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Perenial ryegrass (*Lolium perenne* L.) yield as a response to fitoregulators produced in digestates

Castro-Ramos, Job J.; Castro-Rivera, Rigoberto[1]; Solís-Oba, María M.[1*]; Osorio-Cortes, Guadalupe[2]; Romero-Rodríguez, Angélica[1]; Juárez-Rangel, Ana P.[1]

[1]Instituto Politécnico Nacional, Centro de Investigación en Biotecnología Aplicada, Tepetitla Tlaxcala, México. [2]Instituto Tecnológico Superior de Tlaxco, Tlaxco, Tlaxcala, México.
Corresponding Author: myrobatlx@yahoo.com.mx

ABSTRACT

Objective: To assess the effect on ryegrass (*Lolium perenne* L.) as a response to phytoregulators produced in digestates obtained from the anaerobic digestion of cow manure, at different initial pH.

Design/methodology: Anaerobic cow manure digestions were set up at different initial 5, 6.5, 7.5 and 8.5 pH values and 4, 8 and 20 days of digestion, from these, gibberellic acid (AG_3) and indole acetic acid (IAA) were quantified. The digestates were applied to ryegrass grown in pots: a) on 6 months pastures applying all the digestates and b) on 45 days pastures applying digestates at 4 days of digestion. The assessed variables were height, fresh and dry weight and number of stems. The control was developed on unfertilized soil.

Results: The initial pH of the digestion influenced the production of phytoregulators, being higher at pH 5.5 and 6.5; no IAA production was recorded at basic pH. The application of the digestates had a different effect depending on the pastures age, was greater on the leaf weight variable. In 6 months pastures the increase was between 21 and 24%, in young pastures from 48 to 115% respect to the control. Likewise, there were between 50 and 60% greater number of stems than in the control, applying digestate at 4 days of digestion.

Limitations/Implications: The study took place on ryegrass, it would be of interest in the area to evaluate it in other crops.

Findings/Conclusions: The initial manure pH has a higher effect on the digestates properties as well as the time of digestion. Digestates can be a fertilizer for ryegrass, its effect is better in young grasses. The digestate even with 4 days of digestion has a positive effect on ryegrass development.

Keywords: Lolium perenne, anaerobic digestion, phytohormones

INTRODUCTION

The importance of forage production lies in several aspects, for example they are food production systems for livestock, they influence the mitigation of climate change and serve as prevention and fire control. Forage yield relates to environmental factors and management practices. To increase the profitability of the agricultural sector, producers must make efficient use of the pasture resource, including intensifying forage production per area; as well as the search for forage species that meet the nutritional requirements of animals and establish a harvest system ensuring a constant production throughout the year (Araya-Mora and Bochini-Figueroa, 2005).

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An alternative to increase production is organic fertilizers, such as digestate. This is a by-product of the anaerobic digestion of organic solid waste, classified as a fertilizer due to its nutrient content and the presence of phytoregulators (Xin *et al*., 2016). The latter regulates physiological processes in plants and reduce the effects of biotic and abiotic stress (Vega-Celedon *et al*., 2016). The usage of plant hormones as growth promoters has increased (Sebastian *et al*., 2019); however, due to their high production cost, different production methods have been sought, for example, submerged fermentation or solid-state fermentation (Rodrigues *et al*., 2009), as well as via anaerobic digestion (Moller and Muller, 2012).

The perennial ryegrass (*Lolium perenne* L.) is one of the most used grasses for livestock production in temperate zones of Mexico, due to its high yields, nutritional quality, and ease of growing in different types of soil (Velasco-Zebadúa *et al*., 2002); however, there is little scientific information in the use of phytoregulators contained in digestates as promoters of plant growth in grasses. Therefore, in this research, the development and performance of ryegrass were evaluated as a response to phytoregulators produced in digestates, obtained from anaerobic digestion of cow manure, adjusted to different initial pH values and considering different days of digestion.

MATERIALS AND METHODS

The cow manure was donated by the Instituto Tecnológico del Altiplano de Tlaxcala, collected fresh at the bovine unit. For its characterization, its pH, C/N ratio, total solids and volatile solids were assessed. The evaluations were done following APHA normative (APHA, 2017).

Anaerobic digestions were established in 135 mL serological bottles, by triplicate, with 7% solids and initial pH of 5.5 (DA1), 6.5 (DA2), 7.5 (DA3) and 8.5 (DA4). The oxygen elimination was done by introducing nitrogen in the bottles, hermetically closed and kept for twenty days in incubation at 37 °C. At 4, 8, 12, 16 and 20 days, the pH, indole-3 acetic acid (IAA) and gibberellic acid (AG_3) were measured by high-performance liquid chromatography (HPLC Hewlett Packard) with a diode arrange sensor, an Eclipse XDB-C18 (4.6 mm ID × 250 mm 5) column was used following Teniza-García *et al*. (2015).

Two evaluations of the digestates were made in perennial ryegrass (*L. perenne*):

The first evaluation was in a 3×4 design, using digestates obtained at three different times of anaerobic digestion (4, 8 and 20 days) and at the 4 initial pH values for digestion (5.5, 6.5, 7.5 and 8.5), with five repetitions for each treatment. Ten grass seeds were sown in plastic containers with one kilogram of soil and kept in a greenhouse with irrigation. At 6 months, a uniformization cut was made at 5 cm and later fertilized by supplying 35 mL of digestate at the base of the tiller. Every 5 weeks of the development, a cutting was done again at 5 cm and then fertilized with digestates; this process was repeated every five weeks. The controls were only filled with soil.

The second evaluation was made with digestate at 4 days of the digestion process, and at the 4 initial pH values. The grass grown in plastic containers with one kilogram of soil was used, five grass seeds were sown and kept in the greenhouse with irrigation. After 45 days, a uniformization cut was made at 5 cm and later, 35 mL of the corresponding digestate was added to the base of the tiller. Every 5 weeks, a 5 cm cut was made again along with subsequent fertilization with digestate; this was repeated three times every five weeks. Five repetitions per treatment were made. In both evaluations, the height of the forage was weekly assessed. The forage yield was obtained by weighing the collected material on a Sartorius analytical balance, separating into green and senescent leaves, weighed fresh and later dehydrated in a forced air oven at 65 °C until constant weight, then the value of the dry matter was recorded. At week five, the number of stems in each pot was counted.

The obtained results were analyzed with the PROC GLM procedure of the SAS® Statistical Software Version 9.0 for Windows®. Treatment means were compared using the Tukey test at a 5% significance level.

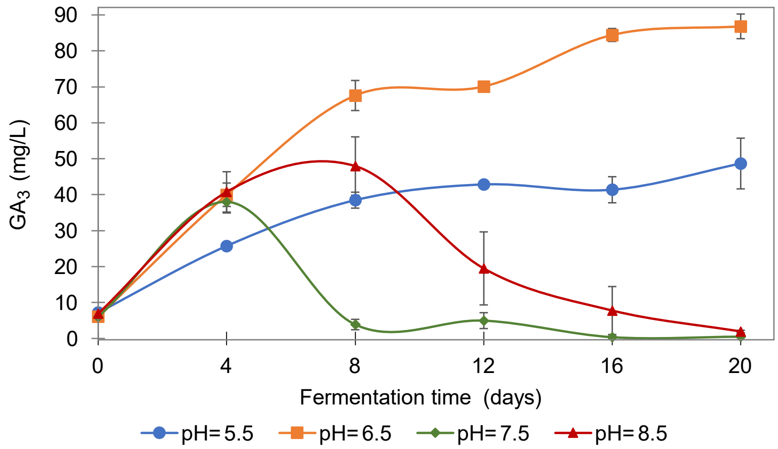

Figure 1. GA_3 quantification in the DA1 (pH=5.5), DA2 (pH=6.5), DA3 (pH=7.5) and DA4 (pH=8.5) digestions.

RESULTS AND DISCUSSION

Anaerobic digestions

Production of gibberellic acid. The measured amounts of gibberellic acid are shown in Figure 1. The modification of the initial pH changed the AG_3 production, this was higher in the DA1 and DA2 digestions, where digestion began in acidic pH values (5.5 and 6.5); in these digestions, this acid was detected from the beginning and until day 20. The maximum AG_3 production was registered in DA2 it was 85.3 mg/L. In all digestions, gibberellic acid was recorded four days after the digestion started, 38.01, 40.07 and 25.8 mg/L for DA2, DA3 and DA4, respectively. Xin *et al*. (2016) reported AG_3 production of 18 mg/L using chicken manure, 16 using cow manure and 47 mg/L with pig manure.

Production of indole acetic acid (IAA). In Figure 2 the values of IAA are shown, the variation of the initial pH affected the IAA production, this was registered only in the two digestions with initial acid pH (DA1 and DA2). In both, the maximum quantified was at day four after digestion started, 50.5 mg/L and 0.98 mg/L were quantified for DA1 and DA2 respectively. In the treatments that started with a basic pH value (DA3 and DA4) there was no IAA formation. Xi *et al*. (2016) performed digestions with different manures, and report IAA production of 12 mg/L, 22 and 21 mg/L using chicken, cow and pig manure respectively; these indicated that the nutritional characteristics of the digestate depend on the initial pH. Scaglia *et al*. (2015) reported 9.94 mg/L of IAA using pig manure.

Evaluation of digestates at 4, 8 and 20 days of fertilization. The average heights of the pasture are shown in Figure 3. Using the digestates obtained after 4 days of digestion in week 5, DA3 produced higher heights than that in the other treatments, these were 14% higher than the control. Fertilizing with the digestate obtained at 8 days of digestion, with DA2, DA3 and DA4, between 11 and 12.6% higher heights were recorded in week 5 compared to the negative control. While in the case of application of the digestate obtained at 20 days of digestion, with DA4 in week 5, they showed a significant difference ($p>0.05\%$), being 12% higher than those of the other treatments and the negative control.

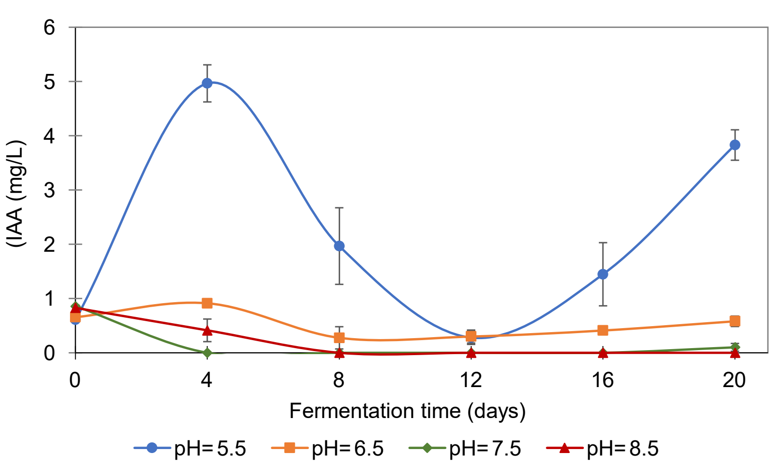

Figure 2. IAA quantification in DA1 (pH=5.5), DA2 (pH=6.5), DA3 (pH=7.5) and DA4 (pH=8.5) digestions.

Figure 4 shows the total accumulated biomass of the pasture, this was significantly higher ($p<0.05$) applying the digestates compared to the negative control, except using the digestate that was obtained after 4 days of digestion with an initial pH of 5.5 (DA1), in this case, the height equal to the control. The maximum biomass values were recorded in DA1, using digestate after 8 and 20 days of digestion, DA3 with digestates of 4 and 20 days of digestion and with DA4 fertilizing with the digestates obtained at the three times of digestion. Using these digestates increased between 21 and 24% respect to the negative control. In DA2, at all digestion times,

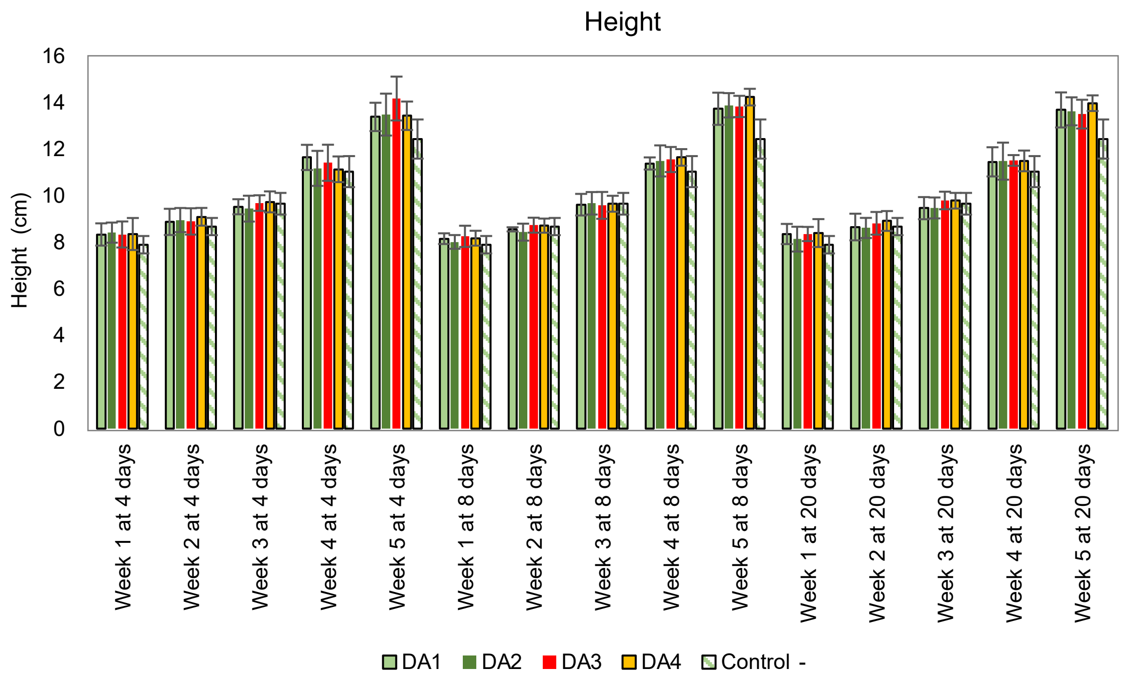

Figure 3. Average height of the grass applying digestate after 4, 8 and 20 days of digestion. Bars represent standard deviation.

lower heights were obtained than the above-mentioned treatments, but higher than with the negative control. This contrasts is observed in Figures 1 and 2: that in DA3 and DA4 at 20 days of digestion there was no AG_3 or IAA presence. This indicates that the digestate in addition to the phytoregulators contains other components that helped the development of the grass, as explained by Xin *et al*. (2016) who indicate that the positive effect of digestate on plants has been explained by its macronutrient content and the presence of phytoregulators. Likewise, Moller and Muller (2012) reported that digestates contain bioactive substances such as phytohormones with the potential to promote plant development.

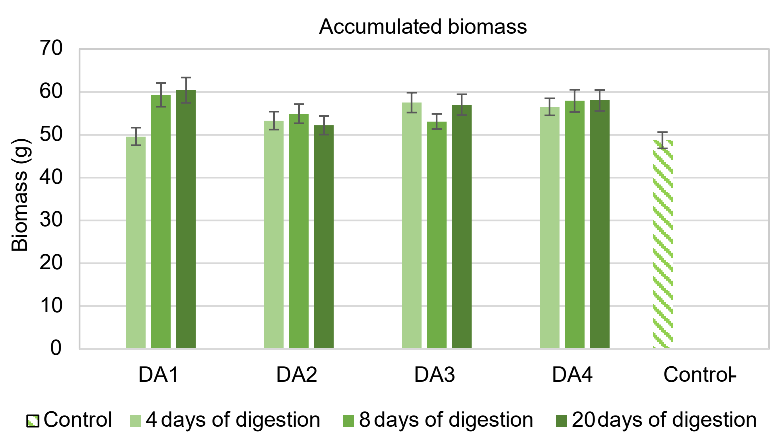

Figure 4. Total accumulated biomass applying the digestates DA1 (pH=5.5), DA2 (pH=6.5), DA3 (pH=7.5) and DA4 (pH=8.5) and in the control. Bars represent standard deviation.

The results concur with those reported by Tempere and Viiralt (2014) and Walsh and Rousk (2012) who found that when applying digestates, there was a higher yield in grasslands than were without no fertilization or chemical fertilizers.

According to the results in figures 3 and 4, 4 days of digestion was selected to make a second ryegrass fertilization evaluation, because fertilizing with the digestate obtained after 4 days of digestion had a similar effect in the development of the grass than using digestate of longer fermentation time (20 days).

Digestate evaluation at 4 days of fermentation

Figure 5 shows that during the second week, all the plants fertilized with digestate were statistically higher than the negative control; During the third and fourth weeks, the DA3 plants were statistically higher than those from the control; while in week 5, using the DA1, DA2 and DA3 digestates, taller plants were obtained compared to the negative control. This increase that was registered during week 5 was 12 to 16% higher than that of the control. This point that digestates, regardless of the digestion process, were useful to fertilize grasses. This is partially explained by that reported by Small *et al*. (2019), who indicate that gibberellins have positive effects on plant development by controlling cell elongation. It is also observed that in the negative control there was no increase in height from week 4 to 5, while applying the digestate the growth was constant.

Table 1 shows the accumulated weight of fresh and senescent leaf and mean stem number. It is observed that there was a significant increase in biomass applying the four digestates, it was 115% applying DA2, 90% with DA1, 81% using DA3 and 48% with DA4 compared to the negative control. In the case of the dead leaves, a greater quantity was obtained in the negative control, between 56 and 69% more compared to the treatments where digestates were applied. It is to be noted that DA1, DA2 and DA3 digestates supply has between 50 and 60% higher stem production compared to the unfertilized control. These results concur with those reported by Eickenscheidt *et al*. (2014), who report that the application of digestate to a plot with grass increased its performance compared to the application of liquid manure.

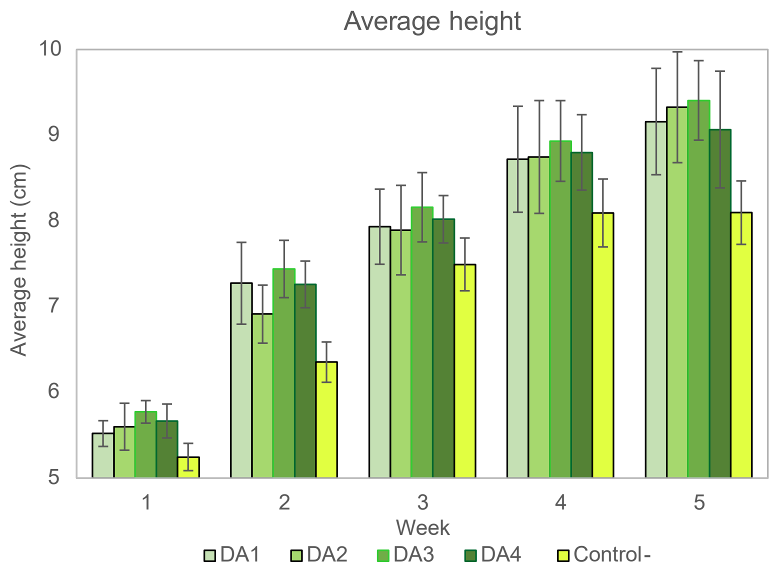

Figure 5. Average heights of the plants supplied with a digestate of 4 days of digestion DA1 (pH=5.5), DA2 (pH=6.5), DA3 (pH=7.5) and DA4 (pH=8.5).

For their part, Eich-Greatorex *et al*. (2018) report that the addition of digestates produced a similar yield in biomass production in two types of grass, Italian grass (*Lolium multiflorum*, var. Macho) and canary red grass (*Phalaris arundinacea*), compared to chemical fertilization; they also reported that the digestate contributed to improving the pH for plant development, reduced soil density of and increased water retention.

It has been reported that the regrowth capacity of a plant, after harvest or defoliation, is influenced, among others, by physiological factors such as the accumulation of carbohydrate reserves in the root, the remaining leaf area and the growth meristems activation (Pérez *et al*., 2002). The increase in the number of stems when applying digestate is a crucial factor since it has been reported that the persistence of the pasture is directly determined by the combined effect of the seasonal pattern and stems mortality. In a perennial ryegrass meadow, both its persistence and forage production depend on the balance between the emergence rates and the death of the stems (Ramírez *et al*., 2011).

As reported by Small *et al*., (2019) the digestates in addition to increasing the grass yield to be used as forage helps grassland areas recovery.

CONCLUSIONS

The production of AG_3 and IAA in the digestates is influenced by the initial pH of the manure, the acid pH being better (5.5 and 6.5). The digestates obtained from the anaerobic digestion of cow manure can be used as fertilizer in perennial ryegrass. Its effect on the development of ryegrass applied in pastures with 6 months or 45 days of the establishment was different, the variable in which had the most influence was the leaf weight. It is shown that the obtained results using digestate after four days of digestion are like those obtained after eight or twenty days, reducing the digestion time to only four days, can have an important economic impact since the expenses to carry out the anaerobic digestion will be less than leaving the process for a longer time.

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Table 1. Weight of fresh and senescent leaf and number of stems of perennial ryegrass plants DA1 (pH=5.5), DA2 (pH=6.5), DA3 (pH=7.5), DA4 (pH=8.5) and the control.

	Green leaf weight (g)	Senescent leaf weight (g)	Average number of steams
DA1	15.47	0.11	38
DA2	16.80	0.16	37.8
DA3	14.20	0.14	38
DA4	11.60	0.16	35.6
control -	7.82	0.37	23.6

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