Scientific Paper

Effect of mineral nutrition on the yield and bromatological characteristics of corn hydroponic green forage

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Abstract

The objective of this study was to determine the effect of mineral nutrition, applied through nutritional solutions, on the fresh yield (FY) and bromatological characteristics of corn hydroponic green forage (HGF). The study was conducted in a greenhouse located in the Agricultural Research Station Fabio Baudrit Moreno, Alajuela, Costa Rica. Two treatments of nutritional solution were applied: 1) with high (N_h), and 2) with low (N_l) concentration of mineral nutrients, and a control with water (T_e), distributed in an unrestricted randomized design. The seed was selected; prepared through washing, disinfection, imbibition, draining and aeration; it was pregerminated in humidity chamber (3 days) on plastic trays (density of 3 kg m^{-2}); and was transferred to the greenhouse, where it remained during 11 days until harvest. In general, no significant differences were found among the treatments, and the average values were: 15,3 kg m^{-2} of FY; 20,01 % of crude protein; 18,95 % of crude fiber; 1,48 % of lignin; 44,27 % of neutral detergent fiber; 0,96 % of nitrogen of the neutral detergent fiber; 22,09 % of acid detergent fiber; 0,24 % nitrogen of the acid detergent fiber; 4,5 % of ash; 7,44 % of ether extract; 88,6 % of dry matter digestibility; and 3,2 Mcal kg DM^{-1} of metabolizable energy. It is concluded that the application of mineral nutrients through nutritional solution did not affect the fresh yield or bromatological indicators, and the potential of utilization of the corn hydroponic green forage as feeding source in animal production was proven.

Keywords: digestibility, crude protein, nutritional solution, nutritional value

Introduction

Forage availability, in the traditional system of animal feeding based on extensive grazing in open field, faces a series of contrasts associated with climate change and the world crisis of water, such as: land flooding, scarcity of arable lands, water salinity, increase in the cost of fertilizers and labor, long growth periods and natural phenomena (Naik *et al.*, 2015).

An alternative in animal feeding can be hydroponic green forage, because it shows a series of advantages with regards to the conventional forage production system in open range. The hydroponic green forage is obtained from the germination of seeds or grains, and can be used as nutritional supplement in different animal species, because it shows and excellent protein percentage (Contreras *et al.*, 2015), an adequate balance in the soluble fiber/insoluble fiber ratio, high DM digestibility (Gómez-Burneo, 2008) and good energy contribution (Bedolla-Torres *et al.*, 2015).

The intensive production of hydroponic green forage in protected environments is less vulnerable to climate changes; allows programmed and periodic production throughout the year, with efficient water use (Al-Karaki and Al-Hashimi, 2012), and a reduction of fertilizers, agrochemicals and labor (Candia, 2014).

In the production of hydroponic green forage different species have been used, among them grasses and legumes. Some studies evaluated the quality of hydroponic green forage in corn [(*Zea mayz* L.) (Naik *et al.*, 2017)], sorghum [(*Sorghum bicolor* L.) (Gonzales-Díaz and García-Reyes 2015)] barley [(*Hordeum vulgare* L.) (Quispe-Cusi *et al.*, 2016)], wheat [(*Triticum aestivum* L.) (Contreras *et al.,* 2015)], rice [(*Oriza sativa* L.) (Maldonado *et al.*, 2013)], and in mixtures of cereals and legumes (Contreras *et al.,* 2015). However, only a reduced number studied the quality of hydroponic green forage in response to the application of nutritional solutions; for example: in corn (Acosta *et al.*, 2016), wheat (Maldonado *et al.,* 2013), barley (Quispe *et al.,* 2016) and sorghum (Gonzales-Díaz and García-Reyes, 2015). In several of these studies, an absolute control with water without nutrients was used; and in some bromatological indicators similar values to others in which nutritional solution in irrigation was applied, were obtained (Naik *et al.,* 2017); which generates uncertainty with regards to the need of using mineral nutrition in the production of hydroponic green forage.

In the reviewed literature, it was found that the concentration of mineral nutrients in the nutritional solution varied widely. For example, in the case of nitrogen, the ranges fluctuated between 5 mg L^{-1} (Rivera *et al.*, 2010) and 250 mg L^{-1} (Vargas-Rodríguez, 2008); while the iron concentrations, from 4,3 mg L^{-1} (Salas-Pérez *et al.*, 2012) to 800 mg L^{-1} (Rivera *et al.,* 2010). This variability is due to the diversity of factors which influence, such as climate, genotype, planting density and days until harvest.

Taking into consideration the above-stated facts, the objective of this study was to determine whether mineral nutrition, applied through nutritional solutions, affected the yield and bromatological characteristics of corn hydroponic green forage, and based on this basis define the need of fertilizer application.

Materials and Methods

Experimental site. The study was conducted in the Agricultural Research Station Fabio Baudrit Moreno, located in San José de Alajuela, Costa Rica (10º 01' N, 84º 16' W, at 840 m.a.s.l.), with monthly average temperature values of 22 ºC, relative humidity of 78% and annual average rainfall of 1 940 mm.

A multi-tunnel greenhouse, 9,75 m wide and 50 m long, and with a height of 6 m at the center of the tunnel and 4 m in the gutter, was used. The greenhouse was built with galvanized iron, cover of trilayer transparent polyethylene (200 µm) and an anti-insect nylon mesh (43 x 28 threads $inch^{-2}$) in the walls and the zenithal opening. The ventilation system was passive, combined with the automated functioning of zenithal windows according to the wind speed, which was monitored with an anemometer.

Within the structure of hydroponic green forage production, the air temperature and relative humidity were monitored (Data logger HOBO U23 Pro v2) every five minutes, recording the hour averages. The maximum, minimum and average temperature and relative humidity were 31,5; 19,7 and 23,9 ºC, and 97,4; 59,8 and 86,0 %, respectively.

Plant material. Corn seed was used, based on its availability, high production volume and low cost with regards to other imported materials (Ramírez-Víquez, 2016); specifically of the local variety Diamantes 8843, of free pollination, white grain, with late maturity (120-135 days), fresh yield of 3-6 t ha^{-1} and a wide range of adaptation to agroclimatic conditions (INTA-AECI, 2005).

Treatments. Two treatments of nutritional solution (table 1) were applied: 1) high nutrient concentration (N_h), and 2) low nutrient concentration (N_l); and a control with water without nutrients (T_e).

The concentration of mineral nutrients in the nutritional solution, for the treatments N_l and N_h, was defined based on the ranges reported in literature (Al-Karaki and Al-Hashimi, 2012; Candia, 2014), and those values considered extreme were discarded. The quantity of nutrients in T_e was in correspondence with the concentration present in water.

Experimental procedure. The process of hydroponic green forage production was carried out according to the proposal made by Vargas-Rodríguez (2008), and included seed preparation, pre-germination and growth stage. In turn, seed preparation included: selection, cleaning, pre-washing, disinfection and imbibition. Disinfection consisted in: immersion of the seeds in a solution of 100 g L^{-1} of calcium hydroxide (8 h) washing of the lime and, finally, immersion for 5 min in Busamart® (TCMTB: benzothiazole) with a dose of 1 ml L^{-1}. Later the TCMTB residue in the seeds was rinsed away; they were aerated under ambient conditions (1 h) and were subject to an imbibition process, submerging them in water during a period of 10 h.

Table 1. Concentration of macro- and micronutrients in each treatment.

Treatment	pH	Macro (mg L^{-1})					Micro (mg L^{-1})						♦EC
		N	Ca	Mg	K	P	Fe	Zn	Cu	Mn	Na	S	(mS cm^{-1})
Control (T_e)	8,3	6,2	12,6	5,4	3,6	0,3	ND	ND	ND	ND	9,3	0,9	0,2
Low nutrition (N_l)	6,7	94,6	94,2	22,3	145,6	18,4	0,3	0,1	0,1	ND	12,1	35	1,3
High nutrition (N_h)	6,6	227	202,7	49,5	341,4	46,1	1,2	0,5	0,5	0,7	16,3	78,8	2,5

♦EC: electrical conductivity (mS cm^{-1}), ND: not detectable.

The production process was carried out in a cultivation cycle of 14 days, which included two stages: I: germination (3 days), and II: growth (11 days). Once the imbibition was concluded, passive runoff was carried out and the seeds were placed on plastic trays, at a density of 3 kg m^{-2} according to the reports for corn (Acosta *et al.,* 2016; Naik *et al.,* 2017). Afterwards, they were put to germinate in dark chamber, with relative humidity higher than 85 % and temperature of 23-25 ºC.

In the growth stage of the hydroponic green forage the protection paper of the germinated seeds on the trays was withdrawn, and they were transferred to a production area located within the greenhouse. Such area consisted in a structure 3 m long; 1,3 m wide and 2 m high, with five horizontal shelves separated by 0,40 m. The structure was vertically divided in three sections, which were randomly assigned to each of the three treatments. Each vertical section was composed by five shelves, and each one contained four repetitions.

The irrigation system was composed by: a) storage tanks, b) feed pumps, c) pipelines (PVC of 19 mm), d) self-compensating micro-sprayers, e) pressure regulators, and f) manometers. Each shelf or level had four irrigation lines (PE of 16 mm), provided with two sprayers each, for a total of 20 lines and 40 sprayers throughout the structure.

For the preparation of the nutritional solutions hydrosoluble fertilizers were used, such as monopotassium phosphate, potassium sulfate, magnesium sulfate, calcium nitrate, potassium nitrate and a pre-mixture of micronutrients. Afterwards, they were stored in two tanks identified as N_h and N_l for the treatments with high and low nutrient concentration, respectively.

The nutritional solutions were applied in each irrigation event, through fertigation by nebulization. The irrigation programming was done by fixed times through a timer, with a duration of 15 seconds and a frequency every 45 minutes, in a time interval between 6 a.m. and 6 p.m. in each treatment the water inputs and outputs in the cultivation systems were recorded, during 11 days of the cultivation cycle (table 2).

Response variables. At the end of the cultivation cycle the yield (kg m^{-2}) was determined, from the fresh weight (FW) obtained per tray (0,165 m^2). As bromatological variables, the following were determined: crude protein (CP), crude fiber (CF), acid detergent lignin (lignin), neutral detergent fiber (NDF), nitrogen of the neutral detergent fiber (NNDF), acid detergent fiber (ADF), nitrogen of the acid detergent fiber (NADF), ash, ether extract (EtE), dry matter digestibility (DMD) and metabolizable energy (ME). The standardized analysis methodologies of the laboratory of the Research Center on Animal Nutrition (CINA, 2015) were used: a) Official Association of Agricultural Chemists (AOAC) 942.05, 2) AOAC 920.39, 3) AOAC 962.69, 4) AOAC 2001.11, and 5) AOAC 996.17.

The DMD (%) was estimated from the ADF content, according to the equation: DMD = 88,9 - (% ADF x 0,779), proposed by Di Marco (2011). The ME (Mcal kg^{-1} DM^{-1}) was estimated from the DMD, according to the procedure described by Di Marco (2011), using the equation ME = 3,61 x (DMD/100).

The samples were taken from the center of each tray, in order to discard the edge effect. In each treatment the sample by repetition corresponded to a composite sample of all the repetitions present in each of the five shelves, in order to obviate the effect of the reduction of sunlight, according to the descending position from the top to the lowest shelf.

Experimental design and statistical analysis. An unrestricted random design was used, with four

Table 2. Water balance for the production of corn hydroponic green forage in the different treatments.

Indicator in the production system		Treatment		
		Control	Low nutrition	High nutrition
Inputs (L m^{-2})	Irrigation	11,7	12,1	8,9
Outputs (L m^{-2})	Lateral losses	4,1	4,1	2,6
	Drainage	3,3	4	0,6
	ET_c	3,6	3,1	5,1
	Total outputs	11	11,2	8,3
Balance (L m^{-2})		0,6	0,9	0,5

repetitions in each of the five shelves within the production structure. Each repetition corresponded to a plastic tray (0,55 m x 0,30 m = 0,165 m^2) with hydroponic green forage. Between treatments, there was a forage tray used as edge. The data of the response variables were subject to the verification of normality and homoscedasticity, using the computer program INFOSTAT (Di Rienzo *et al*., 2017). When those assumptions were fulfilled, the data were subject to variance analysis (ANOVA) and multiple mean comparison using LSD FISHER, with a probability level of 0,05.

Results

At the end of the cultivation cycle, the nutrition treatments did not affect the fresh yield of corn hydroponic green forage, with values of 15,20; 15,27 and 15,37 kg m^{-2} for T_e, N_l y N_h, respectively. There was no effect either on the CP, CF, lignin, NDF and ADF contents.

The averages of the bromatological variables are shown in table 3. For the variables NNDF and NADF, the treatment T_e differed from N_h and N_l, without statistical differences between the last two. The average of all the treatments was 0,95 % for NNDF and 0,24 % for NNDF.

The ash and EtE contents showed statistical differences ($p < 0,05$) among treatments (table 4). N_h showed an ash content slightly higher than that of T_e and N_l, without differences between the last two. For the EtE, there were no significant differences between T_e and N_l, which differed from N_h. On the other hand, there was no effect of mineral nutrition on the DMD or on the ME content, with averages of 88,6 % and 3,20 Mcal kg DM^{-1}, respectively (table 4).

Discussion

The application of the low and high concentrations of nutrients in nutritional solution, through irrigation did not affect the fresh yield or the bromatological quality of the corn hydroponic green forage. The results showed that, to obtain acceptable yields and good bromatological quality of the forage, the application of mineral nutrition was not necessary.

Table 3. Bromatological variables in the corn hydroponic green forage.

Treatment	Variable (%)						
	CP	CF	Lignin	NDF	NNDF	ADF	NADF
Control (T_e)	19,27	19,61	1,30	45,05	0,86^a	22,40	0,21^a
Low nutrition (N_l)	20,06	18,75	1,55	43,20	0,98^b	21,88	0,27^b
High nutrition (N_h)	20,69	18,50	1,58	44,55	1,03^b	21,98	0,25^b
Average	20,01	18,95	1,50	44,27	0,95	22,09	0,24
VC	6,26	4,72	13,51	3,08	4,62	4,64	7,99
P value	0,3221	0,2363	0,1531	0,1955	0,0009	0,7508	0,0028

Values with different letters in the same column indicate significant differences among treatments ($p < 0,05$).

Table 4. Content of ash, ether extract, dry matter digestibility and metabolizable energy.

Treatment	Variable			
	Ash (%)	EtE (%)	DMD (%)	ME (Mcal kg DM^{-1})
Control (T_e)	4,10^a	7,95^b	88,63	3,22
Low nutrition (N_l)	4,07^a	7,50^b	88,60	3,20
High nutrition (N_h)	5,32^b	6,87 ^{a}	88,55	3,21
Average	4,5	7,44	88,59	3,21
VC	7,77	4,28	0,07	0,08
P value	0,0009	0,0032	0,2955	0,2437

Values with different letters in the same column indicate significant differences among treatments ($p < 0,05$).

The root system, during the early growth of corn seedlings, comprises the embryo roots, formed by only one primary root and a variable number of seminal roots (Hochholdinger *et al.*, 2018). These roots were later substituted by a permanent root system that emerges from the stem nodes, composed by lateral roots that substantially increase the absorption surface, because of the appearance of highly differentiated roots called absorbent hairs (Marzec *et al*., 2015).

Thus, during the initial seedling growth, the incipient root system –little differentiated and lacking absorbent hairs– is not efficient in the absorption of mineral nutrients. In studies of corn nutrition, the response to fertilization treatments appeared from two or three weeks (Bertsch, 2009) after planting, at the end of the germination stage, emergence and initial growth. In addition, during such stage the seedling can survive from the seed reserves. The seed endosperm starch and proteins are transformed into simple sugars and aminoacids, which are used for the growth of the embryo, hypocotyl and seedling (Taiz and Zeiger, 2006).

With regards to fresh yield (FY) and the quality of the obtained forage, in similar studies with corn hydroponic green forage acceptable values were reported in such variables, independently from the application of irrigation with water or with nutritional solution.

The yields, including those of the control with water, were similar to the ones reported in corn (16,49 kg m^{-2}) by Salas-Pérez *et al*. (2010) and Naik *et al.* (2015, 2017), who used concentrations of nutrients and seed densities similar to the ones in treatment N_h. In turn, Rivera *et al*. (2010) indicated that the use of nutritional solution for the production of hydroponic green forage does not improve the bromatological quality of the forage, and that it is feasible to use only water without nutrients.

Hydroponic green forage is valued due to its CP levels (Contreras *et al*., 2015). In this sense, Van Soest (1994) stated that the minimum content of crude protein in hydroponic green forage should be around 7 %. The CP contents in the forage of this study, even applying water alone, were higher than the ones reported in similar studies (13,5 -19,2 %) by López and Mcfield (2013) and Acosta *et al*. (2016). In another study, Salas-Pérez *et al.* (2010) proved that the application of nutrients through nutritional solution did not affect the CP content (13,3 %), compared with the treatment in which water without nutrients was applied (12,2 %).

The hydroponic green forage is also valued for a good soluble fiber/insoluble fiber balance, which determines the digestibility of the nutrients contained in it. The NDF and ADF contents in corn hydroponic green forage were not affected by the application of nutritional solution, and acceptable quality was maintained in the control (T_e). The above-explained facts coincide with the report by López and Mcfield (2013) and Acosta *et al*. (2016), who did not find response in the NDF and ADF contents in corn hydroponic green forage, when applying nutritional solution or water without nutrients.

The NDF represents the potentially digestible component of forages (Candia, 2014). In this regard, López and Mcfield (2013) stated that those values higher than 55 % reduce the digestibility of forage, due to the inverse relation between the NDF content and the nutritional value, intake and digestibility. The NDF values (43,2-45,05 %) of this study are within the range reported by Salas-Pérez *et al*. (2010) and Acosta *et al.* (2016): 42,1-57,8 %, which explains the high digestibility.

The ADF is the insoluble fraction of the crude fiber, and an important factor for adequate fermentation in the rumen. According to Maldonado *et al*. (2013), the rations of dairy cattle should contain between 19 and 27 % of ADF, because lower values can reduce the fat content in milk. In this study, there was no effect on ADF when applying nutritional solution (N_h = 21,98 %; N_l = 21,88 %) or water without nutrients (T_e = 22,4 %). Such values were similar to the ones reported by Acosta *et al.* (2016) when applying nutritional solution. On the other hand, López-Aguilar *et al.* (2009) showed that it was feasible to obtain acceptable ADF values (28,5 %) in corn hydroponic green forage even with the application of water alone.

The lignin content shows a negative correlation with the DMD of the forage, because low lignin values increase its digestibility. In this study, the lignin percentage (1,30-1,58 %) in all the treatments was lower than 5 %, value from which it is considered that there is a substantial reduction in the forage digestibility, and for such reason, the DMD (88,6 %) was high. This differs from the findings by Acosta *et al.* (2016), who reported lower DMD values in corn hydroponic green forage (70,0-72,3 %), independently from the application of nutritional solutions or water.

High values of NNDF and NADF indicate that nitrogen appears in a non-utilizable way. The

NNDF (0,95 %) and NADF (0,24 %) were similar to the report by Sánchez and Soto (1998).

The EtE represents the quantity of fat contained in the forage and, thus, the energy component. In this study, the obtained range (6,87-7,95 %) showed that there was no effect of the application of nutrients compared with the control. These values exceed the ones reported in corn hydroponic green forage (2,0-4,6 %) when the response to the application of nutritional solutions was evaluated (Acosta *et al*., 2016; Naik *et al*., 2017).

The studies about the effect of the application of mineral nutrition are focused rather on the evaluation of production and bromatological variables than on the effect on the mineral composition of forage. In this study, the average ash was 4,5 %; while in similar studies, when water without nutrients was applied, 3,6-6,9 % of ash was obtained (Cuesta and Machado, 2009; López-Aguilar *et al*., 2009).

The DMD and ME content are important indicators because they show the quantity of metabolic work of the organism. The average ME, based on the fact that there were no statistical differences among treatments, was 3,2 Mcal kg^{-1}. These results coincide with the ones reported in corn hydroponic green forage, when comparing different nutritional solutions with a control without nutrients (López-Aguilar *et al*., 2009; Acosta *et al*., 2016).

Summarizing, high-quality forage contains approximately 70 % of DMD, less than 50 % of NDF and more than 15 % of protein; while in low-quality forage the DMD decreases to less than 50 %, the NDF increases to more than 65 % and protein decreases to less than 8 % (Di Marco, 2011). In the control treatment, irrigated with water without nutrients, the DMD, crude protein and good balance of the fibers proved that the corn hydroponic green forage has optimum quality, with great potential as complement in diets for animal feeding.

It is concluded that mineral nutrition, through the application of nutritional solutions with different concentrations, did not affect the fresh yield or bromatological quality of the corn hydroponic green forage at 11 days of harvest. Additionally, the values in the variables of response to treatments, compared with the ones reported in different studies, showed the potential of utilization of the corn hydroponic green forage as feeding source in animal production, independently from the application of irrigation with nutritional solution or with water without nutrients.

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