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Agronomic Responses of Forages to the Effect of Acetylsalicylic Acid

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The research investigates the impact of acetylsalicylic acid (ASA) on the agronomic performance and nutritional quality of forage species under varying cutting intervals. Forages, being subject to biotic and abiotic stress, often experience changes in productivity and composition, significantly affecting their utility in livestock nutrition. This study evaluates the growth responses and chemical composition of *Lolium rigidum*, *Poa pratensis*, and *Plantago lanceolata* when treated with three ASA doses (0, 1, and 1.5 L/ha) over cutting frequencies of 25, 35, and 45 days. The experiment employed a bifactorial randomized block design with 3×3 treatment combinations. Results indicate that a higher ASA dose of 1.5 L/ha positively influenced plant height, stem density, vigor, and dry matter yield, with a maximum yield of 1922.73 kg DM/ha at 45 days of cutting. This demonstrates that increased ASA application and extended cutting intervals optimize biomass accumulation and agronomic traits. However, shorter cutting intervals at 35 days improved the forage's nutritional content, including ash, protein, and ether extract, suggesting a trade-off between quantity and quality. *Lolium rigidum* displayed the highest protein content (18.05%) and moisture levels, making it suitable for high-protein diets but less ideal for storage. Conversely, *Poa pratensis* exhibited higher fiber content, impacting digestibility but enhancing its use in maintenance diets. *Plantago lanceolata*, with elevated ether extract content, offers potential for energy supplementation in high-demand scenarios. The findings underscore the importance of tailoring ASA doses and cutting strategies to specific forage species and production goals, emphasizing their role in sustainable livestock management. These results provide valuable insights into optimizing forage quality and yield under challenging environmental conditions.

Keywords: Climate change, nutritive values, organic biostimulants, template forages, small livestock.

INTRODUCTION

Because they are stationary organisms, plants must adapt to their environment and biotic attacks throughout their life cycle to avoid predators and stressors (Kilpeläinen *et al.,* 2011; Yan *et al.,* 2013). As a result, a variety of environmental stresses are applied to plants, lowering and restricting the yield of agricultural products. Biotic stressors are regarded as one of the most significant impediments to plant growth and development and are among the most concerning abiotic stresses to the scientific community (Lautenbach *et al.,* 2012; Pragya *et al.,* 2020; Nicewicz *et al.,* 2021). Stresses cause a variety of plant responses, including altered gene expression, cellular metabolism, and variations in growth (Grando and Macpherson, 2005; Guamán-Rivera, Albanell, *et al.,* 2023). Furthermore, plant stressors are classified into two types: abiotic and biotic (Neugebauer, 1988; Capstaff and Miller, 2018; Schwarz *et al.,* 2022). Abiotic stress put on plants by the environment might be physical or chemical (Benabderrahim and Elfalleh, 2021). Abiotic stressors can be caused by exposure to biological units such as illnesses or insects (Flachowsky *et al.,* 2013; Pragya *et al.,* 2020; Nicewicz *et al.,* 2021). In this regard, research into the use of exogenously administered chemicals as a tolerance strategy

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against the impacts of various abiotic stresses has become important (Soldado and Bessa, 2021).

Plant hormone antioxidants, signaling molecules, polyamines (PAs), and trace elements, among others, are commonly employed to reduce plant damage (Singh and Suresh-Gaikwad, 2020). In the same way, research data has shown that salicylic acid (SA) is responsible for metabolic pathways that are linked to others involved in the creation of many key signaling molecules and metabolites in plants during stress responses (López-Paredes *et al.*, 2024).

Aside from that, SA, which belongs to a large group of substances known as phenolic compounds, has a role in several metabolic functions in plants, including lignin formation, allelopathic activity, and, in some situations, phytoalexin biosynthesis (Missanjo *et al.*, 2011). As a result, growth boosters have proven to be a highly fruitful practice in crops, providing increased yields in green matter, dry matter, and nutritional value, making them a very intriguing tool in adverse settings (Pragya *et al.*, 2020).

In effect, multiple studies have found that plant growth regulators such as SA have a favorable influence on growth and yield (Gutiérrez-Coronado *et al.*, 1998; González-Correa *et al.*, 2007; Khan *et al.*, 2007; López-Paredes *et al.*, 2024). Furthermore, Gutiérrez-Coronado *et al.* (1998) demonstrated that SA improved the number of flowers, pods per plant, and grain yield of soybean. As a result, SA has a positive effect on physiological processes such as cell division, plant development, and cell wall production, as well as increasing the synthesis of numerous pigments such as carotenoids and xanthophylls.

Ecuador's livestock population has risen dramatically in recent years, with 4.13 million cattle meant for meat and milk; the province of Pichincha is the main producer of milk, accounting for 15.90% of the national total (INEC- ESPAC, 2022; Ortiz-Naveda *et al.*, 2023). However, the livestock sector faces challenges in maintaining consistent and sustained development due to the poor and scarce feed offered to cattle (Guerrero-Pincay *et al.*, 2023; Guamán-Rivera *et al.*, 2024), despite our country's favorable characteristics for producing pasture all year (Leon *et al.*, 2018). In addition, as at the global level, environmental variations due to the effect of greenhouse gases in Ecuador have caused significant changes (i.e. little rainfall and long periods of drought), which is why low productivity of pastures used for ruminant feed has been observed by Guamán-Rivera *et al*. (2024).

As a result, the use of growth promoters in pastures could give best practices with a high productive impact, resulting in increased yields in green matter, dry matter, and nutritive value, making it a very attractive strategy in adverse conditions (Morón *et al.*, 2008; Figueroa-Saavedra and Guamán-Rivera, 2023; Guerrero-Pincay *et al.*, 2023). There has been few research on strategies for limiting the biotic stressors of forages under tropical conditions in Ecuador. Aiming this, the goal of this study was to assess the effect of various quantities of acetylsalicylic acid in forages intended for ruminant feeding.

MATERIALS AND METHODS

***Study location*:** The investigation was conducted in Rumiñahui, province of Pichincha, at latitude 0°20′04″S, longitude 78°26′51″O, and altitude 2550 m.a.s.l. Rumiñahui is an Ecuadorian canton, located in the province of Pichincha. In this canton is a large part of the Valle de los Chillos, its cantonal capital is Sangolquí (INAMHI, 2021).

***Experimental conditions*:** Seventy-six experimental plots were delimited by the following dimensions: 3 m broad by 5 m long, with 15 m2 plots and 5 m2 cutting intervals. Then, three forage species were sown: annual rye grass (*Lolium rigidum*), blue grass (*Poa pratensis*), and fodder plantain (*Plantago lanceolata*) (60 g of seed per plot).

***Experimental treatments*:** After 90 days of planting, all plots were trimmed to ensure consistent growth. The experiment followed a completely randomized block design with a bifactorial arrangement (3 × 3) and three replicates for each species under consideration. As a result, the treatments consisted of three doses of SA (0, 1, and 1.5) and three grazing frequencies (GF, 25, 35, and 45 days), as follows:

T1, Lolium rigidum = 0 + 25 GF
T2, Lolium rigidum = 1 litre+ 35 GF
T3, Lolium rigidum = 1.5 litre + 45 GF
T4, Poa pratensis = 0 + 25 GF
T5, Poa pratensis = 1 litre + 35 GF
T6, Poa pratensis = 1.5 litre + 45 GF
T7, Plantago lanceolata = 0 + 25 GF
T8, Plantago lanceolata = 1 liter + 35 GF
T9, Plantago lanceolata = 1.5 litre + 45 GF

Measurement data

***Canopy height*:** The height was measured using a tape measure from the base of the plant to the apex of the flag leaf, and the variable was measured every 10 days till cutting. Ten plants were selected from each net plot of each treatment to determine their height, and the average was recorded in cm in the field book (Guerrero-Pincay *et al.*, 2023).

***Plant vigour*:** The number of tillers in a 1 × 0.25 m2 sampling frame, distributed at the average height of the plot at each treatment and harvesting (Lemaire, 2002), was directly counted to determine the tiller population density (TPD; tiller/m^2) (González Marcillo *et al.*, 2021; Figueroa-Saavedra and Guamán-Rivera, 2023; Guerrero-Pincay *et al.*, 2023).

***Number of stems per plant*:** A sample of 10 plants was collected from each net plot and treatment to count the number of stems per plant; this variable was only measured once before each cutting, such as was described by Figueroa-Saavedra and Guamán-Rivera (2023) and Guaman-Rivera *et al*. (2023).

***Forage dry matter production*:** A sub-sample (200 g) of dry matter was collected, encased, labeled, and dried in an oven at 100°C for 24 hours. The samples were then weighed, and weight differences were used to calculate the dry matter percentage and yield in kg DM ha^{-1} for each treatment (NRC, 1962).

***Chemical composition*:** The sampling technique was used as the first step in an integrated study of forages, adhering to the requirements of uniformity and representativeness. As a result, a 1 kg sample was collected from each treatment. The samples were collected in the field and tested by the AOAC (2000).

***Statistical analysis*:** All data were analyzed with SAS v.9.4 software (SAS Institute Inc., Cary, NC, USA). After checking for normality (PROC UNIVARIATE), the data were subjected to ANOVA tests (PROC MIXED). To do this, our statistical model considered species, dose and grazing frequency as main effects, with blocks and residual error being random effects. The means were expressed as least squares obtained using the PDIFF option of SAS and then compared with a Tukey test. Statistical differences were declared at $P < 0.05$, while trends at $P < 0.10$.

RESULTS

The Tukey test at 5% established that the plants that received the dose (1.5 L. ha^{-1} SA) presented a greater height with 27.27 cm, the lowest height was with the dose of (1 L. ha^{-1} SA; 24.01 cm). Plants cut at 45 days obtained the best response with 28.31 cm, while plants cut at 25 days obtained a height of 24.21 cm (Table 1). In the interaction Dose (SA) × days of cutting, Table 1, three ranges were established, with the best response to treatment T3 (1.5 L. ha^{-1} SA with 45 days) in first place with 26.3 cm, followed by T2 (1 L. ha^{-1} SA with 45 days) with 24.6 cm and in last place T1 with 21.14 cm. Tukey's test at 5%, Table 1, establishes that the plants that received a dose (1.5 L. ha^{-1} SA) obtained the best response with 17 stems/plant, while the 0 dose (without product) obtained 11.11 stems/plant. Plants cut at 25 days showed a higher number of stems with 16.22 stems/plant, the lowest number was in plants cut at 45 days with 11 stems/plant. Therefore, the 5% Tukey test, Table 1, establishes that the plants that received the dose of (1.5 L. ha^{-1} SA) obtained the best vigor with 3.33 (good), the lowest vigor was obtained with the 0 doses (without product) with vigor of 2.44 (fair).

In addition, the Tukey's test at 5%, establishes that when applying a dose (1.5 L. ha^{-1} SA) the highest yield was obtained with 1922.73 Kg of DM ha^{-1}, the lowest yield was obtained when applying a 0 dose (without product) with 1600.00 Kg DM ha^{-1}. Plants that were cut at 45 days have the highest yield with 1981.66 Kg DM ha^{-1}, the lowest yield was in plants cut at 25 days with 1469.74 Kg DM ha^{-1} (Table 1). Regarding stems/plant, the treatment that received a dose of (1.5 L. ha^{-1} SA) had major responses than those observed in the other doses (Table 1). In the same way, plants cut at 25 days showed a higher number of stems when compared to those cut at 45 days (16.22 vs. 11.0 stems/plant).

Table 1. Agronomic responses to the effect of acetylsalicylic acid.

Item	Canopy height	Stems /plant	Plant vigor	Dry matter (kg MS. ha^{-1})
Dose (SA)				
0	24.01^{b}	11.11^{b}	2.44^{b}	1600.09^{b}
1 L	26.38^{ab}	14.44^{ab}	2.78^{ab}	1886.47^{a}
1.5 L	27.27^{a}	17.00^{a}	3.33^{a}	1922.73^{a}
Cutting frequency				
25	24.21^{b}	16.22^{a}	2.56	1469.74^{b}
35	25.14^{b}	15.33^{a}	3.00	1957.90^{a}
45	28.31^{a}	11.00^{b}	3.00	1981.66^{a}
Treatment				
T1, Lolium rigidum	21.14^{c}	12.67	2.67	1274.23
T2, Poa pratensis	24.61^{abc}	11.00	2.33	1836.03
T3, Plantago lanceolata	26.32^{abc}	9.67	2.33	1690.00

$^{a\text{-}b}$ Means con different words in the same line differ a P > 0.05

The Chemical composition of forages by efect of acetylsalicylic acid (ASA) is shown in Table 2. *Lolium rigidum* has the highest moisture content (82.22%), which may influence its digestibility and freshness. On the other hand, *Plantago lanceolata* has an intermediate moisture content (74.00%), while Poa pratensis has the lowest moisture content (71.01%), which could facilitate its preservation. As for ash concentration, the values were similar among the species, ranging from (11.0 to 11.88%), suggesting a comparable mineral composition among the three plants. About crude protein, *Lolium rigidum* shows the highest protein content (18.05%), making it potentially more beneficial for a protein-rich diet. Therefore, although all three species evaluated were above the critical values for ruminants, *Poa pratensis* and *Plantago lanceolata* have protein levels of 15.59% and 13.72%, respectively. In addition, *Poa pratensis* had the highest fibre content (25.30%), which could impact its digestibility compared to the other species.

Table 2. Chemical composition of forages.

Treatment	Chemical composition				
	Humidity	Ash	Ether extract	Protein	Fibre
Lolium rigidum	82.22^{a}	11.88	3.65	18.05^{a}	24.42
Poa pratensis	71.01^{c}	11.03	3.55	15.59^{b}	25.30
Plantago lanceolata	74.00^{b}	11.34	4.63	13.72^{c}	23.80

$^{a\text{-}c}$ Means con different words in the same line differ a P > 0.05

DISCUSSION

The data indicates that as the SA dose is increased from 0 to 1.5 L/ha, there is a significant increase in almost all the growth parameters evaluated. The highest dose (1.5 L/ha) showed the best performance in canopy height (27.27 cm), number of stems per plant (17), plant vigour (3.33) and dry matter yield (1922.73 kg DM/ha). This suggests that the use of higher doses of HS has a positive effect on forage growth and productivity, possibly due to improved nutrient availability and stimulation of physiological processes that increase biomass. However, it is essential to consider that an optimal dose must balance cost and potential effect to avoid overuse that may affect soil or water resources. These results coincide with those described by Ortega-Aguirre *et al*. (2015), who mentions that in their study when applying concentrations of 1.5 ml/L of acetylsalicylic acid, rice plants var. J-104 presented a considerable increase in plant height compared to control plants.

Cutting frequency also significantly affected agronomic parameters (Guaman-Rivera *et al.*, 2023; Guerrero-Pincay *et al.*, 2023). Cutting at 45 days resulted in the highest dry matter production (1981.66 kg DM/ha) and plant height (28.31 cm), indicating that longer cutting periods allow for greater biomass accumulation. However, this practice may have disadvantages in terms of forage nutritional quality, since as the plant ages, its fiber content increases and its digestibility decreases. Therefore, the choice of cutting frequency depends on production objectives: to maximize forage quantity, an interval of 45 days may be adequate, but for high quality grass, shorter intervals may be preferable. Studies by Quintanilla *et al*. (2020), indicate that the number of flowers per plant increased by 56% when salicylic acid was administered to *Gerbera jamesonii* flowers at the first cutting, while in this research the number of stems per plant increased by 64% at the first cutting, increasing green matter production. The results obtained in this study corroborate with those reported by González-Correa *et al*. (2007), who obtained an increase in the vigor of rice plants var. J-104 when applying concentrations of 1.5 ml/L of acetylsalicylic acid. concentrations of 1.5 ml/L of acetylsalicylic acid, by improving vigor the plants presented a greater defense against diseases and pests of this crop.

The species studied show different adaptations and responses to the treatments. *Lolium rigidum* showed lower height and biomass values compared to *Poa pratensis* and *Plantago lanceolata*, which may be indicative of their specific management requirements or their ability to adapt to different soil and climatic conditions. *Poa pratensis*, with a dry matter yield of 1836.03 kg DM/ha, appears to be an efficient species in terms of production under optimal fertilization and cutting conditions. Plantago lanceolata, although not reaching the yield level of Poa pratensis, shows a vigorous growth and can be useful in production systems where an intermediate and stable forage supply is required. Quintanilla *et al*. (2020) reported that the dry matter yield of perennial *Rye grass* with an initial fertilization varies according to the number of cutting obtaining values between 440; 890; 221.0 kg DM. ha^{-1} in the first, second and third evaluation cut; while in this study with the application of acetylsalicylic acid, the yields increased between values of 1469.74; 1957.90; 1981.66 kg DM ha^{-1} in each cutting interval. In the interaction Dose (SA) × days of cutting, Table 1, three ranges were established, with the best response to treatment T3 (1.5 L. ha^{-1} SA with 45 days) in first place with 26.3 cm, followed by T2 (1 L. ha^{-1} SA with 45 days) with 24.6 cm and in last place T1 with 21.14 cm. Studies by Quintanilla *et al*. (2020) indicate that the number of flowers per plant increased by 56% when salicylic acid was administered to *Gerbera jamesonii* flowers at the first cutting, while in this research the number of stems per plant increased by 64% at the first cutting, increasing green matter production.

Lolium rigidum has a significantly higher moisture content (82.22%) compared to *Poa pratensis* (71.01%) and *Plantago lanceolata* (74.00%). This higher moisture content may improve the palatability and digestibility of the forage (Guamán Rivera *et al.*, 2023), but it also implies greater difficulty in storage, as high moisture increases the risk of spoilage and undesired fermentation (Oppong *et al.*, 2008; Stefański *et al.*, 2020). Therefore, *Lolium rigidum* might be preferable in direct grazing systems (Rochon *et al.*, 2004; Molle *et al.*, 2008), where it is consumed fresh, while *Poa pratensis* and *Plantago lanceolata* might be more suitable options for hay storage, due to their lower moisture contents. The ash content, which represents the mineral content, is relatively similar between the species, with *Lolium rigidum* having a slightly higher percentage (11.88%) compared to *Poa pratensis* (11.03%) and *Plantago lanceolata* (11.34%). This factor is important because it indicates the amount of available minerals, such as calcium and phosphorus, essential for animal health (Patil *et al.*, 2009; Dehkordi and Dehkordi, 2011; Plessers, 2015). The slight variations suggest that all species are relatively good sources of minerals, but do not differ greatly enough to prefer one over the other on this basis (Gross *et al.*, 1989; Oscarsson *et al.*, 1996).

Plantago lanceolata stands out with the highest content of ethereal extract (4.63%), followed by *Lolium rigidum* (3.65%) and *Poa pratensis* (3.55%). A higher lipid content in *Plantago lanceolata* could increase the energy intake of the forage (Khan *et al.*, 2011; Mulligan *et al.*, 2016), which is beneficial in diets intended for animals in stages of high energy demand, such as lactation or growth (Guamán-Rivera *et al.*, 2024). Lipid content in forage may also improve the absorption of fat-soluble vitamins (A, D, E and K) in the digestive tract of ruminants (Esposito *et al.*, 2014). However, in conditions where dietary energy needs to be controlled (e.g. in maintenance animals), forages with lower E.E. content, such as *Poa pratensis*, may be preferable.

On the other hand, protein is a critical component in the diet of ruminants, as it is essential for growth and production. In this study, *Lolium rigidum* has the highest protein content (18.05%), followed by *Poa pratensis* (15.59%) and finally *Plantago lanceolata* (13.72%). This suggests that *Lolium rigidum* could be the most suitable choice in diets requiring a high level of protein, especially for young growing animals or dairy cows (Guamán-Rivera *et al.*, 2023; Guerrero-Pincay *et al.*, 2023). Protein is essential for muscle and milk synthesis (Alemneh, 2019; Stefański *et al.*, 2020; de Moura *et al.*, 2021), so choosing a species with higher protein content may improve production efficiency (van der Walt and Meyer, 1983; Lei *et al.*, 2018). *Plantago lanceolata,* with the lowest protein content, could be used in situations where protein is not a major requirement or where it is necessary to balance the diet with other protein sources (Salazar-Cubillas and Dickhoefer, 2021).

Fibre content influences digestibility and the health of the gastrointestinal tract of ruminants (Damiran and Yu, 2012; Tedeschi *et al.*, 2020). *Poa pratensis* has the highest fibre content (25.30%), which may reduce its digestibility compared to *Lolium rigidum* (24.42%) and *Plantago lanceolata* (23.80%). Although fibre is essential for the digestive health of ruminants, excessively high fibre content can limit dry matter intake and reduce digestive efficiency (Lee *et al.*, 2017; Patra, 2017; Amoah *et al.*, 2018). Therefore, *Poa pratensis,* may be more suitable in maintenance diets or where increased fibre intake is sought, while *Lolium rigidum* and *Plantago lanceolata* would be more digestible options for animals with higher energy requirements (Council, 1994; Butler, 2003; Salah *et al.*, 2014). According to Verdecia *et al.* (2008) the reduction of degradability is influenced by the increase of the age of the plant which leads to a thickening of the cell wall and thus reduces the intracellular space where nutrients are found (Van Soest, 1965, 1994), this coincides with the results obtained in fiber content where the lower content causes a low digestibility while plants with a high fiber content do not have a good digestibility (McDonald *et al.*, 2010).

In terms of economics, our study showed that using 1.5 litres of acetylsalicylic acid increased the amount of biomass produced. This means that by having more green forage we will have more receptive capacity for cattle, which in general terms represents a cost benefit of $1:23 versus the control of $1:01.

***Conclusion*:** Based on our results, by increasing the SA dose from 0 to 1.5 L/ha, a significant increase was observed in all growth parameters evaluated, such as canopy height and number of stems per plant. The highest doses, specifically 1.5 L/ha, resulted in the highest dry matter yield (1922.73 kg DM/ha), suggesting that a controlled increase in dose can improve yield. In addition, cutting intervals (25, 35 and 45 days) influence growth characteristics. Cutting at 45 days showed the best results in canopy height and dry matter, reaching a maximum of (1981.66 kg DM/ha). This suggests that a longer cutting interval may allow greater development and biomass accumulation. Therefore, the combination of adequate SA doses and cutting times adjusted to the specific characteristics of each species seems to be key to optimizing yield. In general, higher doses and longer cutting periods were correlated with better results in dry matter and plant vigour.

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***SDGs addressed*:** Zero Hunger, Responsible Consumption and Production, Climate Action, and Life on Land.

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