Increase of phytomass and protein in hydroponic green forage through fertilization in Casanare, Colombia

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ABSTRACT
Objective: To evaluate the effect of two types of fertilizers on the phytomass and protein content of hydroponic green forage (HGF) of corn (*Zea mays* L.), rice (*Oryza sativa* L.), and soybean (*Glycine max* L.).
Design/Methodology/Approach: A 32×32 bifactorial design was implemented. The seeds were immersed in 1% chlorine for 15 min for their disinfection. A hydroponic system with nebulization irrigation was used; the plants were irrigated for 1 minute every 4 hours. Five cm^3 of organic fertilizer (OF) and 5 cm^3 inorganic fertilizer (IF) were applied per liter of irrigation water. Plant height (cm), fresh phytomass production (kg), actual phytomass yield (kg^{-1} m^{2-1}), and crude protein content (%) were measured. The data were analyzed by means of an ANOVA and a Tukey comparison test ($p \leq 0.05$), using the SPSS® Statistics 24 software (IBM).
Results: A phytomass yield of 50.7 kg/m^2 of HGF and a protein content of 17% were obtained using 7.102 kg of corn seed; meanwhile, a yield of 30.53 kg/m^2 and a protein content of 15% were obtained with rice seed. Finally, soybean obtained a yield of 19.17 kg/m^2 of HGF and a protein content of 38%.
Study Limitations/Implications: The nitrogen content of the fertilizers can be considered as the main limitation factor in the production and quality of HGF.
Findings/Conclusions: Inorganic fertilization has a significant effect on phytomass production and the protein production of HGF.

Keywords: soilless forage, HGF, phytomass, protein.

INTRODUCTION

Casanare is one of the 32 departments of Colombia. One of its main economic activities is cattle production: 87% of its surface (3,499,806 ha) is used for this activity. Out of the total of its territory, 2,465,302 ha are used to grow native grasses which feed 1,861,776 cows (ICA 2016). Cattle production in Casanare is divided among breeding stock (73%), fattening livestock (16%), dual-purpose livestock (10%), and dairy cattle (1%) (FEDEGAN 2014). In this region, cattle production is focused on a pastoral system that provides cattle with a food resource. However, the variable and intense weather that prevails in the region —a bimodal pluviometric regime (4 months of drought, from December to March) and an

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intense precipitation regime (which causes floods from April to November)— frequently impacts cattle production (PEGA 2015). This situation causes grass shortage and scarce nutrient content, leading to high mortality and weight loss among cattle. From April 2013 to April 2014, 232 cows and 8,382 capybaras died as a consequence of the dry season that impacted grasses, crops, aquifers, and wild life in four municipalities of Casanare (Contraloría, 2014).

Currently, 30% of the continuous grazing ranches do not have forage or protein banks for the different seasons of the year (PEGA, 2015) and producers prefer to use silos, hay, or other supplements (such as concentrated feeds) to replace grass (FEDEGAN, 2019), raising the price and, consequently, the feeding costs. Therefore, producing an efficient and sustainable feed —through the research of other technologies to implement protein banks that provide nutrients and improve the food supplements for cattle— is fundamental. In this regard, the hydroponic green forage (HGF) is a very efficient technique for the production of vegetal biomass. This technique uses the initial grow of plants in their germination state and the early growth of seeds (pulses and cereals) to produce feeds. The species traditionally used for this technique are barley, wheat, oats, rice, or corn (FAO 2001). This forage is used to feed cattle, sheep, goats, horses, pigs, rabbits, and poultry (Müller *et al.* 2005 a, b; Herrera *et al.*, 2007). Campêlo *et al.* (2007) and Mata (2011) used rice husk as organic substrate, given its moisture-retention capacity. This substrate supports the development of the roots of the seedlings used in the production of HGF. However, Della *et al.* (2002) recorded a 90% silicon content in rice husk: a high amount of this mineral in the forage can inhibit the consumption of dry matter (DM) and fiber digestion. Rocha *et al.* (2007) recommended using only small amounts of substrate in the production of HGF.

Its short harvest time (10-15 days) means that HGF is a sustainable alternative that can be produced all year round. Additionally, not only can it be developed in small areas, but its energy and protein content, as well as its digestibility, are high, while its neutral detergent fiber and lignin contents are relatively lower than in other feeds (FAO, 2001; Müller *et al.*, 2005b). Meanwhile, 1 m^3 of water is required to produce 1-8 kg of dry matter of feed using crops in soil, while the same volume of water used in hydroponic green forage produces about 100 kg (FAO, 2001). The viability, physiological and phytosanitary state of the seed is fundamental (González-Cortés *et al.*, 2015). Similarly, fertilizers applied during irrigation can provide the macro and micronutrients required for plant growth in the production of HGF (Maldonado *et al.*, 2013). This will create a forage layer that includes leaves, stems, and roots, in which 20-30 cm tall plants will grow in an 8-12 days period (Resh, 2001). The harvest of the hydroponic forage must be carried out after 10-13 days (25.4 kg to 2714 kg m^{-2}), because plants have a higher nutrient, lysine, and tryptophane content during this period (FAO, 2001 and García *et al.*, 2017). McDonald *et al.* (1981) pointed out that, the more the HGF grow, the more the protein content decreases; however, this situation can be reversed applying nitrogen fertilizers. Maldonado *et al.* (2013) mentioned that adding nitrogen (in the form of nitrate) increases height, conversion ratio, yield per m^2, protein content, and the amount of nitrate in the plants.

MATERIALS AND METHODS

Geographic location of the experimental area

The research was carried out during summer, in the El Recuerdo ranch, located in the vereda Matapantano, in the municipality of Yopal, department of Casanare, Colombia (05° 37' N and 72° 35' W). The environmental conditions during the experiment were: a 31 °C mean temperature and a 63.8% relative humidity.

Hydroponic material

Three iron vertical shelves with 5 levels were built with angle bars and secured with nuts; the dimensions were 2 m (height) × 1.26 m (length) × 0.61 m (width). They also had a 10° horizontal support inclination which allowed them to drain water. Their storage capacity included 10 black hydroponic polyethylene trays (50 cm long × 25 cm wide × 2.5 cm high). The metal structure was covered with a 6 mm greenhouse plastic sheet. The irrigation system per shelf was made up of a PVC pipeline, a 120 L bin connected to a water pump, 5 stopcocks, 5 tees, 4 degree elbows, and 5 irrigation lines with two nebulizers per line (360° radius), located 0.36 m away from the hydroponic trays (10 nebulizers in total). The crop was irrigated for 1 minute every 4 hours. This procedure took place during 10 days of the crop cycle. Water consumption amounted to 48 L per day, with a volume of 0.2 L/seg^{-1}. Fertilization took place from the first day of sowing (das) until two days before the harvest. Irrigation continued until the tenth day, using drinking water to remove the excess of salts, which makes the forage suitable for consumption (Sousa *et al.*, 2014).

Experimental design

A 32×32 bifactorial design was used. Factor 1 consisted of the following types of fertilization: a) inorganic fertilization; b) organic fertilization; and c) control (drinking water). Factor 2 included the following seed types: a) corn; b) rice; and c) soybean. A total of 9 treatments, with three replicates were used, resulting in 27 experimental units. Each experimental unit consisted of a 0.264-m^2 black polyethylene tray. Table 1 shows the design of the treatments.

Table 1. Design of the treatments for the evaluation of the effect of organic and inorganic fertilization on the production and quality of hydroponic green forage (HGF) prepared with corn (*Zea mays* L.), rice (*Oryza sativa* L.), and soybean (*Glycine max* L.).

Fertilizer type	Plant	Treatment
Organic fertilizer (FO)	Corn	1
	Rice	2
	Soybeans	3
Inorganic fertilizer (FO)	Corn	4
	Rice	5
	Soybeans	6
Water (control)	Corn	7
	Rice	8
	Soybeans	9

The commercial fertilizers were prepared following the instructions of the manufacturer. A 5 cm^3 dose of OF and a 5 cm^3 dose of IF were used per liter of water, adjusting both solutions to a 5.8 pH. According to the analysis carried out by the ACUALIM SQR S.A.S lab, control (drinking water) had the following characteristics: 8.23 pH, 39.9 dS m^{-1} EC, 15.2 total hardness, <1.0 cloudiness, 4.7 mg NO^3 L^{-1}, and a lack of total and fecal coliforms. Table 2 shows the chemical composition of the inorganic and organic fertilizers.

Seed treatment

Certified seeds from commercial brands, without chemical treatments, were used for this experiment: yellow corn variety Corpoica V-114; rice variety Victoriosa 10-39, and soybean variety Panorama 357. The forage was produced in 10 days; sowing density (seed kg per tray) was different for each species: 1.5 kg of corn seed and 300 g of wet rice husk as substrate; 1.8 kg of rice seeds; and 0.165 kg of soybean. The germination of soybean is special, because it does not develop a root layer (Sousa *et al.*, 2014). In order to produce an innocuous HGF, all the impurities and broken seeds were removed, using an indirect flotation method proposed by López *et al.* (2005). Subsequently, the seeds were immersed in a 1% sodium hypochlorite solution (chlorine), for 15 minutes, to disinfect them. Finally, the seeds were washed three more times with drinking water. The same procedure was carried out to disinfect the substrata (rice husk), using hot water instead of drinking water. For the pre-germination of corn and rice, the seeds were immersed in water for 24 h; the water used was changed twice a day (Contreras *et al.*, 2015). Subsequently, the seeds were placed in a perforated bin and covered with a black plastic to shield them from light and to encourage their germination during the next 48 h. Afterwards, the seeds were evenly sown in hydroponic trays, according to the sowing density assigned to each species. Soybean seeds were immersed for 5 h in water, which was changed after the first two hours. Subsequently, seeds were placed in 44 cm long × 32 cm wide × 4 cm deep black polyethylene trays, with 0.025 L of water. Finally, they were covered with a black plastic bag for 67 h and the water was changed every 12 h. This species has an epigeal germination, because its cotyledons grow above ground level. Consequently, soybean was sown directly over a polyethylene net, previously placed on the plastic trays, to encourage the root to grow through the holes of the net. Using this method, the roots can be in contact with the fertilizer received by each irrigation tray.

Table 2. Chemical composition (minerals) of the fertilizers evaluated to produce hydroponic green forage (HGF) prepared with corn (*Zea mays* L.), rice (*Oryza sativa* L.), and soybean (*Glycine max* L.).

Type of fertilizer	N	P	K	Ca	Mg	S	Fe	Mn	B	Cu	Zn	Cl	Si
	%						Mg kg^{-1}						
Organic fertilizer (FO)	1.2	1.1	0.9	1.1	1.7	0.13	10	66	10	1.0	2.0	0.0	0.0
Inorganic fertilizer (FI)	2.0	0.6	2.4	2.1	0.4	0.01	3.0	13	1.0	1.0	11	3.0	0.0

Table developed by the authors based on the data provided by the manufacturers of the commercial products.

Measuring the variables

The crude protein (CP) content was determined using the Kjeldahl method. In each of the 9 treatments, a 200-g sample of HGF was taken from the center of each experimental unit in triplicate, obtaining a total of 27 samples. Seedling height (SH) was measured at 10 days. A sample of 10 seedlings was taken from the center of each hydroponic tray and corn and rice were measured, using a ruler (mm), from the seed to the apex of the leave (cm). Soybean was measured after the opening of the cotyledons (day 5). The length of the foliar area (from the cotyledon to the primary leaves) and the root were measured separately. A Pesatronik® precision electronic scale (0.001 g) was used to determine the fresh biomass (FB) production. The growth of layer was measured on the harvest day. Fresh forage yield was determined, based on fresh biomass and seed weight per tray (Equation 1).

$$Conversion\ efficiency = \frac{Fresh\ biomass\ (kg)}{Weight\ of\ corn\ seed\ (kg)}$$

Statistical analysis

The data from the variables obtained during harvest day were subjected to a normality and homogeneity verification. After both assumptions were determined to be right, the results were subjected to an ANOVA (with a $p \leq 0.05$ significance level) and to a Tukey comparison test, using the SPSS Statistics 24 software (IBM).

RESULTS AND DISCUSSION

Hydroponic green forage production is a feeding strategy for all the breeding livestock (poultry, rabbits, sheep, goats, dairy cows, etc.) during critical drought or flood periods, when grass is scarce. Only water is usually used to produce HGF; however, different seed species and the application of fertilizers in the irrigation systems should be evaluated to increase the production and quality of HGF. This research clearly points out that fertilization and seed species (corn, rice, and soybean) have a significative effect ($p \leq 0.05$) on the production and quality of HGF. From the beginning, the amount of phytomass per species (corn, rice, and soybean) is different for each species (Figure 1).

Plant height of the HGF: Regarding the plant height variable, there were significative differences ($p \leq 0.05$) between the type of fertilizers and seed species. The corn plants of

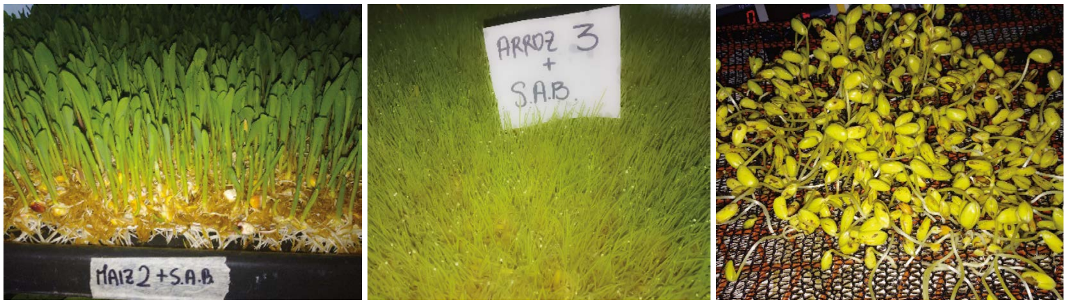

Figure 1. Seeds germinated to produce hydroponic green forage. From left to right: corn (*Zea mays* L.), rice (*Oryza sativa* L.), and soybean (*Glycine max* L.).

the treatment that received a chemical fertilizer reached a height of 42 cm. The corn plant of the treatment fertilized with OF reached a height of 30.8 cm. In contrast, control only received water and reached a plant height of 29 cm. A 45% increase in growth was recorded when fertilizers were applied instead of irrigation systems that only use water. The rice HGF also showed a different growth response depending on the type of fertilization. These plants reached a height of 15.4, 7.4, and 6.2 cm, using IF, OF, and only water, respectively. Nevertheless, the soybean HGF showed no significative differences, reaching a 13.5 cm mean height (Table 3).

Fresh phytomass production (kg) of HGF. There was a significant difference ($p \leq 0.05$) in the HGF production between the types of fertilizers and the seed species. The corn HGF fertilized with IF obtained the highest yield (10.7 kg), followed by the OF treatment (9.4 kg), and the water treatment (9.3 cm). Fertilization did not have a significate impact on the rice HGF. The yields obtained were 7.8 (IF treatment), 7.0 (OF treatment), and 6.7 kg (control). Meanwhile, the soybean HGF recorded a mean yield of 400 g, using 165 g of seeds (Table 3).

Phytomass actual yield ($kg\ kg^{-1}\ m^2$). In order to estimate the forage production for a particular area and the number of animals to be fed, calculating the potential phytomass yield of the HGF per m^2 is fundamental. This research recorded the following yields, using 7.102 kg of corn seed: up to 50.70 kg m^2 of HGF, with corn fertilized with IF; 44.31 kg of HGF, with corn fertilized with OF; and just 43.89 kg when the HGF only received water. These results show that, using the IF treatment, an increase of 7 kg was obtained, while only a small increase (0.42 kg) was recorded with the OF control treatments. In the case of the rice HGF, the highest yield recorded was 30.53 kg (IF treatment), followed by 27.68 kg (OF treatment), and 26.27 kg (control), obtaining a small increase of 1.42 kg. Meanwhile, the soybean HGF recorded 19.17 kg (IF), 17.04 kg (OF), and 14.20 kg (control) yields (Table 3).

Table 3. Effect of organic and inorganic fertilization in the production and quality of corn (*Zea mays* L.), rice (*Oryza sativa* L.), and soybean (*Glycine max* L.).

Fertilizer type	Plant species	Treatment	Plant height (cm)	Production fresh phytomass (kg)	Phytomass real yield ($kg\ m^2$)*	crude protein (%)
Organic	Corn	1	30.8 b	9.40 b	44.31	12.5 ab
	Rice	2	7.40 b	7.00 a	27.69	10.6 b
	Soybeans	3	13.8 a	0.40 a	17.04	35 b
Inorganic	Corn	4	42.0 a	10.7 a	50.70	17 a
	Rice	5	15.4 a	7.80 a	30.53	15 a
	Soybeans	6	15.4 a	0.45 a	19.17	38 a
Water (control)	Corn	7	29.0 b	9.30 b	43.89	11.5 b
	Rice	8	6.20 b	6.70 a	26.27	10.5 a
	Soybeans	9	11.3 a	0.33 b	14.20	30 c

* Yield (kg) of the HGF for every 7.102 kilograms of seeds per m^2.
Measures with different letter show significative statistical differences (Tukey's test, $p \leq 0.05$).

Crude protein content (%) of the HGF. This research recorded an increase of protein in the HGF. Two types of fertilizers were evaluated: organic fertilizer and inorganic fertilizer. The inorganic fertilizer had a higher nitrogen (2%), potassium (2.4%), and calcium (2.1%) content than the organic fertilizer (1.2% N, 0.9 % K, and 1.1% Ca). The analysis of the CP content 10 days after the HGF was harvested recorded a significative increase ($p<0.05$) between treatments (type of fertilizers and seed species). The highest CP increase was recorded with the corn seed + IF treatment (17% protein), followed by the corn seed + OF treatment (12.5%), and the control + water treatment (11.5%); therefore, there is a significant protein increase (up to 48%) in the HGF when the IF is applied than when the HGF only receives water. For its part, the rice HGF recorded a CP content of up to 15% (IF treatment), followed by 10.6% (OF treatment), and 10.5 % (control); therefore, a 42% increase regarding the OF and water treatments was obtained with application of IF. Finally, in the case of the soybean HGF, an increase of CP was also recorded. The following values were obtained: 38% (IF treatment), 35% (OF treatment), and 30% (control). The IF treatment recorded a 26% increase in CP compared with the OF and water treatments.

Fertilization (N, K, and Ca concentration) is a determinant factor of plant growth; in particular, nitrogen causes a better foliar development (Maldonado *et al.*, 2013). Most of the scientific research about HGF production are focused on corn, likely as a consequence of its low cost, easy availability, and high yield. In this study, corn seed recorded excellent results regarding plant height (42 cm), which are higher than the results recorded by other authors. González *et al.* (2015) reported a 14.4 cm maximum height on white corn. Quispe *et al.* (2015) found out that, 11 days before the harvest, the plants reached a 26-30 cm height. Silva (2008) obtained a 28.7 cm, while Ramírez *et al.* (2017) applied nutrients to the water irrigation system and obtained a height of up to 30.2 cm. Maldonado *et al.* (2013) pointed out that height increases along with sowing density, as a result of the competition for light between the plants, which promotes etiolation. Additionally, a 13-16 h period of natural or artificial light is essential to obtain a better HGF. Meanwhile, Vargas (2008) recorded that, at 20 days, rice HGF reached a height of 25 cm and Müller *et al.* (2005b) obtained a 10.6 cm height. In the case of soybean HGF, Jitsuyama (2013) reported that hydroponic soybean is included in the V3-V4 growing stage, with several trifoliated leaves for 1-2 weeks and a 20.2-cm long root.

In this research, the best phytomass yield was obtained with the corn HGF + IF treatment. The rice HGF yield is similar to the yields reported by Müller *et al.* (2005 b). For his part, Vargas (2008) recorded an HGF of 14.35 kg at 20 days; however, 13 days after the sowing, the nutrient value of the HGF starts to decrease. The phytomass of the soybean HGF is lower than with the other species, as a consequence of the sowing density. There are no studies that evaluate this variable; nevertheless, Jitsuyama (2013) studied soybean under hydroponic conditions. Ramírez *et al.* (2017) mentioned that fresh biomass is related to the genotype used, the days of harvest, and the sowing density. According to CONtexto ganadero, a cow eats 1.5-10% of its body weight per day in forage. Consequently, the phytomass variable allows to determine how much forage should be sown to meet the food supplement required by the animal diet. Therefore, production must be cyclical in order to provide food every day of the year. Valdivia (1997) obtained a 5 kg yield of HGF per seed kg; however, up to 7 kg/kg[1] could be potentially produced.

Loomis *et al.* (2012) pointed out that forage with a >16% of CP can be considered a good quality forage that meets the requirements of different types of livestock, although a 7% minimum CP is required for the HGFs. For their part, Albert *et al.* (2016) recorded a 13% CP content in corn HGF, while Naik *et al.* (2012) found a 10.67% CP also in corn HGF. Therefore, the CP content of the control HGF, which received only water, falls within these parameters. These results prove that the N content of the fertilizer promotes not only an increase of phytomass, but also of the CP content, obtaining a significant increase (up to 47%) and surpassing by far the CP content of the corn silage (8.35%) reported by Hazard *et al.* (2001). The results obtained in this research for rice + IF treatment were 7.92% higher than the results recorded by Vargas (2008) and 8.15% higher than the results obtained by Müller *et al.* (2005 b). Currently, rice straw or harvest residues are used as hay to feed animals and they provide 5.1% protein (Engormix, 2009). Olave and Castellar (1987) indicated that soybean crops in open fields produce 18.3% CP in fresh forage. Currently, soybean is the best choice to feed animals, as a result of its high protein content (30-40%) (Garzón *et al.* 2013). Silva (2008) pointed out that sowings with a >2.0 kg/m^{-2} density favor an increase in the protein content of hydroponic forages.

The HGF generated with the evaluated species can supplement the diet of a cow, which requires approximately 2.5% of its weight in dry matter (Fedegan, 2019) —*i.e.*, 12.5 kg of dry matter for a 500 kg cow. The protein percentage is suitable in the three varieties and can be used to feed different livestock species. Cattle reduce their consumption of forage when its protein content falls below 8% (Aregheore *et al.*, 2006). A small fraction of neutral detergent fiber (NDF) in the corn, rice, and soybean HGF indicates a high-quality forage. According to Van Soet (1982), forages with >60% NDF content are considered low-quality forages, because they can interfere with digestion and consumption. Some researchers pointed out that the time of harvest is related to the degree of digestibility of the food, because when forage matures and the cellulose fiber and lignin content increases, the protein content decreases, reducing forage digestibility.

CONCLUSIONS

According to the results obtained in this study, the nutrient solution applied with the inorganic fertilizer had a significant effect in the protein content, biomass, and plant height of the HGF of the three species used in the experiment. The species is a key factor related to the protein content. For instance, soybean had a 38% protein content; this value surpasses by far the protein content of corn (17%) and rice (15%) at 10 days of treatment.

The three species recorded a high fresh biomass content at 10 days. The layer weight and the conversion efficiency were low in corn, rice, and soybean, because sowing density promotes biomass growth.

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