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# Evaluation of the Effect of Two Types of Fertilizer on the Growth, Development and Productivity of Hydroponic Green Forage Oat (Avena sativa L.) and Ryegrass (Lolium multiflorum Lam.) as a Biomass Source

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Hydroponic Green Forage (HGF) is increasingly being internationally recognized as an alternative to ensure sustainable biomass production per unit area in the shortest possible time and at the highest possible quality. This research thus evaluates the effect of two nutrient solutions (with two dilutions in each case) applied in the HGF production system for Oats and Ryegrass on the parameters of growth, development and productivity of both species. To this end, a handmade greenhouse was built in which two seed sowing trays were installed with a fertigation system for oats and ryegrass species. The experimental design consisted of the assembly of two modules of 24 seed trays in which HGF Oat and Ryegrass were grown using two nutrient solutions (which were applied according to the position of the seed trays inside the module at level 2 and level 4). The tests showed for the two species evaluated that the use of organic solutions significantly promote the conversion and yield variables of fresh mass per unit area - an increase in the relative growth rate, the net assimilation rate, the crop growth rate and the absolute growth rate; all of these as a measure of crop development. Likewise, when the analysis was conducted, it was observed that the most economic assemblies turned out to be those which used the liquid humus as nutrient solution to 1/20 v.v. and 1/40 v.v. This supports the conclusion that the use of organic nutrient solution can yield high quality HGF exceeding the development parameters of those produced using conventional (synthetic) nutrient solutions

# 1. Introduction

Biomass production of different crops is limited by various factors, such as the decrease of land available for sowing, adverse weather conditions (Carneiro et al., 2014), high labour costs. Along of other factors, in the case of the natural pastures, these are, in addition, being affected by the previously mentioned factors and also by the costs in the concentrates and by the use of abundant quantities of irrigation water (which is increasingly scarce and expensive). Due to the above, it forces agricultural producers to seek other options for the biomass production containing a high protein content (ryegrass, sorghum, oat), fresh and plentiful, that are not impacted by climate changes (Weinwurm et al., 2014) and which do not require large areas and which have especially low cost (Rivera et al., 2010). Despite these problems the agriculture is confronted with another even more alarming situation: climatic events as drought and early frosts. The effect of these phenomena is being decreased in protected agriculture, also affects the agroproductive systems in terms of the production efficiency, biomass yields and profitability per unit area. Among the alternatives to this situation one finds beneficial to use of plasticultures. To be more specific - the plastic greenhouses can facilitate the conditions to obtain crops with a high efficiency of used water and reducing the maximum effects of climatic factors. In addition, they are able to provide a constant and excellent quality production annually in reduced

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spaces. All above mentioned factors justify their employment. So far, this alternative has been mostly applied for vegetables and flowers, and recently it has been also applied for crops like tomatoes, chili pepper, cucumber and strawberry (Fuentes et al., 2011). However, an important option is possibility to employ greenhouses in the production of hydroponic green forage (HGF) that can provide biomass of a quantity and quality in the desired time. The HGF is a technique for obtaining vegetal biomass with high nutritional quality and health. It produces within a few days (between 15-20) regardless of the time of year in any field with the minimum conditions need to be considered for its use (Cerrillo et al., 2012). HGF systems is complementary (not competitive) to the conventional forage production from suitable species such as oats, clover and grass mixtures, alfalfa, etc. (Sánchez et al., 2013).

## 2. Materials and methods

## 2.1 Experimental conditions

HGF Oat and Ryegrass production were developed on two modules of 24 seed trays in parallel and located in a semi-controlled environment (this was achieved with a plastic greenhouse that protected the crop from a rain, wind and other external factors that could affect the optimal growth of forage)..

## 2.2 Preselection of seeds

The criterion for the seed preselection was the cost of seeds and their forage potential. The italian ryegrass was chosen for its high forage potential in a cattle and lack of tests in the production system of HGV. The cayuse oat was chosen due to nutritional benefits that offers in the animal diets and to be the cheapest among the commercial varieties. Later, the broken grains and foreign materials that can affect crop development were removed as they can result in rottening and the spread of diseases

# 2.3 Seeds disinfection and moisturizing

Seeds were soaked in a bucket with three fourth parts of water during 24 hours. A total of 16 pounds were hydrated and were divided in 8 pounds of ryegrass and 8 pounds of oats per module. Once the germination process started, the seeds were moved to trays and were scattered one pound of seed by each tray forming a homogeneous layer. The evaluation of morpho-physiological characteristics was performed in 4 trays per level. A total of 24 trays per module were studied

## 2.4 Watering sprouts

The irrigation was made with water during the first 8 days and afterwards the previously diluted nutrient solution was used. For this, 4 tanks with a capacity of 200 litters were used and their total volume was sufficient to make the fertigation until making the harvest of the FGH. The pulses of irrigation ware established six times per a day in intervals of 2 hours. This was done as the temperature in the greenhouse was increased in comparison with the environment outside and also a higher evaporation of water was there.

## 2.5 Nutrient solution

For the evaluation, two different nutrient solutions (one of the synthetic origin and the second one of the organic origin) were used at different dilutions. The synthetic nutrient solution was compound of a premix of major and minor elements which were in different bags; each package was diluted in 5 litters of water to form a stock solution. This stock solution was evaluated in two dilutions 1/10 v.v. and 1/20 v.v. On the other hand, the organic nutrient solution (liquid Humus) has a very rich composition in macro and micro elements. This was evaluated in dilutions of 1/20 v.v. and 1/40 v.v.

#### 2.6 Harvest

The harvesting of HGF Oat and Ryegrass was made in a period of 15 days from the germination of seeds. The average height of plants by tray for the harvest was not taken as reference because this is one of effects that has been evaluated in the experimental process

## 2.7 Measurement of parameters of growth and development

During a production of HGF Oat and Ryegrass the following morpho-physiological characteristics were measured:

a) Germination percentage is given by the ratio between the number of seeds sown and germinated. To determine the percentage the following formulate was used. Germination percentage = (sprouts / total number of seeds in a test) X 100 (Constantino et al., 2010.); b) Plant height (cm) was measured using a measuring tape (or square) from the stalk base to the tip of the last completely formed leaf; c) Leaf Area (cm<sup>2</sup>): three plants for each treatment (each one independent) were used. For these plants we have marked on a bond paper (belonging at same ream of paper) the silhouette of all leaves of high photosynthetic activity. The silhouettes were cut out and the weight of each unit was determined by the analytical balance. Besides, a (1) cm<sup>2</sup> of type of paper used was weighed, and by the rule of three the leaf area of each plant for each treatment

was determined; d) Fresh matter (g): the fresh weight of three plants was determined with an analytical balance for each of treatments. This measurement was carried out within fifteen (15) days following the start of germination and thirty days after the first measurement; e) Dry matter (g) was determined according to the procedures (Cortés-Castillo et al., 2010); f) Growth rates and development. These are reflected in Table 1

Table 1: Physiological indices, adapted of Silva et al., 2009

Growth rate	Symbol	Instantaneous	Average value in a time interval	Units
		value	(T2-T1)	<u> </u>
Relative Growth Rate	RGR	1 dw	$RGR = \frac{(Ln W2 - Ln W1)}{(T2 - T1)}$	g/gd
		w dt	(T2 - T1)	
Net Assimilation Rate	NAR	1 <i>dw</i>	(W2 - W1)	g/cm <sup>2</sup> d
		LA dt	(T2-T1)	5
			$NAR = \frac{\frac{(W2 - W1)}{(T2 - T1)}}{(LnLA2 - LnLA1)}$	
			(LA2 - LA1)	
Leaf Area Index	LAI	LA	(LA2 + LA1)	Dimensionless
		$\overline{FA}$	$LAI = \frac{\frac{(LA2 + LA1)}{2}}{1}$	according to the
			1	units
			FA	
Crop Growth Rate	CGR	1 dw	$CGR = \frac{1}{FA} \times \frac{(W2 - W1)}{(T2 - T1)}$	g/cm <sup>2</sup> d
		FA dt	$FA \wedge (T2 - T1)$	
Absolute Growth Rate	AGR	dw	$AGR = \frac{W2 - W1}{T2 - T1}$	g/d
		dt	$AGR = \frac{T2 - T1}{T2 - T1}$	
Leaf Area Duration	LAD	_	$(LA2 + LA1) \times (T2 - T1)$	cm/d
			$LAD = \frac{(LA2 + LA1) \times (T2 - T1)}{2}$	
Specific Leaf Area	SLA	LA		cm <sup>2</sup> /g
		$\overline{DL}$	$SLA = \frac{\frac{LA2}{W2} + \frac{LA1}{W1}}{2}$	······g
			$SLA = \frac{2}{2}$	

(dw/dt= Derived of the function, LA=leaf area, FA= area of the floor, DL= dry mass foliar, T= time, W= dry mass).

#### 2.8 HGF productivity parameters

In the moment when both tested species reached the 20 cm of height, the following determinations were made:

a)Conversion: a fresh forage-seed conversion (FSC) was calculated, which indicates the kg forage produced per kg seed used; b)Yields: is a magnitude of the forage in kg m<sup>-2</sup> or kg ha<sup>-1</sup> based on fresh weight (FM).

#### 2.9 Statistical analysis

A single analysis of variance (ANOVA) between the averages of the samples by treatment at a significance level of 95% ( $\alpha = 0.05$ ) was carried out to establish whether any differences exist for the variables under evaluation (Suárez, 2011). If there was no significant difference between the samples, a multiple range test was performed using the statistical package Statgraphics Centurion.

# 3. Results and discussion

# 3.1 Germination percentage HGF Oat and Ryegrass

When making the assessment of germination percentage in oat seeds, it was established that of the 100 placed seeds in the sprouter only 10% did not germinate. Everything seems to indicate that specific seed factors that interfere in the dormancy and embryo viability could be affecting this process. On the other hand, for the ryegrass seeds it was established that of the 100 placed seeds in the sprouter only 7% did not germinate. Rivera et al., (2010) points out that a good quality seed is characterized by a high germination capacity and vigour. To evaluate these components the result shows that germination was equal or higher than 90% in both species. This is because the grains have developed and matured physiologically as normally caused by adequate moisture conditions.

## 3.2 Plant height in HGF

The greater height of the oat coleoptiles was achieved with the treatment T5 (Oat with the synthetic nutrient solution dilution 1/10 v. v within the module Level 2) exceeding 18 cm followed by the T6 (oat the synthetic nutrient solution dilution 1/20 v.v within the module Level 2). Both nutrient solutions are based on synthetic products rich in nitrogen (nitrogen stimulates vegetative growth of the plants). Additionally these (plants grown under the T5 and T6 treatments) did not show statistically significant differences between the averages of their samples. On the other hand, the treatment with a low outcome of measurements of growth variables resulted to be the T1 (Oat with organic nutritious solution dilution 1/20 v.v. within the Level 2 module), apparently due

to the low content of nitrogen that can be assimilated and can be found in suspension of the nutrient solution reference. In Ryegrass coleoptiles, the highest point was reached with the treatment T13 (Ryegrass with the synthetic nutrient solution 1/10 v.v. within the Level 2 module) exceeding 18 cm and followed by T14 (ryegrass with the synthetic nutrient solution dilution 1/10 v. v within the module Level 2). Both nutrient solutions are based on synthetic products rich in nitrogen (nitrogen stimulates vegetative growth of the plants). On the other hand, the treatment with a low outcome in presented measurements proven to be the T9 (Ryegrass with the organic nutrient solution dilution 1/20 v.v. within the Level 2 module). Apparently due to the low content of nitrogen that can be assimilated found in the suspension in the nutrient solution reference.

## 3.3 Behaviour of leaf area (LA)

The leaf areas of HGF Oat and Ryegrass (initial-LAi and final-LAf) were determined in the days 6 and 15 after germination. For oats, the best development on LAf was achieved by the treatment T8 (oat with the synthetic nutrient solution dilution 1/20 v.v. within the module Level 4). It is possible see the same trend in the treatment T7 (oat with the synthetic nutrient solution dilution 1/10 v.v within the module level 4) but the LAi of these two treatments resulted to be smaller than for the treatment T1 (oat with organic nutrient solution dilution 1/20 v.v. within the module level 2) and the treatment T2 (oat with organic nutrient solution dilution 1/40 v.v within the module Level 2) which obtained similar LAi among all treatments. Apparently, this can be the impact of the organic nutrient solution, mainly by the presence of considerable traces of humic acids which have biostimulating action (have been reported auxin content) that promotes the formation and development of plant tissues. On the other hand, the synthetic nutrient solution lacks these stimulants (presence of humic substances) which prevents to generate the same amount of biomass. That could be compensated if there were in cellular expansion and simultaneously being enriched in nitrogen (mainly urea). Similar behaviour was observed for the Ryegrass, where the treatment T11 (Ryegrass with the organic nutrient solution dilution 1/20 v.v within the module level 4), achieved the best development in the LAf. It can appreciate the same trend in the treatment T10 (Ryegrass with the organic nutrient solution dilution 1/40 v.v. within the module level 2). Regarding the LFi these two treatments were less effective than the treatment T9 (Ryegrass with the organic nutrient solution dilution 1/20 v.v. within the module level 2). The treatment with low A-Fi and A-Ff among of all treatments was the T15 (Ryegrass with the organic nutrient solution dilution 1/10 v.v within the module level 4). It had the minor LAi and LAf and apparently this behaviour can be due to the impact of the organic nutrient solution that contains humic acids which have biostimulants elements such as auxins that help formation to plant tissues. However the synthetic nutrient solution does not possess these stimulants which prevents to generate the same amount of biomass

## 3.4 Fresh matter (FM) and dry matter (DM)

From the analysis of these variables for oat was found that the T1 treatment was who showed the greatest gain in the fresh matter (initial-FMi and final- FMf) and in the dry matter (initial-DMi and final- DMf). This behaviour can be influenced by the luminosity that trays received (meaning by an effective photosynthesis process). This treatment involves a more efficient accumulation of biomass than the other treatments. Additionally, it appears that the organic nutrient solution had a positive effect on a dry matter accumulation, having in its composition compounds as phytohormones and other asimilatos that help to improve the metabolism of the plant, resulting in the plant tissue formation reflected in the weight that accumulates during its development. The treatment T5 has achieved the greatest accumulation of fresh matter (initial and final) and dry matter (initial and final) similar to treatments T6, T7 (oat with the synthetic nutrient solution dilution 1/10 v.v. within the module level 4) and T8. These results may be due to the effect of the nutrient solution of synthetic origin. There was an analysis for ryegrass and it was clear that the T9 treatment obtained a great constant gain in fresh matter (FMi and FMf) and in dry matter (DMi and DMf). This behaviour was similar to the T1 of oat testing. Likewise it suggests that the incidence of the organic nutrient solution with its composition of active compounds, as phytohormones, were able to help to speed up and promote plant metabolism to give rise to plant tissues. This is reflected in the weight stored for in our development process. The T13, T14 and T15 treatments presented similar behaviour in the accumulation of FMi, FMf, DMi and DMf. The previous results for oat and Ryegrass may be caused by the nutrient solution of synthetic origin together with exposed seeds. In its composition contains nitrogen and other elements that contribute to growth in length but not to the formation of plant tissue.

## 3.5 Development indices

When observing the relationship between RGR and NAR (see Figure 1) with the accumulation of dry mass in all oat crops, it was evident that the treatments T1 followed by T3 were responsible for a high dry matter content. This was obviously related to the T1 treatment, which also showed a large leaf area. This relationship indicates that the organic nutrient solution not only stimulates the accumulation of fresh and dry matter, but

also impacts upon the RGR rates and the NAR development. The NAR decrease according Álvarez-Solís et al., (2010) it is due to, in part, the gradual increase in non-assimilatory tissues.



Figure 1: Behaviour of relative growth rate - RGR and net assimilation rate - NAR for FVH crop of oat

A distinct and well marked behaviour is obtained for the same indicators (RGR and NAR) for FVH crop of Ryegrass. The RGR and NAR (see Figure 2) show that the treatment that received a higher dry matter content was the T12 (Ryegrass with synthetic nutrient solution dilution 1/40 v.v. within the Level 4 module) followed by the T10. This suggests that the organic nutrient solution helps to the formation of plant tissues by its biostimulating compounds as phytohormones in which involved cytokinins that promote the leaf growth. The treatment that has obtained a low dry matter was T16 (Ryegrass with synthetic nutrient solution dilution 1/20 v.v. within the Level 4 module). This could be due to the synthetic nutrient solution have a lack of organic stimulants.



Figure 2: Behaviour of relative growth rate - RGR and net assimilation rate - NAR for FVH crop of ryegrass

On the other hand, T1, T2 and T3 (oat with the organic nutrient solution dilution 1/20 v.v. within the Level 4 module) for LAI show a similar behaviour. This could be influenced by a genetic expression of species or by the composition of the nutrient solution in terms of active compounds such as phytohormones, aminoacids and organic acids that facilitate the leaf development. When relating the LAI with LAD, it was evident that the pattern of behaviour is very similar, which can indicate that the plant had more metabolize energy for the growing of large leaf area. This is backed by analyzing SLA where it was noted that probably the plant saves unnecessary energy in moments of stress and use this energy for the formation of leaf tissues; results more notorious in the T2 and T3, both corresponding to organic solutions. While analyzing Ryegrass LAI, LAD and SLA, it can be observed that T9, T10 and T11 treatments show a similar behaviour compared to LAI that relates to the LAD, which may indicate that the plant has more metabolize energy for the formation of a large leaf area. Analyzing the CGR and AGR in the cultivation of oats, it was observed that the T1 has the largest dry weight obtained for both variables, which seems to indicate that the nutrient solution is one that promotes a greater efficiency in the dry matter. This does not happen with T6 and T7 as they were fertilized with the synthetic nutrient solution. These have a low accumulation of the dry matter obtained when harvesting. Similar behaviour was shown in Ryegrass for the T12 having the largest dry weight obtained in both the CGR and AGR. Apparently, this indicates that the solution nutrient is the one that promotes greater efficiency in the dry

mass, which does not happen with the treatments T15 and T16 when fertilized with the synthetic nutrient solution. These have also a low accumulation of the dry matter obtained when harvesting

## 3.6 FVH productivity parameters

The treatment that generated the highest quantity of the fresh biomass in the cultivation of oats at harvest was the T3 with more than 6 kg. The following treatments that obtained near values were T2 and T4 with 6 kg of the fresh biomass in the area of 2400 cm2. Compared to T6 and T7 where the biomass accumulation did not exceed 4 kg on the area of 2400 cm2. Ryegrass showed a similar behaviour to oat where the treatment that generates the highest quantity fresh biomass at the harvest time was the T10 with 4 kg in the area of 2400 cm2. The following treatments that obtained close to similar values obtained were the T9, T11 and T12 with 3.5 kg of the fresh biomass. Compared to treatments T13 and T16 where the biomass accumulation did not surpass 3 kg.

## 4. Conclusions

It was showed that an increase of the vegetative growth (height of the plant) is more pronounced for those treatments that were fertilized with the synthetic nutritious solution indistinctly to the used species (Oat and ryegrass). This can be influenced by the content of nitrogen of the same that provides a greater cellular but a lower expansion accumulation in fibres and reservations. The treatments T1 and T3 have showed results more significant regarding indexes of development for the oat crop, whereas while making the evaluations in the Ryegrass crop showed that the treatments T10 and T12 were more effective. They showed the CGR, AGR, RGR and NAR higher to the rest of the treatments. The previous indicates that the position inside the modules did not influence significantly these variables. When performing the analysis of the indices of LAI, SLA and LAD, it can be noted that the results were representative for growing Ryegrass treatments T9 and T11. For the analysis of conversion rate (expressed in kg) and performance \* kg ha-1 is evident that for both species, the best results were shown in plants that were fertilized with the organic solution. It fits to highlight that this generates a remarkable environmental impact with the possible replacement of the synthetic fertilizer by the organic one with a possible consequent social impact when reducing the ingestion by part of the animals of chemical products that in short, average and long term will be consumed by the human beings.

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