

Agronomic Responses and Nutritive Values of Savoy Grass (*Megathyrsus Maximus*) Handled with Different Fertilization Strategies

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Abstract

The utilisation of artificial nitrogen fertilisers in tropical regions is presently a subject of considerable discourse within the context of implementing adaptive strategies in response to climate change. Moreover, considering the exorbitant expenses and substantial carbon emissions associated with livestock farming practises in the northern region of Ecuador, it can be deemed impractical. A research study has been conducted to assess the potential of utilising biol as a viable alternative to partially substitute synthetic chemistry. Consequently, a completely randomised block design was employed to establish many trials, including a control group, a group without fertilisation, a group treated with 100% urea, a group treated with 100% biol, and a group treated with a 50:50 combination of urea and biol. The agronomic characteristics measured for urea were found to be somewhat greater than those for biol ($P < 0.001$). Significant differences were found in the crude protein (CP) content between urea and biol, with urea exhibiting higher CP content ($11.3\% \pm 0.46\%$; $P < 0.001$). Additionally, biol exhibited higher dry matter (DM) content and lower neutral detergent fibre (NDF) content compared to urea. Similar nutritional values were found for gross energy (GE) (4.65 ± 0.008 Mcal kg/DM; $P = 0.43$), PDIM (52.49 ± 1.15 g kg/DM; $P = 0.33$), and RPB (-17.61 g kg/DM; $P = 0.45$) in terms of content. Hence, the utilisation of biofertilizers would represent a sustainable approach for the delicate ecosystems in the northern region of Ecuador. However, it is advisable to conduct long-term investigations in order to substantiate our findings.

Keywords: Amazon Region, Chemical Composition, Fertilization, Nutritive Values.

INTRODUCTION

The primary approach to beef and dairy cow production in tropical locations is predominantly based on grass-based systems, as indicated by research undertaken by Portugal *et al.*^[1], Nascimento *et al.*^[2], Villarreal *et al.*^[3], and O'Brien *et al.*^[4]. The utilization of nitrogen (N) fertilizers is of utmost importance in maintaining productivity and protein levels in pastures, thereby enabling optimal animal performance in the current context.^[5-8] Multiple studies^[9-11] have identified carbon dioxide (CO₂), methane (CH₄), and nitrous oxide (N₂O) as the three primary greenhouse gases (GHGs) of utmost importance inside the Earth's atmosphere. Livestock production systems have been identified as a substantial contributor, accounting for around 42% of total greenhouse gas (GHG) emissions.

Within the realm of emissions, it is observed that 28% of these emissions can be ascribed to direct emissions that arise from enteric fermentation, specifically methane (CH₄). Conversely, the remaining 14% is linked to the management of manure, encompassing its handling, storage, and utilization as fertilizer, which consequently results in the release of both methane (CH₄) and nitrous oxide (N₂O). The results of this study have indicated the problem of pollution caused by agricultural practices

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has become a prominent global issue, especially in economically challenged countries.^[12-16]

Plants demonstrate temperature sensitivity and manifest discernible growth patterns within tropical regions.^[17,18] The diversity in nutritional needs might be observed in the aggregate nutritional requirements, the particular types and proportions of nutrients needed, or a combination of both the amount and quality of nutrients.^[5] Previous studies have indicated that tropical grasses demonstrate greater efficiency in the utilization of nitrogen (N), calcium (Ca), and phosphorus (P) in comparison to legumes. This advantage is mostly attributed to their possession of the C4 photosynthetic pathway.^[19-21] However, it is crucial to adopt enhanced nitrogen (N) management practices that encompass the utilization of suitable amounts of fertilizer forms and formulations, while also carefully evaluating the timing and manner of application.

Da Costa Leite *et al.*^[22] found that the application of nitrogen fertilization substantially increases the costs related to grass production. The primary factor contributing to this situation is the heavy dependence on fossil fuel resources for the production of nitrogen fertilizers, with a significant proportion of the necessary inputs currently being imported. Furthermore, it is important to acknowledge that the benefits of nitrogen fertilization in tropical soils are constrained to the immediate term as a result of heightened losses through leaching and volatilization. Furthermore, the introduction of nitrates may pose a possible hazard of soil and water contamination, as indicated by studies conducted by

Gomes *et al.*^[23], Oliveira *et al.*^[24], and Stanley *et al.*^[25]. Therefore, given the previously described context, it is crucial to promote the adoption of sustainable agricultural practices to maintain or maybe improve agricultural productivity, while simultaneously reducing the reliance on agrochemicals that have a substantial impact on carbon emissions. A study was undertaken to evaluate the feasibility of organic fertilizers as a feasible substitute for chemical nitrogen fertilization, with the major objective of this inquiry.

MATERIALS AND METHODS

Study Area

The present study was conducted in the Francisco de Orellana Canton, specifically in the San José de Guayusa Parish, which is situated in the Province of Orellana (Figure 1). According to estimates from INEC-ESPAC^[26], the province of Orellana utilises agricultural land spanning a total area of 606,307 hectares (ha). This land is distributed across various terrains, including mountains and forests, which account for 485,039 ha (80%) of the total area. Permanent crops occupy approximately 43,582 ha (7.2%), while other uses encompass 28,049 ha (4.6%). Cultivated pastures cover 25,162 ha (4.2%), natural pastures occupy 19,034 ha (3.1%), and transitional crops and fallow land account for 4959 ha (0.82%). The climatic conditions prevalent in the area are typified by the presence of humid tropical forests.^[27-29] The mean annual precipitation is recorded at 2942 millimetres, while the average yearly temperature is at 29.7 degrees Celsius.

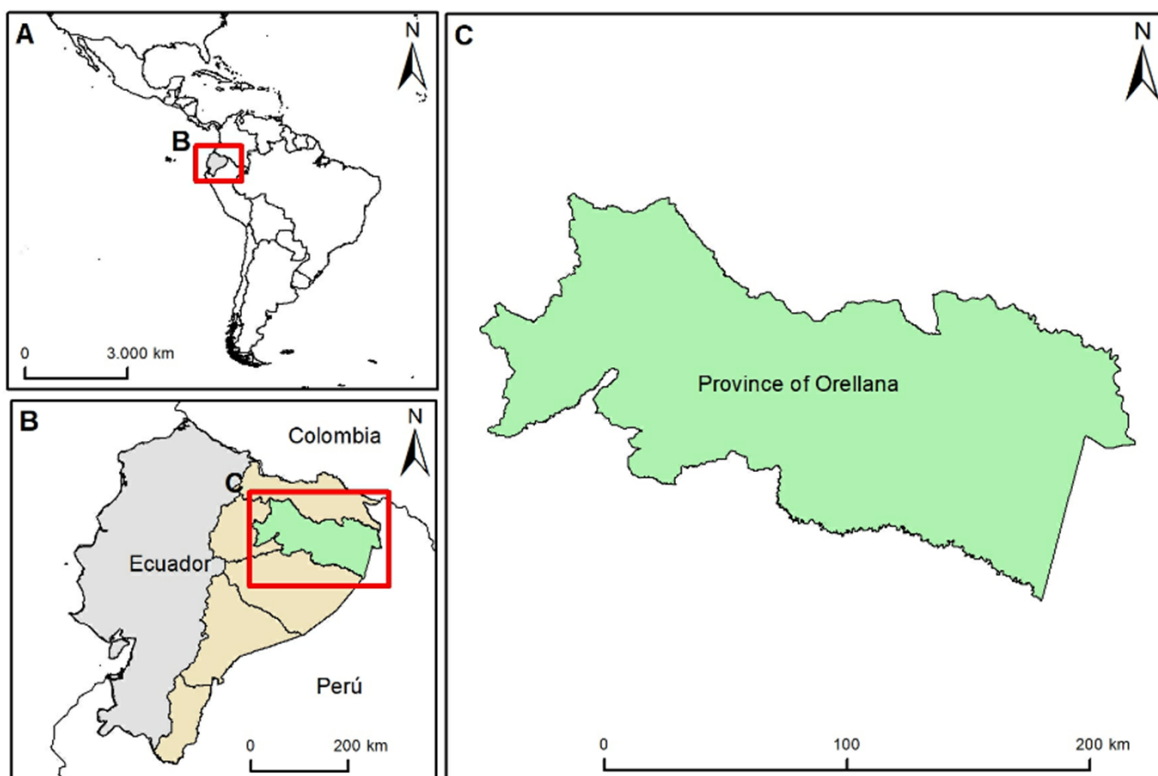


Figure 1. Study Area. A) Geographical Location of Ecuador in America. B) Ecuadorian Amazonia. C) Province of Orellana.

In the year 2020, the cultivation of Savoy grass (*Megathyrus maximus*) commenced with a seeding rate of 3 kg per hectare and an initial application of 195 kg of nitrogen per hectare. The soil exhibited physicochemical characteristics including 7% organic matter content, 15.1 ppm phosphorus (P), 0.26 meq potassium (K), and a base saturation of 57%. The biol production practise was conducted at a specific farm, where the substances used for its formulation were bovine dung (46%), *Gliricidia sepium* leaves (20%), commercial yeast (1%), whole milk (3%), cane molasses (3%), and water (27%). The biol was harvested three months subsequent to its preparation and administered via foliar application using a manual backpack pump.

Table 1. Experimental Scheme.

Variation Sources	Freedom Degree	Mean Squares
Treatments	$t - 1$	3
Block	$r - 1$	2
Error	$(t - 1)(r - 1)$	6
Total	$rt - 1$	11

Experimental Design and Trials

Under a completely randomized block design, 2×2 m plots were established with a 0.5 m path between plots, as shown in Table 1 with the trials such as control without fertilization, 100% nitrogenous Urea, 100% biol and Urea + biol (50:50). The different fertilization trials were applied to supply a total amount of N, equivalent to 150 kg ha⁻¹/year, according to the needs for the species according to Berça *et al.*^[30]. Therefore, all trials were applied after each cut, which in our case was determined every 35 days, as recommended by González Marcillo *et al.*^[31] and Guamán Rivera *et al.*^[32].

Sampling and Data Determination Dossal Height

Throughout the study, dossal height was measured and recorded using a 1 m graduated ruler at several random points with respect to each treatment before each harvest (i.e., every 35 days).^[33]

Tiller Population Density

Tiller population density (tillers/m²) was obtained by directly counting the number of tillers in a 1 × 0.25 m² sampling frame, assigned to the average height of the plot in each trial and harvest.^[34,35]

Punctuation of Plant Coverage

The punctuation of the plants in the plots was realized to determine the degree of forage coverage. To achieve such purpose, for the subjective evaluation, we have used a five-point scale suggested by Onyeonagu and Asiegbu^[36]. The degree of coverage was evaluated as, 1 (< 20%) labeled as very low, 2 (20 to 39%) labeled low, 3 (40 to 59%) labeled medium, 4 (60 to 79%) labeled as high and 5 (80 to 100%) labeled as very high.

Green Forage

Green forage per ha was evaluated using the double

sampling technique described by National Research Council^[37] and National Research Council^[38]. Thus, for direct measurements, a 0.25 m² square was used to collect random samples in each treatment before each grazing throughout the experimental period. Green forage was estimated by cutting and weighing the forage. While, as an indirect measurement method, the visual technique was considered. The scale was from 1 to 3, meaning that 1 is the lowest forage mass and 3 is the highest.^[39] Additionally, during direct forage determination, two samples of approximately 0.5 kg were collected per treatment. The first sample was dried in a forced air circulation oven at 60°C for 72 h to determine dry matter (DM kg ha⁻¹), while a second sample of fresh forage was frozen at -20 °C for subsequent determination. of the chemical composition.

Chemical Composition

The chemical analysis of the grass regarding its trial was analyzed in duplicate in the animal nutrition laboratory of the Higher Polytechnic School of Chimborazo in central Ecuador. Following the techniques described by the AOAC^[40] and expressed based on dry matter (DM), it was determined proximal, DM, ash, crude fiber, organic matter and nitrogen-free extractives. While the fiber contents (FND, FAD and LAD) according to Van Soest *et al.*^[41] adding Sodium sulfite and alpha amylase in a Fiber Analyzer equipment (Hanon advanced technology Group Co., Jinan, China).

Nutritional Values

The chemical composition previously determined in each treatment served to estimate the nutritional values of savoy grass (*Megathyrus maximus*), using the equations proposed by Inra^[42]. In the energy values and expressed in kcal/kg MS, gross energy (GE), digestible energy (DE), metabolizable energy (ME) and net energy for meat production (ENV) were calculated using a conversion of 1 UFV = 1.76 Mcal/kg MS. Regarding protein values (g/kg DM), it was estimated as digestible protein in the intestine of food origin (PDIA), digestible protein in the intestine of microbial origin (PDIM) and digestible protein in the intestine (PDI) as the sum of (PDIA + PDIM). Additionally, the protein balance in the rumen (RPB) was estimated using the equation $RPB = -84.5 + 0.61 \times CP$.^[42]

Statistical Analysis

Data were analyzed using the PROC MIXED procedure for repeated measures SAS, version 9.4 (SAS Institute Inc., Cary, NC, USA). Normality in all continuous variables was checked visually using the UNIVARIATE procedure of SAS and homogeneity of variance with the Kolmogorov-Smirnov procedure.^[43] Next, different structures of the variance-covariance matrix were tested and the composite symmetry structure was chosen based on the smallest Bayesian information criterion.

Analysis of variance (ANOVA) was performed using the SAS MIXED procedure.^[44,45] The statistical model considered the type of fertilization, sampling period

and their interaction as fixed effects, with the block and residual error taken as random effects. Differences between means by least squares were obtained by t tests using the PDIFF option of SAS and adjusted with a Bonferroni test.^[46,47] Main effects, least squares mean differences, and interaction terms were considered significant at $P < 0.05$ and trends at $P < 0.10$.

RESULTS

Agronomic Characteristics

The agronomic data related to the principal influences and

their interplay is presented in Table 2. The therapy had a substantial impact on all of the identified agronomic factors ($P < 0.001$). The study's findings indicated that the treatment including urea-based fertiliser led to a growth rise of 10% (70.30 ± 1.12 cm) in comparison to the utilization of biol alone or in conjunction with urea, which exhibited an average growth of (63.21 ± 1.12 cm; $P = 0.30$). On the other hand, notable disparities were detected in the heights of the Control group ($P < 0.05$), indicating that they were comparatively shorter (Table 1).

Table 2. Least Square Means of Agronomic Variables Determined According to Treatment, Period and Interaction.

Item	Treatments				Period				SEM	P = value		
	Con	Urea	Biol	U + B	I	II	III	IV		Trat	P	Trat×P ¹
Plant height, cm	59.83 ^c	70.30 ^a	62.41 ^b	64.0 ^b	49.65 ^c	50.30 ^c	69.40 ^B	87.14 ^A	1.12	0.001	0.001	0.001
Tillers, #/m ²	116 ^b	264 ^a	119 ^b	230 ^a	165 ^B	170 ^A	211 ^A	190 ^A	21.84	0.001	0.03	0.07
Herbage mass, kg/ha	8506 ^c	16439 ^a	13850 ^{bc}	11894 ^y	12763	11733	13521	12672	830	0.001	0.39	0.41
DM ha ⁻¹	1680 ^c	2976 ^a	2486 ^b	2209 ^b	2336	2171	2507	2338	162	0.001	0.40	0.50
HBD kg cm ⁻¹ ha ⁻¹	106.6 ^c	176.3 ^a	145.0 ^b	136.4 ^b	0.126d	246a	189b	129c	9.82	0.001	0.001	0.001
TUH, kg DM ha ⁻¹	1176c	2083 ^a	1740 ^b	1546 ^b	1635	1520	1755	1637	114	0.001	0.40	0.50
HA, kg DM/100 kg BW	4.29 ^c	7.60 ^a	6.35 ^b	5.64 ^b	5.97	5.55	6.40	5.97	0.41	0.001	0.40	0.50
Partial Factor productivity ²	56 ^d	109 ^a	92 ^b	79 ^c	85	78	90	84	9	0.001	0.41	0.43

^{a-c} means with different letter in the same line differ at $P < 0.05$ for treatment effect; ^{A-C} means with different letter in the same line differ at $P < 0.05$ for period effect; ¹ treatment × period interaction; SEM, standard error of the mean; ² Partial Factor productivity = Yield /Amount of Nitrogen Applied

There was no statistically significant difference in tiller density per square meter between the treatment of Urea and Urea + Biol, with an average of 247 21.84 tillers/m² ($P = 0.22$). In a same manner, it was observed that the Control and 100% Biol treatments displayed comparable mean tiller densities, with an average of 118 ± 21.84 tillers/m² ($P = 0.45$). Significant discrepancies were seen in the quantity of green biomass per hectare, with a p-value of less than 0.001. The treatment that incorporated urea, which has a nitrogen (N) content of 46%, demonstrated a notably greater amount of green forage at the time of acquisition in comparison to the other evaluated treatments. In particular, the use of urea resulted in a green forage yield of 16,439 kg ha⁻¹, while the alternative treatments yielded 850 kg ha⁻¹ and 12,872 ± 830 kg ha⁻¹, respectively. Based on the aforementioned contrasts, the utilization of Urea as a fertilizer led to a greater production of dry matter per hectare, regardless of the other fertilization techniques employed (see Table 1).

The experimental study involving the application of Urea resulted in statistically significant differences in the apparent density of the fodder (176.33 ± 9.8 cm ha⁻¹; $P < 0.001$). Nevertheless, when either a pure biol solution or a mixture containing 50% biol was applied together with Urea, the observed density of the forage exhibited a similar numerical value ($P = 0.32$; 141 ± 9.8 cm ha⁻¹). The results suggest that there are similar effects of the period on the perceived density of fodder, as indicated by the statistical analysis ($P < 0.001$; Table 1). The results demonstrated a significant increase following the introduction of fertilizers, but experienced a considerable decrease during the fourth period, possibly due to reduced rates of assimilation for organic fertilizers. The Urea trial replicated grazing conditions and saw a 21% increase in the quantity of grass available to the animals by using a quotient of 0.70 to calculate the total amount of usable grass.

The experimental findings suggest that the trials involving Urea demonstrated a greater daily grass intake per cow (7.60 ± 0.41 kg DM/100 BW), followed by Biol and Urea + Biol (6 ± 0.41 kg DM/100 BW). On the other hand, it can be observed that the Control group had the lowest values, displaying an average of 4.29 ± 0.41 kg DM/100 BW.

Statistically significant differences were observed solely in the parameters of plant height and the number of tillers ($P = 0.03$ to 0.001) with respect to the time factor. In the context of plant height, a 50% increase was seen during the fourth period in comparison to the first period (87.14 cm compared to 49.65 cm). After conducting an analysis of the regression model for height, it was observed that there is a significant polynomial relationship ($r = 0.63$) ($P < 0.001$; Figure 1A). However, the regression analysis that was performed to investigate the correlation between the number of tillers and time yielded no statistically significant association ($r = 0.02$). In addition, it was found that there were no statistically significant differences identified ($P < 0.60$; Figure 1B). Table 1 demonstrates a linear increase in the percentage of tillers as compared to the Control. Hence, the dissimilarities indicated above, which arise from the influence of Urea, correspond to the observed associations between Trat × P with respect to plant height and tiller count. The partial factor productivity (PFP) shown substantial variability across the different treatments ($P < 0.001$; Table 1). The therapy employing Urea demonstrated a significantly greater PFP (109 ± 9) in comparison to alternative treatments. Furthermore, the utilization of biol led to a significantly elevated PFP value of 92, in contrast to the Control treatment which exhibited a PFP value of 56 ± 9 . On the other hand, the probability of failure to perform (PFP) did not demonstrate any substantial fluctuation with respect to the period impact ($P = 0.41$) or the interaction between treatment and period ($P = 0.43$). Moreover, it was revealed that Urea had a greater

degree of agronomic efficiency (52) in comparison to Biol (36) and Urea + Biol (23).

Chemical Composition

With the exception of the FAD concentrations ($P = 0.82$), all other chemical components displayed statistically significant differences when comparing the trials (Table 3). Table 2 presents the higher dry matter (DM) concentration of 103 °C and neutral detergent fiber (NDF) concentrations observed in the Control and Biol treatments, as compared to the Urea and Urea + Biol treatments. The crude protein (CP) content displayed slight differences between Urea and Biol, with percentages of 11.3% and 10.8% respectively, accompanied

by a standard deviation of $\pm 0.46\%$. Nevertheless, it is crucial to acknowledge that diminished levels were detected in both the Control and Urea + Biol groups, exhibiting an average percentage of 10.03% and a standard deviation of $\pm 0.46\%$. There was no statistically significant difference seen between these groups, as shown by a p-value of 0.20. After doing an analysis of the regression adjustments illustrated in Figure 2, it becomes apparent that despite the observed fluctuations in data patterns, no statistically significant correlation was found (varying from $r = 0.008$ to 0.42). However, a strong and statistically significant correlation ($r^2 = 0.65$ to 0.74; $P < 0.001$) was seen between the concentrations of dry matter (measured at 103°C) and the quantities of FAD and lignin.

Table 3. Minimum Mean Squares of the Chemical Composition of Savoy Grass (*Megathyrus Maximus*) Evaluated with Different Fertilization Strategies.

Item	Treatments				Period			SEM	P = valor		
	Con	Urea	Biol	U + B	I	II	III		Trat	P	Trat×P ¹
DM, 60°C	19.76 ^a	18.09 ^y	19.27 ^x	18.54 ^x	19.01	18.38	18.60	0.76	0.059	0.54	0.62
DM, 103°C	90.58 ^a	90.43 ^c	90.61 ^a	90.56 ^b	90.43 ^b	90.59 ^A	90.63 ^A	0.05	0.001	0.001	0.99
Ash, %	10.20 ^b	8.90 ^c	10.30 ^b	20 ^a	12.24	12.32	12.45	0.50	0.001	0.95	0.87
OM, %	89.8 ^a	91.1 ^a	89.7 ^a	80.4 ^b	87.8	88.0	87.9	0.15	0.001	0.76	0.66
CP, %	10.0 ^c	11.3 ^a	10.80 ^b	10.05 ^c	10.23 ^c	10.47 ^B	10.78 ^A	0.46	0.001	0.001	0.96
NDF, %	59.44 ^a	58.93 ^b	59.74 ^a	58.32 ^b	59.87 ^A	59.22 ^A	58.24 ^B	0.37	0.001	0.001	0.92
ADF, %	22.58	22.09	23.73	23.35	18.58	26.36	23.90	2.39	0.82	0.007	0.99
Lignin, %	5.90 ^b	5.81 ^d	5.96 ^a	5.85 ^c	5.85 ^C	5.87 ^B	5.92 ^A	0.001	0.001	0.001	0.97

^{a-c} means with different letters in the same line differ at $P < 0.05$ for treatment effect; ^{A-C} means with different letters in the same line differ at $P < 0.05$ for period effect x- and different letters in the same line differ at $P < 0.10$; ¹ treatment × period interaction; SEM, standard error of the mean.

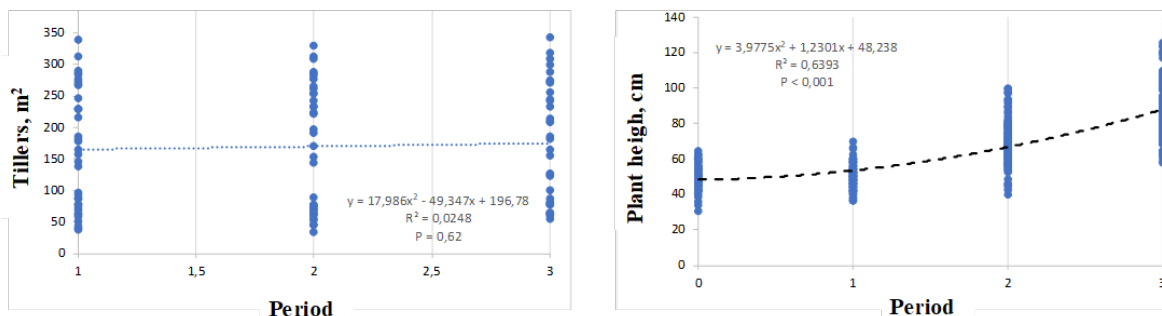


Figure 2. Regression Fits for Plant Height (A) and Number of Tillers (B).

The chemical composition values of savoy grass (*Megathyrus maximus*) were considerably influenced by the period effect ($P = 0.001$ to 0.007), except for DM (60°C) which had an average value of $18.66 \pm 0.76\%$. Based on the information provided in Table 2, it can be observed that there was a 5% rise in the crude protein (CP) concentration during the third sampling in comparison to the beginning phase of the experiment (10.78% as opposed to 10.23%). However, there was a significant decrease in FND contents between sampling times 1 and 3 (59.87 vs. 58.23), even though there were no observed correlations with PB contents ($r = 0.03$; $P = 0.83$). The differential behavior of FAD and lignin levels in comparison to NDF is a salient observation that will be thoroughly explored in forthcoming talks. Despite observing significant differences in the main effects, such as trials and period, no significant interaction was detected in the chemical composition of Savoy (*Megathyrus maximus*) ($P = 0.62$ to 0.99).

Nutritive Values Energetic

Table 4 presents the gross energy (GE) values, expressed as Mcal/kg DM. The genetic expression (GE) content demonstrated a similar level between Urea and Biol, with an average value of 4.65 ± 0.008 ($P = 0.32$). The experimental group, which included Urea + Biol, as well as the control group, demonstrated a decrease of 3% in gross energy (4.63 ± 0.008). Nevertheless, there were no statistically significant disparities detected in the computed energy levels throughout the several experimental iterations ($P = 0.75$ to 0.77; Table 3). In general, the mean values recorded were as follows: the standard deviation (SD) was 3.03 ± 0.12 , the mean error (ME) was 2.99 ± 1.11 , the environmental variation (ENV) was 2.95 ± 0.15 , and the ultimate fuel value (UFV) was 1.68 ± 0.08 Mcal/kg DM. In regard to the particular temporal scope examined in this study, it was observed

that, with the exception of the experimental group contents, there were elevated values at the concluding sampling period (4.65 vs. 4.64 ± 0.008). No statistically

significant differences were observed for any of the analyzed interactions in regard to energy values (P > 0.05; see Table 3).

Table 4. Estimated Energy and Protein Values in Savoy Grass (*Megathyrus Maximus*) According to the Type of Fertilization.

Item	Treatments				Period			SEM	P = valor		
	Con	Urea	Biol	U + B	I	II	III		Trat	P	Trat×P1
Energy values, Mcal/ kg MS											
GE	4.63 ^b	4.65 ^a	4.65 ^a	4.63 ^b	4.64 ^B	4.64 ^B	4.65 ^A	0.008	0.001	0.001	0.98
DE	3.04	3.10	3.0	2.99	3.25 ^A	2.86 ^B	2.99 ^B	0.12	0.75	0.0009	0.99
ME	2.99	3.05	2.95	2.96	3.20 ^A	2.81 ^B	2.95 ^B	1.11	0.77	0.0009	0.99
ENV	2.95	3.03	2.91	2.91	3.23 ^A	2.73 ^B	2.89 ^B	0.15	0.75	0.0009	0.99
UFV	1.68	1.72	1.65	1.65	1.83 ^A	1.55 ^C	1.64 ^B	0.08	0.75	0.0009	0.99
Protein values, g/kg MS											
PDIA	22.45 ^c	27.27 ^a	25.91 ^b	22.56 ^c	23.40 ^B	24.48 ^{AB}	25.77 ^A	1.98	0.001	0.001	0.90
PDIM	52.36 ^a	53.11 ^a	51.86 ^a	49.13 ^b	53.63 ^A	50.02 ^C	51.19 ^B	1.15	0.003	0.0009	0.98
PDI	74.81 ^c	80.36 ^a	77.77 ^b	71.65 ^d	77.01 ^A	74.47 ^C	76.96 ^B	1.62	0.001	0.009	0.99
RPB	-23.50 ^a	-16.59 ^b	-18.62 ^b	-23.35 ^a	-22.12 ^A	-20.62 ^{AB}	-18.81 ^B	2.79	0.001	0.001	0.98

^{a-b} Means with different letters on the same line indicate statistical differences at P < 0.05 between treatments; ^{A-C} Means with different letters on the same line indicate statistical differences at P < 0.05 for the period effect; ¹ Treatment × period interaction; SEM, standard error of the mean.

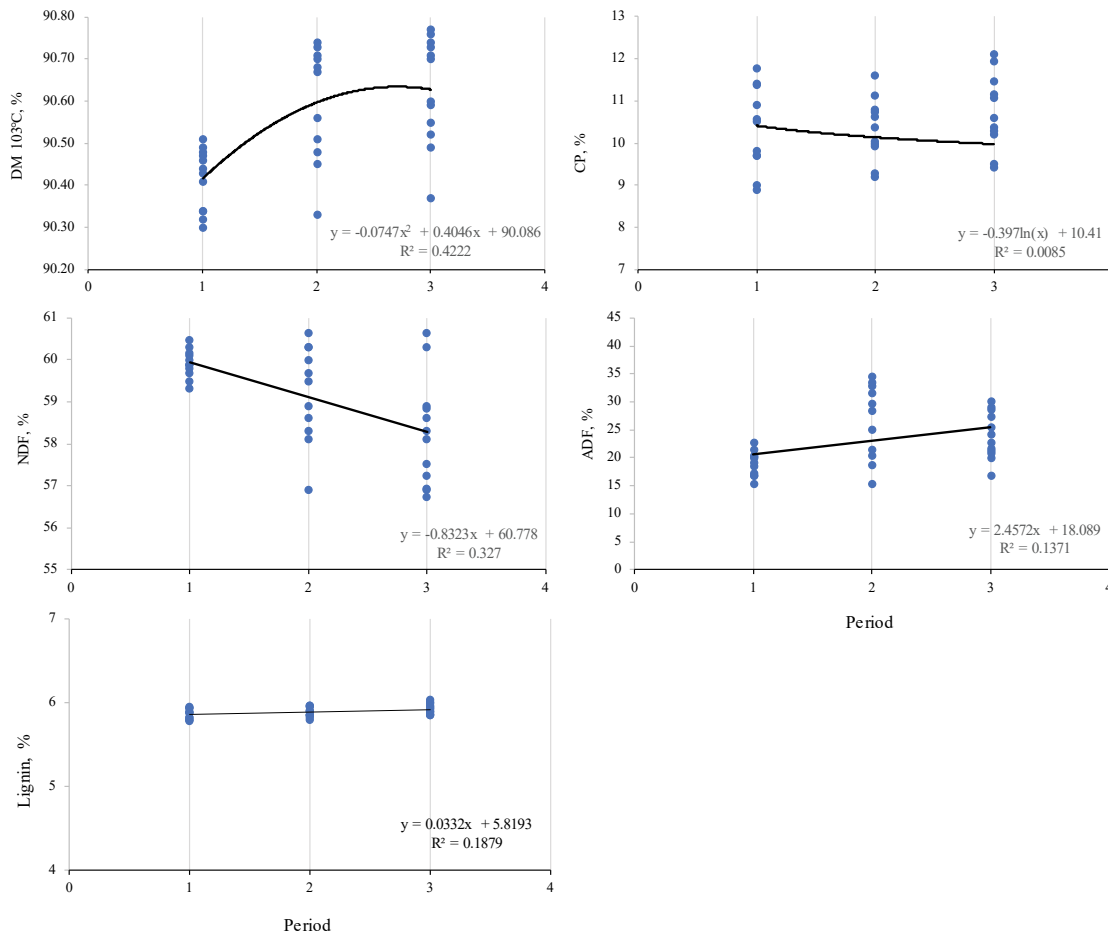


Figure 3. Regression Fits of the Chemical Components of Savoy Grass (*Megathyrus Maximus*).

The protein composition of Savoy grass (*Megathyrus maximus*) is presented in Table 3. Significant statistical differences were seen for PDIA, with a p-value of less than 0.001. The utilization of Urea led to significantly higher

levels in comparison to Biol (P = 0.002), as well as for the Control and Urea + Biol treatments (P < 0.001). The levels of PDIM content that were observed to potentially reach the small intestine were found to be comparable across the

Control, Urea, and Biol treatments, with an average value of 52.44 ± 1.15 g/kg DM. This observed similarity in PDIM content among the treatments was not statistically significant ($P > 0.05$). The measured quantity of protein obtained from both dietary and microbiological sources, known as PDI, demonstrates a significantly greater value when pastures are solely fertilized with Urea ($P = 0.002$ to 0.004). Despite the differences described above, it was observed that the rumen protein balance (RPB) displayed a similar trend when using Urea or Biol (-17.61 ± 2.79 g/kg DM) compared to the Control and Urea + Biol treatments (-23.42 ± 2.79 g/kg DM). The protein values determined in the current experiment were conditioned by the timeframe ($P = 0.001$ to 0.009). Within this particular context, it is observed that the values of PDIM, PDI, and RPB demonstrated elevated levels throughout the beginning period ($P = 0.002$), except for PDIA. No statistically significant differences were seen for the interaction between the trial and period ($P > 0.005$).

DISCUSSION

Agronomic Responses

The economic activities in the northern region of Ecuador are significantly influenced by livestock production, notwithstanding the challenges related to soil quality.^[48] The research area has been extensively recorded to demonstrate a noteworthy susceptibility of biodiversity. Nevertheless, the preservation of natural resources is presently confronted with a serious concern as a result of human activity, including deforestation and the exploitation of oil. The aforementioned issue has been brought to attention in recent academic research undertaken by Fuentes-Quisaguano *et al.*^[49], González-Marcillo *et al.*^[48], Heredia-R *et al.*^[50], and Lozano *et al.*^[51]. The present study presents findings that support the notion that the unregulated application of synthetic chemical fertilizers produces prompt outcomes, considering the rate at which they evaporate and the acidic pH levels of soils specifically intended for cow farming. Furthermore, as indicated by the MAGAP^[52] study, a considerable percentage (75%) of the agricultural production units in Orellana function within subsistence systems that are distinguished by minimal or insignificant utilization of technology. Nevertheless, these agricultural systems are extremely dependent on the use of agrochemicals, leading to a significant carbon footprint. Therefore, in light of these conditions, the administration of livestock agriculture not only generates limited financial benefits but also acts as an indirect contributor to the release of nitrous oxide, as demonstrated in prior research conducted in various geographic areas.^[53]

The analysis revealed that the observed inequalities in tiller density per square meter were similar when Urea was used alone or in combination with Biol. The grazing behavior of ruminants is influenced by two important factors: the height of the pasture and the perceived density of the fodder.^[54-56] In contrast to the aforementioned findings, Berça *et al.*^[30] observed an increase in the quantity of tillers in tropical grasslands with the use of Urea. Based on extant

literature, the application of Biol has been found to elicit the promotion of fresh tiller development, hence resulting in a notable augmentation of leaf biomass within grassland environments. Furthermore, it is crucial to limit the emission of leachates and/or substantial quantities of nitrous oxide that are generated during the volatilization mechanism of Urea. Furthermore, Berça *et al.*^[30] have posited that the application of synthetic chemical fertilizers possesses the capacity to amplify the loss of ammonium and the release of nitrous oxide, so exerting a substantial influence on the environment. As predicted, the plant's height demonstrated a discernible augmentation due to the application of Urea. The phenomenon in question can be elucidated by the role of nitrogen as the predominant macronutrient responsible for the synthesis of many organic components, such as proteins and enzymes. Moreover, the metabolic activities of plants are directly impacted by nitrogen, as demonstrated in research conducted by Zanine *et al.*^[57] and de Lima Veras *et al.*^[58]. The current study demonstrated that the utilization of Urea as a fertilizer led to a 16% augmentation in dry matter (DM) yield in contrast to the utilization of Biol. In contrast, the Biol treatment demonstrated a 32% increase in dry matter (DM) yield compared to the Control treatment, with respective values of 2486 and 1680 DM/ha-1. From a production and environmental standpoint, the experimental group exhibits a greater biomass in comparison to the control group, leading to an increased quantity of leaves. Leaves serve as the major location for the process of photosynthesis in plants, hence making a substantial contribution to their overall nutritional content. Delevatti *et al.*^[59] reported similar results to those obtained in our study with Panicum grass while applying a nitrogen rate of 180 kg N/ha-1. This conclusion is consistent with our own data at a nitrogen rate of 150 kg N/ha-1. In contrast, the study conducted by Berça *et al.*^[30] revealed a diminished production of green fodder in comparison to our own research when utilizing a nitrogen (N) rate of 150 kg/ha-1. The study conducted by Nascimento *et al.*^[2] shown that environmental conditions have a considerable influence on the agronomic responses of Brachiaria spp. In comparison, the daily amount of available forage per individual (HA) in our study ranged from 5.6 to 7.60% of the animal's body weight for the Urea + Biol, Biol, and Urea treatments. According to the findings of Machado *et al.*^[60], reference values have been established to indicate that animal consumption generally falls within the range of 8% to 12% of live weight. Nevertheless, upon assessing the ingestion of dry matter (DM) equivalent to 2% of an animal's body weight, our research indicates that the observed intake in our investigation is twice as substantial as the reference values provided. Consequently, the incorporation of hydroponic agriculture (HA) possesses the capability to optimize leaf consumption to its maximum potential, thereby facilitating enhanced individual animal productivity in grazing situations. The Control group's results provide support for the findings, as they exhibited a harmful accumulation (HA) that was below the set threshold

of 5% of live weight. This finding provides additional support for the proposition that organic fertilizers have the potential to serve as viable alternatives to synthetic chemicals in the agricultural practices employed in the Province of Orellana, either to a certain extent or completely.

Chemical Composition

Multiple scientific studies have provided evidence indicating that the application of chemical fertilizers enhances the concentration of crude protein (CP) in plants through the promotion of soluble protein synthesis.^[59,61,62] Previous studies conducted in the study region have documented the crude protein (CP) contents of *Panicum maximum* within the range of 70 to 146 g/kg DM. These studies have also highlighted the significant impact of the rainfall regime on CP levels.^[31,32,48] In contrast, Ruggieri *et al.*^[63] reported a crude protein (CP) content of 136 g/kg dry matter (DM) when applying a fertilizer rate of 90 kg N/ha-1. This finding differs with the results obtained in our study, where a fertilizer rate of 150 kg N/ha-1 was utilized. However, our most significant chemical properties exhibit exceptional performance while utilizing 100% organic nitrogen (Biol) or when partially substituting 50% of synthetic nitrogen. The findings of this study indicate significant favorable outcomes in relation to organic fertilization in the Ecuadorian Amazonian context, as compared to the Control group. Regardless, all of the fertilization procedures employed in this investigation demonstrated crude protein (CP) levels over 8% on a dry matter (DM) basis, which is considered optimal for ruminant nutrition.^[64]

According to the findings of Berça *et al.*^[30] and Ademar Pereira *et al.*^[9], it has been observed that the augmentation of chemical nitrogen (N) levels leads to a consistent decline in neutral detergent fiber (NDF) concentrations, following a linear relationship. However, in our study, it was shown that the combination of Urea and Biol resulted in lower NDF levels when compared to the Control group, and slightly lower values when compared to the group that received chemical nitrogen fertilization. The decrease in neutral detergent fiber (NDF) levels can be attributed to the development of new tillers and the reduction in the proportion of plant area occupied by structural carbohydrates, as discussed by Santini^[65] and López *et al.*^[66]. The findings indicate that the application of Biol or Urea + Biol resulted in a significant decrease in NDF levels below 600 g/kg DM. This reduction in NDF values could potentially restrict the forage intake of ruminant animals. Consequently, the utilization of organic fertilizers as a fertilization approach may present a promising alternative for livestock farmers in the northern region of Ecuador.

Nutritive Values Energetic Values

Based on basic knowledge, most of the energy available in forages is in the form of structural carbohydrates.^[67,68] Consequently, the increase in the values of structural fibers would condition the energy values of the pastures. Despite not having detected correlations between CP and NDF

contents ($r = 0.005$; $P = 0.95$), a slight significant association was observed with ADF contents ($r = 0.33$; $P = 0.05$). At the Ecuadorian level, few studies have been reported on the nutritional values of the *Megathyrus maximus* grass.^[32] In any case, the GE values obtained are similar to those reported for this grass that is widely spread in livestock farms as the main food resource. Olomonchi *et al.*^[69] reports lower ME contents (1.23 to 1.28 Mcal/kg DM), than those observed in our study (2.95 to 3.05 Mcal/kg DM). According to Inra^[42], the reported values of UFV (0.41 to 0.70 Mcal/kg DM) differ from ours (1.65 to 1.72 Mcal/kg DM), as a consequence of a higher chemical composition of our pasture. But it is important to consider what was revealed by Guamán Rivera *et al.*^[32] who observed that in *Megathyrus maximus* grass all energy values were conditioned by climatological factors.

Protein Values

Aumont *et al.*^[70] documented the PDI values at 28 g/kg DM, however Guamán Rivera *et al.*^[32] reported a higher value of 73 g/kg DM. In the current investigation, we have noted elevated PDI concentrations compared to the aforementioned values, maybe attributable to an increased chemical composition resulting from the implementation of reduced grazing frequencies (45 vs. 35 days). According to Bach *et al.*^[71], nitrogen serves as the primary substrate for microbial protein synthesis in the rumen. A low crude protein (CP) content would result in increased dry matter (DM) consumption and decreased cellulite activity among bacteria. In a study conducted by Guamán Rivera *et al.*^[32], a strong positive connection was observed between the contents of PB and PDIA ($r = 0.99$; $P < 0.001$). However, this study did not demonstrate a significant association between these contents ($r = 0.99$; $P < 0.001$). Nevertheless, a moderate positive correlation was discovered between the contents of FAD and the variables under investigation ($r = 0.46$; $P < 0.05$). In their reference tables, Inra^[42] presents PDI values ranging from 71 to 86 g/kg DM. However, it is important to take into account the findings of Guamán Rivera *et al.*^[32], who discovered significant fluctuation in the protein levels of *Megathyrus maximus* grass as influenced by environmental variables. The RPB values for *Megathyrus maximus* have been documented by Inra^[42] and Guamán Rivera *et al.*^[32], with reported values ranging from -5 to -44 g/kg DM. The Rumen-Protected Protein (RPB) is a measure of the discrepancy between the amount of crude protein (CP) consumed and the non-ammoniacal CP that enters the duodenum, which includes undegraded dietary CP, microbial CP, and endogenous CP.^[72] Hence, the crude protein (CP) content in the diet, specifically when the rumen-protected protein (RPB) value is zero, is approximately 140 grams per kilogram of dry matter (DM). Additionally, when considering DM consumption, it is seen that, on average, around 60% of the variations in protein intake do not reach the duodenum in the form of protein or ammonia (NH₃). In contrast, Arias *et al.*^[73] have documented a decline in organic

matter (OM) digestibility as the relative proportion of rumen-protected bypass (RPB) declines to values below zero. Nevertheless, while observing a modest correlation coefficient of -0.37 between PB contents and RPB, this relationship did not reach statistical significance ($P = 0.12$). Due to this rationale, the utilization of net energy and protein assessment systems (FES) provide an opportunity to develop rations by modifying feed values, such as net energy and metabolizable protein, to align with the specific requirements of the animals. However, the quantification of different digestive and metabolic reactions of animals to dietary changes is crucial for ensuring accuracy and precision.^[74] Based on the available research, it has been observed that the utilization of organic fertilization has the potential to partially substitute nitrogenous chemistry in agricultural practices. This substitution can lead to the attainment of protein values that are deemed satisfactory for the purpose of ruminant feeding.

Conclusions

Based on the findings of our study, it can be concluded that the agronomic variables examined exhibited satisfactory responses when organic fertilization was implemented. This suggests that organic fertilization has the potential to serve as a viable alternative to partially substitute nitrogenous chemical fertilizers. While the use of Urea resulted in slightly greater crude protein (CP) levels, the utilization of Biol in *Megathyrus maximus* grass led to increased dry matter (DM) contents while simultaneously reducing concentrations of neutral detergent fiber (NDF) compared to the observed effects of Urea. From a nutritional perspective, the Biol application produced comparable levels of genetically engineered (GE) components as the nitrogenous chemistry. Similarly, despite Urea exhibiting higher levels of PDI, no significant disparities were detected in the levels of PDIM and RPB as compared to Biol. Therefore, the utilization of biofertilizers produced within the same agricultural premises leads to the implementation of a sustainable approach for these vulnerable ecosystems. In any event, it is advisable to conduct long-term investigations in order to substantiate our findings.

Conflict of interests

All authors declare that they have no conflict of interest.

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ABBREVIATURE

CP: Crude protein

NDF: neutral detergent fiber

ADF: Acid detergent fiber

GE: gross energy

DE: digestible energy

ME: metabolizable energy

ENV: net energy for meat production

PDIA: digestible protein in the intestine of food origin

PDIM: digestible protein in the intestine of microbial origin

PDI: digestible protein in the intestine

RPB: protein balance in the rumen