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Nutritional quality of five grasses associated to *Lotus uliginosus* Schkuhr in the high tropic of Colombia

Edwin Castro-Rincón[1] *https://orcid.org/0000-0001-9841-8242*, Juan Evangelista Carulla-Fornaguera[2] *https://orcid.org/0000-0002-8854-1850* and Edgar Alberto Cárdenas-Rocha[2] *https://orcid.org/0000-0002-4279-844X*

[1]Corporación Colombiana de Investigación Agropecuaria, AGROSAVIA. Nariño, Colombia. [2]Universidad Nacional de Colombia. Facultad de Medicina Veterinaria y de Zootecnia. Bogotá, Colombia. E-mail: ecastro@agrosavia.co, jecarulla@unal.edu.co, eacardenasr@unal.edu.co

Abstract

Objective: To evaluate the nutritional quality and acceptability of five cold-climate grasses: *Holcus lanatus* (L), *Bromus catharticus* (Vahl)*, Festuca arundinacea* (Schreb)*, Cenchrus clandestinus* Hochst. ex Chiov (naturalizado), *Dactylis glomerata,* associated with *Lotus uliginosus* (Schkuhr) in the high tropic of Colombia.

Materials and Methods: The work was conducted in the Marengo Agricultural Center, of the National University of Colombia. A randomized block design was used, with split-plot arrangement. The association and regrowth age of 45 and 70 days was taken into consideration. The nutritional quality of each accession was analyzed: crude protein, neutral detergent fiber and acid detergent fiber and *in vitro* dry matter digestibility. After one year of establishment, a cafeteria test was carried out with heifers (300 kg of live weight) to calculate the relative acceptability index).

Results: Due to their high crude protein content, associated *C. clandestinum* (naturalized), *D. glomerata* and *F. arundinacea*, stood out ($p < 0,01$), compared with the other grasses and the pure control. In general, all the samples of *L. uliginosus* showed good nutritional content, with high values of protein (26,5 %) and *in vitro* dry matter digestibility (68,6 %), especially.

Conclusions: The association that showed the highest relative acceptability index was *C. clandestinus* (naturalized), being also the one with the best CP content and digestibility. *F. arundinacea* stood out as the promising material for the establishment in associations with legumes, due to its nutritional quality and compatibility with the legume.

Keywords: forages, legumes, feed quality, acceptability

Introduction

From the research perspective, in the selection of optimum forages for each ecosystem nutritional quality is evaluated, as supplementary of the biomass production, phenology, adaptation and acceptability by the animals (Enciso *et al*., 2019; Portillo-López *et al.,* 2019).

When forage selection is made, it is not the same to evaluate pure grasses as associated to a legume. In the latter, the effect of the companion legume on the variables of the grasses object of study, on production or its quality, can be observed (Lok-Mejías *et al.,* 2017; Momberg *et al.,* 2017).

Through this analysis species of grasses can be selected susceptible to be adequately mixed with legumes, and which offer desirable nutritional characteristics, complementary with the relative acceptability by the animal, from the premise that the nutritional value is a product of its quality and its relative acceptability (Arcos-Álvarez *et al*., 2019; Portillo-López *et al*., 2019).

In studies on cold-climate forages, works have been conducted like the one carried out by Jaime (2002), who evaluated in an exploratory way the species found in the Unit of Forage Genetic Resources of the National University of Colombia, in the Marengo Agricultural Center (CAM, for its initials in Spanish), in Mosquera, Cundinamarca. This author proved that there are grasses which, due to their adaptation and biomass production, would be materials of potential evaluation for their later use in animal feeding.

Portillo-López *et al.* (2019) conducted works that included in the evaluations *Lolium multiflorum* Lam, annual and biannual, *Cenchrus clandestinus Hochst. ex Chiov*; the legumes *Trifolium repens* L.*, Trifolium pratense* L., *Vicia sativa* L. and the non-legume creeping plants *Cichorium intybus* L. and *Plantago major* L. During the rainy and dry seasons, *L. multiflorum* aubade and italiano; the perennial cultivars Yorkshire fog, columbia and boxer; the legume *V. sativa*, and the non-legume creeping plants *C. intybu* and *P. major* showed in the evaluated localities the best values of dry matter (DM), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF) and nitrogen-free extract (NFE).

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As alternative, the evaluation of the legume *Lotus uliginosus* Schkuhr has also been indicated which, compared with other legumes, such as *Medicago sativa* L., shows advantages due to its adaptation to acid and infertile soils, high production of aerial biomass, high protein contents and, besides, presence of tannins in favorable levels for protein digestion (García-Bonilla *et al.,* 2015; Santacoloma-Varón, 2017). These characteristics profile this legume with potentialities for its use in milk production systems in the high Andean tropic of Colombia.

From these reasons, the objective of this research was to evaluate the nutritional quality and acceptability of five cold climate grasses: *Holcus lanatus* (L), *Bromus catharticus* (Vahl)*, Festuca arundinacea* (Schreb)*, Cenchrus clandestinus* Hochst. ex Chiov (naturalized), *Dactylis glomerata,* associated with *Lotus uliginosus* (Schkuhr) in Colombia.

Materials and Methods

Location of the study. The work was conducted during 2004, at the Marengo Agricultural Center, located in the San José district, Mosquera municipality, Cundinamarca, Colomia. The municipality is located at 4º 42´ North latitude and 74º 12´ West longitude.

Edaphoclimatic conditions. Its altitude corresponds to 2 650 m.a.s.l., with average temperature of 13 ºC, fluctuations between 0 and 20 ºC and presence of frosts in January, February and early August. The average annual rainfall is 528,9 mm, with bimodal distribution of two rainy seasons: one between April and May, and another from September to November. The region is classified as life zone of low mountain dry forest (Holdridge, 1982). The months with average rainfall equal to or higher than 50 mm (October, March, April and May) were considered as rainy season and dry season, those months in which at least 50 mm of rainfall as average was not recorded (January, February and June).

Treatments and experimental design. In a first phase, 11 materials of grasses associated to the legume *L. uliginosus* were used, which were evaluated regarding adaptation, production and relative acceptability with cattle. From such evaluation, the best five grass materials were selected: *H. lanatus*, *B. catharticus* var. Banco, *F. arundinacea* var. Festorina, *Cenchrus clandestinus* (naturalized), *D. glomerata* var. knaulgrass, associated with the legume *L. uliginosus*, and a pure control *C. clandestinus* (naturalized). The materials were selected at the Unit of Forage Genetic Resources (URGF, for its initials in Spanish) of the National University of Colombia, for their higher adaptation and production of aerial biomass. A randomized block design was applied with strip-plot arrangement. Each plot constituted the experimental unit, which contained each accession, and the strip, cutting time (45 and 70 days). In total, six grass materials, and five of them associated with *L. uliginosus*, were gathered. From the six plots established for the experiment, each one had three repetitions. The evaluations were conducted in the rainy (October, March, April and May) and dry season (January, February and June).

Experimental procedure. The evaluations were carried out at regrowth age of 45 and 70 days. These ages were established because, in the current productive systems of the Colombian high Andean tropic, 70 days is the most widely used regrowth for grazing *L. multiflorum* in the region, and 45 days is the regrowth that is considered optimum for the harvest in this zone (Correa *et al.,* 2016).

Evaluated variables. For each association the acceptance was evaluated with Holstein heifers (300 kg of average weight) in 12 plots and the relative acceptability index (RAI) used by Maass *et al.* (1999) was estimated. For such purpose, observations were made every five minutes, between 8:30 a.m. and 3:00 p.m., during two days in each repetition. The former was conceived as adaptation. With the result of the observations the RAI was calculated for each accession, dividing the grazing frequency observed in each specific accession by the total grazing frequencies, relative to the total included accessions. This is:

$$RAI = \frac{\text{Accession intake}}{\dfrac{\text{Intake all accessions/Repetitions}}{\text{No. of accessions}}}$$

For the best materials the nutritional quality was determined. For that purpose, a 200-g subsample was taken, of which the following was analyzed: crude protein (CP) according to AOAC (2016), neutral detergent fiber (NDF) by Van Soest (1963) and acid detergent fiber (ADF) through the methodology proposed by Van Soest *et al.* (1991) and *in vitro* dry matter digestibility (IVDMD) by the Tilley and Terry (1963) method. The plots were uniformed, at the end of the production cuts (1 year post-establishment) and at 45 days of regrowth in the dry season.

Statistical analysis. The nutritional quality variables CP, NDF, ADF and IVDMD were analyzed

through the program GLM/ANOVA (SAS, 2009); while the RAI was shown as the absolute value obtained. The mean comparison was done by Tukey's test.

Results and Discussion

For the 12 plots, in the regrowth of 45 days, the RAI varied from 0,43 to 1,82 among associations, and *F. arundinacea* (1,82) and *C. clandestinus* (1,76) stood out, these associations being highly acceptable, unlike for 50 % of them (RAI < 1,0). The associations of lower acceptability were *F. pratense*, *H. lanatus* and *P. pratense*, with values of 0,43; 0,45 and 0,46, respectively (table 1). This can be associated to the high proportion of legume in these associations.

In a study conducted by Leep *et al.* (2002) similar results were reported, when evaluating the acceptability of grass mixtures with *L. uliginosus.* These authors found that the mixture with *F. arundinacea* was the most accepted by the animals, in terms of percentage of pasture utilization, with values of up to 50 and 54 %, with regards to 13 and 15 % obtained in other mixtures. They are also similar to another work that evaluated acceptance and milk production, and where good intakes were recorded for *F. arundinacea* and increases in milk production of up to 20 % (Roca-Fernández *et al.,* 2016). It should also be taken into consideration that, in other cases, low intake of *F. arundinacea* is reported due to little preference, especially in sheep (Cougnon *et al.,* 2018).

Nutritional quality. In the rainy season no differences appeared among grasses in the CP content, at 45 days of regrowth, with average of 15,1 %. Meanwhile, at 70 days of regrowth, differences were observed among grasses ($p < 0,01$), with average of 12,0 % (table 2).

The CP content in *C. clandestinus* (naturalized) at 45 and 70 days of regrowth, stands out among all the grasses, with 17,3 and 14,3 %, respectively (table 2). Nevertheless, Portillo-López *et al.* (2019) referred higher values, with CP content of 23,3 % in the Pasto locality and 18,7 % in Sapuyes, Nariño, Colombia, in the rainy season. Jaime (2002) reported for *C. clandestinus,* at 45 days of regrowth, 21,5 % of CP, higher value than the one recorded in this study.

In the companion legume, the CP content did not vary between the regrowth ages, but it did between seasons ($p < 0,01$), with general average among associations of 26,1 and 24,0 % in the rainy season, at 45 and 70 days of regrowth. For the CP content in the legume, values were found between 21,7 and 30,8 %. The association with *C. clandestinus* (naturalized) stands out, with CP values of 30,8 and 29,1%, at 45 and 70 days (table 3).

In the dry season, at 45 days of regrowth, the grasses did not show significant differences, with a general average of 14,4 %. Meanwhile, at 70 days, significant differences were found ($p < 0,01$) with general average of 13,0 %. Among all the grasses, the CP content in *C. clandestinus* (naturalized) stood out, at 70 days of regrowth, with 16,4 % (table 4).

These values are lower than or similar to the ones reported by Correa *et al.* (2016), who referred

Table 1. Relative acceptability index (RAI) by grazing heifers of grass accessions associated to *L. uliginosus* and an accession of pure grass.

Accession	Relative acceptability index
C. clandestinus (naturalized pure control)	1,46
B. catharticus	0,74
F. rubra	0,47
D. glomerata	0,94
F. arundinacea	1,82
P. pratense	0,46
C. clandestinus (int)	1,42
A. odoratum	1,35
H. lanatus	0,45
D. glomerata (var Knuaulgrass)	0,71
F. pratense	0,43
C. clandestinus (naturalized)	1,76
Average	1,00
Standard deviation ±	0,58

Table 2. Nutritional quality of five grasses associated to *L. uliginosus*, at the cut of 45 days and 70 days, during the rainy season in Mosquera, Cundinamarca

Material/days of regrowth	CP		NDF		ADF		IVDMD	
	45	70	45	70	45	70	45	70
C. clandestinus (naturalized pure control)	16,6	14,2^{a}	59,3^{ab}	57,8^{ab}	27,2^{c}	29,4^{b}	64,6	62,3^{ab}
D. glomerata	14,9	11,6^{ab}	52,9^{c}	57,3^{ab}	34,5^{a}	34,2^{a}	58,9	65,7^{a}
F. arundinacea	15,9	12,2^{ab}	54,2^{c}	52,0^{c}	31,6^{b}	34,8^{a}	69,5	63,8^{ab}
H. lanatus	13,1	10,0^{b}	60,2^{a}	60,7^{a}	34,0^{ab}	34,3^{a}	62,1	58,4^{c}
B. catharticus	12,8	10,2^{b}	56,0^{bc}	56,5^{b}	34,7^{a}	34,3^{a}	68,1	61,6^{bc}
C. clandestinus (naturalized)	17,3	14,3^{a}	59,6^{a}	59,8^{ab}	27,2^{c}	26,8^{c}	68,7	62,4^{ab}
SE ±	1,49	0,55	0,66	0,7	0,78	0,36	0,8	10,32
P - value	0,07	0,0003	0,0002	0,0001	0,00001	0,00002	0,08	0,004

Small-case letter corresponds to significance among grass averages
Means followed by equal letters in the same column are not significantly different ($p < 0,05$), according to Tukey's test

Table 3. Nutritional quality *L. uliginosus* associated to grasses, at the cut of 45 days and 70 days, during the rainy season.

Material/days of regrowth	CP		NDF		ADF		IVDMD	
	45	70	45	70	45	70	45	70
D. glomerata + *L. uliginosus*	26,6^{ab}	24,0^{ab}	38,0^{b}	40,2^{b}	24,2^{b}	24,9^{d}	64,4^{d}	61,9^{b1}
F. arundinacea + *L. uliginosus*	26,6^{ab}	22,5^{b}	34,6^{c}	42,2^{a}	23,2^{c}	25,3^{d}	71,6^{a}	60,2^{c}
H. lanatus + *L. uliginosus*	22,9^{b}	23,1^{ab}	39,0^{a}	40,3^{b}	22,3^{c}	26,9^{a}	67,0^{c}	70,4^{a}
B. catharticus + *L. uliginosus*	23,3^{b}	21,7^{b}	38,9^{ab}	41,9^{a}	25,4^{a}	28,8^{a}	60,3^{e}	58,7^{c}
C. clandestinus (naturalized) + *L. uliginosus*	30,8^{a}	29,1^{a}	35,2^{c}	35,1^{c}	16,6^{d}	26,0^{c}	68,8^{a}	65,4^{ab}
SE ±	1,19	2,07	0,12	0,048	0,013	0,046	0,006	1,3
P - value	0,0002	0,03	0,0002	0,002	0,00001	0,00002	0,00006	0,004

Small-case letter corresponds to significance among legume averages
Means followed by equal letters in the same column are not significantly different ($p < 0,05$), according to Tukey's test

Table 4. Nutritional quality of five grasses associated to *L. uliginosus,* at the cut of 45 days and 70 days, during the dry season.

Material/days of regrowth	CP		NDF		ADF		IVDMD	
	45	70	45	70	45	70	45	70
C. clandestinus (naturalized pure control)	14	15,9^{a}	62,5	59,7	31,5	29,5^{b}	64,8	71,2^{a}
D. glomerata	15,5	13,8^{ab}	53,2	55,1	34,7	35,2^{ab}	64	54,2^{c}
F. arundinacea	15,1	12,1^{ab}	54,9	59,7	35,7	37,1^{b}	67,6	57,4^{bc}
H. lanatus	13,6	10,1^{b}	61,9	65,4	34,9	39,6^{a}	61,8	56,6^{bc}
B. catharticus	13,1	9,7^{b}	57,9	60,3	35,7	40,0^{a}	66,4	67,9^{a}
C. clandestinus (naturalized)	15	16,4^{a}	62,6	59,6	29,3	28,8^{b}	62,5	62,1^{b}
SE ±	0,41	1,2	8,05	4,95	8,94	2,40	15,48	1,81
P - value	0,07	0,0003	0,09	0,08	0,07	0,002	0,08	0,0004

Small-case letter corresponds to significance among grass averages
Means followed by equal letters in the same column are not significantly different ($p < 0,05$), according to Tukey's test

CP content of 19,2 and 14,5 % for this species in monoculture systems, at 45 and 79 days of regrowth. They are also similar to those obtained by Portillo-López *et al*. (2019), when they reported CP content of 18,7 % in the Pasto locality and 19,0 % in Sapuyes, Nariño, Colombia, in the dry season. Flórez-Gómez and Correa (2017) indicated for these species CP content of 20,3 % in the dry as well as in the rainy season.

Quiroga and Barreto (2002), when evaluating the nutritional quality of *C. clandestinus*, at frequencies of 45 and 75 days, in the rainy and dry seasons, obtained values between 12,6 and 17,9 % in the CP content, which are also very similar to the ones recorded in this study. In this same research line, Correa *et al*. (2018) when evaluating the residual height and its relation to the nutritional quality of *C. clandestinus*, concluded that *L. multiflorum* improves its quality and production until 35 days of regrowth, when a remnant of 15 cm is left in the pastureland. In this study, in the companion legume, the CP content did not vary between the regrowth ages, but it did between seasons, with general average among associations of 28,1 and 28,1 %, at 45 and 70 days, respectively, in the dry season.

For the CP content in the legume, values that varied between 26,7 and 30,6 % were recorded, according to the grass with which it was associated ($p < 0,01$). The association with *C. clandestinus* (naturalized) stood out, with CP values of 30,0 and 30,6 %, at 45 and 70 days (table 5), the content being high in general, if compared with studies conducted for this same legume and for *M. sativa*.

For the grasses, during the dry and rainy seasons, the CP content was higher at the regrowth of 45 days compared with that of 70 days. When comparing the two periods, there was higher average content in the rainy season, although without significant differences among associations.

The NDF and ADF contents in the grasses, at 70 days, were higher with regards to 45 days of regrowth, with 57,3 and 59,9 % for the rainy and dry seasons, respectively. The lowest values were obtained in the grasses *D. glomerata*, *F. arundinacea* and naturalized *C. clandestinus* as pure control, with content of 57,3 and 52; 57,3 and 55,1; 59,7 and 59,7%, respectively. This agrees with the high values recorded in the IVDMD content: 65,7 and 63,8; 62,3 and 54,2; 57,4 y 71,2; respectively (table 2 and 3).

The results of this research were in correspondence with and, in some cases, higher than the ones reported by Castro *et al*. (2008). These authors evaluated two pastures: a mixture of *C. clandestinus* and *F. arundinacea,* with NDF, ADF and IVDMD contents of 59,1 and 28,9; 65,7 and 58,6; 34,3 and 65,6 respectively, and a mixture of *F. arundinacea* and *L. uliginosus,* with NDF, ADF and IVDMD contents of 58,3 and 34,2; 66,4 and 38,7; 23,9 and 68,9 respectively.

Sleugh *et al.* (2000) found CP contents of 18,3 % in *L. uliginosus* and of 18,1 % in *M. sativa.* Jaime (2002) also evaluated cold-climate legumes, such as *T. pratense, M. sativa*, *T. repens* and *L. uliginosus,* and found in the two last ones the highest CP contents, of 27,9 and 27,8 %, respectively. Santacoloma-Varón (2017) evaluated the nutritional quality of *L. uliginosus,* and concluded that it responds very well to biofertilization, with CP content of 22 %. Likewise, Murillo (2003) reported CP values between 18,9 and 21,8 % for *L. uliginosus* with different geographical origins. This author

Table 5. Nutritional quality of *L. uliginosus* associated to grasses, at the cutting of 45 and 70 days, during the dry season in Mosquera, Cundinamarca.

Material/days of regrowth	CP		NDF		ADF		IVDMD	
	45	70	45	70	45	70	45	70
D. glomerata + *L. uliginosus*	27,8	28,4	29,0^{b}	36,3^{a}	22,2^{a}	20,9^{bc}	74	70,1^{ab}
F. arundinacea + *L. uliginosus*	27,6	28,7	34,8^{a}	25,4^{d}	19,0^{b}	24,0^{a}	72,7	74,7^{ab}
H. lanatus + *L. uliginosus*	26,7	26,6	27,3^{c}	28,2^{c}	18,9^{b}	21,5^{b}	70,9	76,8^{a}
B. catharticus + *L. uliginosus*	28,6	26	29,2^{b}	33,4^{b}	19,4^{b}	20,3^{c}	68,3	72,9^{ab}
C. clandestinus (naturalized) + *L. uliginosus*	30	30,6	27,0^{c}	36,9^{a}	20,0^{b}	24,2^{a}	75,7	67,6^{b}
SE ±	1,85	1,15	0,009	0,05	0,075	0,111	9,24	3,61
P - value	0,06	0,09	0,0002	0,002	0,0001	0,0002	0,1	0,004

Small-case letter corresponds to significance among legume averages
Means followed by equal letters in the same column are not significantly different ($p < 0,05$), according to Tukey's test

corroborated that *L. uliginosus* is considered a forage legume of high tropic, as important as *M. sativa*. In addition, the inclusion of *L. uliginosus* in ruminant feeding is associated to a decrease from 16 to 25 % in the methane emissions per unit of consumed DM (Christensen *et al.,* 2017; Narváez-Herrera, 2017).

In the Bogotá savanna, Quiroga and Barreto (2002) recorded in *C. clandestinus* fertilized with compost 72 % of IVDMD, 67,4 % of NDF and 35,5 % of ADF at 45 days of regrowth, in the rainy season. Meanwhile, at 75 days, they obtained 75,9; 62,5 and 36,3 % of IVDMD, NDF and ADF, respectively.

Correa *et al.* (2018) referred NDF contents that varied from 59,55, at 14 days of regrowth, to 62,7 % at 35 days. Likewise, Jaime (2002) found for cold-climate grasses, harvested at 45 days, average contents of NDF (55,3 %); ADF (36,9 %) and IVDMD (70,9 %) which coincide with those values that stood out the most in the associations evaluated in this research.

In turn, Burns and Chamblee (2000a) reported for *F. arundinacea* 56 and 71,7 % of NDF and IVDMD, respectively, at 60 days of regrowth, in the winter. Burns and Chamblee (2000b), also for *F. arundinacea,* obtained 51,5 and 64,2 % of NDF and IVDMD, respectively, at 60 days, in the summer.

Naydenova and Vasileva (2016) reported in *D. glomerata* 56,0 and 29,6 % of NDF and ADF, respectively, in pure pastures and in association with *Trifolium subterraneum* L. In Turkey, Tenikecier and Ates (2019) evaluated *D. glomerata* at different altitudes above sea level, and recorded 36,49 % of ADF and 5,2 % of NDF, at 35 days, at low altitude.

The IVDMD, in grasses, was generally higher at 45 days, in the rainy as well as the dry season ($p < 0,05$). Meanwhile, in the legumes, significant differences were found ($p < 0,01$) between seasons, the IVDMD being higher during the rainy season. With regards to the regrowth age, at 70 days, the IVDMD was lower compared to 45 days, with 63,3 and 66,4 %, respectively. This agrees with the NDF 39,9 and 37,1 % and ADF content 31,5 and 22,3 %, for 70 and 45 days of regrowth, respectively.

For the legume, the contents of NDF, ADF and IVDMD recorded in this study (34,6; 22,7 and 68,6 %, respectively) were better than the ones referred by other authors, like Jaime (2002), who reported 34,8; 24,6 and 75 % of NDF, ADF and IVDMD, respectively. Meanwhile, Peiretti *et al.* (2016) indicated 36,2 and 29,7 % of NDF and ADF in *L. corniculatus* and *T. repens,* respectively.

Conclusions

Due to its high protein content, the grasses *C. clandestinus* (naturalized), *D. glomerata* and *F. arundinacea* stood out. *L. uliginosus*, which confirms the idea that it is a promising species for the milk production systems in Colombia.

The association that showed the best RAI was *C. clandestinus* (naturalized), also being the one with higher CP content and digestibility. *F. arundinacea* stood out as the promising material for the establishment in associations with legumes, due to its high nutritional quality and compatibility with the legume.

More studies should be conducted focused on identifying species that stand out because of their adaptation to the environment, biomass production, persistence, nutritional quality and, finally, acceptability by the animals.

Conflict of interests

The authors state that there is no conflict of interests among them.

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Authors' contribution

- Edwin Castro-Rincón. Development of the research, data analysis and writing of the original draft.
- Juan Evangelista Carulla-Fornaguera. Methodology design, manuscript writing, revision and edition.
- Alberto Cárdenas-Rocha. Methodology design, manuscript writing, revision and edition.

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