Influence of dairy slurry dry matter contents and electrical conductivity on two legume species germination

Gutierrez Guadalupe, Volpe Susana, Sardi Graciela, Flores Miriam, Calcagno Lisandro, Pastorino Florencia, Herrero María A.*
Universidad de Buenos Aires, F.Cs.Veterinarias, Chorroarín 280 (1427) Buenos Aires, Argentina
*Corresponding author: aherrero@fvet.uba.ar

Abstract
The aim was to evaluate the effect of slurry solutions with different electrical conductivity and dry matter contents on *Medicago sativa* (ALF) and *Trifolium repens* (WCl) germination stages. Two CRD were used, with five treatments (seven replicates) for both species. Treatments consisted in two slurry dry matter contents (DM% = 5% and 15%) each with four electrical conductivity (EC) (5, 10, 15, 20 $mS\cdot cm^{-1}$), and a control. A combination of standard germination protocol (%G at 96 hour and 10 day) with phytotoxicity bioassay protocol (Germination Index, %GI, at 72 hours) was used. In ALF no significant differences were found in G% with different EC and DM% and no phytotoxic effects were detected, showing its tolerance in early germination stages. WCl presented lower %G and %GI with higher EC for both DM% solutions ($p<0.05$). Results show that slurry application near imminent sowing is not recommended in areas where water and slurry salinity is higher than 10 $mS\cdot cm^{-1}$.

Introduction
Accumulation of slurry due to the high production and intensification in dairy farms is at present one of the main environmental problems to be solved. Slurry may have different characteristics, such as composition (minerals and % of dry mater (%DM)) and salinity. Its composition depends on the type of diet and the amount of water that ends up in the slurry which affects its %DM. Salinity may increase mainly due to the composition of the water used, not only for farm facilities hygiene, but also from pen manure hose down. These aspects may have a harmful effect on germination when slurry is used as a nutrient source, and can also have certain phytotoxic effects that can affect plants in different growth stages [1 y 2].

Tolerance to salinity may differ between different stages (germination, seedling and adult) within the same species. There are few studies that have considered phytotoxic effects during different forage species growth stages. Germination is a critical stage as it involves the initial root growth of the future crop. The seeds of different botanical species have dissimilar hydration levels, below which germination processes may be depressed or completely suppressed. Therefore, forage species germination studies are necessary in order to understand possible impacts when slurry is applied at the time of sowing.

In Argentina, the use of cattle slurry as a fertilizer is an emerging practice which is increasingly being used on forages in different stages with different application rates. It is usually applied to temperate species adapted to different soils types, although not necessarily taken into consideration the doses and application timing that these species require. Likewise, the variability of slurry quality is also something that is not always taken into account. One major source of this variability is due to available groundwater quality in use (electrical conductivity between 1 up to 20 mS·cm-1), specially between the different milk production regions [4].

The aim was to evaluate the behavior of two forage legumes (*Medicago sativa* and *Trifolium repens*) during germination stage, with slurry having different electrical conductivity and dry matter contents.

Materials and methods
Two forage legumes *Medicago sativa* (ALF) and *Trifolium repens* (WCI) were selected according with their different salinity tolerance. They are also the most common forage legumes species that are used in template pasture composition in dairy farms in the region.

Two completely randomized designs were used, with five treatments and seven replicates for both selected species. Treatments consisted in two concentrations of slurry (5% and 15% of dry matter contents) with four different electrical conductivity (EC) (5, 10, 15, and 20 $mS \cdot cm^{-1}$), and a control with distilled water. Both of these characteristics (EC and %DM) were selected according to the values determined in water quality and slurry characteristics of different dairy farms in a previous study in the dairy basins [4].

Fresh manure from the milking shed holding yard was collected (around 50kg), during two successive milkings. This dairy farm had average feeding programs and water handling, in accordance to national statistics. The dung was thoroughly homogenized, and then dried at 60 °C, to constant weight. Dry matter (%) was determined, and pH, electrical conductivity, total nitrogen, phosphates, and ammonium were analyzed by standard methods [2].

Different EC slurry solutions were prepared with distilled water in order to avoid adding other minerals, and manure was added to achieve the required %DM. Saline solutions were obtained by adding Sodium chloride (MERK, Analytical Reagent). Solutions pH, nitrate, phosphates, and ammonium concentrations were analyzed [2], their results were: 5%DM solution, pH= 7.68; nitrates= <5 ppm; phosphates= 31 ppm, and ammonium= 0.19 ppm, and for the 15%DM solution pH= 7.68; nitrates= 74 ppm; phosphates= 64 ppm, and ammonium= 0.81 ppm.

Due to the interest of evaluating the influence of slurry in the initial germination stages a combination of the standard germination protocol [3] with phytoxicity bioassay protocol evaluation proposals [2] was used. To evaluate germination, the percentage of germinated seeds (G%) was determined at 96 hours and 10 days. To evaluate phytotoxicity the Germination Index % (GI%) and the percentage of germinated seeds (G%) was determined at 72 hours [3]. This index can be calculated by using the formula: GI% = (RSG x RRG)/100 in which: RSG (%) (Relative seed germination %) = (number of seeds to have germinated in the slurry extract/number of seeds to have germinated in the control treatment)*100; and RRG (Relative root growth)= (mean root length of germinated seeds in slurry extract/ mean root length of germinated seeds in control)*100.

The germination test was carried out in disposable petri dishes with 25 seeds placed on a double layer of filter paper over a cotton base moistened to saturation with distilled water. Four ml of the different solutions were added to the slurry treatments and the same amount distilled water to the control. A growth chamber was used for incubation, at 20ºC±2 with 8 hours of natural light, throughout the trial period. A digital caliber (Wembley de 0- 150 mm) was used to measure roots length. Seeds with roots reaching 5mm were considered germinated. Germination percentages (G%) were recorded at 72 and 96 hours, and at 10 days. Measured roots length and G% taken at 72 hours were used to calculate the Germination index (GI%) [2].

Parametric or non-parametric tests were run as needed. Multiple comparison tests were performed when significant differences were found.

Results and discussion

Nitrates, phosphate, and ammonium results for the slurry solutions were lower than those found in dairy farms presented in other studies [2].

In the case of ALF, no significant differences ($p>0.05$) were found between treatments for G% mean values at each count (72 hours, 96 hours or 10 days) neither between EC nor slurry DM contents. Mean values of G% were found to be between 70 to 80% both at 96 hour as for 10 day count for all treatments including control. These values are slightly lower than those recommended as acceptable for pasture implantation (80-90%).

These results show that ALF in early germination stages is tolerant to a wide salinity range. On the other hand, no phytotoxic effects were registered as no significant differences ($p>0.05$) were detected for GI% at 72 hours, and all values were over 99%.

In the case of WCl significant differences ($p<0.05$) were found between treatments for G% mean values at each count. The lowest values were found with higher EC slurry solutions (15 and 20 $mS.cm^{-1}$), for both 5%DM (Table 1) as for 5% de DM (Table 2).

Table 1. White Clover mean values of germination (%) ± standard deviation with 5% of dry matter slurry solution with different electrical conductivity.

	72 h	96 h	10 days
Control	68.57 ± 7.81^{a}	77.14 ± 4.45^{a}	80 ± 8.33^{a}
5	61.71 ± 12.83^{a}	66.28 ± 15.98^{a}	76 ± 9.52^{a}
10	$49.14 \pm 14.55^{a\ b}$	$60 \pm 8.00^{a\ b}$	$71.42 \pm 10.18^{a\ b}$
15	38.86 ± 16.77^{b}	41.14 ± 14.74^{c}	$53.71 \pm 11.04^{b\ c}$
20	36.57 ± 10.69^{b}	$47.42 \pm 9.64^{b\ c}$	48 ± 17.59^{c}

Different letters within the same column show significant differences ($p<0.05$) according to Tuckey Test

Table 2. White Clover mean values of germination (%) ± standard deviation with 15% of dry matter slurry solution with different electrical conductivity.

	72 h	96 h	10 days
Control	68.57 ± 7.81^{a}	77.14 ± 4.45^{a}	80 ± 8.33^{a}
5	60.57 ± 3.60^{a}	77.14 ± 5.52^{a}	78.86 ± 10.25^{a}
10	40.57 ± 6.29^{b}	55.43 ± 9.64^{b}	56.57 ± 15.39^{b}
15	24 ± 7.30^{c}	26.86 ± 11.48^{c}	28 ± 6.53^{c}
20	22.67 ± 4.84^{c}	28.00 ± 6.20^{c}	26.67 ± 14.24^{c}

Different letters within the same column show significant differences ($p<0.05$) according to Tuckey Test

White clover IG% showed significant differences ($p<0.05$) between treatments median values. For both DM% contents the 5 $mS \cdot cm^{-1}$ solution showed values of 100%. With the highest EC solution (15 and 20 $mS \cdot cm^{-1}$) values lower than 55% were found with slurry solution of 5%DM, and lower than 28% with the highest DM% content. These results show that high EC and the phytotoxicity of the higher DM% contents affect WCl germination. Similar findings were observed by other authors [2] for dairy slurry with EC up to 10 for another legume species.

Conclusion and perspectives

It can be concluded that slurry composition (DM of 5 and 15% with EC up to 20 $mS \cdot cm^{-1}$) can vary considerably in its impact on germination and phytotoxicity.

Alfalfa was not affected by these slurry characteristics, and therefore slurry with different salinities and composition could be applied immediately prior to planting in alfalfa pastures.

In the case of WCl, it is advised that both salinity and slurry composition be analyzed before its application prior to sowing. This species' sensibility to salinity values higher than 10 $mS \cdot cm^{-1}$ and to different DM contents may affect its growth. According to these results the application of slurry when sowing is imminent is not recommended in areas in which water salinity and therefore slurry salinity is expected to be higher than 10 $mS \cdot cm^{-1}$.

Future studies will be necessary to evaluate these effects on different grasses and legumes in germination and in early growth stages.

Although it was not contemplated in this study, this protocol combination could be useful to select the best genotypes for the development of new forage cultivars adapted to a certain soil and water salinity.

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