	100	60	60

As nutrient sources, ammonium nitrate, triple superphosphate, muriate of potash, ammonium sulfate and magnesium oxide, were used.

was conducted in the summer, between June and September.

The variables biomass production, crude protein (CP) and normalized differential vegetation index (NDVI) were evaluated. The biomass production was determined by the quadrant method, with sizes of 0,3 x 0,3 m and random sampling in the plots. When making a low cutting, all the green matter on this surface was collected and weighed to take a subsample later and dry it in a stove during 24 hours, at 70 ºC (Mejía-Taborda *et al*., 2014). Finally, the dry matter (DM) content was determined and the total production per hectare, expressed in kg DM ha^{-1}, was calculated (AOAC, 2019).

The protein analysis was carried out at leaf level, from a DM sample obtained from the ground DM sieved in a 750-micra mesh, by the semimicro-Kjeldahl method, according to the official procedure of reference AOAC 2001.11. The result was expressed in percentage (UNAM, 2019).

To determine the NDVI the GreenSeeker equipment was used, which through an optical sensor measures the intensity of green color and plant size. These data were used in an algorithm and a value between 0 and 1 was determined (Trimble, 2019).

Statistical analysis. Variance analysis was carried out, after testing the variance and normality homogenization assumptions. The Shapiro-Wilks test, modified to determine normality, and Levene's test of variance homoscedasticity, were used. Tukey's test was applied for separating the means of the variables that indicated statistical significance ($p < 0,05$). Finally, through comparisons and orthogonal polynomials it was determined whether the associated data in each variable had a lineal, quadratic or cubic component. The equations were corroborated with the determination coefficient, adjusted determination coefficient and standard error. The program INFOSTAT® was used.

Results and Discussion

The biomass production indicated statistically significant differences ($p < 0,05$) for the studied factors species and N doses, but not for the interaction of such factors. All the species showed a positive response, with quadratic trend to increasing nitrogen doses. *D. glomerata* and *F. arundinacea* showed the lowest biomass production values, compared with *L. perenne*, which had moderate yield. *F. arundinacea* x *L. multiflorum* and *L. perenne* x *L. multiflorum* reached the highest biomass values

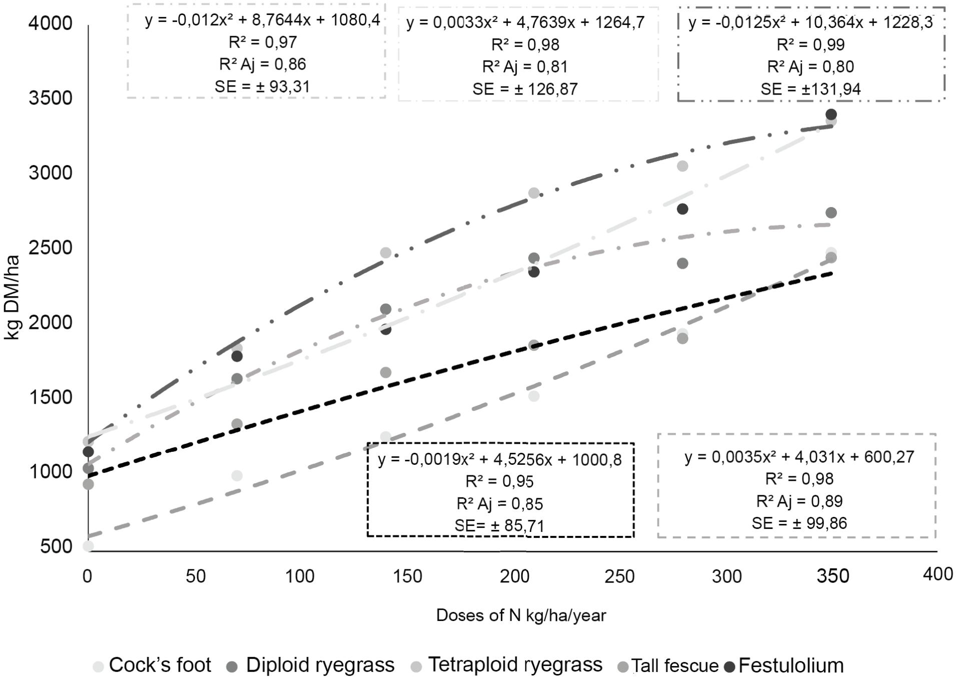

Figure 1. Biomass production, expressed in kg DM/ha, in five pasture species fertilized with different doses of nitrogen.

D. glomerata (cock's foot), *L. perenne* (diploid ryegrass), , *L. perenne* x *L. multiflorum* (tetraploid ryegrass), *F. arundinacea* (tall fescue), *F. arundinacea* x *L. multiflorum* (festulolium)

Table 2. Biomass production in five pasture species fertilized with different nitrogen doses (kg DM ha^{-1}).

Species	0	70	140	210	280	350
F. arundinacea x *L. multifloru*m (festulolium)	1 164a	1 800a	2 488a	2 885a	3 065a	3 407a
L. perenne x *L. multiflorum* (tetraploid ryegrass)	1 233a	1 853a	2 113ab	2 453ab	2 779a	3 364a
L. perenne	1 052a	1 651a	1 979ab	2 361ab	2 418ab	2 754ab
F. arundinacea	946ab	1 350ab	1 693bc	1 875bc	1 951b	2 490b
D. glomerata	535b	1 003b	1 265c	1 537c	1 919b	2457b
P - value	0,035	0,032	0,031	0,032	0,032	0,032
SE ±	96,77	75,68	93,72	103,88	105,91	111,02

Different letters in the same column differ for $p < 0,05$ according to Tukey

(fig. 1 and table 2). The low yields of *D. glomerata* and *F. arundinacea* are explained by the little adaptation of these species to the edaphoclimatic conditions of the zone, and also by the repeated presence of rust, which did not occur with the other evaluated species.

The results agree with the report by Villalobos (2016), who refers that N is the most important nutrient in pasture production, and that the fertilization doses used should optimize the production of biomass of high nutritional value with low cost. In this regard, Campillo *et al.* (2007) indicate that N is used to enhance grass productions. These authors reported significant effects of the nitrogen dose on the productivity and harvest of such grasses as *Triticum aestivum* L.

Méndez *et al.* (2016) found that the nitrogen fertilization increased the production of *F. arundinacea,* from 5 500 to 8 900 kg DM/ha, with N doses of 0 and 350 kg N/ha. These doses allowed to change the distribution of forage in the seasons, which is important in grazing systems.

Duran *et al.* (2016) stated that nitrogen fertilization could improve the plant productivity, but it should be managed carefully to prevent environmental damage. In this sense, Suter *et al*. (2015) reported about the current challenges for the world food security, which require sustainable intensification of agriculture through initiatives that include a more efficient use of N and the reduction of its losses to the environment. Rechiţean *et al*. (2018) propose that the response of grasses can be optimized, if they are sown in a mixture with legumes like clover (*Trifolium* sp.).

The CP content at leaf level showed significant differences ($p < 0,05$) among treatments for the two studied factors, but not for their interaction. There was positive response to the N doses with a lineal trend. Regarding the factor species, the CP content was lower for cock's foot, and the highest one was reached by festulolium. Meanwhile, *F. arundinacea*, *L. perenne* and *L. perenne* x *L. multiflorum* had moderate values (figure 2 and table 3).

The results of this research indicated crude protein contents that did not reach 23,0 %, for each of the studied species, even with the highest dose of nitrogen fertilization. In this regard, Fulkerson *et al.* (2010) reported that normally protein is adequate in pastures, if they are fertilized with N. However, once the protein content exceeds 23 %, nitrates start to accumulate, and this could cause rejection and intake decrease, as well as reduction in the rumen efficiency, which should not occur under the conditions of this experiment.

Cerdas-Ramírez (2018) found that, by increasing the N doses, the CP concentration increases, but the obtained response was not lineal. He only achieved that until 200 kg of N, higher doses did not increase the CP concentration. Velazco *et al.* (2019) stated that the protein content has a lineal performance with the applications of nitrogen fertilizer. Soto *et al.* (2004) established that protein is increased by 32 %, when the N doses increase from 200 to 400 kg N ha^{-1}.

Pautasso *et al.* (2020) refer that, although the utilization of fertilizers is an acknowledged technology, it is not correctly applied, and that the response of a crop varies under different agroclimatic conditions. Newell and Hayes (2018) found that the hybrid varieties of wheat have higher protein concentration at the same dose of fertilizer. One way to increase the CP content in the pastures, without demanding high quantities of fertilizers, is the incorporation of legumes in the pastures (Bozhanska and Churkova, 2020).

The NDVI indicated that there are statistically significant differences ($p < 0,05$) for species and

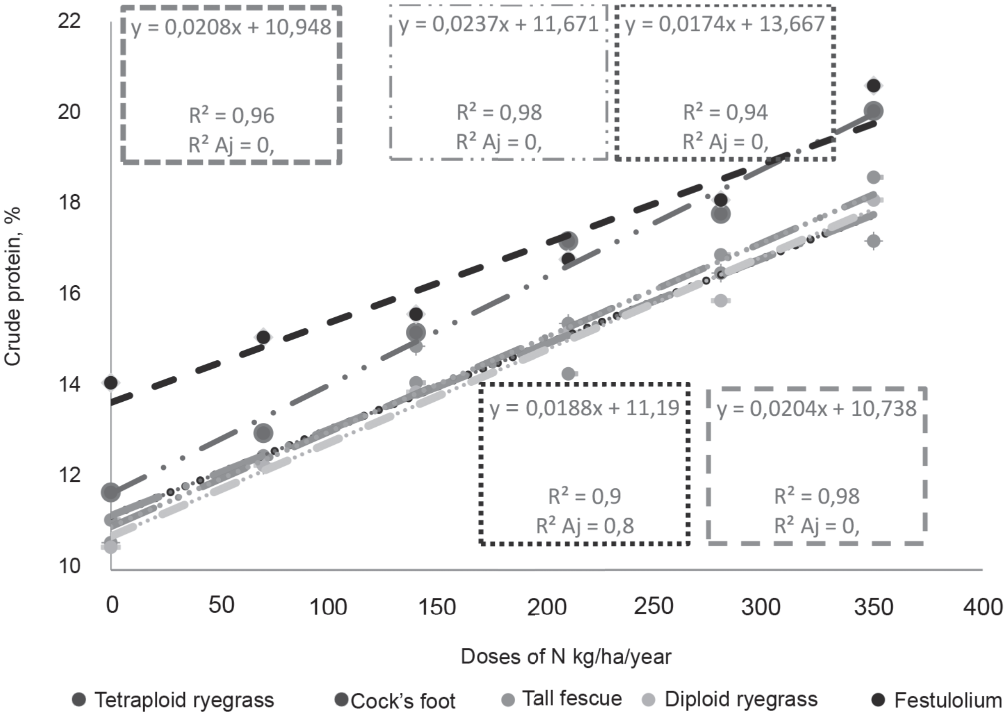

Figure 2. Crude protein content in five species of fertilized pastures with different nitrogen doses.

D. glomerata (cock's foot), *L. perenne* (diploid ryegrass), *L. perenne* x *L. multiflorum* (tetraploid ryegrass), *F. arundinacea* (tall fescue), *F. arundinacea* x *L. multiflorum* (festulolium)

Table 3. Crude protein content in five pasture species, fertilized with different nitrogen doses.

Species	Crude protein, %					
	0	70	140	210	280	350
F. arundinacea x *L. multiflorum* (festulolium)	11,4a	15,1a	15,6a	16,8a	18,1a	20,6a
L. perenne x *L. multiflorum* (tetraploid ryegrass)	9,6ab	13ab	15,2ab	17,2a	17,8a	20ab
L. perenne	9,6ab	12,5ab	13,9ab	15,2ab	15,9ab	18,1ab
F. arundinacea	9,6ab	12,3ab	14,1ab	14,3ab	16,5ab	18,6ab
D. glomerata	7,7b	9,7a	11,58b	15,4ab	16,9ab	17,2b
P - value	0,032	0,032	0,031	0,032	0,032	0,031
SE ±	0,41	0,44	0,27	0,43	0,39	0,4

Different letters in the same column differ for p < 0,05 according to Tukey

N doses. All the species showed positive response to the N doses. Their changes in growth, determined through the NDVI, responded to a quadratic model. The species *F. arundinacea* x *L. multiflorum*, *L. perenne* x *L. multiflorum* and *L. perenne* had higher values of NDVI, when fertilized with 280 and 350 kg of N, with regards to *F. arundinacea* and *D. glomerata*, which showed the lowest ones (fig. 3 and table 4).

The use of NDVI and the color indexes (spectral images) to determine changes in plant color and morphology have been tested by Filippa *et al.* (2018). Also Yao *et al.* (2014) concluded that NDVI can be used to estimate the nutritional status of a crop in a non-destructive way.

In studies conducted to establish the factors that control the photosynthetic efficiency of the plant, Ma *et al.* (2017) determined that the temperature, humidity and soil nutrients, mainly nitrogen, are the most important factors that determine photosynthetic efficiency, for which NDVI has been used as a tool to

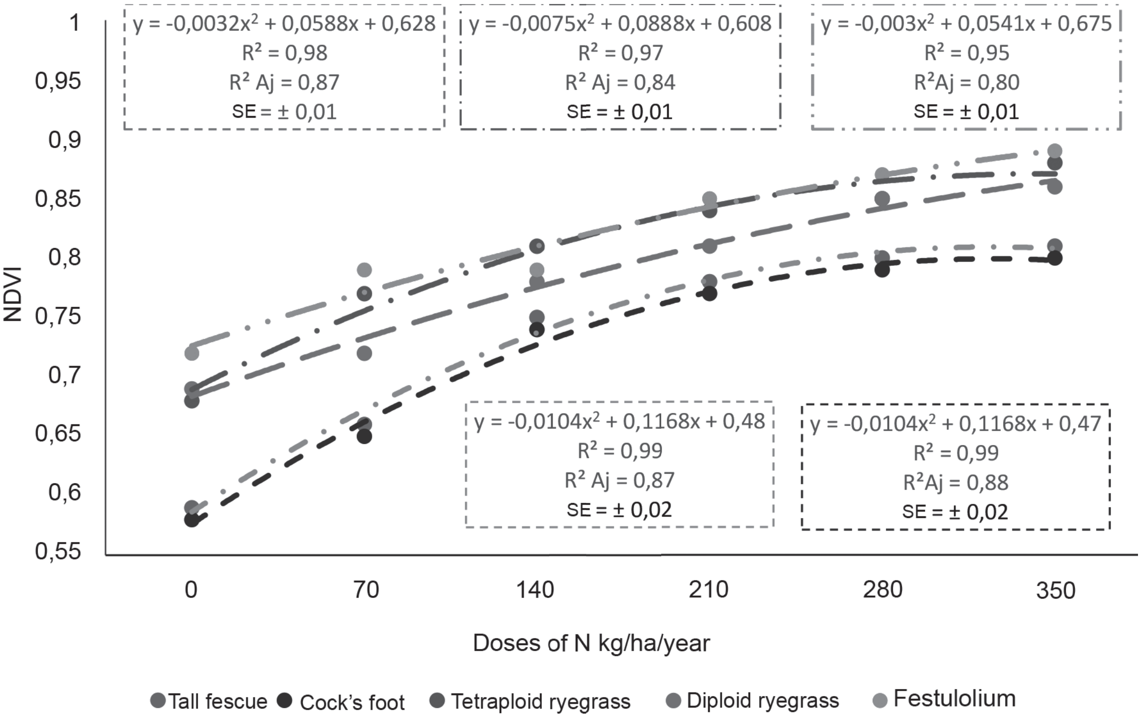

Figure 3. Normalized differentiated vegetation index in five pasture species fertilized with different nitrogen doses.

D. glomerata (cock's foot), *L. perenne* (diploid ryegrass), *L. perenne* x *L. multiflorum* (tetraploid ryegrass), *F. arundinacea* (tall fescue), *F. arundinacea* x *L. multiflorum* (festulolium)

Table 4. Normalized differentiated vegetation index (NDVI) in five pasture species fertilized with different nitrogen doses.

Species	NDVI					
	0	70	140	210	280	350
F. arundinacea x *L. multiflorum* (festulolium)	0,72a	0,79a	0,79a	0,85a	0,87a	0,89a
L. perenne x *L. multiflorum* (tetraploid ryegrass)	0,69ab	0,77a	0,81a	0,84a	0,85a	0,88a
L. perenne	0,68ab	0,72ab	0,78ab	0,81ab	0,85a	0,86a
F. arundinacea	0,58b	0,65b	0,74b	0,77b	0,79b	0,8b
D. glomerata	0,58b	0,65b	0,74b	0,77b	0,79b	0,8b
P - value	0,032	0.031	0,032	0,035	0,032	0,031
SE ±	0,02	0,01	0,01	0,02	0,01	0,01

Different letters in the same column differ for $p < 0,05$ according to Tukey

improve the application and efficiency of nitrogen fertilizers (Lapidus *et al*., 2017; Umesh *et al*., 2018). In turn, Chim *et al*. (2017) determined that the NDVI allows to establish the potential response of N in the crops, which is corroborated in the results of this study.

Rahetlah *et al*. (2014) and Karlsen *et al*. (2018) concluded that the biomass of pasturelands can be estimated from the NDVI. These authors referred their correlation is variable (between 0,6 and 0,8) and depends on the method that is used and on the species that is to be measured, which coincides with the data obtained in this research.

Vleugels *et al*. (2017) determined that the highest levels of NDVI (0,86) in ryegrass pasture for seed production were reached with 140 kg N ha^{-1}. Higher doses of N did not increase significantly the NDVI levels or seed production. However, (Fagundes *et al*., 1999) concluded that different cultivars show different light use efficiency, leaf areas, and, thus, different growth and forage productions.

Conclusions

All the species responded positively to the N application doses. There was increase in the biomass production, protein content and NDVI. Nevertheless, there were differences among species. *F. arundinacea* x *L. multiflorum* and *L. perenne* x *L. multiflorum* had better results, *L. perenne* reached moderate values; while *F. arundinacea* and *D. glomerata* achieved low performance, which is related to the fact that they did not adapt to the agroclimatic conditions of the essay.

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Authors' contribution

- Francisco Adolfo Gutiérrez-León. Writing of the scientific paper and research project, management of the funds, sample analysis, tabulation and statistical analysis.
- Brenda Pamela Benalcázar-Carranza. Evaluation of the varieties of *L. perenne*; *Lolium perenne* x *Lolium multiflorum* var. Shogum and *F. arundinacea*.
- Vanessa Fernanda López-Fiallos. Evaluation of *D. glomerata* and *F. arundinacea* x *L. multiflorum* var. Premium.
- Soraya Alvarado-Ochoa. Writing of the project and revision of the database.
- Arnulfo Rigoberto Portilla-Narváez. Sample drying and laboratory analyses.

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