LA GRANJA:
REVISTA DE
CIENCIAS DE LA VIDA

Editorial

ABYA YALA | UNIVERSIDAD POLITÉCNICA SALESIANA

Dear reader:

After a collaborative and uninterrupted work, the UPS has continued to improve one more year as a Research University, and has gone from the position 6,611 in the world and 13 in Ecuador (Webometrics, 2013) to the position 2627 (Webometrics, 2020) worldwide, it is the 170 on the continent and 7 in Ecuador. The continuous improvement of all teams and the strategic vision have made progress in the World Ranking, allowing to go to 3984 positions in 7 years and 366 new positions compared to 2019. Within this global strategy of improvement of the UPS as a Research University, LA GRANJA, belonging to the area of Life Sciences, was the first university publication of Ecuador to be included in SCOPUS, Elsevier publishing house and indexed in the Emerging Source Citation Index of the Web of Science (WOS).

This edition presents articles by authors from 5 countries and 16 Universities and Research Centers. We started this number 31 with the topic of weather monitoring, in an effort between the Sciences Department of Environmental Systems of Switzerland and Universidad de Cuenca, led by Dr. Ryan Padrón and his excellent team. In addition, researchers from Universidad de las Fuerzas Armadas, led by Teófilos Toulkeridis, analyze the problem of perceptions in the important and current topic of Climate Change.

From Mexico, Nayeli Martínez and Erick de la Barrera, researchers from Benemérita Universidad Autónoma de Puebla and Universidad Nacional Autónoma de México, present studies of urban weeds. While Salomé Araujo and the joint team from Universidad Nacional de Loja, Universidad de las Américas and Universidad Técnica Particular de Loja, analyze cadmium contamination on cocoa almonds with spectroscopic techniques.

In the field of conservation and biotechnology, Paola Jiménez and her interdisciplinary team from Universidad Politécnica Salesiana and the National Institute of Agricultural Research present effective techniques for the in vitro propagation of Quishuar. Also, addressing the topic of livestock systems, specifically goats, Araceli Solís and her team from Universidad Estatal de la Península de Santa Elena present a comprehensive study of the classification of these mammals.
Additionally, an important area of La Granja is that of Veterinary Sciences, Jimmy Quisirumbay, from Universidad Central, presents a meta-analysis of the effects of glutamine on piglet feeding. Meanwhile from Peru, Victor Carhuarpoma De la Cruz and his team of researchers from Universidad de Huancavelica, present studies of antibiotic resistance on alpacas.
Finally, with the topic agricultural production, Rosa Pertierra and Jimmy Quispe, of Universidad Estatal de la Península de Santa Elena, present an economic analysis of the production system of hydroponic lettuces. And Carlos Abad and his team from Universidad Técnica Particular de Loja, analyze the mircro-tunnel technique in the productivity of strawberry crops.

We thank all of them for their collaboration and work to continue improving the Journal and contribute to the improvement of society from the dissemination of science and international research in the field of Environmental and Earth Sciences.
Those who are part of La Granja, Revista de Ciencias de la Vida, are confident that this volume will surely be of great use and interest in the scientific community.

Sincerely,

Ph.D Ignacio de los Ríos Carmedano
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WEATHER MONITORING

Rain gauge inter-comparsion quantifies differences in precipitation monitoring

Comparación entre pluviómetros cuantifica diferencias en el monitoreo de la precipitación

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Resumen

Por décadas se ha trabajado para corregir las medidas de precipitación, sin embargo estos esfuerzos han sido escasos en zonas tropicales montañosas. Cuatro pluviómetros de balancín (TB), con distinta resolución y comúnmente utilizados en las montañas de los Andes, fueron comparados en este estudio: un DAVIS-RC-II, un HOBO-RG3-M, y dos TE525MM (con y sin una pantalla Alter contra el viento). El desempeño de estos pluviómetros, instalados en el Observatorio Ecohidrológico Zhurucay, sur del Ecuador, a 3780 m s.n.m., fue evaluado en relación al sensor de mejor resolución (0,1 *mm*), el TE525MM. El efecto de la intensidad de precipitación y condiciones del viento también fue analizado utilizando 2 años de datos. Los resultados revelan que (i) la precipitación medida por el TB de referencia es 5,6 % y 7,2 % mayor que la de pluviómetros con resolución de 0,2 *mm* y 0.254 *mm*, respectivamente; (ii) la subestimación de los sensores de menor resolución es mayor durante eventos de baja intensidad—una máxima diferencia de 11 % para intensidades ≤ 1 *mm* h^{-1}; (iii) intensidades menores a 2 *mm* h^{-1}, que ocurren el 75 % del tiempo, no pueden ser determinadas con exactitud para escalas menores a 30 minutos debido a la resolución de los pluviómetros, e.g. sesgo absoluto $> 10\,\%$; y (iv) el viento tiene un efecto similar en todos los sensores. Este análisis contribuye a mejorar la exactitud y homogeneidad de las medidas de precipitación en los Andes mediante la cuantificación del rol clave de la resolución de los pluviómetros.

Palabras clave: Pluviómetros de balancín, análisis comparativo, exactitud de medición, efectos de intensidad y viento, tropical.

Abstract

Efforts to correct precipitation measurements have been ongoing for decades, but are scarce for tropical highlands. Four tipping-bucket (TB) rain gauges with different resolution that are commonly used in the Andean mountain region were compared-one DAVIS-RC-II, one HOBO-RG3-M, and two TE525MM TB gauges (with and without an Alter-Type wind screen). The relative performance of these rain gauges, installed side-by-side in the Zhurucay Ecohydrological Observatory, south Ecuador, at 3780 m a.s.l., was assessed using the TB with the highest resolution (0.1 *mm*) as reference, i.e. the TE525MM. The effect of rain intensity and wind conditions on gauge performance was estimated as well. Using 2 years of data, results reveal that (i) the precipitation amount for the reference TB is on average 5.6 to 7.2 % higher than the rain gauges having a resolution of 0.2 *mm* and 0.254 *mm* respectively; (ii) relative underestimation of precipitation from the gauges with coarser resolution is higher during low-intensity rainfall mounting to a maximum deviation of 11 % was observed for rain intensities $\leq$1 *mm* h^{-1}; (iii) precipitation intensities of 2 *mm* h^{-1} or less that occur 75 % of the time cannot be determined accurately for timescales shorter than 30 minutes because of the gauges' resolution, e.g. the absolute bias is $>$10 %; and (iv) wind has a similar effect on all sensors. This analysis contributes to increase the accuracy and homogeneity of precipitation measurements throughout the Andean highlands, by quantifying the key role of rain-gauge resolution.

Keywords: Tipping-bucket rain gauge; comparative analysis; measurement accuracy; intensity and wind effect; tropical.

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1 Introduction

Hydrological studies require precipitation as input (Vuerich et al., 2009; Savina et al., 2012; Seo et al., 2015; Muñoz et al., 2016) and in response to this several rainfall sensors, featuring different operational and technological principles, have been developed. Among point-based recording sensors the tipping bucket, weighing and floating gauges are the most widely used (Nystuen, 1999; WMO, 2008; Grimaldi et al., 2015). In particular, the tipping bucket (TB) gauge is a very popular device, used all over the globe (Humphrey et al., 1997; Habib et al., 2001; Tokay et al., 2003; Molini et al., 2005; Vuerich et al., 2009; Mekonnen et al., 2014; Chen and Chandrasekar, 2015; Dai, 2015; Keller et al., 2015). TBs are in general low-cost, but depending on the manufacturer can have different resolution and accuracy for measuring rainfall. According to Savina et al. (2012) inaccuracies in measurements of TB gauges are primarily due to the precipitation variability and sensitivity to environmental conditions, as well as calibration and mechanical errors. According to the World Meteorological Organization (WMO, 2008) the principal sources of inaccuracy of TB gauges are: evaporation and wetting losses, and wind-induced errors. Since errors in rainfall measurements can lead to the failure of hydraulic infrastructure or wrong conclusions in research (Willems, 2001), considerable international efforts were made to quantify and limit the uncertainty in rainfall measurements (Lanza and Stagi, 2008).

Several comparative studies have been conducted to define differences in the precipitation depth captured by rainfall gauges, and to develop guidelines for the correction of measurements. Since 1955, the WMO has conducted four international high-quality comparative studies (Sevruk et al., 2009) to assist the multiple users of rain gauges in the correct interpretation of precipitation measurements. In the WMO intercomparison studies (Sevruk and Hamon, 1984; Vuerich et al., 2009), data from pit gauges are used as reference for quantifying the deviations of the measurements of the sensors with respect to actual rainfall depth. Nonetheless, relative inter-comparison studies are also valuable, hence the extensive volume of literature dedicated to comparing the performance of rain sensors with varying technology, accuracy and resolution (Krajewski et al., 1998; Nešpor and Sevruk, 1999; Nystuen, 1999; Krajewski et al., 2006; Lanzinger et al., 2006; Rollenbeck et al., 2007; Duchon and Biddle, 2010). Even though more precise gauges are expected to overall perform better than less precise ones, the effect of rainfall intensity and wind conditions on the sensor measurements is still insufficiently known.

Comparative studies of rain gauges in tropical mountain areas are few. In the Andes, the longest continental mountain range in the world, one study has analyzed the performance of rain sensors in a tropical mountain forest in southeastern Ecuador located at an elevation of 1960 m a.s.l. (Rollenbeck et al., 2007), and another study analyzed the performance on rain gauges in a high-elevation tussock grassland ecosystem, locally called páramo, at 3780 m a.s.l. (Padrón et al., 2015) Both of these studies used specialized sensors, such as disdrometers and micro rain radars, that are rarely available at standard monitoring stations. Meanwhile, there are several monitoring initiatives in the highlands above 3000 m a.s.l., such as the Initiative for the Hydrological Monitoring of Andean Ecosystems (Iniciativa para el Monitoreo Hidrológico de Ecosistemas Andinos), a Northern Andes network of Non-Governmental Organizations, Public Institutions and universities that are conducting basic hydrological monitoring in small catchments as to gain knowledge about their hydrological functioning and the impacts of global change, and that use a variety of commercial rain gauges with different resolutions. Using these heterogeneous data can affect hydrological applications, highlighting the need to understand the differences between the gauges under the particular rainfall and climate conditions of the ecosystem.

This study assesses the relative performance between TB rain gauges in the páramo ecosystem in southeastern Ecuador. This ecosystem is a vital year-round water provider (for agricultural, urban and energy production purposes) for Ecuador, Colombia and Venezuela (Buytaert et al., 2006b,a; Célleri and Feyen, 2009; Ochoa-Tocachi et al., 2016), regions characterized by low intensity rains throughout the year. Specific aspects also studied are the effect of rainfall intensity and wind on the measurements of the tested rain gauges.

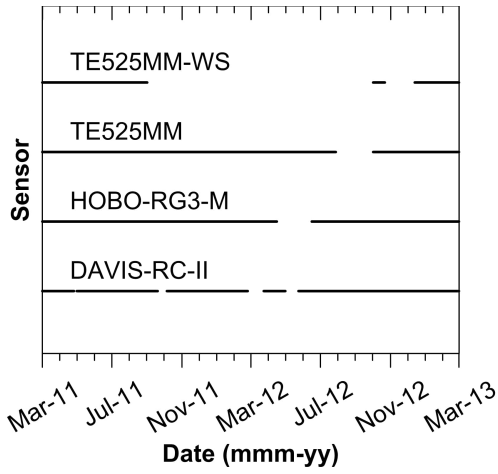

Figure 1. Available data of the Tipping-Bucket (TB) rain gauges used in the comparative analysis are depicted by continuous lines (gaps in the lines represent periods of missing observations).

2 Materials

Four tipping-bucket rain gauges were installed in the Zhurucay Ecohydrological Observatory, situated on the western cordillera of the Andes in southern Ecuador (3°03′S, 79°14′W): one Davis Rain Collector II (DAVIS-RC-II), one Hobo Data Logging Rain gauge RG3-M (HOBO-RG3-M), one TE525MM sensor (TE525MM) and one TE525MM gauge equipped with the 260-953 Alter-Type Wind Screen (TE525MM-WS). The windscreen consists of an iron-zinc shield concentric with the TB gauge. A full description with technical details of the windshield and installation considerations is available in previous studies (Alter, 1937; Duchon and Essenberg, 2001). Table 1 lists the main features of the four rain gauges used in the comparative study.

The tested rain sensors are commonly used in the Andean region for operational and research purposes, e.g. by the Ecuadorian National Institute of Meteorology and Hydrology (Instituto Nacional de Meteorología e Hidrología, INAMHI), the Initiative for the Hydrological Monitoring of Andean Ecosystems (Buytaert and Beven, 2011; Crespo et al., 2012; Muñoz et al., 2016, 2018; Sucozhañay and Célleri, 2018). The gauges were installed on a mutual distance of 2 m, with the surface area of the gauges opening 1 m above the ground surface, on an extended flat area, at an elevation of 3780 m a.s.l. Precipitation data were recorded during 2 years; the number of tips per minute was stored in an automatic data logging system for the TE525MM rain sensors, whereas for the other gauges the timestamp (hh:mm:ss) of each tip was recorded. The average temperature during the observation period was 6 $°C$, the relative humidity 91 % (Córdova et al., 2015), and the wind speed was on average 3 $m\ s^{-1}$ in the period October–April and 4.5 $m\ s^{-1}$ in the period May–September. Climate data were recorded with an interval of 5 minutes, in an adjacent automatic weather station.

The gauges were subjected to a static calibration, prior to installation. Given the low intensity of the frequent rains and in line with the findings of (Vasvári, 2005) and gauge manufacturer recommendations, the application of a dynamic calibration would be needless. Using a high-resolution pipette the real water volume to tip the bucket of the rain sensors was measured. The correction factor varied between $-6{,}56$ and $+4{,}29$ %. Due to a malfunctioning of the electronic connection to the datalogger for the TE525MM-WS, more than half of its data were disregarded. Figure 1 depicts the data available for each instrument; missing data varied between 8 and 10 % for the gauges DAVIS-RC-II, HOBO-RG3-M and TE525MM, and amounted to 61 % for the TE525M-WS gauge.

Table 1. Manufacturer specifications of the compared Tipping-Bucket (TB) rain gauges.

Sensor	Capturing diameter (cm)	Resolution (mm)	Intensity (mm h^{-1})	Accuracy (%)
DAVIS-RC-II	16.5	0.254	$0-50$	± 1
			$50-100$	± 5
HOBO-RG3-M	15.4	0.200	$0-20$	± 1
TE525MM TE525MM-WS	24.5	0.100	$0-10$	± 1
			$10-20$	$+0,-3$
			$20-30$	$+0,-5$

3 Methods

3.1 Statistical indices for the assessment

For the quantitative assessment of differences in performance between the rain gauges the following set of statistical indices, similar to that proposed by Tokay et al. (2010), was used: the coefficient of determination (R^2), the Spearman's non-parametric correlation (ρ), the standard deviation (σ), the percent bias and the percent absolute bias. The statistical indices were calculated with respect to the rain data collected by the TE525MM sensor. This sensor was considered in this study as reference because of its better technical features compared to the other rain gauges, and its larger data set compared to the TE525MM-WS. The percent bias and percent absolute bias, Equations (1) and (2) respectively, were calculated as follows:

$$percent\,bias = \frac{1}{\bar{y}}\left(\frac{1}{n}\sum_{i=1}^{n}(x_i - y_i)\right) \quad (1)$$

$$percent\,absolute\,bias = \frac{1}{\bar{y}}\left(\frac{1}{n}\sum_{i=1}^{n}|x_i - y_i|\right) \quad (2)$$

where x and y are defined as the precipitation depth registered by one of the gauges and the TE525MM for any time interval, n is the number of values or intervals with recorded rainfall, and $\bar{y}$ is the average precipitation depth measured by the TE525MM for the considered timescale. For rating the performance of the gauges the following categories of the percent bias were defined: excellent $\leq 2\%$, $2\% <$ good $\leq 5\%$, $5\% <$ regular $\leq 10\%$, and poor $>10\%$.

3.2 Overall performance

The precipitation records of the different gauges were compared to check if they were working properly. The functioning of a sensor was characterized by R^2 and ρ. The latter was calculated to test the validity of R^2 that normally is affected by the non-normal distribution of rainfall. For the TE525MM data at an hourly timescale, the non-normal distribution was confirmed by finding p-values of less than 0.01 for both the Kolmogorov-Smirnov and Shapiro-Wilk's tests. Nonetheless, R^2 was calculated to compare our results with those of other studies. To assess the effect of extreme values on R^2, the difference between ρ and R^2 was considered as indicator. According to Tokay et al. (2003); Rollenbeck et al. (2007); Tokay et al. (2010), R^2 values are expected to be greater than 0.95 for hourly and daily data from TB gauges located at the same site.

Following this, differences in precipitation depth were analyzed between each of the rain sensors and the reference gauge using the percent bias as indicator (Equation 1). Additionally, given the hydrological relevance of precipitation data for short timescales (Ciach, 2003; Rollenbeck et al., 2007; Buytaert and Beven, 2011), the accuracy of the rain gauges was also determined for respectively the 5 min, 10 min, 30 min, hourly and daily timescale. For this, absolute bias (obtained with Equation 2) was compared to regular bias, and this information was complemented with the standard deviation of the differences between measurements from the sensors (σ_{x-y}).

3.3 Rainfall intensity effect

For the assessment of the rainfall intensity effect on the accuracy of the rain gauges, measurements from the sensors corresponding to the following

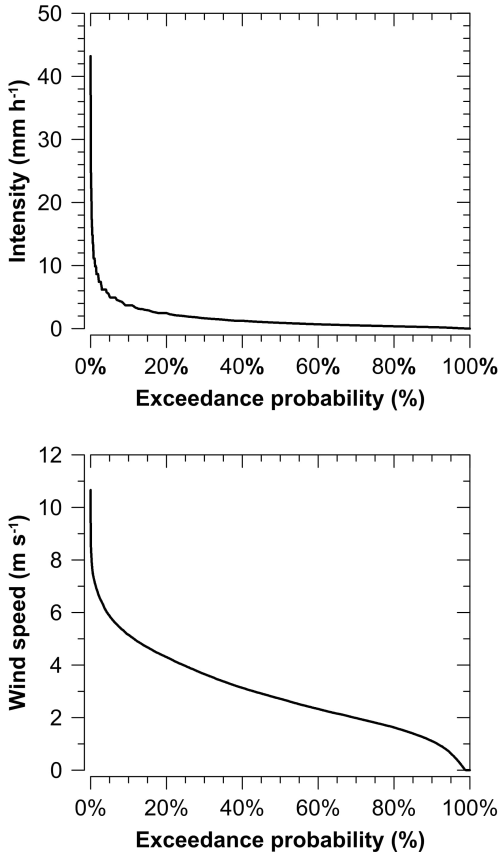

Figure 2. Rainfall intensity exceedance probability curve of the 5-min rainfall data collected by the TE525MM gauge (**top**), and wind speed exceedance probability curve of the corresponding 5-min intervals (**bottom**).

categories: 0–1 *mm* h^{-1}, 1.01–2 *mm* h^{-1}, 2.01–5 *mm* h^{-1}, 5.01–10 *mm* h^{-1} and >10 *mm* h^{-1} were compared. These categories were established by trying to have for each of them a flat distribution, and a representative percentage of the total amount of data (Figure 2 top). For the separation of the data per intensity category, the intensities measured by the TE525MM gauge were within the limits of each specific category, whereas the corresponding intensities measured by the other gauges were not necessarily within these limits. For data belonging to each intensity category and timescales of 5, 10, 30, and 60 minutes, the percent absolute bias was computed to define the effect of these variables on the accuracy with which TB gauges estimate actual rainfall intensity. Percent bias was also calculated for the different intensity categories to understand and quantify how the deviations between the measurements of the gauges vary as a function of actual rainfall intensity.

The intervals with rain intensities for the comparison were between exact hours, without time overlap and with rain during their entire length. For example, for an event that had its first tip at 19:08 UTC and its last one at 21:17 UTC of the same day, the used intervals for a 30 minute timescale were: 19:30–20:00, 20:00–20:30, and 20:30–21:00 UTC. The intervals 19:00–19:30 and 21:00–21:30 UTC were discarded because it did not rain during the entire interval. Although, this approach reduced the volume of data, the useable dataset was still very representative—e.g. for the 5 min timescale the cumulative rainfall of the used intervals represented 85 % of the total precipitation volume of the TE525MM database.

3.4 Wind speed effect

Only the effect of wind speed on the accuracy of the rain gauges was analyzed because there were not any evident obstacles to suggest an effect of wind direction. Similar criteria as for the rainfall intensities were used to classify wind speed (Figure 2 bottom), establishing the following categories: 0–2 $m\ s^{-1}$, 2.01–4 $m\ s^{-1}$ and 4 $m\ s^{-1}$. The wind speed data and selected categories are similar to those used by Sevruk and Hamon (1984) in their worldwide study. Also, wind speeds registered in other mountain highlands, like the Swiss-Austrian Alps or the Bolivian Altiplano, did not differ much from the recorded wind speeds for the páramo (Vacher et al., 1994; Draxl and Mayr, 2009). Percent bias and percent absolute bias were used for the data corresponding to each category to analyze the effect of wind speed on the measurements from the sensors.

The selected timescale had to provide good accuracy of the precipitation depth captured by each gauge, while assuring at the same time that a sufficient amount of data per wind speed category was available. The accuracy requirement was determined from the overall assessment and intensity effect analyses. To distinguish the individual effect of wind from the effect of rainfall intensity, the distribution of the rainfall intensity data corresponding to each of the wind speed categories was determined. The rain depth intervals for the comparison of the sensors with respect to the effect of wind were selected employing the same criteria used for doing this when examining the effect of rainfall intensity—i.e. intervals between exact hours, without time overlap and with rain during their entire length.

4 Results and Discussion

4.1 Overall performance

Table 2 depicts the coefficient of determination (R^2) and the Spearman's correlation (ρ) of the DAVIS-RC-II, HOBO-RG3-M and TE525MM-WS gauges with respect to the precipitation recorded by the TE525MM sensor. Both coefficients are presented for the timescales 5, 10 and 30 min, hourly and daily. The data clearly reveal that the value of R^2 and ρ increases with timescale, which according to Nystuen (1999) is due to the fact that for longer intervals it is less important how it rains. This also illustrates that the importance of accuracy and resolution of the rain gauges is more relevant for time aggregations of 30 minutes or less. Values of R^2 tend to suggest a better agreement between sensors than expected, due to the effect that extreme values have on this index. This effect can be seen clearly for the 5 and 10 minute timescales, where R^2 is much higher than ρ. Correlations between all gauges and the TE525MM were statistically significant; the probability that measurements from two different gauges were not correlated was always less than 1 % ($p<0.01$). Several other studies (Tokay et al., 2003; Rollenbeck et al., 2007; Tokay et al., 2010) also found high correlations ($R^2 >0.95$) between the measurements of two side-by-side gauges for respectively hourly and daily timescales.

The percent bias of the difference between the total precipitation captured by the three gauges relative to the TE525MM gauge, which is independent of the used timescale, varied among $-7{,}2\,\%$ (DAVIS-RC-II), $-5{,}6\,\%$ (HOBO-RG-3) and $-2\,\%$ (TE525MM-WS). The negative values indicate an underestimation of the gauges in relation to rainfall volume caught by the reference sensor. Based on the aforementioned criteria, the performance of the DAVIS-RC-II and HOBO-RG-3 gauges is regular, whereas that of the TE525MM-WS is excellent. The obtained results are in line with the deviation range found by Rollenbeck et al. (2007) for gauges from different manufacturers, and by Tokay et al. (2003, 2010) for identical gauges.

Figure 3 shows the percent absolute bias of the TB gauges as a function of the timescale. There is a clear influence of gauge resolution, with the DAVIS-RC-II sensor having the largest percent absolute bias and the coarsest resolution. There is also an effect of timescale on the gauges' accuracy that can be explained by the fact that during short intervals, given the overall low rainfall intensity, some sensors do not register rain, while others do, and in the next time interval the opposite often occurs. Indeed, in nearly 50 % of all 5 min intervals considered, when one of the compared TB gauges recor-

Table 2. Coefficient of determination (R^2) and Spearman's correlation coefficient (ρ) for the Tipping-Bucket (TB) rain gauges in relation to the TE525MM sensor for different timescales

Sensor	Timescale	R^2	ρ
DAVIS-RC-II		0.603	0.364
HOBO-RG3-M	5 min	0.690	0.395
TE525MM-WS		0.809	0.495
DAVIS-RC-II		0.826	0.590
HOBO-RG3-M	10 min	0.876	0.624
TE525MM-WS		0.933	0.716
DAVIS-RC-II		0.953	0.792
HOBO-RG3-M	30 min	0.969	0.826
TE525MM-WS		0.986	0.887
DAVIS-RC-II		0.975	0.860
HOBO-RG3-M	hourly	0.985	0.893
TE525MM-WS		0.993	0.935
DAVIS-RC-II		0.993	0.992
HOBO-RG3-M	daily	0.997	0.996
TE525MM-WS		0.997	0.997

ded precipitation the other did not. For hourly and shorter timescales the standard deviation of the differences with the TE525MM were smaller than the resolution of the sensors; values of 0.165 *mm*, 0.130 *mm* and 0.085 *mm* were obtained respectively for the DAVIS-RC-II, HOBO-RG3-M and TE525MM-WS gauges at the hourly timescale. These results imply that in regions with frequent low-intensity rains such as the wet páramo, and when interested in rainfall behavior during time periods of 1 hour or less, the importance of sensor resolution is strongly amplified.

4.2 Rainfall intensity effect

As evidenced by values of percent absolute bias in Table 3, the accuracy of the rain gauges decreases significantly for low intensities and short timescales. This reduction in accuracy (expressed as percentage) happens under the mentioned conditions because precipitation depth is extremely low, boosting the effect of gauge resolution. However, accuracy is still especially poor for rainfall intensities of 2 $mm\ h^{-1}$ or less (which are typical conditions, as shown in Figure 2) and timescales of 10 minutes or shorter. These results agree well with the findings from Habib et al. (2001); Ciach (2003); **?**.

Considerable errors between actual and calculated rainfall intensity affect the analysis of how the deviations between the measurements of the gauges vary depending on rainfall intensity. Therefore, different timescales for each intensity category had to be used to obtain data with acceptable accuracy, while still counting with an appropriate number of time intervals for which rainfall was measured by the sensors. The data categories with bold text in Table 3 were used to determine the percent bias of the TB gauges, in relation to the TE525MM gauge, for the different intensity categories (Figure 4).

Percent bias results depict a general tendency of higher values for lower intensities and coarser gauge resolution. The results for the DAVIS-RC-II correspond to a poor performance for the lowest intensity category, and a regular rating for all other categories. For the HOBO-RG3-M, the value of the percent bias was near the transition from regular to good performance for intensities $\leq 5\ mm\ h^{-1}$, and corresponded to an excellent rating for intensities >5 $mm\ h^{-1}$. The TE525MM and TE525MM-WS gauges registered very similar measurements for all intensities, but performance was rated good for the ≤ 1 $mm\ h^{-1}$ category, and excellent for all other categories. As a general conclusion, the larger value of the

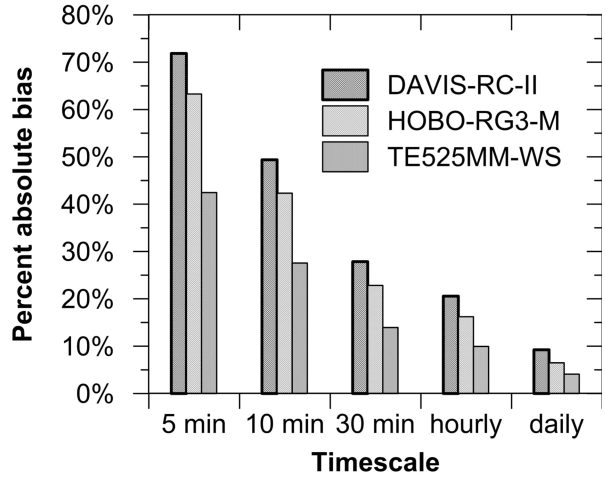

Figure 3. Percent absolute bias of the Tipping-Bucket (TB) rain gauges in relation to the TE525MM sensor as a function of timescale.

percent bias for lower intensities is attributed to intrinsic features of the studied gauges that influence wetting and evaporation losses (WMO, 2008). It is important to notice that an overall comparison of the same TB gauges used in this study at a location with rainfall intensities different from those typical of the páramo, would reveal completely different results.

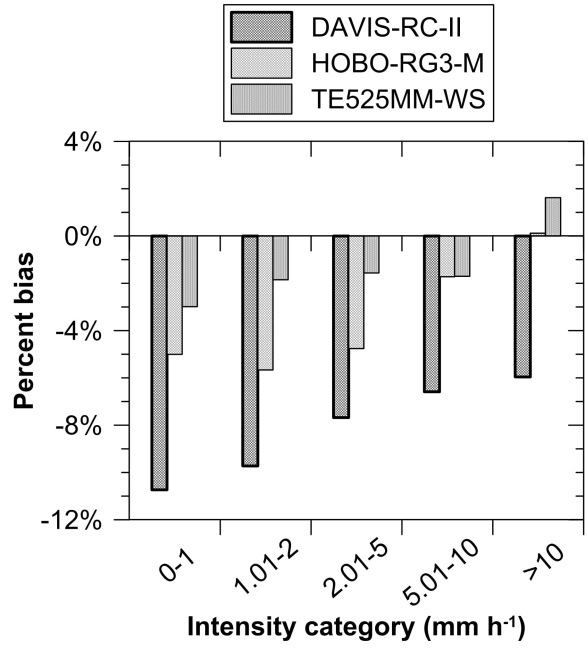

Figure 4. Percent bias of the Tipping-Bucket (TB) rain gauges in relation to the TE525MM sensor as a function of rainfall intensity. The data used for this figure corresponds to the highlighted categories in Table 3.

Table 3. Percent absolute bias (%) and number of data points (#) per intensity category and timescale, for each of the Tipping-Bucket (TB) rain gauges in relation to the TE525MM sensor.

Intensity category (mm h^{-1})	Timescale							
	5 min		10 min		30 min		60 min	
	DAVIS-RC-II							
	%	#	%	#	%	#	%	#
0−1	72	4864	56	2192	27	549	**15**	**165**
1.01−2	37	2618	29	1341	**14**	**411**	13	181
2.01−5	25	2492	16	1170	**10**	**320**	8	111
5.01−10	17	530	12	255	**7**	**50**	7	18
> 10	12	158	**10**	**62**	4	10	5	2
	HOBO-RG3-M							
	%	#	%	#	%	#	%	#
0−1	62	5130	47	2323	18	580	**10**	**180**
1.01−2	30	2569	20	1325	**10**	**404**	8	181
2.01−5	20	2444	12	1169	**7**	**322**	6	112
5.01−10	16	520	10	256	**4**	**49**	4	18
> 10	12	155	**8**	**62**	4	10	2	2
	TE525MM-WS							
	%	#	%	#	%	#	%	#
0−1	39	3000	27	1325	10	334	**7**	**105**
1.01−2	20	1537	11	799	**6**	**254**	5	120
2.01−5	12	1338	7	618	**4**	**171**	3	62
5.01−10	9	260	**4**	**122**	3	28	2	8
> 10	**4**	**71**	4	29	2	4	3	2

Note: Categories with **bold** text were used for the percent bias analysis in Figure 4.

4.3 Wind speed effect

The percent bias and percent absolute bias of the TB gauges in comparison with the TE525MM sensor were calculated for each wind speed category (Table 4), using the 30-min data. This timescale was used since it provided an acceptable accuracy for estimating rainfall intensity, and because a sufficient amount of 30-min rainfall data was available. The absolute percent bias showed an increment for all the gauges when wind speed was greater than 4 $m\ s^{-1}$. This surprising behavior is most likely related to the fact that rainfall intensities, in general, were lower for the highest wind speed category (Mekonnen et al., 2014). The percent bias results for the DAVIS-RC-II and HOBO-RG3-M gauges hardly change for the different wind speed categories. This does not mean that those gauges are not prone to wind-induced errors; instead it shows that these errors are practically identical for the DAVIS-RC-II, the HOBO-RG3-M and TE525MM sensors. We hypothesize that the similarity in shape, dimension and installation heights of the gauges generate a similar airflow around them, leading to dismissible differences between the sensors due to wind speed (Nešpor and Sevruk, 1999).

Given the presence of an Alter-Type windshield around the TE525MM-WS sensor, a noticeable difference in rainfall depth may have been expected with the technologically identical gauge (TE525MM) without windshield. A dismissible bias, less than 1%, was found for the precipitation data that occurred with wind speeds $\leq 4\ m\ s^{-1}$, suggesting that the Alter windshield does not really reduce the under catch of rainfall in the páramo for these wind speed conditions. A similar result was found by Duchon and Essenberg (2001), although at a site likely with a different rainfall drop size distribution. Moreover, Duchon and Biddle (2010) concluded that the Alter windshield does not significantly reduce wind-induced errors by also using a ground-level gauge. Meanwhile, for wind speeds $>4\ m\ s^{-1}$, results show that the shielded gauge recorded even less precipitation than the unshielded gauge.

Table 4. Percent bias and percent absolute bias per wind speed category for each of the Tipping-Bucket (TB) rain gauges in relation to the TE525MM sensor.

Wind speed (ms^{-1})	DAVIS-RC-II		HOBO-RG3-M		TE525MM-WS	
	% bias	% absolute bias	% bias	% absolute bias	% bias	% absolute bias
0−2	−8.6	11.4	−4.3	8.1	−0.2	3.7
2.01−4	−8.2	12.2	−4.4	8.2	−0.7	5.0
> 4	−8.4	16.7	−2.4	11.0	−6.6	9.0

5 Conclusions

The performance of four tipping-bucket rain gauges, installed side by side in an Andean headwater catchment, was analyzed using 2-year data. The installation site is situated in the tropical Andes, southern Ecuador, at an elevation of 3780 m a.s.l., and is representative for the wet páramo region. Rain events are most of the time characterized by a low intensity: 95%, 76%, and 53% of the 5-min intervals have intensities lower than 5, 2, and 1 $mm\ h^{-1}$, respectively. These rainfall characteristics differentiate the present research from previous intercomparison studies. In addition for the study site, precipitation intensities are lower than average under windy conditions ($4\ m\ s^{-1}$), which occur during 25% of the rainfall events.

A clear relation between gauge resolution and total precipitation depth was found: a coarser resolution corresponds to less registered rainfall depth. The results showed an underestimation of 7.2% and 5.6% for gauges with a resolution of 0.254 *mm* and 0.2 *mm* respectively, when compared to a gauge with 0.1 *mm* resolution. The differences between the sensors are principally attributed to a combination of wetting and evaporation losses, because of the relation between these sources of error and gauge resolution. The higher underestimation of the DAVIS-RC-II gauge could be related to higher evaporation caused by the black color of the funnel absorbing more ambient heat. Additionally, evaporation losses are boosted by the frequent low-intensity rain events.

It was found to be very common for timescales of 5 and 10 minutes that a TB gauge registers a tip within an interval when another gauge does not for that same interval, but for the one that follows. This phenomenon caused results to show an extreme difference among sensors when actual differences in measured precipitation depth were much lower. Therefore, sensor resolution is a critical aspect to consider for rainfall monitoring in the wet páramo, or any other ecosystem with similar precipitation characteristics.

Wind speed has a similar effect on the analyzed unshielded TB gauges. Meanwhile, the Alter windshield did not reduce wind-induced losses.

The quantitative knowledge of the differences between the sensors obtained in this study is an important step to homogenize rainfall data from multiple sites within the Andean highlands. Additionally, the fact that rainfall in the wet páramo is underestimated in at least 5% by commonly used gauges is pivotal for water-related studies in these landscapes and guide the selection of adequate equipment for monitoring networks.

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CLIMATE CHANGE

ABYA YALA | UNIVERSIDAD POLITÉCNICA SALESIANA

Climate Change according to Ecuadorian academics–Perceptions versus facts

Cambio Climático según los académicos ecuatorianos - Percepciones versus hechos

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Resumen

El cambio climático se ha convertido en uno de los temas principales en las agendas en diferentes países. Los efectos actuales requieren de acciones climáticas efectivas ya establecidas en el Acuerdo de París con el objetivo de reducir las emisiones de gases de efecto invernadero. Sin embargo, los principales cambios para enfrentar y reducir el cambio climático dependen de las decisiones de cada país y no sólo de los acuerdos mundiales, ya que los impactos y magnitudes varían localmente. Uno de los componentes clave para una mejora efectiva es el papel que el comportamiento de la población puede tener sobre la política nacional y las decisiones posteriores. Por esta razón, el nivel de conciencia y conocimiento sobre el cambio climático es vital. El objetivo de la investigación fue comparar la percepción de los académicos ecuatorianos sobre el cambio climático global y nacional con la evidencia científica y los hechos históricos, y cómo su vulnerabilidad puede afectar a los efectos del cambio climático. Los resultados muestran que los académicos ecuatorianos están conscientes de los hechos ocurridos mundialmente sobre el cambio climático, como la existencia, la gravedad y la responsabilidad de los seres humanos. Sin embargo, hay un conocimiento limitado sobre el origen del problema, ya que el 67,2 % cree que este es el primer cambio climático en la historia de la humanidad. Los principales efectos del cambio climático en Ecuador presentan percepciones heterogéneas, como sequías más frecuentes (34,36 %) y lluvias escasas pero intensas (21,41 %) como sus mayores preocupaciones. En cuanto a las regiones más afectadas en Ecuador, las sierra y los valles interandinos representan el 45,6 %, mientras que Galápagos sólo alcanza 1,6 % a pesar de ser una insignia ecológica con alta vulnerabilidad climática. Parece que los encuestados carecen de conocimiento

sobre la situación en otras regiones y creen que su propio entorno se ve más afectado.
Palabras clave: cambio climático, calentamiento global, vulnerabilidad, desastres, ecosistemas, paleoclimatología.

Abstract

Climate change has become one of the main issues in the countries government agendas. The current effects demand effective climate actions which were set out in the Paris Agreement with the global goal of reducing greenhouse gas emissions. However, the main changes to face and mitigate climate change depend on each countrys decisions and not only on global agreements as the impacts and its magnitudes vary locally. One of the key components for an effective adaption and mitigation is the role that the behavior of the population may have over national politics and subsequent decisions. For this reason, the level of awareness and knowledge about climate change is vital. . The objective of the current study was to compare the perception of Ecuadorian academics regarding global and national climate change with the scientific evidence and historical facts, and how it may affect their vulnerability to the climate change effects. The results show that Ecuadorian academics are well aware of globally known facts of climate change such as existence, gravity and responsibility of humans. However, there is limited awareness about the origin, since 67.2% believes that this is the first climate change in human history. The main effects of climate change in Ecuador exhibit heterogeneous perceptions, with the more frequent droughts (34.36%) and rarer but more intense rains (21.41%) as their greater concerns. Regarding the regions more affected in Ecuador, highlands and Inter-Andean valleys sum up 45.6% while Galapagos only reaches 1.6% despite being an ecological flagship with high climate vulnerability. It seems that respondents lack knowledge about the situation in other regions, and believe that their own environment is more impacted.
Keywords: climate change, global warming, vulnerability, disasters, ecosystems, paleoclimatology

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1 Introduction

The occurring and potential impacts that climate change has over both nature and societies have converted it into a complex topic to approach if thorough researches and institutional cooperation are not linked (Luterbacher et al., 2004). Although the causes of climate change are globally averaged by the climatic system (UNDP, 2009), they are actually, local, and highly depend on the level of industrialization and habits of consumption of each country. Frequently the data of diverse countries are reported considering the highest emitters of cumulative carbon dioxide. Considering the most recent available data from 2016 (Agency, 2018), the top emitters of total CO_2 are, by far, China with 9056.8 metric megatons (MT), which almost doubles the following being the United States with some 4833.1MT. The USA is followed by India, Russian Federation and Japan. However, if the countries are ranked in terms of the carbon dioxide emissions per capita, instead of the total emissions, the results change dramatically and China, the major emerging economy and most populated country, does not lead the rank any longer. By 2016, the top five countries in the list of CO2 emitters per capita are Saudi Arabia, Australia, USA, Canada and South Korea, with values ranging between 16.3 and 11.6 Metric Tons (T). Hereby, China occupies the 12th place in per capita emissions, with approximately 6.4 T, while India ranks 20, with 1.6 T, ten times less than the average citizen of Saudi Arabia or Australia and, at only 40% of the global average.

These uneven current contributions together with differentiated historical responsibilities for climate change have long been discussed (Rajamani, 2000; Page, 2008; Müller et al., 2009; Baatz, 2013; Friman and Hjerpe, 2015), and are at the core of the challenges the world faces in reaching agreements and achieving commitments in the international climate change negotiations. In a further study it has been discussed the most controversial issue of the Brazilian proposal, which has led to a methodology of calculating shares of responsibility as opposed to the shares in causal contribution considering two conceptions of responsibility being 'strict' or 'limited' (Müller et al., 2009). Other studies focused on specific policy options to compensate those vulnerable to climate change in developing countries, analyzing the applicability of the Beneficiary Pays Principle rather than the Polluter Pays Principle (Baatz, 2013).

In any case, such commitments need to be nationally-tailored, and developing countries should endorse meaningful participation and assume their share while claiming climate justice and compensation for climate change loss and damage (Calliari, 2018; Page and Heyward, 2017). In fact, the developing and poorest countries are facing greater climate change loss and damage because they are at higher risks (Zenghelis, 2006; Mertz et al., 2009; Hedlund et al., 2018). It has been demonstrated that the increase of environmental deterioration through the usage of natural resources is indirectly related to the culture, education, policy decisions, social movements, and economic incomes of each country (Luterbacher et al., 2004). The economic underprivileged people tend to live in areas of even higher risk, re-enforcing the statement that vulnerability is correlated to poverty. For this reason, the socioeconomic inequality and political instability that Latin America faces, added to a multi-hazard geographic location and continuous environmental deterioration in their attempt to reach development, only increases the risks of vulnerability of these regions. Thus, the mitigation of the effects of global and local climate change coincides with the reduction of poverty and social inequalities, the application of sustainable regulation of natural resources, and an in-depth planning that promotes development and reduces risks (Rojas, 2016; Goworek et al., 2018; Furley et al., 2018). Ecuador, in particular, is affected by the regional South American aspects previously mentioned and its specific issues such as socio-economic differences among the Coast, Highlands and Amazon regions, and the way in which energy and soil are being used. Those aspects hence threat the mitigation and adaptation attempts that Ecuador is implementing to face climate change and reduce its impacts (Reuveny, 2007; Buytaert et al., 2010; Luque et al., 2013; Luterbacher et al., 2004).

Scientists have been informing the society about the impacts that our activities are having over the planet during the last decades. The monitoring of different substances started around 32 years ago with the signature of the first protocol after the leaders of the main developed countries realized the impact that humanity had on triggering an irrever-

sible climate change. The first global agreement was the Montreal Protocol, signed on 1987, that aimed to protect the ozone layer by reducing and stopping the usage of the main gases that deplete the layer (Ibárcena and Scheelje, 2003). These gases included the chlorofluorocarbon (CFC) and the hydrochlorofluorocarbon (HCFC) (Manzer, 1990; Prather and Spivakovsky, 1990). The second important agreement was the Kyoto Protocol, which was adopted in 1997 but only entered into force in 2005. It targeted the reduction of the Green House Gases (GHG) such as carbon dioxide (CO_2), el metano (CH_4), nitrous oxideo (N_2O), hydrofluorocarbon (HFC), perfluorocarbons (PFC), sulphur hexafluoride (SF_6) (Prather and Spivakovsky, 1990). Lastly, the most recent protocol is the Paris Agreement, sealed in 2015 and with 185 state parties to date. The Paris Agreement looked out for reducing the carbon emissions in order to keep the upcoming increase of global temperature below de 2°C (Ibárcena and Scheelje, 2003; Enkvist et al., 2007; Van Vuuren et al., 2007; Friel et al., 2009; Hoegh-Guldberg et al., 2018).

As the achievement of a global commitment and support requires a trustworthy source of information about the ongoing changes around the world, the UN Environment Agency and the World Meteorological Organization created the Intergovernmental Panel on Climate Change (IPCC) in 1988. The research that the IPCC has been realizing over the past 25 years has confirmed the severity and undeniable effects of the climate change around the globe. Some of these effects include: the temperature increase of 0.85°C between 1880 – 2014, a sea level rise of 19 cm between 1901 and 2010, the decrease of 1,07 106 km^2 every 10 years of the Arctic ice, the absorption of the thermal energy by the oceans, increase of the greenhouse gases (GHG) in the atmosphere, the increase of 40% CO_2 concentration, the acidification of oceans due to their further absorption of CO_2, the loss of ecosystems, and the longer droughts and intense precipitations (Doney, 2006; McNeil and Matear, 2008; Knapp et al., 2008; Frank et al., 2015; Hoegh-Guldberg et al., 2018). The latest special report presented by the IPCC in 2018 confirms that the principal driver of the current climate change is the anthropogenic unsustainable development thus they propose an immediate reduction of CO_2 emissions to keep the global temperature increase below the 1.5°C (Hoegh-Guldberg et al., 2018).

On the other hand, according to the most recent report by the World Biodiversity Council (IPBES), one million species will be threatened with extinction in the coming years and decades if there are no major changes in land use, environmental protection and mitigation of climate change (on Biodiversity and Services, 2019). Hereby, as the most important factor in the extinction of species, the report names the effects of agriculture. The detailed report indicates that a) some 85 percent of wetlands are already destroyed; b) since the late 19th century, around half of all coral reefs have disappeared; c) Nine percent of all livestock breeds are extinct; d) between 1980 and 2000, 100 million hectares of tropical rainforest were cut down and another 32 million hectares between 2010 and 2015 alone; e) 23 percent of the planet's land is considered to be ecologically degraded and can no longer be used; e) the loss of pollinators threatens food production worth 235 to 577 billion a year; and f) destruction of coastal areas such as mangrove forests threatens the livelihoods of up to 300 million people among other facts (on Biodiversity and Services, 2019).

The local effects of the ongoing climate change implies that each country has to face them in different ways compared to others countries. The risk that Ecuador has to tackle is not only due to the hazards linked to its geographic location along the equatorial line, but also due to its economic and cultural vulnerability, its preparation towards upcoming disasters and the importance of climate change for its society (O'Brien and Wolf, 2010). In order to implement effective mitigation and adaption plans against climate change, cooperation and commitment are needed among multiple actors, mainly, the government as law makers and enforcers, industry, corporations, and population as main GHG emitters, and academia as knowledge producers. In the case of Ecuador, its government ratified the Paris Agreement in 2017, and, later in March 2019 presented its First Nationally determined contributions (NDCs) to the United Nations Framework Convention on Climate Change (UNFCCC). While the NDCs are not legally binding, they are subject to required normative expectations of progression Rajamani and Brunnée (2017)and to the evaluation of their progress by technical experts to assess achievement toward the NDC. The NDC is the national plan to reduce national emissions and to adapt to

the impacts of climate change.

In particular, Ecuadors NDC has set the target to reduce GHG emissions by 9% in the sectors of energy, industry, waste and agriculture. Furthermore, Ecuador plans to reduce an additional 4% of GHG emissions in change of land uses, that is, deforestation and land degradation. Regarding adaption to climate change, the Ministry of Environment will incorporate actions in seven sectors being natural and water heritage, health, production, human settlements and agriculture (MAE, 2019). The period of implementation of the NDC is 2020-2025, hence, has not yet started. Then, in 2025, an evaluation will be performed to monitor to what extent the targets were reached. The Ecuadorian government considers that, being a developing country with many socio-economic needs, its NDC is an ambitious yet fair plan to tackle climate change. However, the successful implementation of the Ecuadorian NDC requires the generation of strategic alliances and the financial support, especially from the private sector and international cooperation.

Corporation driven lobbied politics of Climate Change should be rejected and overcome in pursuit of ambtious emission policies. Achieving the commitment of the industry in CC plans is arguably one of the main challenges, yet, these policies should incorporate the industry as a key pole of enforcement by identifying the economic opportunities and mobilizing the co-benefits linked to climate actions such as mitigation and adaptation plans, climate change risks, incentive types, and incentivized stakeholders (Huang-Lachmann et al., 2018; Helgenberger and Jänicke, 2017). In facing the current climate crisis, with the active participation of the different actors, there is a need to shift paradigms: from burden-sharing to opportunity-sharing. In all cases, as efficient policies require scientific bases, the different actors need clear and accurate information from the academics.

Therefore, a survey was performed inquiring Ecuadorian academics about their perceptions and knowledge about the climate change processes and its associated vulnerabilities in the Ecuadorian territory with the objective of comparing those with the facts and evidences about the global climate change occurrence in Ecuador. The long term aim or intention is that the results help to determine the degree of preparation about climate change of a well-educated community of academicis, and how this could contribute to the implementation of measures for mitigation and adaptation to climate change whose success would depend on their daily habits. The link between a well-informed community and adequate policies implementation is missing because it's not necessarily true develop argument and state of the art literature on this respect. In countries where there is a limited interrelation between academia and governmental institutions the "well-informed community" indicator may be inadequate.

2 Methodology and data collection

Considering that there is little research in Ecuador about climate change perceptions (Valdivia et al., 2010; Crona et al., 2013) and misconceptions, the first reason for conducting this survey (Appendix) was to collect quantitative baseline data from a relatively homogeneous population. The second reason was to prove or disprove the hypothesis that people with higher education has a more accurate knowledge about climate change, and are more likely to share common perceptions about climate change, as proposed by Crona2013. The third reason was to use the results of this first survey in the future to monitor changes in the perceptions over time. Furthermore, the survey was conducted along the Ecuadorian highlands with the objective of identifying and measuring possible geographical differences, and asses if they are linked to their degree of affectation. Taking into account that this study focuses on perceptions, the possible methodological approaches were a survey, a case study through qualitative interviews with emphasis on openended questions, or a method mixing both.

Qualitative research is often used to explore poorly known or understood topics or to clarify issues of meaning which is not the case of climate change. It was opted for the survey because the goal was to pursue a quantitative research which includes measurement, comparison and hypothesis testing. Good statistical estimates within quantitative research require a big number of samples or surveyed individuals; hence, in order to reach a larger audience, maximize response rate and still be able to analyze a big amount of data, a simple and

short survey was considered the most adequate approach.

The survey consisted on 14 closed-ended questions in a single sheet, that were based on both global and local appearances of climate change and their respective hazards. The inquired universities are located in four sites of the Ecuadorean Highlands (Fig. 1). A total of 7803 inquiries were administered in person to students and lecturers, from which 56% correspond to the capital, Quito. The fourteen questions have been divided into two main groups, the global and the local effects. The group of global climate change questions consists of knowledge background about phenomenon origin, scope and global manifestations. Meanwhile, the second group con-sists of selected Ecuador-related questions about the local vulnerabilities and issues.

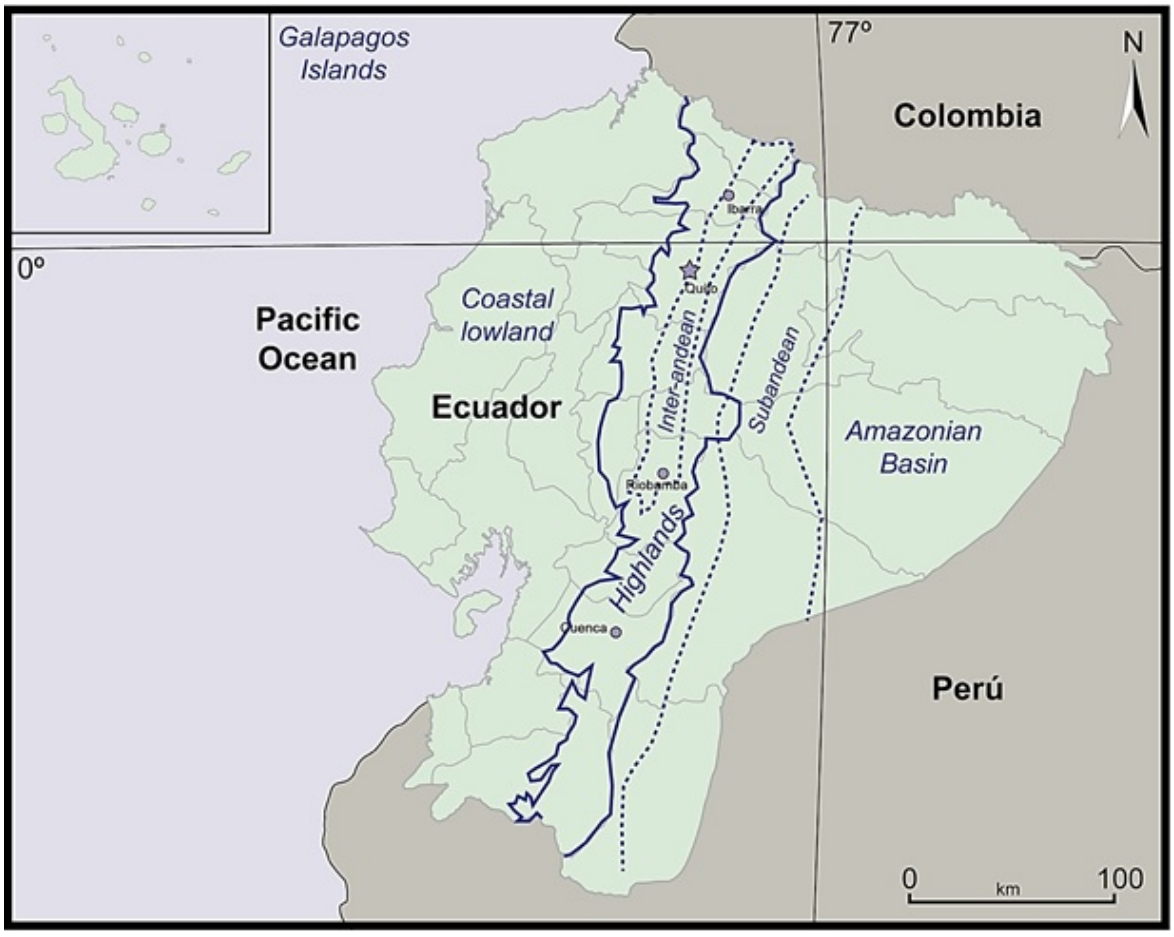

Figure 1. Map of Ecuador with the most important morphological areas and the sites where the survey has been performed.

3 Results and discussion

A) The global perspective. The first three questions of the survey (#1, 2 and 3) focused on global issues by asking about the existence and the seriousness of the climate change, as well as the involvement or not of humans in the rise of this phenomenon. The results to these three questions were affirmative and exposed a major agreement in their responses, as more than the 75% agreed with the given statements regarding the real existence and the gravity of the climate change and the involvement or res-

ponsibility of humans. Those topics are commonly researched worldwide and are regularly presented on the daily news. Therefore, it was assumed that the broad and worldwide inputs infer on the common agreement. According to the PNDU2009, the high importance of climate change is due to the risks that humanity will face at the current trend of CO_2 concentrations. The risks include: (1) reduced agricultural productivity, (2) increased water stress and insecurity, (3) rising sea levels and exposure to climate disasters, (4) collapse of ecosystems, (5) increased health risks, (6) flooding and (7) hunger.

Although there is still a lack of a reliable quantification of the cumulative impacts of climate change on the global-scale agricultural productivity, there is no doubt that there are direct negative effects of an increase of CO_2 on plant physiology and increase resource use efficiencies (Olesen and Bindi, 2002; Battisti and Naylor, 2009; Gornall et al., 2010). Therefore, food insecurity will most likely rise due to the present climate change, especially if societies are unable to cope rapidly with ongoing developments (Lobell et al., 2008; Brown and Funk, 2008). Overall, local biota and human livelihoods are threatened by changing climates and the associated changes in terrestrial ecosystems (Verchot et al., 2007).

Clean water resources are essential for man, society, its life-support system and its industrial develop-ment (Sullivan, 2002; Milly et al., 2005; Falkenmark, 2013). However, increasing temperatures are stressing the existing water resources and the ecosystems which provide this important element. Reduction of glaciers, evaporation of water deposits and high-use of subterranean water resources have led to the overall reduction of water in arid and semi-arid areas (Messerli et al., 2004; Greenwood, 2014; Zografos et al., 2014). An increase of such vulnerability may cause significant social and territorial problems between societies or even among countries (Allouche, 2011; Adano et al., 2012; Gleick, 2014).

An increase of the average worldwide temperature results into rising sea levels (Harley et al., 2006). Such climate-induced changes lead to more damaging flood conditions in coastal areas as well as other vulnerable zones close to the sea level (Watson et al., 1998; Berz et al., 2001; Hoegh-Guldberg et al., 2007). Furthermore, hydrometereological disasters such as hurricanes or cyclones tend towards longer duration and greater intensity being correlated with the rise of tropical sea surface temperatures in the last decades, even in regions which have not been affected in their past (on Climate Change, 2007; Dasgupta et al., 2011; Brecht et al., 2012). Furthermore, the rise of the mean sea-level may also result in the direct collapse of a variety of ecosystems (Worm et al., 2006; MacDougall et al., 2013). Such dramatic effects are especially reported in island regions or states such as those in the Caribbean and southern Pacific areas, where the environmental conditions and coastal communities are alike (Pelling and Uitto, 2001; Dolan and Walker, 2006). A consequence of ocean warming is the enhancement of ocean circulation driven atmosphere-ocean phenomenon such as the ENSO and cyclones. Example of this appears to be the 2015/16 El Niño episode registered as one of the strongest in the history, although it has been also alternatively interpreted (**?**Mato and Toulkeridis, 2017; Brainard et al., 2018). The strong 2015/16 El Niño coincides with the global average temperature in 2015, reaching values of 1 $^{\circ}C$ above preindustrial level for the first time, labeling this year as the warmest so far (P. et al., 2016).

Climate change may affect health through a huge range of forms like more intense and more frequent heat waves as well as changes in the distribution of vector-borne diseases, among many others (Patz et al., 2005; Haines et al., 2006). The effects of global warming have been observed in the temporal increase in temperatures that occur more frequently and by subsequent enhanced hot waves as well as the rise of temperatures in the oceans (Meehl and Tebaldi, 2004). Thus, long-lasting heat waves which occurred in 1995 in Chicago, USA and in 2003 in Paris, France caused 35.000 deaths (Karl and Knight, 1997; Luterbacher et al., 2004; Stott et al., 2004). Further heat waves are expected in a variety of regions all over the planet, based on several modeled scenarios (Lhotka et al., 2018; Frölicher and Laufkötter, 2018; Guo et al., 2018). Additionally, there are many scientific evidences stating that the climate variability of the last decades has given rise to vector-borne diseases, as a result of several droughts and or flooding as well as fires (Martens et al., 1995; Githeko et al., 2000; Amiro et al., 2001; Flannigan et al., 2009; Moritz et al., 2012; El Universo, 2018).

Changes in climate in different regions allows the migration of a high variety of insect and bird

species, who may carry several vector-borne and emerging infectious diseases with them (Patz et al., 1996; Kovats and Hajat, 2008; Tol and Dowlatabadi, 2001; Epstein, 2001; Jones and Mann, 2004; Wu et al., 2016). Based on the compilation by the World Health Organization (WHO), the current global climate change may cause up to 150.000 deaths per year (Cifuentes Lira, 2008).Additionally, as consequences of the current trade of pollution, the global temperature is predicted to rise de 1.5 a 4.5 $^{\circ}C$. This rise will cause the melting of many glaciers, resulting to a sea level rise of about around 50 cm, impacts on the biological systems like the coral reefs, further damage or slower recuperation of the ozone layer, and the spread of tropical diseases such as malaria and paludism (Ibárcena and Scheelje, 2003). The temperature rise and the heavy rainfall are the main causes for the spread of diseases. For example, the malaria distribution model is based on temperatures (Rogers and Randolph, 2000). The effects on people health due to climate change include malnutrition, undernourishment, and infectious diseases (McMichael and Haines, 1997). Examples of this are the health impacts such as the epidemics and diarrhea which occurred after the seasonal variability of the El Niño-Southern Oscillation (ENSO) (Patz et al., 2005).

The responses to question #4, which regard to the level of agreement that scientists have about the occurrence or not of climate change, showed a majority of 60% that believe there is agreement in the scientific community. From the research undertaken in several reports and scientific papers, the response of the survey is supported as most of the scientists agree in the imminent and mostly dangerous effects of climate change and global warming. Both processes have been accelerated by humans and their industrial activity. Moreover, they agree on the effects that pollution and global warming have had and will have over the ecosystem and the atmosphere, which includes ice poles melting, enhanced ENSO, droughts, flooding, coral reef bleaching and the extinction of some species (Timmermann et al., 1999; Clauer et al., 2000; Walther et al., 2002; Thomas et al., 1999; Cazenave, 2006; Brook et al., 2008; Markus et al., 2009; Ibárcena and Scheelje, 2003; Chen et al., 2013; McClanahan et al., 2018; Cai et al., 2019).

Question #5 of the survey focuses on past climate changes during human history, as the idea was to encounter the knowledge background of climate variability by the academics of Ecuador. Based on scientific studies, there have been several climate and temperature changes since the existence of humans, and also significant rapid climate changes during the current Holocene (Mayewski et al., 2004). Such climate fluctuations, even the abrupt ones, seem to have been triggered by changes in earth orbital insolation, volcanic aerosols and associated albedo feedback processes rather than by a significant increase of antrophogenic greenhouse gases (Crowley et al., 1993; Rampino and Self, 1993; Overpeck et al., 1997; Crowley, 2000; Atwell, 2001; Bay et al., 2004; Christensen et al., 2019; Guanochanga et al., 2018; Fuertes et al., 2019).

In a more detailed approach, it should be reminded that since the appearance of hominins over 2,000,000 years ago, the history of human evolution has been intrinsically linked to Earths climate fluctuations, which have helped to shape our species. The origins of bipedalism were in a period of climatic transition, and *Homo erectus* emerged in the time of the colder Pleistocene Epoch and survived various glacial-interglacial cycles (de Menocal, 1995; Rightmire, 2008). *Homo sapiens* domination initiated during the last glacial period and accelerated over the last glacial-interglacial transition (Groucutt et al., 2015; Williams et al., 2016; Asrat et al., 2018; Skillington, 2018). The modern human species has successfully experienced nearly two full glacial-interglacial cycles due to its worldwide geographical distribution, extensive population expansion, and global ecological domination together with superior technology, and more dynamic social relationships (Shea, 2008; Schramski et al., 2015).

Such climate changes have impacted human evolution and dispersal, and are believed to have deter-mined and even stimulated the emergence of agriculture. Animal domestication seems to have initiated in western Asia around 11,000 years ago when goats and sheep were first herded, while plant domestication started some 2000 years later as wheat, lentils, and rye were grown by first time (Diamond, 2002; Terrell et al., 2003). This shift from a nomadic hunter-gatherer lifestyle to agrarian-based settlements took place during a phase of climatic transition after the last glacial period (Richerson et al., 2001). It seems that, although climate change affected nomadic societies by causing dras-

tic decreases in natural resources, it also brought opportunities as new plants and animals spread or appeared. Although certain civilizations may have collapsed due to abrupt and intense climatic changes, humans have adapted and survived climate variations through history. These climate changes range from decadal to centennial and millennial variations, and have been well documented through historical and proxy records, particularly annual growth rings in trees, cave stalagmites, corals, cores of lake and deep-sea sediments and ice cores (McManus et al., 1994; Jones et al., 2009; Esper et al., 2002). Interannual and decadal climate oscillations include El Niño–Southern Oscillation, the North Atlantic Oscillation, the Pacific Decadal Oscillation or the Atlantic Multidecadal Oscillation (Henley et al., 2015; Geng et al., 2017; Kayano et al., 2019). They have also been associated to the collapse of the Maya civilization in Mesoamerica between the 8th and 9th centuries, suggesting that intense droughts may have led to social stresses (Hodell et al., 1995; Haug et al., 2003; Douglas et al., 2015; Beach et al., 2016). As for centennial timescales, the Little Ice Age (LIA) was a period of relative cooling which spanned from early 14th through the mid-19th centuries (Jones and Mann, 2004; Rubino et al., 2016). It is characterized by a mountain glaciers expansion in regions such as the European Alps, New Zealand, and the Patagonian Andes, and a decline of 0.6 °C in the mean annual temperatures across the Northern Hemisphere (Svarva et al., 2018). The effects of LIA include bad harvests and famines over most of Europe due to the increase of rain during summers (Appleby, 1980), as well as the collapse of cod fisheries in the North Atlantic resulting from a pronounced decreased of sea temperatures. The LIA began after the Medieval Warm Period (9̃50–1100 CE) (Cronin et al., 2010), whose warmer winters and summers led to good crop yields over most of Europe, with wheat and vineyard being grown in colder regions than today. In the Northern Hemisphere, the period around ad 1000 to 1100 featured high temperatures-similar to those recorded between 1961 and 1990 while minimum temperatures about 0.7 $^{\circ}C$ below the average of 1961-90 may have occurred around ad 1600 (Moberg et al., 2005). However, neither the Little Ice Age nor the Medieval Warm Period were climatically homogeneous periods nor did they exhibit uniform temperatures everywhere in the Earth. Instead, they featured regionally complex spatial rain patterns and asynchronous warming, most probably as a result of coupled ocean–atmosphere processes (Cronin et al., 2010). For instance, during the LIA, temperatures of areas like eastern China or the Northern Andes did not experience a significant reduction. Nonetheless, past climate changes have been different compared to the recent one, as the climate changes have not been recorded worldwide unlike the current (Neukom et al., 2019a,b; Brönnimann et al., 2019).

In this respect, and based on the aforementioned studies, the perceptions recorded in the survey does not match with the facts, since most of the inquired academics (6̃7.2 %) believe that the current climate change is the first climate change humans are experiencing. Hence, there has been no clear recognition of earlier climate changes since humans appeared on the earths surface (Fig. 2). The options of totally and mostly agree reach an average of 39.77 % of the results, while the options of totally and mostly disagree sum up only 32 % on average; furthermore, 27.42 % of the answers correspond to the option probably. However, among the four cities surveyed, Ibarra and Riobamba are the ones with a higher percentage (38 %) of totally and mostly disagree answers, hence, with a better knowledge of the facts, while Quito is the city with the lowest percentage (20,5 %) in these options, showing a worse correspondence with the reality. Independent of the city, the diverse results clearly indicate that there is lack of agreement in the perception of this issue, most likely linked to a lack of knowledge or less to no access on facts about paleoclimatology.

B) The Ecuadorian perspective

The second part of the survey aims to evaluate the knowledge and perceptions of the Ecuadorian academics about many visible or perceived changes in the climate of their own country. Therefore, the first question of the second part (#6) has been if the inquired people think that Ecuador experiences a climate change. The answers agreed with about 60 % to 80 %, having a concurrent perception in all four sites of the survey. From past events, it is commonly known that the geographic location of

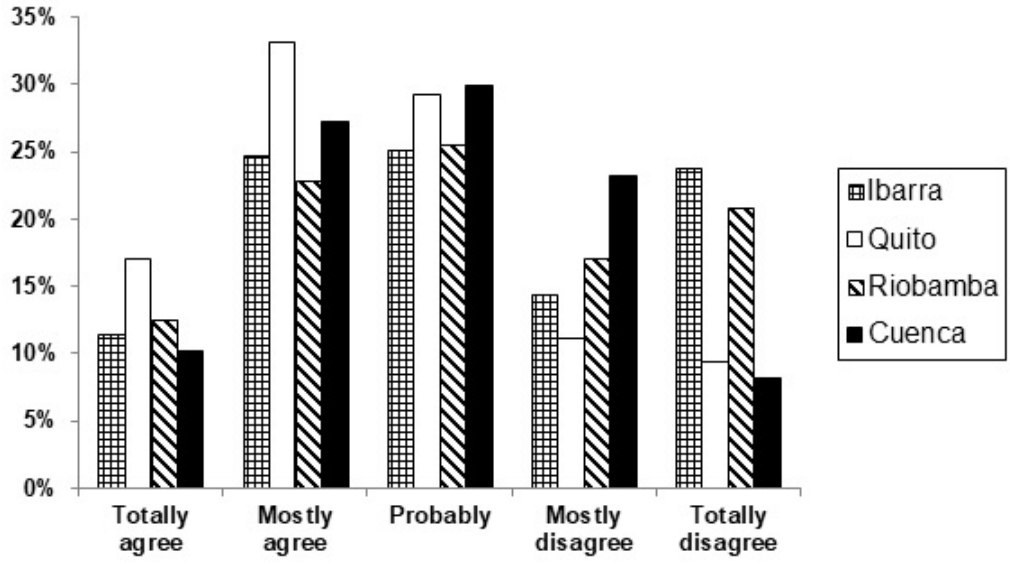

Figure 2. Answers with a histogram to the question #5 of the survey with the question-affirmation that since the existence of human history (humanity) it is the first time that there is a climate change impacting the planet.

Ecuador makes it a vulnerable country to climate change due to the effects that the ENSO has over the Latin-American coasts but also more inland, in the Highland and even the Amazonian Lowland, although with opposite effects since thousands of years (Rodbell et al., 1999; Riedinger et al., 2002; Terneus and Gioda, 2006). These effects, together with corroborating observations of an increasing trend in temperature, and increases in intra and inter-annual variations, may be some of the main reasons to agree that a climate change is occurring in Ecuador.

In Ecuador, the impacts of climate change are highlighted in a variety of observations. There is first the intensification of extreme climatic events, such as those that occurred as a result of the ENSO phenomenon; specifically, the El Niño extreme events of 1982-83 and 1997-98, and in later years, causing significant damage to livelihoods, agriculture and infrastructure (Rossel and Cadier, 2009; Aceituno et al., 2009; Bendix et al., 2011). Secondly, a certain rise in sea level has been determined during the aforementioned climatic variations (Cucalon, 1987; Rodbell et al., 1999). The increase of the sea level threats to coastal flooding of near-coast towns and further towns as the effect of the ENSO was also enhanced. Third, the retreat of the glaciers which is most visible particularly in summer, with tremendous retreat ranges (Francou et al., 2000; Jordan et al., 2005; Francou et al., 2005). Fourth, a determined decrease in annual runoff, as indicated in a variety of studies (Yates, 1997; Poulenard et al., 2001; Laraque et al., 2007; Zubieta et al., 2015). Furthermore, in Ecuador there has been an increase in the distribution range of dengue, malaria and other tropical diseases (Gueri et al., 1986; Ruiz and Roeger, 1994; Kovats et al., 2001; Gabastou et al., 2002; Stewart-Ibarra and Lowe, 2013; Stewart-Ibarra et al., 2014; Padilla et al., 2017), as a consequence of temperature and humidity rise, which create greater areas suitable for the expansion of tropical diseases (Reguero et al., 2015; Ibárcena and Scheelje, 2003). Additionally, the expansion of populations of invasive species in Galapagos and other sensitive ecosystems of continental Ecuador (Schofield, 1989; Mauchamp, 1997; Roque Albelo and Causton, 1999; Wikelski et al., 2004; Chaves, 2018; Rueda et al., 2019; Urquía et al., 2019) and finally even the extinction of several species (Haase, 1997; Bataille et al., 2009; Moret et al., 2016).

Regarding the question #7, about global climate change, a few aspects must be mentioned about this issue. The geographic location of Ecuador at the equator allows higher solar radiation, which added to a rise of the global temperature may also represent a threat for the human health of this region

such as skin cancer (Farmer et al., 1996; Duro Mota et al., 2003; Torre et al., 2015; Echegaray-Aveiga et al., 2018). Global warming, besides affecting the crops and vegetation, also contributes to the sea level rise due to thermal expansion in the ocean and the glaciers melt (Solomon et al., 2009). The elevation of the glaciers in Ecuador is higher than in other countries, for this reason, a change in the Equilibrium Line Altitude (ELA) may have critical impacts in glacier regression and even its disappearance. Many provinces in Ecuador, such as Pichincha and Cotopaxi obtained part of their water from the glaciers; hence, its reduction will cause serious social issues (Rojas, 2016). Some of the problems that Ecuador would have to face with an ongoing climate change and increase of the average temperatures would be the scarcity of water in some cities, such as Quito, fact similar to Lima in Peru, where their water supply derives predominantly from glaciers (Buytaert et al., 2017; Beeman and Hernández, 2018; Johansen et al., 2018). The glacier in Latin America, like almost all glaciers worldwide, are in constant reduction (La Frenierre and Mark, 2017; Milner et al., 2017). Several studies have concluded that particularly the tropical glaciers below an altitude of 5500 meters above sea level will be vanished in less than a decade (Chadwell et al., 2016; Veettil et al., 2017; Wu et al., 2019). Several glaciers have been already reduced dramatically or disappeared completely such as those in Bolivia and the one-third of the Quelccaya ice cap in Peru (Miranzo, 2015; Veettil et al., 2016; Yarleque et al., 2018). In Ecuador its effects are also seen in the drought of the Andes Cordillera and flood of the coastal area (MAE, 2017). Furthermore, the nature impacts or issues in Ecuador include expansion of the invasive species in Galapagos islands and other protected areas, a decrease of ecosystems and extinction of species (Diaz, 2012).

On the other side, also beneficial issues may arise by the appearing climate change conditions worldwide as well as in Ecuador (Bonan, 2008; Wassmann et al., 2009). There are areas where precipitation have been very low and the surrounding population struggle in their agronomic outcome for decades may benefit from higher precipitations and up to the potential use of dry and or unfertile areas for future exploitation for agricultural purposes (Cassman, 1999; Barrow, 2012; Junk, 2013). This would also allow a variety of plants and also animals to thrive in these new evolved warmer ecological niches. Farmers will be able to plant crops earlier in the year, having the opportunity to obtain potentially more harvest than before (Grau and Aide, 2008; Sissoko et al., 2011). Trees may be able to be planted at higher altitudes due to the now given warmer conditions, which simultaneously may allow farmers to use higher altitudes for pasture and livestock (Grace et al., 2002; Hemery, 2008; Hemery et al., 2010; Mathisen et al., 2014). Based on higher temperatures worldwide, there is a less need of cutting trees or even entire forests for firewood, which will subsequently lead to a decelerated evolution of global warming with less wood burning (Herrero et al., 2013; Rahn et al., 2014). Warmer conditions lead to a less use of fossil fuels, in order to warm up homes (Edwards et al., 2004; Sathre and Gustavsson, 2011). Additionally, warmer environments are more conductive to human health against health related sicknesses of cold climates (Kalkstein and Greene, 1997; Khasnis and Nettleman, 2005; Epstein, 2000; Kovats and Hajat, 2008; Séguin et al., 2008; Wilke et al., 2019).

By comparing the scientific facts and the perceptions registered in the survey about question #7 ″ the climate change only generates disasters for Ecuador″, a fairly good correlation was identified be-tween facts and perceptions, since a majority of the surveyed academics (55 %) chose the options of mostly agree and probably. Therefore, there is a general belief that climate change mostly brings disasters or negative effects to Ecuador, although, at the same time, they also recognized that climate change may have some few positive effects in their country, which coincide with the facts exposed above (Fig. 3). The results are distributed among all categories, with each option reaching more than 10% of the responses. The options of totally and mostly agree reach an average of 44.08% of the results, while the options of totally and mostly disagree sum up only 27.35% on average; furthermore, 26.66 % of the answers correspond to the option probably. An analysis of the responses of the four locations surveyed shows that three out of the four cities, Ibarra, Quito and Riobamba, has its highest percentage in the option of mostly agree, in particular, 32.3% of academics from Quito chose this option, demonstrating a better knowledge of the facts. On the other hand, Ibarra and Cuenca exhibit divergent patterns: Ibarra is the city with highest percentage of both to-

tally agree and totally disagree, revealing more extremist perceptions, whereas Cuenca is the city with lowest percentage of both totally agree and totally disagree, and highest percentage of probably, indicating a conservative approach regarding the issue of effects of climate change in Ecuador. Overall, the diverse results indicate a lack of consensus in the perception of this issue, hence, a need of more detailed knowledge on facts about both negative and positive effects of climate change in their country.

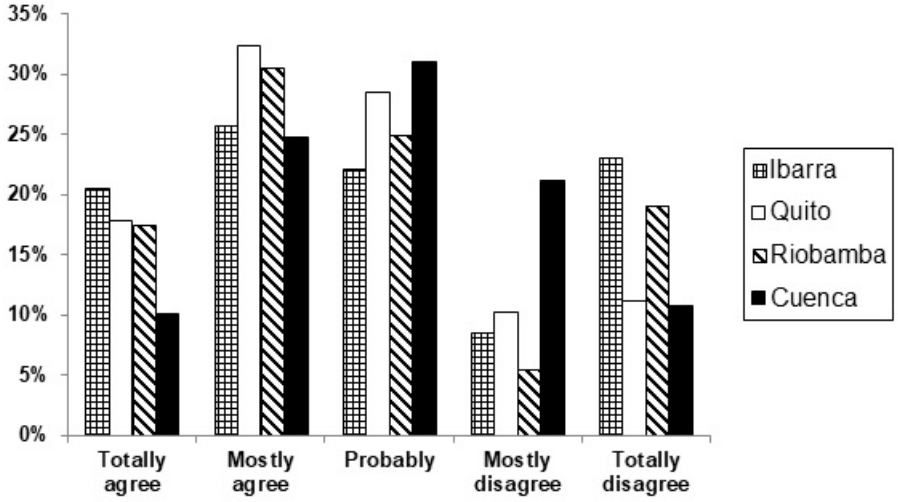

Figure 3. Histogram with the results based on question #7 of the survey with the question-affirmation if global climate change only generates disasters for Ecuador.

The question #8 of the survey pointed out about the features which may demonstrate the most the global warming and the climate change in Ecuador. The NOAA in 2015 determined that a new record in greenhouse gas emissions and global increase in temperatures was reached. As results, impacts on water and terrestrial ecosystems have been evident in all regions of the world. Ecuador has suffered several changes over time due to climate change, and it has been evident on the increasingly intense flooding, beach erosion, decreased biodiversity in the Andean paramos, retreat of glaciers, reduction of agricultural productivity and freshwater supplies. The city of Quito has undergone an increase of 1.1 $^{\circ}C$ from 1880 to 2017. The collateral effects of this increase have produced intense winter seasons that have increased in the last 30 years (García-Garizábal et al., 2017).

As for perceptions (Fig. 4), the academics believe that the feature which mostly shows climate change in Ecuador is more frequent droughts reaching 34.36% of the responses, the four cities coincided in choosing this option as the first. The second feature is the rarer but more intense rainfall with the 21.41% on average. These two options are indeed the extreme ends of the spectrum, and may seem opposite; however, as Martin2018 explains, when it gets warmer, water vapor will build up in the atmosphere, so when it does rain it rains a lot, but there will be longer periods between rain events so droughts will become worse, and more frequent.

Contrary to expectations, only 18% of the surveyed academics chose the option of increase in temperature, which indicates that Ecuadorians do not perceive the temperature as the most affected variable; on the contrary, they believe that rainfall is the main feature. However, this option exhibits divergent results among cities, Ibarra and Riobamba show high values around 26-27% while Quito and Cuenca report very low values of 9.6 and 8.4% , respectively. Some 9.98% on average opted for the

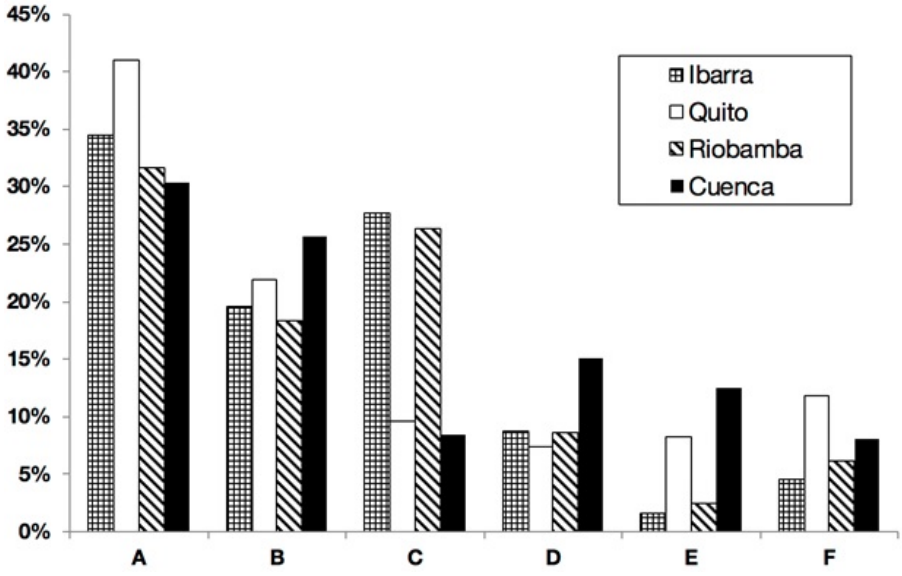

Figure 4. Histogram with the results based on question #8 of the survey with the multiple choice answers about which may are the main effects of climate change in Ecuador. A = more frequent droughts; B = rarer but more intense rains; C = increase in temperature; D = glacier retreat; E = sea level rise; F = lack of water.

feature of glacier retreat, but with marked differences between 7.4% in Quito, and Cuenca which doubles the percentage of Quito (15 %). Although the reduction of ice caps is a visible reality in Ecuador; it seems that the role of glaciers is not considered so relevant, especially in Ibarra, Quito and Riobamba. These results are more surprising considering that Quito and Riobamba are two cities which obtain part of its water from glaciers while Cuenca does not depend on glacier water.

The sea level rise was the option with lowest average percentage (6.22%) since it is minor and difficult to observe in Ecuador. Still, the results show a wide range, with higher val-ues for Cuenca (12.45 %) and Quito (8.3 %), and very low values for Riobamba (2.5 %) and Ibarra (1.63%), despite being all of them highland cities with similar distances from the coast. Regarding the lack of water, Quito shows the highest percentage with 11.78%, probably because it has a higher population. Therefore, the diversity in the inquiries demonstrated a coincidence with the heterogeneity of the real circumstances. Overall, the rainfall, due to its changing frequency and intensity trends, seems to be the feature that concerns the academics the most.

In early 2015, the Ecuadorean Ministry of Environment, conducted technical visits to demonstrate the effects of climate change in the Cuyabeno Lagoon and the retreat of the Chimborazo glacier. During these visits, the direct relationship between the low flow of this lagoon and the glacier was confirmed. It was determined in this study that one of the causes of this phenomenon is the deforestation, which alters the hydrological cycle, influencing the flow of nearby water sources. It was concluded that climate change directly affects the ecological flow in a decrease of 10% along with the reduction of biodiversity in these sites. Additionally, it was verified that the Chimborazo glacier has decreased by 58.9% between 1962 and 2010, with unusual landslide records of ice and stone material attributed to the increase in temperature in that area (Telégrafo, 2016).

In the southern region of the country, studies have been carried out about the vulnerability to climate change and potential impacts on ecosystems, biomass production, and water production. It was evident that Ecuador has a high vulnerability index to environmental changes related to global climate change due to anthropogenic and climatic factors as it has a diversity of ecosystems. The area studied has 41 ecosystems, all of these are strongly threatened by the exploitation of resources and changes

worldwide (Aguirre et al., 2015). Similarly, the effect in El Oro includes higher levels of precipitation while in Zamora Chinchipe it includes an increase in temperature. These patterns of temperature increase and precipitation in the study area could affect the physiology, phenology, dynamics, structure, the productivity of communities and the functioning of ecosystems. This evolution demonstrated the sensibility of the area to the anthropogenic activity and its negative effect (Aguirre et al., 2015).

A recent study carried out in the province of Santa Elena determined predictions of temperature increase in the coastal area of 2.7% by the end of the 21st century. The results were based on theoretical models and historical climate data. An increase of 8.2% could possibly lead to a reduction in the degree of aridity of the region, passing from arid semi-desert to a Mediterranean semi-arid, and from Mediterranean semi-arid to sub-humid (García-Garizábal et al., 2017).

The final question #9 focused more in detail about which area in Ecuador shows the most of the effects of global warming and climate change. Hereby, a relatively high percentage has indicated the coastal lowland (29%) with similar values, close followed by the Inter-Andean Valley with the 17.26 %, that are apparently the regions where the strongest effects of global warming may have occurred and still occur (Fig. 5). While inquiries of Cuenca opted also highly for the Subandean region (23 %), such site with 12.37 % as well as the Amazon basin with 10.07% on average have been less a target of global warming according to the majority of the opinions. The Galapagos Islands obtain only the 1.6% on average, demonstrating that academics in highlands do not consider them a target probably due to its distance, despite being a biodiversity hotspot with high climate vulnerability.

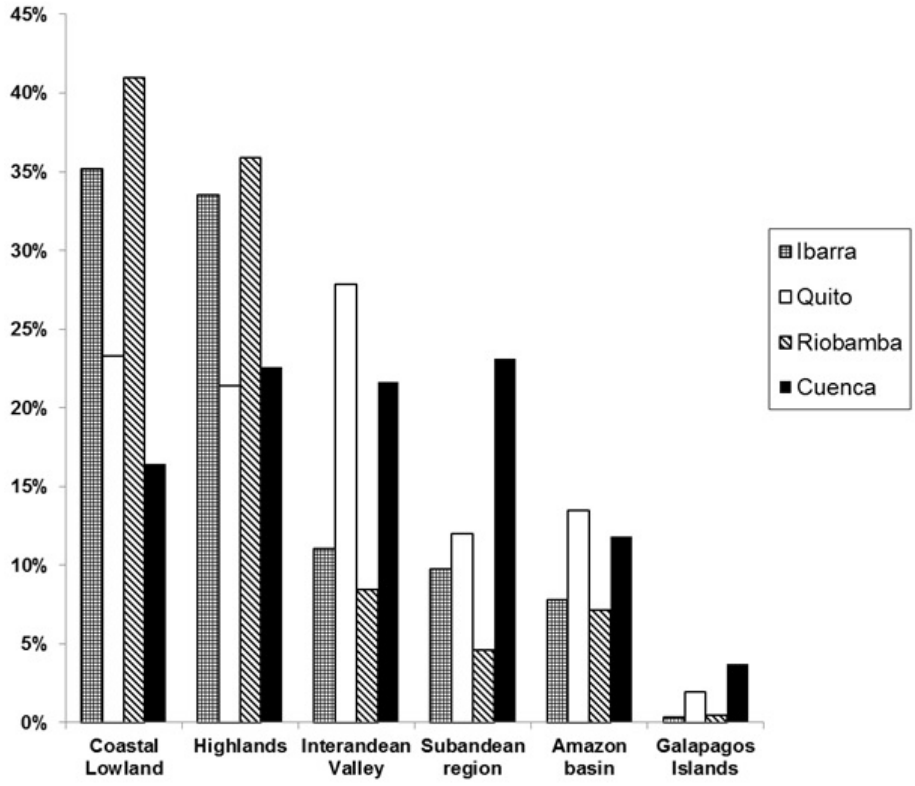

Figure 5. Histogram with the results based on question #9 of the survey with the question-affirmation about which regions of Ecuador are more affected by climate change.

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The results have indicated that people apparently tend to see that their own environment is having some severe effects as also previously demonstrated with #8 of the survey (Fig. 4). This allows to potentially interpret that people most likely do not know well or less frequent visit other regions in order to be able to have a better view and more aspects for a better evaluation of potential effects of global warming and also other atmospheric effects based on climate change. However, the coastal lowlands, with the highest percentage, especially in Ibarra and Riobamba (35.2 y 41 %), was an exception since it was generally perceived as highly impacted by climate change for the inhabitants of the highlands, who often watch on TV and newspapers the effects of El Niño and the recurrent floods in the Ecuadorian coast.

Finally, an overall view based on the survey shows that the two main patterns have been the tendency to choose options 'b= Mostly Agree' and 'c=Probably' which are ambiguous, but only in specific questions like 1 and 10 the options 'a= Totally agree' or 'e= Totally disagree' that are specific, were chosen. These two questions are highly spoken about in the media and institutes; hence, it is a clear choice. However, this 'certainty' is dependent on information that is broad and vague where no reference to scientific papers or primary source is given. The omission of the source in the publications of some newspapers in Ecuador was verified in 18 news about climate change. It was noticed that the answers of the academic population agreed with the scientific investigation performed around the world, however most of this knowledge lack of deepness. In addition, about the other 50 % indicates doubt. This is most likely related to the lack of information and poor research and data information about climate change worldwide in general and particularly about Ecuador.

4 Conclusions

There is lack of a wider perspective, discussion of the role of the government, corporations, academics and community in emission, mitigation and adaptation policies implementation.

The Ecuadorean academic community of the highlands is aware of many globally known facts of global warming and climate change. This includes the potential damages and disasters for a variety of environments. However, there is a clear lack of deepness of the origin and reach of such climate issues. There is also an obvious reduced awareness of adaptation issues as well as mitigation and personal preparation against eventual disasters as result of climate change.

Such conditions increase the vulnerability of Ecuador to the Climate change effects, as only few reflected knowledge about the real impacts and how they will affect the future of the country. There is a lack of think ahead planning, as demonstrated in the given results of the survey. However, the effects of the climate change in Ecuador are undeniable, and it is perceived mainly in the more frequent and intense flooding and droughts, and the retreat of the glaciers in the highlands.

Appendix

Figure 1A shows the questions corresponding to the survey conducted to generate the collection of information developed in Section 2.

GEO1 Geodinámica Interna y Externa
Universidad de las Fuerzas Armadas

ESPE
ESCUELA POLITÉCNICA DEL EJÉRCITO
CAMINO A LA EXCELENCIA

Survey on the level of perception and information of academics in Ecuador on Climate Change and Global Warming
Responsible Prof. Dr. Theofilos Toulkeridis GEO1 Research Group of DECTC-ESPE and ECUATORIAL NETWORK OF CLIMATE CHANGE

MARK ONLY ONE ANSWER PER CITY **QUESTION:** **AGE:**

1) Currently we do have climate change in the world
a) Totally agree b) Mostly agree c) Probably d) Mostly disagree e) Totally disagree

2) Climate change is generated from the activities of human beings
a) Totally agree b) Mostly agree c) Probably d) Mostly disagree e) Totally disagree

3) Climate change is a serious problem
a) Totally agree b) Mostly agree c) Probably d) Mostly disagree e) Totally disagree

4) Scientists unanimously agree that climate change exists
a) Totally agree b) Mostly agree c) Probably d) Mostly disagree e) Totally disagree

5) Since humanity exists, it is the first time that climate change occurs on Earth
a) Totally agree b) Mostly agree c) Probably d) Mostly disagree e) Totally disagree

6) We currently have a climate change in Ecuador
a) Totally agree b) Mostly agree c) Probably d) Mostly disagree e) Totally disagree

7) Climate change brings only disasters for Ecuador
a) Totally agree b) Mostly agree c) Probably d) Mostly disagree e) Totally disagree

8) Climate change and global warming can be seen in Ecuador more:
a) With the lack of drinking water b) With more frequent droughts c) With rarer rains but more torrential
d) With the reduction of glaciers e) With rising sea level f) With the increase in temperature every year

9) Climate change and Global warming is more noticeable in Ecuador in:
a) The Amazonian Basin b) The Highlands c) The Inter Andean Valley d) The Subandean Region e) The Coast f) Galapagos

10) The main cause of climate change and global warming is / are:
a) The increase of carbon dioxide in the atmosphere b) The increase of water vapor in the atmosphere c) Solar Explosions
d) The rise of Methane in the Atmosphere e) Generated from the astronomical constellation f) Generated from the Earth's Magnetic Force

11) Garbage recycling helps to curb climate change:
a) Totally agree b) Mostly agree c) Probably d) Mostly disagree e) Totally disagree

12) Forest fires accelerate climate change in Ecuador
a) Totally agree b) Mostly agree c) Probably d) Mostly disagree e) Totally disagree

13) Climate change is accelerating the gradual destruction of the ozone layer
a) Totally agree b) Mostly agree c) Probably d) Mostly disagree e) Totally disagree

14) There are changes at the genetic level in humans due to global warming
a) Totally agree b) Mostly agree c) Probably d) Mostly disagree e) Totally disagree

Figure 1. A. Survey about perception of climate change in Ecuador.

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URBAN ECOLOGY

Germination ecophysiology for three peri-urban ephemeral weeds from Morelia, Michoacán, Mexico

Ecofisiología de la germinación de tres malezas efímeras periurbanas en Morelia, Michoacán, México

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Resumen

Se determinaron las características ambientales que conducen a la germinación de tres especies comunes encontradas durante la temporada de lluvias entre junio-octubre de 2009 en un área periurbana de Morelia, Michoacán, México, donde se llevaba a cabo la construcción de un campus de la Universidad Nacional Autónoma de México (UNAM). En particular, se evaluaron en el laboratorio las respuestas a la estratificación a baja temperatura, la temperatura del aire en el día/noche y el potencial de agua para los nativos *Onagraceae Lopezia racemosa* y *Ludwigia octovalvis*, y las exóticas *Polygonaceae Rumex crispus*. La estratificación a baja temperatura no tuvo ningún efecto sobre la germinación por *L. racemosa*, para lo cual la germinación máxima promedio 88% fue óptima a 25/15 y 30/20 °*C*. La germinación a 21 d se redujo a la mitad a –0,5 MPa e inhibió por completo a –1,0 MPa. Las semillas de *L. octovalvis* tampoco mostraron sensibilidad a la estratificación a baja temperatura y su germinación nunca superó el 70%, siendo las dos temperaturas más altas de 30/20 y 35/25 °*C* las óptimas. Para esta especie la germinación fue máxima a 0,0 MPa, disminuyendo significativamente bajo cada tratamiento con una germinación mínima del 21% para las semillas incubadas a –0,1 MPa. La germinación para *R. crispus* se retrasó por la estratificación a baja temperatura, aunque todas sus semillas germinaron independientemente de la temperatura o el tratamiento potencial de agua. Si bien los requisitos ambientales para la germinación de especies efímeras a menudo coinciden con el clima típico de su temporada de crecimiento, las respuestas diferenciales encontradas para las especies consideradas en el presente estudio proporcionan una cierta visión de los mecanismos que conducen a cambios en la composición de las especies para las comunidades de ambientes perturbados, incluyendo el desplazamiento de especies nativas y la proliferación de plantas exóticas y potencialmente invasoras.

Palabras clave: Especies invasoras, ecofisiología reproductiva, estratificación, temperatura, ecología urbana, potencial hídrico.

Abstract

The environmental requirements leading to germination were determined by three common species found during the June-October 2009 rainy season in a peri-urban site from Morelia, Michoacán, Mexico, where the construction of a campus of the Universidad Nacional Autónoma de México (UNAM) was underway. In particular, we evaluated responses in the laboratory to low-temperature stratification, day/night air temperature, and water potential for the native Onagraceae *Lopezia racemosa* and *Ludwigia octovalvis*, and the exotic *Rumex crispus*. Low-temperature stratification had no effect on germination by Polygonaceae *L. racemosa*, for which maximum germination averaging 88% was optimal at 25/15 and 30/20 °*C*. Germination at 21 d was halved at –0.5 MPa and completely inhibited at –1.0 MPa. The seeds of *L. octovalvis* were also insensitive to low temperature stratification and their germination never exceeded 70%, with the two highest temperatures of 30/20 and 35/25 °*C* being the optimum. For this species germination was maximal at 0.0 MPa, decreasing significantly under every treatment with a minimum germination of 21% for seeds incubated at –0.1 MPa. Germination for the exotic *R. crispus* was delayed by low-temperature stratification, although all its seeds germinated regardless of the temperature or water potential treatment. While the environmental requirements for germination of ephemeral species often match the typical climate of their growing season, the differential responses found for the species considered in the present study provide some insight into the mechanisms leading to changes in species composition for communities from disturbed environments, including the displacement of native species and the proliferation of exotic, potentially invasive plants.

Keywords: Invasive species, reproductive ecophysiology, stratification, temperature, urban ecology, water potential.

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1 Introduction

The species composition of annual and ephemeral plant communities can change substantially from year to year (Cousens and Mortimer, 2009; Chaideftou et al., 2012). The identities of the species observed in any given growing season depends, of course, on which species are present in the seed bank or have dormant regeneration structures in the soil, but their emergence is greatly modulated by their interactions with the physical environment. Indeed, the prevalent temperature and available water can differentially trigger germination at various times during the growing season of a particular year or along various years (De la Barrera et al., 2009), mediated by structural (such as a thick testa) (Debeaujon et al., 2000; Dübbern de Souza and Marcos-Filho, 2001; Rowarth et al., 2007) or physiological seed traits (such as the need for cold-vernalization) (Larcher, 2001; Fenner and Thompson, 2005) that determine both the moment and the rate of germination.

In this respect, the environmental conditions created in habitats resulting from the clearing of vegetation for agricultural purposes, roads, urban development, etc., can restrict the persistence of native species in plant communities, while favoring the establishment of species, native or not, that can tolerate and even thrive under disturbance (Cousens and Mortimer, 2009; Dekker, 2016). Indeed, landscape modification by human actions is a major threat to global biodiversity (Sala et al., 2000; Rockström et al., 2009). This is an issue of special concern in Mexico, one of twelve megadiverse countries, which is, however, experiencing a rapid loss of vegetation cover. Indeed, while 62% of the country's surface had vegetation cover in 1976, a mere 38% remained with vegetation by 2002 (De la Barrera and Andrade, 2005; Challenger and Dirzo, 2009).

Three species that are commonly found in such disturbed sites in Mexico, as agricultural weeds, are the native Onagraceae *Lopezia racemosa* and *Ludwigia octovalvis*, and the exotic Polygonaceae *Rumex crispus* (Calderón and Rzedowski, 2004; Vibrans and Tenorio-Lezama, 2012). *Lopezia racemosa* can be found in various vegetation types including conifer, oak, and cloud forests, in addition to grasslands and thorn scrub. *Ludwigia octovalvis* is mostly restricted to sites with high soil humidity such as riparian ecosystems and along irrigation canals. In turn, *R. crispus*, which is native to Eurasia, has become a common weed throughout the world, especially in the temperate zones of the Northern Hemisphere. These three species were abundant elements of the ephemeral flora of our university campus, during the summer growing season of 2009 (unpublished observations). Located in a peri-urban site, the campus was established in 2005 in a former eucalyptus plantation and has been undergoing construction to house various academic entities, which makes its ephemeral flora an interesting research subject as it changes over the years.

In order to determine the environmental conditions leading to seed germination by *L. racemosa*, *L. octavalvis*, and *R. crispus*, three ephemeral plants from a peri-urban site with different ecological niches, a series of controlled-environment experiments were conducted to determine the temperature relations and the influence of water potential on the time and rate of germination.

2 Materials and Methods

Seeds of *Lopezia racemosa* Cav. (Onagraceae; hereafter referred to as *Lopezia*), *Ludwigia octovalvis* (Jacq.) Raven (Onagraceae; *Ludwigia*), and *Rumex crispus* L. (Polygonaceae; *Rumex*) were collected during the growing season of June-October 2009 from Universidad Nacional Autónoma de México, Campus Morelia (19°38′55.9″ N; 101°13′45″ O; 1967 m), where a lack of construction and landscaping allowed the establishment of spontaneous vegetation. Seeds of at least 7 individuals per species were collected by shaking the main stem of plants. The released seeds were placed in cloth bags and mixed into a compound sample. Seeds were kept in black paper bags and stored in the Agrarian Ecophysiology Laboratory, Instituto de Investigaciones en Ecosistemas y Sustentabilidad, UNAM (in the dark at 23 °*C*, 40% relative humidity) until utilized within 6 months. Seeds were rinsed with running water during 48 h immediately before the beginning of each experiment.

Germination was studied for experimental units consisting of 25 seeds placed in covered plastic petri dishes (55 mm in diameter, 15 mm high) with two

layers of sterile filter paper as substrate. Usually 5 ml of sterile distilled water were added to each petri dish, with additional water being added over the course of the experiments to keep the filter paper saturated. The petri dishes were placed in an I-35LL germination chamber fitted with fluorescent light tubes (Percival Scientific, Boone, Iowa, USA) under a photoperiod of 12 h, usually at a day/night temperature of 20/10 °*C*. Germination, which was scored as seeds with radicle protrusion, was evaluated daily, until no further germination occurred.

Germination responses to low-temperature stratification were evaluated by placing a sample of seeds at 8 °*C* during 3 months and their germination was compared to that of a control of untreated seeds. The effect of air temperature on germination was evaluated for seeds incubated under day/night air temperatures of 20/10, 25/15, 30/20 o 35/25 °*C*. In turn, the effect of water potential on germination was evaluated for seeds incubated under water potentials of 0.00, –0.01, –0.05 y –0.1 MPa that were created with aqueous solutions of polyethylene glycol (PEG; molecular weight of 20.000) of various concentrations that were determined following Michel and Radcliffe (1985).

Twelve replicates of 25 seeds each were utilized for each measurement. Statistical analyses for the onset of germination, i.e., the first day significantly higher than 0%, consisted of a Mann-Whitney U test (alpha = 0.05) for stratification and a Kruskal-Wallis test (alpha = 0.05) for temperature and water potential, which were performed with Statistica 7.0 (StatSoft Inc., Tulsa, Oklahoma, USA). In addition, the process of germination was analyzed with a repeated measures Friedman test followed by post hoc Tukey tests (alpha = 0.05) performed with SigmaStat 3.5 (Systat Software, Richmond, California, USA).

3 Results

3.1 *Lopezia racemosa*

Low-temperature stratification had no effect on germination for *Lopezia* (Table 1; Fig. 1A). For both the control and treated seeds the onset of germination averaged 6.2 ± 0.7 days after the start of the experiment. In turn, the maximum germination for this species averaged 78.7 ± 1.6 % after 21 days of the start of the experiment.

The temperature regime, in turn, had a significant effect on the germination of *Lopezia* (Table 1; Fig. 1D). For instance, the onset of germination occurred after one day of the start of the experiment for seeds incubated under 25/15 °*C*, after two days for those under 30/20 y 35/25 °*C*, while six days were required to trigger the germination for seeds of *L. racemosa* incubated under 20/10 °*C*. Final germination averaged 87.7 ± 2.7 % for seeds incubated under 25/15 and 30/20 °*C* and they were 79.0 ± 2.0 and 76.0 ± 1.3 % lower for those under the extremes of 20/10 and 35/25 °*C*, respectively.

Germination by *Lopezia* responded to the water potential (Table 1; Fig. 1G). The onset of germination occurred at 2 days after the start of the experiment for seeds incubated under 0.0 MPa, the following day for those under –0.01 MPa, and at day six for those under –0.05 MPa, while the seeds incubated under –0.10 MPa failed to germinate. A final germination of 91.33 ± 1.69 % observed after 21 d of the start of the experiment was maximal for seeds incubated under 0.00 MPa, decreasing with increasingly negative water potential until a minimum that was 41.67 ± 1.94 % lower was observed for seeds incubated under –0.05 MPa.

3.2 *Ludwigia octovalvis*

Low-temperature stratification improved germination for *Ludwigia* (Table 1; Fig. 1B). Indeed, the onset of germination occurred at 11.1 ± 0.7 days after the start of the experiment for the control, while treated seeds germinated 2.3 days earlier. Also, while the final germination after 21 days of incubation was similar for both groups of seeds, it occurred at a faster rate for stratified seeds.

The temperature regime had a significant effect on germination by *Ludwigia* (Table 1; Fig. 1E). In particular, the onset of germination increased with air temperature and it ranged from 2.0 ± 0.0 days after the start of the experiment for seeds incubated at 35/25 °*C* to 9 days later for seeds under the lowest temperature regime of 20/10 °*C*. Final germination at 21 days after the start of the experiment was also higher and occurred at a faster rate with increasing temperature. For instance, 68.0 ± 3.0 % of seeds germinated when incubated under 35/25

$°C$, while a mere 40.3 $\pm$ 3.5 % germinated under 20/10 $°C$.

Water potential significantly affected germination by *Ludwigia* (Table 1; Fig. 1H). The onset of germination, which occurred at 3.5 $\pm$ 1.6 for seeds exposed to 0.0 or –0.01 MPa, was delayed up to 4 days at lower water potentials. In turn, final germination at 21 days after the start of the experiment was also higher and more rapid at lower water potentials, ranging from 76.0 $\pm$ 3.4 % for seeds incubated under 0.0 MPa to 20.7 $\pm$ 1.1 % for those under –0.1 MPa.

Table 1. Germination parameters for the ephemeral weeds *Lopezia racemosa*, *Ludwigia octovalvis* and *Rumex crispus*. The onset of germination (first day statistically different from zero) was evaluated with Mann-Whitnney U tests (alpha = 0.05) for stratificationn and Kruskal-Wallis tests (alpha = 0.05) for the temperature and water potential experiments. In turn, the final germination was evaluated with Tukey tests following a Friedman repeated measures test. Data are shown as mean $\pm$ S.E. (n =12 petri dishes with 25 seeds each). For each parameter and experiment different letters indicate statistical differences (alpha = 0.5).

	Seed germination			
Lopezia racemosa	Onset (days after the beginning of the experiment)		Maximum percentage of germination	
Control	6.5 $\pm$ 0.31	a	79.0 $\pm$1.98	a
Stratification	6.0 $\pm$ 0.0	a	77.3 $\pm$ 2.62	a
20/10$°C$	6.5 $\pm$ 0.31	a	79.0 $\pm$ 1.98	a
25/15$°C$	1.0 $\pm$ 0.0	b	85.3 $\pm$ 2.48	b
30/20$°C$	2.3 $\pm$ 0.14	c	90.0 $\pm$ 3.53	b
35/25$°C$	2.0 $\pm$ 0.0	c	76.0 $\pm$ 1.3	c
-0.00 MPa	2.3 $\pm$ 0.1 4	a	91.3 $\pm$ 1.69	a
-0.01 MPa	3.0 $\pm$ 0.0	a	88.0 $\pm$ 1.78	b
-0.05 MPa	6.1 $\pm$ 0.47	b	41.7 $\pm$ 1.94	c
-0.10 MPa	11.0 $\pm$ 0.58	b	1.0 $\pm$ 0.52	d
Ludwigia octovalvis				
Control	11.1 $\pm$ 0.74	a	40.3 $\pm$ 3.46	a
Stratification	8.8 $\pm$ 0.65	b	41.3 $\pm$ 3.28	b
20/10$°C$	11.1 $\pm$ 0.74	a	40.3 $\pm$ 3.46	a
25/15$°C$	4.6 $\pm$ 0.15	ab	43.7 $\pm$ 2.85	b
30/20$°C$	3.8 $\pm$ 0.11	bc	61.7 $\pm$ 4.52	c
35/25$°C$	2.0 $\pm$ 0.0	c	68.0 $\pm$ 2.99	d
-0.00 MPa	3.2 $\pm$ 0.24	a	76.0 $\pm$ 3.38	a
-0.01 MPa	3.8 $\pm$ 0.17	ab	64.0 $\pm$ 1.97	b
-0.05 MPa	5.2 $\pm$ 0.24	bc	47.3 $\pm$ 2.73	c
-0.10 MPa	7.2 $\pm$ 0.41	c	20.7 $\pm$ 1.08	d
Rumex crispus				
Control	1.0 $\pm$ 0.0	a	100.0 $\pm$ 0.0	a
Stratification	1.0 $\pm$ 0.0	a	100.0 $\pm$ 0.0	b
20/10$°C$	1.0 $\pm$ 0.0	a	100.0 $\pm$ 0.0	a
25/15$°C$	1.0 $\pm$ 0.0	a	100.0 $\pm$ 0.0	b
30/20$°C$	1.8 $\pm$ 0.37	a	99.3 $\pm$ 0.45	a
35/25$°C$	1.1 $\pm$ 0.08	a	99.7 $\pm$ 0.33	b
-0.00 MPa	2.2 $\pm$ 0.17	a	100.0 $\pm$ 0.0	a
-0.01 MPa	2.4 $\pm$ 0.15	a	100.0 $\pm$ 0.0	ab
-0.05 MPa	2.8 $\pm$ 0.11	ab	99.7 $\pm$ 0.33	b
-0.10 MPa	3.2 $\pm$ 0.11	b	100.0 $\pm$ 0.0	c

3.3 *Rumex crispus*

Low-temperature stratification delayed germination for *Rumex* (Table 1; Fig. 1C). The onset of germination occurred at the first day after the start of the experiment regardless on whether the seeds had been exposed to air temperature of 8 $^{\circ}C$ for three months or not. Maximum germination after 21 days of incubation was not affected either, reaching 100.0 $\pm$ 0.0 %. However, the maximum was reached faster by untreated seeds.

Incubation temperature did not affect the onset of germination by *Rumex*, which occurred at 1.2 $\pm$ 0.1 days after the start of the experiment regardless of the treatment, nor the final germination at 21 days of incubation, reaching 99.7 $\pm$ 0.2 % (Table 1; Fig. 1F). However, higher air temperatures led to a faster germination rate, as the seeds incubated at 25/15 and 35/25 $^{\circ}C$ reached the germination maxima up to one week faster than those exposed to 20/10 $^{\circ}C$ of day/night air temperatures.

The onset of germination for *Rumex* averaged 2.5 $\pm$ 0.1 days after the start of the experiment, and it only was significantly delayed for seeds incubated under a water potential of –0.1 MPa, which took up to a day longer (Table 1; Fig. 1I). The final germination of 99.9 $\pm$ 0.1 % after 21 days of incubation was also unaffected by the water potentials tested, but the rate was slower under more negative water potentials. For instance, it took 3-5 days to reach final germination by seeds exposed to 0.0 or –0.01 MPa and up to 12 days for those incubated under –0.10 MPa.

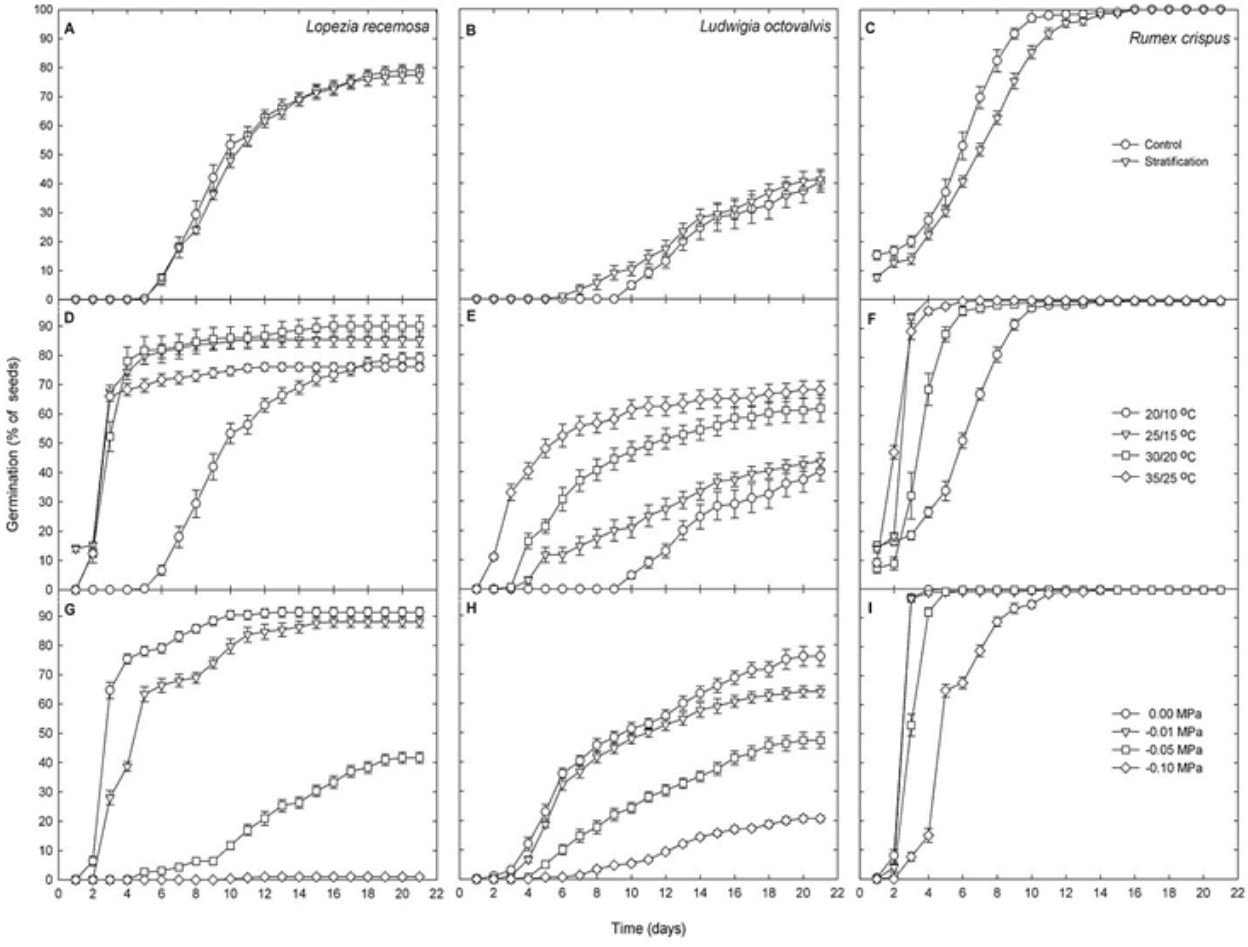

Figure 1. Germination for the ephemeral weeds *Lopezia racemosa* (A, D, G), *Ludwigia octovalvis* (B, E, H) and *Rumex crispus* (C, F, I), in response to low-temperature stratification (A-C), day/night air temperature (D-F), and water potential (G-I). Data are shown as mean $\pm$ SE (n =12 12 petri dishes with 25 seeds each).

4 Discussion

The identity of the species that persist in a community subjected to disturbance depends on which species were originally present in the seed bank and how they respond to the new environmental conditions. Indeed, germination for generalists and species with rapid germination and preference for open sites are likely to be favored by disturbance (Grime, 2006; Del-Val et al., 2015; Gorgone-Barbosa et al., 2016). In the present study, both the exotic weed *Rumex* and the generalist native *Lopezia* germinated under all the experimental conditions, although their germination was delayed or diminished under the most extreme treatments. In contrast, germination for *Ludwigia*, a specialist of high-humidity environments only exceeded 50 % under the most benign conditions. These differential responses of co-occurring species can provide some insight on potential trends of community composition following disturbance.

Species from regions with marked seasonality, i.e., with distinct dry and wet seasons or with prolonged sub-freezing periods, often display an embryonic dormancy that can delay the onset of germination until the season with environmental conditions, favoring seedling establishment (Donohue et al., 2010). For these plants, germination is often improved by exposing seeds to low temperatures during several weeks, by which germination is avoided during the winter, when the prevailing low air temperatures can be lethal for seedlings, even if some precipitation occurs.

This appears to be the case for *Ludwigia* whose stratified seeds germinated 2 days faster than untreated seeds, in agreement with previous studies with its congenerics *L. alternifolia* and *L. decurrens* (Baskin and Baskin, 1988). However, this was not the case for *Lopezia* not for *Rumex*, suggesting that they either lack primary dormancy or that it had been overcome during storage (Totterdell and Roberts, 1979; Chi, 2006; Finch-Savage and Leubner-Metzger, 2006).

Higher temperatures tended to hasten and increase final germination for the species considered in the present work. However, the highest temperature resulted in the lowest germination for *Lopezia*.A similar response, where germination improves with temperature until an optimum, is reached followed by an inhibition of germination observed for the seeds of *Ludwigia* which were the most sensitive. In turn, the exotic weed *Rumex* reached maximum germination regardless of the incubation temperature, confirming observations for many weeds, which are able to tolerate and even thrive under high temperatures, an advantageous trait for survival in the extreme environmental conditions of naked soil (Dahlquist et al., 2007; Baskin and Baskin, 2014).

Germination tended to decrease as the incubation water potentials became more negative, especially at and below –0.05 MPa, similar to responses of other species such as *Campsis radicans* (Chachalis and Reddy, 2000). In contrast, germination for *Rumex* was maximal regardless of the water potential, in agreement with the germination behavior of its congeneric *R. acetosella*, which reaches 90 % germination under –0.1 MPa (Fani et al., 2013). Considering that substantial amounts of water are required to sustain cell elongation for plants following germination, especially for species that escape or avoid drought by completing their lifecycle during the rainy season, it may be advantageous to restrict germination to times when an adequately high water potential signals that the rainy season is well underway or that at least sufficient water is stored in the soil, allowing seedling development (De la Barrera et al., 2009). However, the water potentials assayed here correspond to a mere ca. 7 days after suspending irrigation for a sandy loam, well above the permanent wilting point of –1.5 MPa, which indicates the sensitivity of germination (Young and Nobel, 1986; Nobel, 2009).

5 Conclusions

The environmental requirements for germination of ephemeral species are often predictable to match those resulting from the typical weather of their growing season (De la Barrera and Nobel, 2003; Donohue et al., 2010; Baskin and Baskin, 2014). However, interspecific differences, however small, can determine the identities of the plants that grow in a community during a given year, contributing to the high interannual species variation that has been observed for ephemeral and annual floras (Cousens and Mortimer, 2009; Chaideftou et al., 2012; Dekker, 2016; Gorgone-Barbosa et al., 2016). The differential responses found in the present study illustrate these interspecific idiosyncrasies and provide insight, for instance, into the mechanisms that enable exotic species to become dominant or for native species to be displaced. The emergence of novel environments resulting from human interventions poses a risk to the prevalence of native floras. Further studies considering larger community subsets or even whole communities are required, including long-term observations aimed at characterizing the rate at which native floras may be shifting in response to anthropic environmental changes.

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POLLUTANT ANALYTIC

Verification of the atomic absorption spectroscopy with graphite furnace analytical method for the quantification of cadmium in cocoa almonds (Theobroma cacao)

Verificación del método analítico de espectroscopía de absorción atómica con horno de grafito para la cuantificación de cadmio en almendra de cacao (Theobroma cacao)

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Resumen

El método de espectroscopía de absorción atómica (AA) de llama para la determinación de cadmio (Cd) en almendra de cacao (*Theobroma cacao*) utilizado por Agrocalidad es tóxico para el ser humano y el ambiente; por ello, se pretende utilizar el método de espectroscopia de absorción atómica con horno de grafito (GFAAS) por ser más confiable y seguro. Así, se realizó la verificación de cuatro parámetros de desempeño del método GFAAS para cuantificar Cd en almendra de cacao utilizando material de referencia certificado (MRC) y muestras provenientes de cuatro fincas (A, B, C, D) ubicadas en la zona cacaotera de Ecuador, cantón Flavio Alfaro, provincia de Manabí. Se realizó una prueba inter-laboratorios y finalmente se elaboró el protocolo (PEE/B/14). Sobre el MRC (Cód. 07206B y 07167A) se verificó: linealidad, precisión, veracidad e incertidumbre de acuerdo con la *Guía Eurachem* de Eurolab España et al. (2016), y con el estándar IRAM 35050 (2001) se encontró linealidad entre 0 y 8 ppb con R^2=0.9988; desviación estándar de 0.0005 y 0.0022 respectivamente; sesgo en 0.007 y porcentaje de recuperación de 109.75; la incertidumbre estándar de 0.00013 y 0.00082. El contenido de Cd en las muestras de la finca A con 0.54 ppm, las Fincas B-D con 0.26 ppm y 0.15 ppm en la finca C. En la prueba inter-laboratorios se estableció la misma concentración de cadmio para la muestra C3 y, de acuerdo con lo estipulado por la Unión Europea, el cacao de las cuatro fincas podría ser exportado sin restricciones.
Palabras clave: Cadmio, cacao, verificación, análisis, método.

Abstract

The flame atomic absorption (AA) spectroscopy method for the determination of cadmium (Cd) in cocoa almond (*Theobroma cacao*) used by Agrocalidad is toxic to humans and the environment, reason for which the atomic absorption spectroscopy method with graphite furnace (GFAAS) was used, because it is more reliable and safer. Thus, four performance parameters of GFAAS method were used to quantify Cd in cocoa almond, by using certified reference material (MRC) and samples from four farms (A,B,C,D) located in the most important cocoa area of Ecuador, Flavio Alfaro city, province of Manabi. An interlaboratory test was performed and finally a protocol (PEE/B/14) was developed. Using the MRC (Code 07806B and 07167) was verified: linearity, precision, veracity and uncertainty in accordance with international standards, *Eurachem Guide* of Eurolab España et al. (2016), and with the standard IRAM 35050 (2001) were found linearity between 0 and 8 ppb with R^2 = 0.9988; standard deviation 0.0005 and 0.0022, respectively; slant was 0.007 and the recovery percentage was 109.75; standard uncertainty was 0.00013 and 0.00082. The content of Cd in samples from farm A was 0.54 ppm, B and D farms 0.26 ppm and 0.15 ppm in farm C. In the interlaboratory test, the same concentration of Cd was established for simple C3 and, in accordance with the stipulated by the European Union, cocoa from the four farms could be exported without restrictions.
Keywords: Cadmium, cocoa, verification, analysis, method.

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1 Introduction

Cocoa beans are the seeds of the *Theobroma cacao* tree (native to the Amazon Region of South America), which are widely consumed worldwide (Almeida and Valle, 2007). It is an important neotropical, perennial crop growing at 20° north and 20° south of the equatorial line (Shavez Beg et al., 2017). It is grown at a height of less than 400 m.a.s.l; the optimal temperature ranges from 22 $°C$ to 30 $°C$, depending on the variety (Damatta et al., 2018), and rain should be at least 1500 to 2500 mm of water per year. The almonds are brown and are covered on the outside by a sweet white mucilage that is edible (Waizel-Haiat et al., 2012); grains are processed to obtain chocolate liqueur, cocoa powder and cocoa butter, which are the main ingredients of chocolate and a wide range of products such as cocoa drinks, ice cream, bakery products that distribute a distinctive flavor to derivatives (Dasgupta and Klein, 2014). Cocoa beans are the raw material for the multibillion-dollar industry that produces chocolate and confectionery products, the economic importance of the chocolate industry in the world market has recently been revised (Squicciarini and Swinnen, 2016) rising 13% from 2010 to US$101 billion in 2015, with Switzerland as the highest consumer rate (Wickramasuriya and Dunwell, 2018).

Ecuador, because of its geographical conditions and its richness in biological resources, is the quintessential producer of fine-scented cocoa (63% of world production) from the national variety, whose flavor has been recognized for centuries on the international market. This type of grain is used in all refined chocolates. Of the total Ecuadorian export, 75% is estimated to be fine-scented cocoa while the remaining 25% belong to other varieties such as CCN51. Ecuador ranks as the most competitive country in Latin America in this field, followed by Venezuela, Panama and Mexico, which are countries that have gradually increased their share of the global market (Anecacao, 2019).

Various attributes of grain quality, both physical and chemical, are required by manufacturers, cocoa buyers and control bodies to encourage the cocoa community towards better quality production (CAOBISCO/ECA/FCC, 2015). These quality characteristics include taste, purity and health (e.g. bacteria-free, infestation, allergens, mycotoxins, heavy metals and pesticide residues), physical characteristics (e.g. consistency, yield of grain edible material, size and uniformity, shell content, fat content and moisture content) and the characteristics of cocoa butter (e.g. free of fatty acid) (Dasgupta and Klein, 2014). Some of the attributes of grain quality, such as the total fat content, acidity, total phenols, organic acids, heavy metals, amino acids, caffeine, theobromine, pH, sugars, macro content and micronutrients, have been considered in the proposal Cocoa Quality Index (CQI) for some types of grain (Araujo et al., 2014).

Heavy metals are defined as elements with a density of more than 5 g/cm^3 (Navarro-Aviñó et al., 2007). Copper, iron, magnesium and zinc in low concentrations are essential for biochemical and physiological processes in plants, while arsenic, cadmium and lead do not have a known role in them (Ali et al., 2013). The accumulation of heavy metals in plants is affected by several factors such as pH, organic matter content and soil texture, plant genotype and heavy metal content in the growth medium. Latin America has the highest levels of heavy metals in cocoa beans, especially cadmium and lead (Bertoldi et al., 2016), compared to other producers in the world. The presence of heavy metals in cocoa beans represents a threat to cocoa producers, as a high heavy metal content could affect the export of the grains.

Chocolate has been attributed to the achievement of optimal human health and development, due to its high content of flavonoids that are crucial to reducing the risk or in turn delaying the development of cardiovascular disease, cancer and others related to age (Cooper et al., 2008).

In 2014, the European Union announced, through the Regulation (EU) No. 488/2014, plans to implement regulations on chocolate and cocoa products containing excessive levels of cadmium (Cd), which entered into force on January 1, 2019 (Commission Regulation EU, 2014). Non-compliance with regulations would have significant economic and social consequences for cocoa-producing countries, such as Ecuador, from the date of entry into force of that regulation. The Cd has received attention in the last decade due to its importance in food quality/safety, and in human health, since the consumption of foods high in this heavy metal could

lead to renal tubular dysfunction, the formation of kidney stones, the alteration of calcium metabolism and skeletal, endocrine, reproductive and respiratory defects (Järup and Akesson, 2009).

Analytical techniques for the determination of Cd are based on the use of reagents that act on the grain digestion and that are highly hazardous. One of the reagents of frequent use is *Agua Regia* $(HCl : HNO_3)$, which is subjected to heat resulting in irritating and corrosive vapors; the protocol used corresponds to an adaptation of the official AOAC method: 999.11 (AOAC, 2005).

As a more effective analytical method and less toxic to humans and the environment, this paper verified the performance of the graphite furnace atomic absorption spectroscopy (GFAAS) method in the determination of Cd in cocoa almond (*Theobroma cacao*), by determining linearity, accuracy and veracity (Eurolab España et al., 2016) and uncertainty (IRAM 35050, 2001) on certified reference material, and the analytical protocol for the determination of the concentration of Cd in cocoa almond samples was validated; in addition, an inter-lab comparison test was performed.

2 Materials and methods

First Phase: the verification of the analytical method was performed by evaluating the linearity, accuracy, veracity and uncertainty parameters, based on certified material MRC 07206B and 07167A.

Second phase: cocoa samples (ears) were collected by designated AGROCALIDAD personnel, on four farms in the country's cocoa area: Flavio Alfaro-Manabí, Ecuador, referred to as A, B, C, D. Subsequently, the corresponding samples were analyzed in the Nutritional Science and Microbiology Laboratory to determine their Cd content, after having verified the calibration status of the instruments and volumetric materials to be used.

Third phase: the PEE/B/14 protocol for the determination of Cd in cocoa almond by GFAAS was developed, containing the sample preparation process (cocoa ear) from their reception, drying, homogenization, microwave digestion and subsequent reading in the graphite oven spectrometer.

2.1 Verification of the analytical method

The verification of the analytical method was done as reference to the Eurolab Spain Eurachem Guide Eurolab España et al. (2016), and to the IRAM 35050 (2001), following the PEE/B/14 protocol.

2.1.1 Linearity

A curve with 10 concentrations was developed from the Cd standard of 20 ppb, the concentrations used were 0 to 18 ppb. In the curve, the theoretical concentration was expressed in $\mu g/L$ on the axis of the "x", and on the axis of the "y" the calculated concentration was expressed in $\mu g/L$; from the resulting curve, the linear range was chosen and a new curve was drawn. In this linear range, a white, six different dilutions of the standard of 20 ppb and finally the MRC 07206B and 07167A were evaluated by triplicate.

2.1.2 Accuracy

Fifteen readings were made for each MRC 07206B and 07167A and the standard deviation (s) of each material was calculated, using the following formula:

$$s = \sqrt{\frac{1}{n-1}\sum_{k=1}^{n}(x-\bar{x})^2} \quad (1)$$

Standard deviation (s)
Where:
$n =$ number of total measurements of the reference material.
$\bar{x} =$ average of the readings.
$x =$ each measurement performed.

2.1.3 Veracity

Ten readings for MRC 07206B, ten readings for three digested targets and ten readings for three whites added with 2, 3 and 4 ppb of Cd were done; in addition; ten readings were also performed on three samples analyzed. In the first instance, by the method normally used by the laboratory, using acid digestion with *Agua Regia* $(HCl : HNO_3)$, followed by a reading using flame atomic absorption spectroscopy (AA) and, secondly, by the verified GFAAS method and the analysis protocol proposed in this research.

The data obtained for the MRC 07206B proceeded to perform the calculations with the following formulas provided by Eurolab España et al. (2016):

$$b = \bar{x} - x_{ref} \tag{2}$$

Bias

$$b(\%) = \frac{\bar{x} - x_{ref}}{x_{ref}} \times 100 \tag{3}$$

Bias in Percentage

$$R(\%) = \frac{\bar{x}}{x_{ref}} \times 100 \tag{4}$$

Relative Recovery
Where:
$\bar{x}$ = mean of the readings of the MRC 07206B.
x_{ref} = theoretical value that the certificate grants to the reference material.
The calculated values obtained a measure of bias taking into account the effects of the laboratory compared to the bias method data.
For the analysis of the data obtained from the measurements of digested and added whites, the following formula Eurolab España et al. (2016) was used:

$$R'(\%) = \frac{\bar{x}' - \bar{x}}{x_{addition}} \times 100 \tag{5}$$

Relative retrieval of additions
Where:
$\bar{x}'$ = average value of the added whites.
$\bar{x}$ = is the average value of the digested whites.
$x_{addition}$ = concentration value added.
Finally, with the data obtained from the reading of samples by the method normally used in the laboratory, the verified GFAAS method and the proposed analysis protocol, the calculations were performed based on the following equations Eurolab España et al. (2016):

$$b = \bar{x} - \bar{x}_{ref} \tag{6}$$

Bias

$$b(\%) = \frac{\bar{x} - \bar{x}_{ref}}{\bar{x}_{ref}} \times 100 \tag{7}$$

Bias in Percentage

$$R(\%) = \frac{\bar{x}}{\bar{x}_{ref}} \times 100 \tag{8}$$

Relative Recovery
Where:
$\bar{x}$ = means of the sample readings by the old method.
$\bar{x}_{ref}$ = average sample readings by the proposed new method.
The measurement of bias was obtained according to the verified GFAAS method and the proposed analysis protocol, in order to demonstrate its effectiveness and replace the method normally used by AGROCALIDAD.

2.1.4 Uncertainty

10 readings of the concentration of the reference material were performed and the uncertainty values were calculated with the following formulas (IRAM 35050, 2001):

$$\bar{I} = \frac{1}{n} \sum_{k=1}^{n} I_k \tag{9}$$

Average (I) of readings
Where:
n = number of measurements of the reference material.
I_k = each measurement of the reference material

$$S(I_k) = \sqrt{\frac{1}{n-1} \sum_{k=1}^{n} \left(I_k - \bar{I}\right)^2} \tag{10}$$

Standard deviation (I_k)
Where:
n = number of total measurements of the reference material.
$\bar{I}$ = the average of the readings.
I_k = each measurement performed.

$$S\left(\bar{I}\right) = \frac{S(I_k)}{\sqrt{n}} \tag{11}$$

Standard uncertainty $S(I)$
Where:
$S(I_k)$ = the standard deviation
n = number of total measurements of the reference material.

2.2 Determination of cadmium in cocoa almond

With samples obtained from Farms A, B, C and D, the elaborate protocol (Procedure PEE/B/14) was done for the determination of Cd in cocoa almonds, detailing:

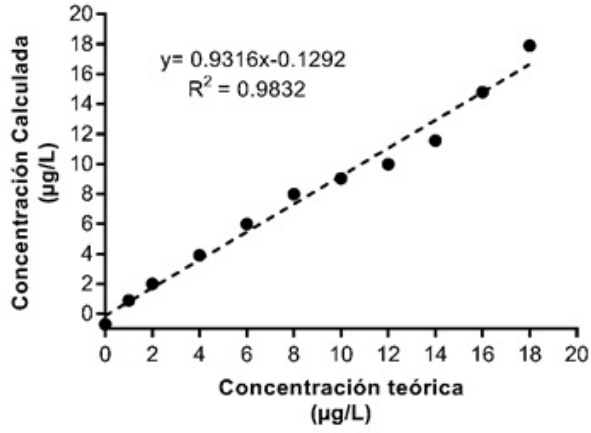

Figure 1. Linear range evaluated with different Cd standard concentrations

1. *Sample processing:* The cocoa almonds were dried on the stove at a temperature of 150 $^{\circ}C$, for a period of 24 hours and it was verified that the shell is easily peeled off, otherwise drying was continued for an additional 12 or 24 hours and a new verification was performed before removing cocoa from the stove. The peeling was done manually with the hot samples to make it easier to husk. To peel the grain, it was pressed with the fingertips or gently striking with a mortar. Approximately 20 g of sample was processed in the mill, until a homogeneous product was obtained. The processed sample was stored in polypropylene bottles with the sample code.
2. *Digestion:* Accurately 0.5 g of dry sample was weighted in the digestion container and microwave digestion was subjected to the parameters indicated by the equipment.
3. *Preparation of standards:* 20 ml of 1000 ppb Cd mother solution was prepared. 2 ml of 1000 $\mu g/ml$ Cd standard was taken with volumetric pipette and graduated to 20 ml with 1 % of nitric acid. From this solution, the graphite furnace atomic absorption equipment automatically performed the corresponding dilutions (1-5 ppb).
4. *Reading of the samples:* The concentration of each encoded sample on the computer was measured. The equipment was programmed to perform triplicate readings on the same sample and the result was expressed in parts per billion (ppb).

2.3 Statistical analysis

The statistical analysis was carried out taking into account each method verification parameter: linearity, accuracy, veracity and uncertainty, each of which provides formulas for the calculation of means, bias, relative bias and recovery. In addition, a variance analysis was performed in the determination of Cd in cocoa almonds to determine whether there are significant differences between the samples obtained on each farm. The statistical program Graphpad Prism 7 was used.

3 Results

3.1 Verification of the analytical method

3.1.1 Linearity

In Figure 1, the theoretical concentration (0-18 ppb) was plotted versus that provided by the equipment

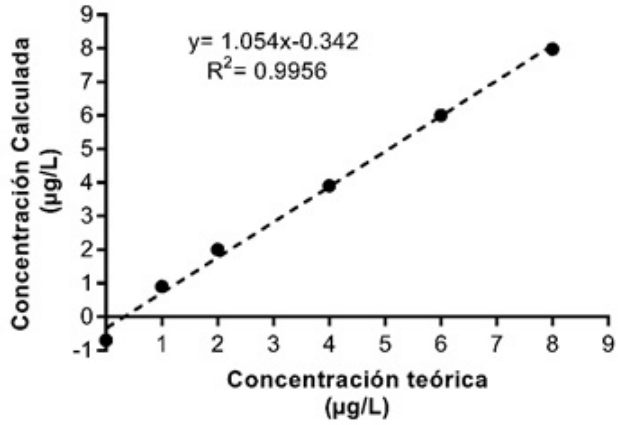

Figure 2. Linear range of the method 0-8 ppb.

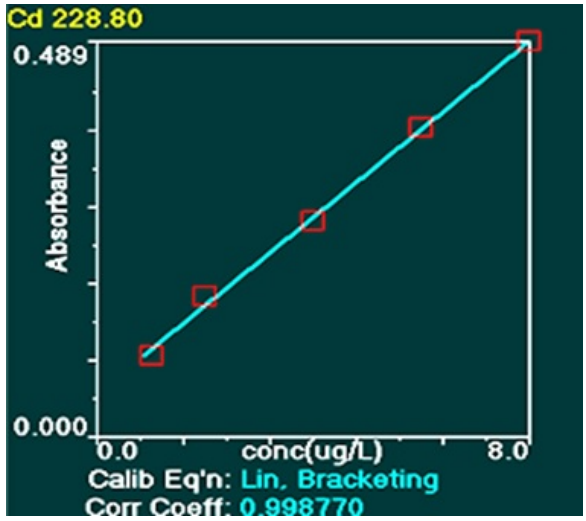

Figure 3. Linear range obtained by the equipment

(calculated), and a visual analysis was performed in which the linear range was established to be between 0 and 8 ppb (Figure 2 and 3).

The linear range is between 0 and 8 ppb (Figure 2), with a residuality coefficient of 0.9956, acceptable for analytical procedures.

The GFAAS method yielded a calibration curve (Figure 3), which presented a residuality coefficient of 0.998770, much more accurate than the one calculated, thus it only uses the points of interest, eliminating background errors. To confirm the linearity of the method and following the procedure of the Eurachem Guide (2016), a target and six known concentration solutions were measured in the linear range given by the equipment, which is represented in Figure 4. Figure 4.A. shows that the residuality coefficient was 0.9964; Figure 4.B. 0.9976 and Figure 4.C. 0.9952, confirming the linearity of the method.

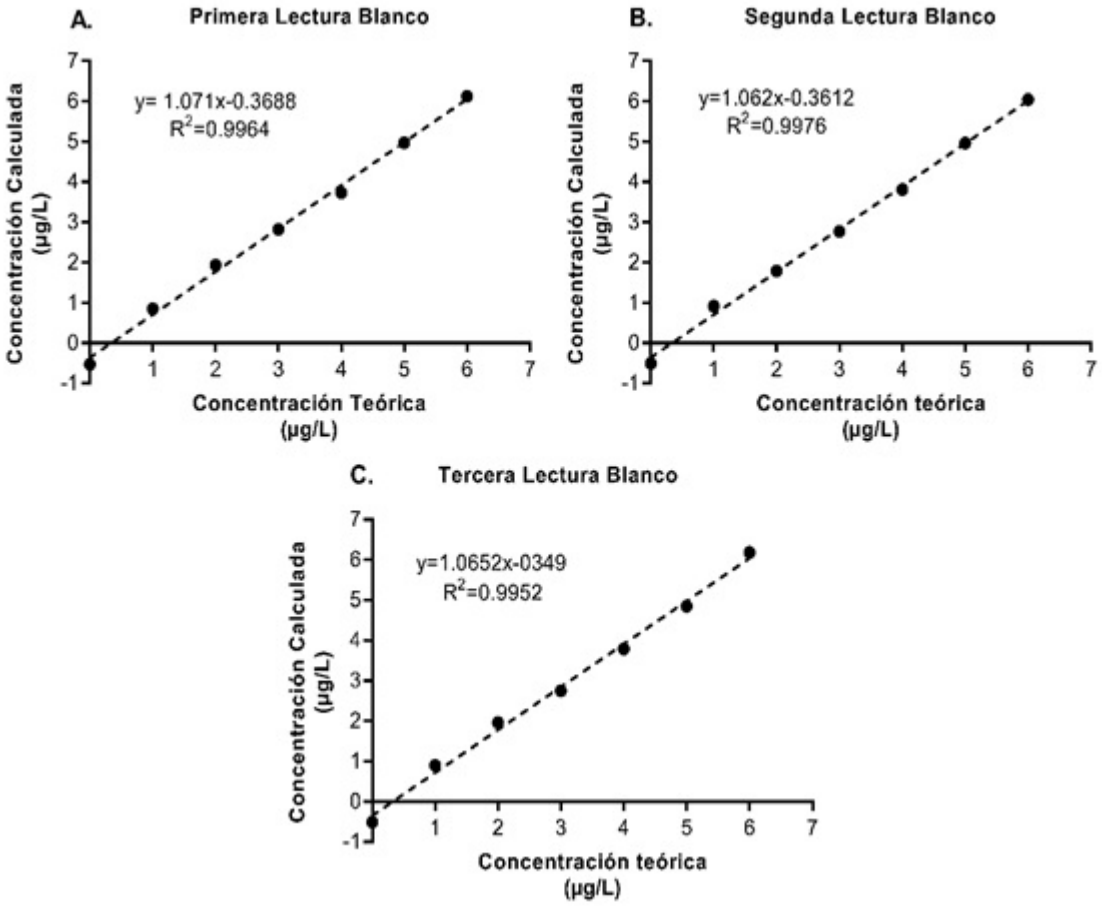

Figure 4. A. First reading of a white and six Cd standards; B. Second reading of a white and six Cd standards; C. Third reading of a white and six Cd.

3.1.2 Accuracy

The accuracy of the analyst, equipment and analytical method were measured by 15 readings of two reference certified materials 07206B and 07167A, obtaining a minimum and less standard deviation compared to the obtained by the manufacturer of the equipment, which was 0.02 as seen in Table 1.

3.1.3 Veracity

The readings of the certified reference material 07167A were made in the measurement of veracity; on the basis of the data obtained the mean, bias, relative bias in percentage and relative recovery were calculated, using as a reference value the data provided by the reference material certificate, as shown in Table 2.

In addition, ten readings of three whites and ten readings of the same white added with 2, 3 and 4 ppb of Cd were made, thus obtaining the mean and percentage of recovery, as shown in Table 3.

Three sample readings were finally made by the method normally used by the laboratory (AA), by the verified GFAAS method and the proposed protocol, as shown in Table 4.

Table 4 shows that the bias in the reading of the three samples by the two methods is minimal and the recovery percentage is high, which gives reliability to the GFAAS method.

Table 1. Concentration of cadmium obtained from 15 readings of two certified cocoa powder reference materials.

Reading number	CERTIFIED REFERENCE MATERIAL			
	Reading obtained by the equipment		Calculated Final Concentration	
	07206B ($\mu g/L$)	07167A ($\mu g/L$)	07206B (mg/kg)	07167A (mg/kg)
1	1.608	1.656	0.0803	0.0820
2	1.605	1.794	0.0801	0.0888
3	1.612	1.604	0.0805	0.0794
4	1.610	1.626	0.0804	0.0805
5	1.609	1.656	0.0803	0.0820
6	1.632	1.614	0.0815	0.0799
7	1.605	1.637	0.0801	0.0810
8	1.615	1.644	0.0806	0.0814
9	1.610	1.655	0.0804	0.0819
10	1.603	1.645	0.0800	0.0814
11	1.608	1.656	0.0803	0.0820
12	1.603	1.702	0.0800	0.0842
13	1.611	1.668	0.0804	0.0826
14	1.622	1.644	0.0810	0.0814
15	1.631	1.656	0.0814	0.0820
Standard deviation (*s*)			0,0005	0,0022

Note: Values are reported in milligrams per kilogram. The final calculated concentration was based on the reading of the equipment by the capacity volume and dilution factor, divided for the weight of the sample per thousand.

3.1.4 Uncertainty

Cd concentration of two reference materials 07206B and 07167A was measured ten times, and the mean, standard deviation and standard uncertainty (Table 5) was calculated.

The uncertainty calculated by measuring the Cd concentration of MRC (07206B and 07167A) is 0.00013 and 0.00082, respectively (Table 5), indicating that measurements made were not affected by systematic errors in the process.

3.2 Determination of cadmium in cocoa almonds

Samples from four farms located in Alfaro-Manabí estates named A, B, C and D (Table 6) were analyzed, collecting 15 samples from farm A, 9 samples from farm B, 4 from farm C and 5 of farm D, according to internal agroquality protocols, establishing the following hypothesis:

Null hypothesis: the concentration of Cd in cocoa almonds from the four farms is not significantly different.

Alternative hypothesis: the concentration of Cd in cocoa almonds of at least one farm differs significantly from the others.

After performing the analysis of variance (Table 7) being $p < 0,05$, the alternative hypothesis is accepted, i.e., the concentration of Cd in cocoa almond of at least one farm differs from the others.

Farm A that has on average 0.546 mg/Kg, being the highest value compared to the other farms. It should be mentioned that between Farm B and D there are no significant differences since in the two farms the concentration of Cd in cocoa almond was 0.260 mg/Kg. Farm C has the lowest concentration of Cd in cocoa almond, being 0.146 mg/Kg.

Table 2. Cadmium concentration obtained from 10 readings of the certified cocoa powder reference material

Reading	Reference Material	
	07167A $(\mu g/L)$	07167A (mg/Kg)
1	1.614	0.080
2	1.637	0.081
3	1.655	0.082
4	1.655	0.082
5	1.645	0.081
6	1.656	0.082
7	1.612	0.080
8	1.668	0.083
9	1.623	0.080
10	1.644	0.081
Media		0.081
Reference Value		0.074
Bias		0.007
Relative bias in percentage		9.75
Relative recovery		109.75

Note: The values reported by the equipment are in $\mu g/L$. The calculated final concentration (mg/kg), is based on the reading of the equipment by the volume of capacity and dilution factor, divided for the weight of the sample per thousand.

Table 3. Reading of three whites and whites added with 2, 3 and 4 ppb of cadmium

No. Reading	WHITES $(\mu g/L)$			ADDED WHITES $(\mu g/L)$		
	B2V	**B3V**	**B4V**	**BA2V**	**BA3V**	**BA4V**
1	−0.5278	−0.7799	−0.4077	2.098	3.115	3.780
2	−0.5017	−0.7791	−0.3903	2.084	3.185	3.898
3	−0.5106	−0.7442	−0.4038	1.962	3.247	4.165
4	−0.5613	−0.7247	−0.4415	1.939	3.025	4.036
5	−0.4176	−0.7153	−0.4453	1.962	3.004	4.054
6	−0.5200	−0.7298	−0.4692	1.963	3.027	4.076
7	−0.6586	−0.7062	−0.4406	1.876	3.057	4.185
8	−0.5363	−0.7423	−0.4894	1.878	2.947	4.196
9	−0.5810	−0.7847	−0.4962	1.890	3.030	4.223
10	−0.5134	−0.7700	−0.4194	1.800	3.137	4.263
Media	−0.53283	−0.74762	−0.44034	1.945	3.077	4.088
Recovery $(\%)$				123.902	127.501	113.199

Note: The units are reported in $\mu g/L$. in the B2V code means: White number two veracity. BA2V stands for, BA2: White added with 2 ppb of Cd; V: veracity.

3.3 Inter-laboratory test

The sample with code C3 was sent to UBA Laboratories located in the city of Guayaquil, Ecuador. The reported result was 0.11 ppm of Cd, which coincides with the result obtained in Laboratory of Nuritional Science and Microbiology of Agroquality.

Table 4. Cadmium concentration in three samples evaluated by GFAAS and AA

Reading	GFAAS Sample Reading			AA Sample Reading		
	A8A	**B23A**	**D6A**	**A8A**	**B23A**	**D6A**
1	1.356	0.174	0.991	1.348	0.178	0.985
2	1.348	0.177	0.996	1.387	0.174	0.981
3	1.358	0.178	0.989	1.356	0.180	0.964
4	1.363	0.175	0.983	1.364	0.173	0.994
5	1.361	0.176	0.987	1.377	0.178	0.997
6	1.371	0.174	0.981	1.374	0.180	0.999
7	1.376	0.180	0.998	1.371	0.171	0.997
8	1.368	0.182	0.993	1.350	0.178	0.986
9	1.373	0.182	1.001	1.336	0.173	0.992
10	1.366	0.180	0.989	1.342	0.173	0.988
Media	**1.364**	**0.178**	**0.991**	**1.361**	**0.176**	**0.988**
Bias	0.004	0.002	0.003			
Bias %	0.26	1.16	0.29			
R %	100.26	101.16	100.29			

Note: Units are expressed in mg/Kg. The code A8A means: A: The name given to the Farm; 8A: sample numbering.

Table 5. Cadmium concentration of two reference materials

Reading number	CERTIFIED REFERENCE MATERIAL			
	Reading obtained by the equipment		Calculated Final Concentration	
	07206B $(\mu g/L)$	07167A $(\mu g/L)$	07206B (mg/Kg)	07167A (mg/Kg)
1	1.608	1.656	0.0803	0.0820
2	1.605	1.794	0.0801	0.0888
3	1.612	1.604	0.0805	0.0794
4	1.610	1.626	0.0804	0.0805
5	1.609	1.656	0.0803	0.0820
6	1.632	1.614	0.0815	0.0799
7	1.605	1.637	0.0801	0.0810
8	1.615	1.644	0.0806	0.0814
9	1.610	1.655	0.0804	0.0819
10	1.603	1.645	0.0800	0.0814
Media			0.0804	0.0818
Standard deviation			0.0004	0.0026
Standard uncertainty			0.00013	0.00082

Note: Values are expressed in milligrams per kilogram. The calculated final concentration is based on the reading of the equipment by the capacity volume and dilution factor, divided for the weight of the sample per thousand.

4 Discussion

The verification of the analytical method of atomic absorption spectroscopy with graphite furnace in the Laboratory of Nutritional Science and Microbiology of Agroquality, complied with the performance parameters: linearity, precision, veracity according to the Eurachem Guide (Eurolab España et al., 2016) and uncertainty according to the Guide of the Uncertainty Calculation of the Argentine Institute of Standardization and Certification IRAM 35050 (2001). Linearity was worked with a curve in

Table 6. Concentration of cadmium in cocoa almonds of Farms A, B, C and D

Sample	Cad concentration (mg/Kg)			
	Farm A	Farm B	Farm C	Farm D
1	0.60	0.60	0.12	0.12
2	0.79	0.10	0.18	0.11
3	0.60	0.01	0.11	0.24
4	0.88	0.34	0.17	0.30
5	0.74	0.30		0.54
6	0.89	0.40		
7	0.50	0.40		
8	0.54	0.09		
9	0.55	0.11		
10	0.49			
11	0.54			
12	0.15			
13	0.13			
14	0.40			
15	0.39			

Note: Values are expressed in milligrams per kilogram. The sample numbers do not correspond to the numbering expressed to the samples on the labeling

a range of 0 to 8 ppb, whose $R^2 = 0.998770$, which by its proximity to $+1$ indicates that there is a "perfect" correlation between the variables being acceptable for analytical measurements (Gaddis and Gaddis, 1990).

Accuracy for two certified reference materials (07206B and 07167A) yielded a standard deviation of 0.0005 and 0.0022, respectively, implying that the measurements made by the analyst were accurate and repeatable. As for the veracity, it was found that the proposed methodology yields result similar to the non-standardized and toxic methodology that the Laboratory of Nutritional Science and Microbiology Agroquality used for the analysis of Cd, since the bias between the two methodologies was minimal and the recovery rate was greater than 100%. This is in agreement with what Ospina and Zapata (2012) mentioned, who point out that the recovery percentage in an analytical method should be in a range of 80% to 120%. In addition, it is comparable with the validation of (Lo Dico et al., 2018) in which they obtain a recovery percentage of 110%. The standard uncertainty was 0.00013 and 0.00082, which gives reliability to the proposed method and leads to the assumption that working in a closed environment and with the other laboratory equipment turned off leads to minimizing errors as mentioned (Gonzaga, 2016).

Table 7. Summary of the analysis of variance

Groups	Count	Sum	Average	Variance
Farm A	15	8.190	0.546	0.051
Farm B	9	2.350	0.261	0.038
Farm C	4	0.585	0.146	0.001
Farm D	5	1.310	0.262	0.031

A related investigation is that of Acosta and Pozo (2013) in which the official Method AOAC 999.11 was applied, where the verification of performance parameters in the Agroquality laboratory is not evident, thus the results are unreliable. However, the same author recommends using for the digestion of the sample, a type of microwave digestion which is faster and more effective; therefore, in this research this methodology of digestion based on the standard was used (AOAC, 2010), obtaining good results. In related research, the technique of flame-coupled spectroscopy with perchloric digestion such as Mite et al. (2010), and a pre-coupled nitric digestion followed by atomic emission spectroscopy reading is used (ICP-MS) from Chavez et al. (2015). However, ICP-MS can be used after microwave digestion, which gives greater specificity of the analysis (Lo Dico et al., 2018).

As stipulated by the European Union (0.8 ppm of Cd in cocoa powder), which entered into force in January 2019, the cocoa from the four farms presented in this study could be exported without restriction, having been at its levels (0.546 A; 0.261 B; 0.146 C; 0.262 D) within the allowed range. They also comply with the codex Committee on Food Pollutants (CCCF), which, at the 9th session in the Netherlands, stipulates a limit of 1.5 ppm (CCCF, 2015).

According to Chavez et al. (2015) the difference in the content of Cd in the four farms can be attributed to the existence of heavy metal contamination in the irrigation water or soil contamination (Rankin et al., 2005). On the other hand, Acosta and Pozo (2013) refer to the location of the crops, i.e., if they are near the paved road, heavy metals are more likely to accumulate in both soil and aerial parts of the plants, because of emissions from vehicles circulating into the atmosphere, which would not happen with farms far from the road. In addition to the above, Mite et al. (2010) note that Cd contamination of cocoa almonds can also be caused by burning urban waste, use of urban sludge in agriculture, agrochemicals and, pollution by petroleum derivatives when drying cocoa on the roads. Argüello et al. (2019) in a recent investigation mention that the concentration of Cd in cocoa almonds may vary from farm to farm with respect to the genotype cultivated (CCN-51 vs. National).

Although this study did not focus on the determination of Cd in cocoa almonds but on a validation of the analytical method for quantifying this metal, it has been possible to contribute significantly to the development of subsequent studies where GFAAS is used and, investigate the traceability of the Cd in the almonds, it being clear that the soil is the largest supply of this metal, being able to implement mitigation strategies, such as bioremediation.

5 Conclusions

The performance parameters of the analytical method of atomic absorption spectroscopy with graphite furnace were successfully verified, thus ensuring that the method is reliable and that it can be used in the Laboratory of Nutritional Science and Microbiology of Agroquality. The developed protocol (PEE/B/14) allows the correct application of the method in the analysis of Cd in cocoa Almond, since it defines a standard operating procedure that ensures faithful compliance with the test requirements for reliable results. Furthermore, differences were evident in the concentration of Cd in cocoa almonds of the farms under study, which are related to the source of the samples. However, all the samples analyzed revealed lower concentrations of Cd than the stipulated by the European Union (0.8 ppm of Cd in cocoa powder); in addition, they comply with the CCCF's standard, a limit of 1.5 ppm, so this cocoa could be exported unrestricted.
The inter-laboratory test carried out confirms the validity of the method verified in this investigation. In view of the difference in the concentration of Cd according to the source of the samples, it is advisable to carry out other investigations concerning the factors involved in the contamination of this element in cocoa.

Acknowledgments

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BIOTECHNOLOGY

Propagation in vitro of qusihuar (Buddleja incana Ruíz & Pav)

Propagación in vitro de quishuar (Buddleja incana Ruíz & Pav)

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Resumen

Quishuar es una especie forestal apreciada por sus usos. La explotación intensiva y la oferta insatisfecha de plantas han sido limitantes para cumplir con programas de reforestación. La micropropagación es una técnica que ayudaría a erradicar este problema ya que el propósito es producir mayor cantidad de plantas en menor tiempo. El objetivo de esta investigación fue desarrollar protocolos para la desinfección, establecimiento y multiplicación *in vitro*, para el efecto se realizaron ensayos partiendo de semillas y brotes de plantas. La primera fase se realizó con semillas, utilizando kilol y benomil, junto con NaOCl al 3 % (10 min). Los resultados indican que el porcentaje de germinación fue de 100 % en MS (Murashige y Skoog medium) y el porcentaje de contaminación y oxidación de 0 %. Por otro lado, los brotes sometidos a fungicidas con adición de antioxidantes y NaOCl al 1 % (10 min) no presentaron contaminación ni oxidación. El porcentaje de brotación fue de 100 % en WPM (Woody Plant Medium). En la segunda fase en medio MS sin adición de hormonas se observó una mayor longitud de brote (1.95 cm), número de nudos (1.94 nudos) e índice de multiplicación (2.47). Basándose en los resultados, se sugiere que los protocolos son efectivos para la propagación *in vitro*.

Palabras clave: contaminación, in vitro, micropropagación, oxidación, Quishuar

Abstract

Quishuar is a forest species which is well known for its uses. Intensive farming and unsatisfied plant supply have been limited to meet reforestation programs. Micropropagation is a technique that would help eradicate this problem as the purpose is to produce more plants in less time. The objective of this research was to develop protocols for disinfection, establishment, and multiplication *in vitro*; for this reason, tests from seeds and plants sprouts were carried out; the first phase with seeds, using kilol and benomil, together with NaOCl at 3 % (10 min). The results indicate that the germination percentage was 100 % in MS (Murashige y Skoog medium) and the contamination, oxidation percentage was 0 %. On the other hand, plants sprouts exposed to fungicides, antioxidants and NaOCl at 1 % (10 min), did not have contamination or oxidation. The sprouting percentage was 100 % in WPM (Woody Plant Medium). In the second phase in MS medium without the addition of hormones, plant sprouts (1.95 cm), knots number (1.94 knots) and multiplication rate (2.67) were observed. Based on the results, it is suggested that protocols are effective for *in vitro* propagation.

Keywords: contamination, in vitro, micropropagation, oxidation, Quishuar

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1 Introducion

Ecuador is considered a country with a forest vocation and yet this sector contributes little to the nation's economy. There are about 7 million hectares of forests with a forest management potential lower than 10 %, but which are economically eligible to be subjected to sustainable forest management (Sánchez, 2012).

Forest genetic resources FGR are being lost at an alarming rate due to indiscriminate use and lack of incentives for the preservation and its sustainable use. The knowledge of FGR is still precarious and insufficient, studies and institutions carrying out activities for their protection are scarce, and the current availability of specific information on the situation, trends and recovery of FGR is deficient (Ministerio del Ambiente-MAE, 2005).

Thus, Quishuar (*Buddleja incana*) is a tree of the Scropulariaceae family with 15 m of height approximately, with flowers grouped on small heads and fruits of 5-6 mm of length. This tree is a forest species native to Ecuador, located in the provinces of Chimborazo, Pichincha, Tungurahua, Azuay, Loja and Imbabura (Grijalva et al., 2012). Wood is used for the making of plows, rudders, hoe capes, poles, stakes, crafts, house building and corrals. In addition, the infusion of leaves is used for medicinal purposes such as antirheumatic, healing, antibacterial and antifungal, as well as to stimulate the proliferation of the endometrium and regenerator of the skin; in mice it has been shown to inhibit cyclooxygenase (COX_2) (Gómez, 2006).

The propagation through the conventional methods established for Quishuar has several problems; the multiplication in nursery requires 5 months, resulting in higher production costs and less plant production per year (Gárate, 2010). The amount of vegetal material available is insufficient for afforestation, reforestation in degraded areas of wastelands and watersheds, and one of the strategies for overcoming conventional propagation difficulties is *in vitro* cultivation of vegetable tissues (Delgado et al., 2008). *In vitro* culture is a technique of plant production under totally aseptic conditions; it is based on cell potency, i.e., the ability of a plant cell to form a complete plant under certain conditions. Thus, the rapid and massive propagation of plants identical to the original is achieved from any part of the plant, whether pieces of tissues, meristhematic apices or even isolated cells (Reyes and y Hewstone, 1994).

According to Vallejo (1988), the spread of this species at the laboratory level presents some problems such as the length of shoots 8.6 mm, low percentage of number of knots per plant (1.5 knots), but there are no reported data regarding pollution. However, Cárdenas (2011), states that pollution is the main limiting in the *in vitro* development of this forest species, since the survival rate does not exceed 54 %, mainly due to fungal and bacterial conditions.

Due to the difficulties of conventional propagation and the results obtained in the laboratory by Cárdenas (2011) and Vallejo (1988), it has been proposed to establish a method for the disinfection, establishment and multiplication of Quishuar through the application of *in vitro*, culture techniques, because it is essential to generate strategies that allow the propagation and conservation of this species.

The aim of this research was to develop a protocol for the *in vitro* propagation of Quishuar (*Buddleja incana*).

2 Methodology

This study was conducted in the plant tissue culture laboratory of the National Department of Biotechnology at INIAP (National Agricultural Research Institute), Cutuglahua, Mejía parish, Pichincha Province and in the Kichwa communities located in the mountain range.

3 Vegetal Material and Explant Preparation

Seeds and buds (axillary and apical) of Quishuar plants in the Kichwa San Juan community, Chimborazo, were used. Prior to the research the plants were moved to the greenhouse of the National Department of Biotechnology, fumigated every 8 days with an application of Carbendazim 1 $ml\ l^{-1}$ and Skul Fe (Thiodicarb) 1 $ml\ l^{-1}$ to prevent contamination, the

plants were fertilized every 15 days with Stimufol 1 $g\ l^{-1}$ and irrigation was performed twice a week with 200 $ml\ l^{-1}$ of drinking water per plant (Laboratorio de Cultivo de Tejidos (LCT-INIAP), 2014).

4 Propagation Phases *in vitro*

Disinfection and establishment phases. The collected seeds were placed in a container with a solution of water and detergent, then were rinsed with running water until all residues were removed. Once washed, they were immersed in a 1 % Povidone solution (Iodine-povidone) for 60 minutes, then were transferred to a Benomyl solution (Benzimidazole) 0,10 $g\ l^{-1}$ adding 30 drops of Kilol (Citrus paradisi). The seeds were taken to a laminar flow chamber, where 2 rinses with distilled water were made and immediately were immersed in a solution of Sodium Hypochlorite (NaOCl) 3 % for 10 minutes, subsequently 5 rinses were performed with distilled sterile water. The seeds were sown in the culture medium MS (Murashige and Skoog medium) and WPM (Woody Medium Plant), incubated 18 $°C$ for 30 days.

On the other hand, buds (axillary and apical) were also washed with a solution of water with detergent and running water to remove all detergent residues. Subsequently, these were disinfected in a Povidone solution (Iodine−povidone) 1 % for 60 minutes, and then were transferred to a fungicide solution (Phyton 0,5 $ml\ l^{-1}$ + Carbendazim 0,5 $ml\ l^{-1}$), antioxidants (Ascorbic acid + Citric acid 0,1 $g\ l^{-1}$),30 drops of Kilol *Citrus paradisi* and activated charcoal 0,5 $g\ l^{-1}$ for 20 minutes. Buds were placed in a laminar flow chamber with two solutions of Sodium Hypochlorite (NaOCl), the first 0,5 % for 60 minutes and the second 1 % NaOCl with the addition of 3 drops of Tween 20 for 10 minutes, with 5 rinses of sterile distilled water. Buds were planted in two MS and WPM culture media and incubated at 21 $°C$ for 30 days (Laboratorio de Cultivo de Tejidos (LCT-INIAP), 2014).

Multiplication phase. Explants of 2-3 cm of length were cut from the plants obtained from seeds, which were transferred to different culture media, MS and WPM without the addition of hormones and with the addition of 0,5 $g\ l^{-1}$ and 0,1 $g\ l^{-1}$ of BAP (Benzyl adenine) and gibberellic acid AG_3 (Hernández et al., 1999). 10 observations were made, with evaluations at 30 and 45 days.
Explants of 1-3 cm of length were cut from plants obtained from shoots, then were transferred to culture media MS and WPM without the addition of hormones and with the addition of 0.5 $g\ l^{-1}$ and 0,1 $g\ l^{-1}$ of BAP (Benzyl adenine) and gibberellic acid AG_3 (Hernández et al., 1999).

Incubation and photoperiod of vitroplants. During the multiplication phase, 5 explants were placed per bottle, sealed with parafilm paper to avoid contamination, kept in the growing room with light intensity of 2000 luxe at a temperature of 18 $°C$ and 21 % humidity. Plants were evaluated every 30 days.

5 Experimental Design and Statistical Analysis

A split plot design was used for the analysis, for the first phase the plot design was (3×2) with six treatments and a second phase with a plot design (2×2×3) with 12 treatments. 10 observations were used in each stage.

PFor the data, a variance analysis (ADEVA) and mean separation were performed at 95 % de probabilidad. probability. To establish statistical differences between treatments, the Tukey Multiple Range Test (p <0.05). was performed. The data that had a value of 0 were transformed with the formula $\sqrt{x+1}$, in order to decrease the variation coefficient and adjust the data to the normal distribution (Vinueza, 2013).

6 Results and Discussion

6.1 Disinfection and Seed Setting Phase

In several researches conducted by the Statistics Division of the Nutrition Institute of Central America and Panama (INCAP) it is stated that laboratory studies using the following control measures: homogeneous experimental material, external factor control, the allocation of few experimental units to treatments and the refinement of the techniques applied in the research, result in the continuation of

Table 1. Variables evaluated in the disinfection stage and *in vitro* establishment of *B. incana* seeds.

Treatment	Protocol	Medium	Contamination percentage	Oxidation percentage	Germination percentage	Bud length (cm) 30 days	Budlength (cm) 45 days
T1×10REP	Povidone solution 1 % 60′ Fungicides (Phyton 1 *ml* l^{-1} + distilled water + Carbendazim 1 *ml* l^{-1} distilled water) + 60 NaOCl 1 % Tween 20 10′.	MS	0	0	100	1.22±0.03	1.37±0.05
T2×10REP	Povidone solution 1 % 60′ Fungicides (Phyton 1 *ml* l^{-1} + distilled water + Carbendazim 1 *ml* l^{-1} distilled water) + 60 NaOCl 1 % Tween 20 10′.	WPM	20	0	100	1.22±0.03	1.44±0.05
T3×10REP	Povidone solution 1 % 60′ + Kilol 30 drops l^{-1} + distilled water 30′ + Benomyl 1 *g* l^{-1} + NaOCl 3 % 10′.	MS	0	0	100	1.33±0.03	1.47±0.05
T4×10REP	Povidone solution 1 % 60′ + Kilol 30 drops l^{-1} + distilled water 30′ + Benomyl 1 *g* l^{-1} + NaOCl 3 % 10′.	WPM	0	0	100	1.27±0.03	1.40±0.05
T5×10REP	Povidone soap 1 % 30′ + Fungicides (Benomyl 1 *g* l^{-1} water + Phyton 0.5 *ml* l^{-1} distilled water) + 30′ + NaOCl 1 % 8′.	MS	20	0	100	1.07±0.03	1.14±0.05
T6×10REP	Povidone soap 1 % 30′ + Fungicides (Benomyl 1 *g* l^{-1} water + Phyton 0.5 *ml* l^{-1} distilled water) + 30′ + NaOCl 1 % 8′.	WPM	10	0	100	1.15±0.03	1.35±0.05

the same experimental error or that it is feasible to reduce the error factor and keep it under control. Research of the experimental error used as an example of processes clearly indicates that the investigation of the error cannot be just a mere part of a complete process, but it should be considered as one more activity within the framework of general techniques to be applied during any kind of investigation (Guzmán, 1975).

The variables evaluated at this stage were percentage of contamination, oxidation, germination and bud length.

Contamination by fungi and/or bacteria was observed in T2, T5 and T6 treatments with a percentage of 100 %. Treatments T1, T3 and T4 resulted in explants without contamination within 30 days of evaluation. The oxidation rate for treatments T1, T2, T3, T4, T5 and T6 was 0 %.

Treatments T1, T3 and T4 reported 100 % seed germination at 30 days, and this percentage was maintained for up to 45 days. In T1 and T3 treatments the MS culture medium was used as well as the WPM culture medium for T4 treatment. For the variable bud length, Tukey 5 % test was performed to establish significance ranges, for T3 (MS) increased growth was observed with an average of 1.33 ± 0.03 cm. . For T4 (WPM) a growth of 1,27 ± 0.03 cm and for T1 (MS) a growth of 1.22 ± 0.03 cm at 30 days of evaluation. At 45 days, growth was evident for T3 (MS) with an average of 1.47 ± 0.05 cm. For T4 (WPM) a growth of 1.40 ± 0.05cm , and for T1 (MS) a growth of 1.37 ± 0.05 cm.

The results obtained at this stage agree with those mentioned by the authors. In Billard et al. (2014), NaOCl 1 % 10′ 10′ was used in *Oncidium bifolum* seeds reporting 0 % of contamination and oxidation; it further mentions that when using MS culture medium, the germination rate was 100 %. However, the bud length was smaller compared to the T3 and T4 treatment, this may be due to González (2010), in his study with *Musa paradisiaca* mentions that the use of fungicides/bactericides (Phyton) at doses higher than 0.5 *ml* l^{-1} could slow the growth of explants, this is because when the product is rapidly absorbed by the leaves and roots, it causes plant toxicity, reducing branching and causing plant deterioration. These results would be corroborated by those obtained from T1 treatment.

On the other hand, Soto et al. (2010), used NaOCl 3 % 10′ in *Cedrela salvadorensis* seeds, reporting 0 % of contamination and oxidation; NaOCl is

recommended as a surface disinfectant as its mechanism of action allows damage to the cell membrane of bacteria, causing the lysis of the microorganism. In addition, they mentioned that seed germination was 65 % for the WPM culture medium and 100 % for the MS culture medium. The MS culture medium contains a high concentration of salts while the WPM culture medium contains reduction of the total mineral salts. Bud length in T3 was longer compared to T1 and T4 treatment.

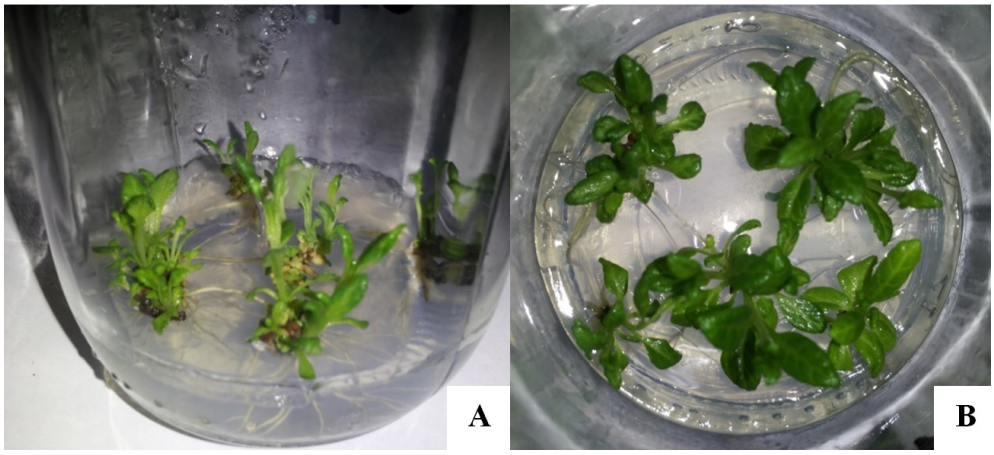

Figure 1. A and B) Treatment T3. Plants in the MS culture medium at 30 days of evaluation.

7 Dissinfection and Settins of Buds

The variables evaluated in this phase were percentage of contamination, oxidation, sprouting, number of knots and bud height.

Contamination by fungi and/or bacteria was observed in T1, T2, T3 and T4 treatments with a percentage of 70, 50, 40 and 80 %,respectively. For T5 and T6 treatments, the results showed explants free of contamination at 30 days of evaluation. The oxidation rate for T1, T2, T3, T4, and T6 treatments was 0 %, while for T5 was 20 %. The percentage of sprouting for treatments T5 (MS) and T6 (WPM) was 80 and 100 %, respectively. Tukey 5 % test was used for the variable number of knots and bud height to differentiate significance ranges, obtaining an average 1.61 $\pm$ 0.1 knots and one mean in bud length for the MS culture medium of 1.45 $\pm$ 0.08 cm, and 1.55 $\pm$ 0.08 cm. for the WPM culture medium. Sodium hypochlorite was effective for disinfection of buds during *in vitro* establishment of *C. spinosa*. In this phase of *in vitro* different compounds can be used for the disinfection of plant material, and solutions of sodium hypochlorite and alcohol at different percentages are the most common products (Azofeifa, 2009).

The results obtained in this phase are higher compared to those mentioned by Cárdenas (2011), who used buds of Quishuar from mother plants that were two years old, and stated that the disinfection process was with NaOCl 2,5 % for 10 min, obtaining 70 % survival. This research used 1-year-old buds and the modified LCT-INIAP disinfection protocol, obtaining 100 % survival. Oxidation in the T5 treatment may have been due to environmental factors such as light intensity, cuts, senescence, heavy metals and lesions that can trigger oxidative stress (Luna et al., 2003). Parada (2009), mentions in his study with *Prunus persica* that a large number of knots (3-4) were obtained when using the WPM culture medium and the length was 13.7 mm, this because the high concentration of salts in the MS culture medium can cause a delay in sprouting and can be toxic to tissues, as is the case of *Vaccinium corymbosum*. This species responded better in the WPM culture medium as it contains reduction of the total amount of mineral salts (Sedlak and Paprstein, 2009). The best results were observed in T6 treatment (WPM).

Table 2. Variables evaluated at the disinfection stage and *in vitro* establishment of *B. incana* buds

Treatment	Protocol	Medium	Contamination Percentage	Oxidation Percentage	Bud Percentage	Percentage of knots (30 days)	Bud length (30 days)
T1×10REP	Povidone Solution 1 % 20′ + Fungicides (Benomyl 1 $g\ l^{-1}$ + Phyton 1 $ml\ l^{-1}$ + Rinfapicina 0.5 $ml\ l^{-1}$) 60′ + Alcohol 70 % 1′ + NaOCl 2 % 15′.	MS	70	0	30	1.22±0.1	1.17±0.08
T2×10REP	Povidone Solution 1 % 20′ + Fungicides (Benomyl 1 $g\ l^{-1}$ + Phyton 1 $ml\ l^{-1}$ + Rinfapicina 0.5 $ml\ l^{-1}$) 60′ + Alcohol 70 % 1′ + NaOCl 2 % 15′.	WPM	50	0	50	1.30±0.1	1.27±0.08
T3×10REP	Povidone Solution 1 % + Kilol 20 drops l^{-1} + distilled water 30′ + Benomyl 1 $g\ l^{-1}$ 90′ + Alcohol 70 % 30″ + NaOCl 1 % 15′ + Activated carbon (0,5 $g\ l^{-1}$).	MS	40	0	60	1.41±0.1	1.33±0.08
T4×10REP	Povidone Solution 1 % + Kilol 20 drops l^{-1} + distilled water 30′ + Benomyl 1 $g\ l^{-1}$ 90′ + Alcohol 70 % 30″ + NaOCl 1 % 15′ + Activated carbon (0,5 $g\ l^{-1}$).	WPM	80	0	20	1.11±0.1	1,11±0.08
T5×10REP	Povidone solution 1 % 60′ + Activated carbon 0.5 $g\ l^{-1}$ + Fungicides (Phyton 0.5 $ml\ l^{-1}$ + Carbendazim 0.5 $ml\ l^{-1}$) + Kilol 30 drops/ 100 ml + Ascorbic acid 0.1$g\ l^{-1}$ 20′ + NaOCl 0.5 % 60′ + NaOCl 1 % 10′ + Tween 20	MS	0	20	80	1.55±0.1	1.45±0.08
T6×10REP	Povidone solution 1 % 60′ + Activated carbon 0.5 $g\ l^{-1}$ + Fungicides (Phyton 0.5 $ml\ l^{-1}$ + Carbendazim 0.5 $ml\ l^{-1}$) + Kilol 30 drops l^{-1} + Ascorbic acid 0.1$g\ l^{-1}$ 20′ + NaOCl 0.5 % 60′ + NaOCl 1 % 10′ + Tween 20	WPM	0	0	100	1.67±0.1	1.55±0.08

8 Multiplication Phase

The variables evaluated in this phase were bud height, number of knots, multiplication rate and root length. Plants obtained from the seed phase of the T3 treatment were used. The MS culture medium without the addition of BAP (6-Bencilaminopurin) showed the best result, obtaining a bud height of 1.95 ± 0.05 cm and number of knots 1.94 ± 0.04, followed by MS without addition of AG_3 ((Gibberellic acid) with an average of 1.78 ± 0.05 cm and number of knots of 1.79 ± 0.04 after 30 days of evaluation. These results are higher than those mentioned by Cárdenas (2011), in which the bud height obtained was 0.4 cm and the number of knots 1.25, using MS without the addition of hormones. In the study with *C. espinosa*, the concentration of BAP (6-Bencilaminopurin) did not significantly influence the number of initial explants regarding the control treatment. However, the addition of 0.25 and 0.50 $mg\ l^{-1}$ significantly favored the number of buds obtained from an initial explant. The lowest concentration of BAP (6-Bencilaminopurin) 0.25 $mg\ l^{-1}$ was selected as the best treatment for this phase of the process, with 96.6 % sprouting and in 30 days of cultivation an established vigorous explant was achieved, with an average of 2.07 buds of 6.71 cm long (Núñez et al., 2017).

The multiplication rate is the arithmetic mean of the number of buds generated at 30 days in each treatment. It was 2.60 for the culture medium MS without the addition of BAP (6-Bencilaminopurin), and 2.38 for MS without the addition of AG_3 (Gibberellic acid). The MS culture medium without the addition of hormones guaranteed a multiplication rate of 2.60.

García et al. (2018), mentions that culture medium supplemented with hormones such as BAP (6-

Bencilaminopurin) promoted proliferation of axillary buds, but also caused physiological effects (abundant callus growth at the base of seedlings being this negative) as evidenced in treatments with the addition of BAP (6-Bencilaminopurin) at 0.1 $g\ l^{-1}$. Different authors refer to the positive effect of 6-BAP and ANA (1-naftalenactic acid) for *in vitro* multiplication of other tree legume species, such is the case of Rahman et al. (1993), who during the *in vitro* establishment of nodal segments of *C. pulcherrima*, reported a multiplication coefficient of 5.8 new buds/explant with a MS culture medium combined with 1.0 $mg\ l^{-1}$ of BAP (6-Benaminocilpurin) and + 1,0 $mg\ l^{-1}$ of ANA (1-l-naftalen acetic acid).

Agramonte et al. (2001), when evaluating the effect of different doses of 6- BAP (0; 0.35; 0.50 and 1.0 $mg\ l^{-1}$) on *in vitro* multiplication of *Eucaliptus grandis*, observed a tendency to increase the values of the variable number of buds per explant, and a reduction in their length with increased concentration, thus demonstrating that relatively high doses induce a high axillary tilling and reduce the size of the bud, resulting in an affectation of the multiplication coefficient.

The highest average of root length was for the MS culture medium without the addition of hormones with an average 2.25 $\pm$ 0.11 cm. Saucedo et al. (2009), in the research with *Xanthosoma sagittifolium*, mentions that the use of cultured medium without the addition of BAP (6-Bencilaminopurin) in the rooting phase developed longer roots, obtaining an average of 4.5 cm. The use of BAP (6-Bencilaminopurin) caused symptoms of hypehydricity in plants.

In general, Murashige and Skoog (1962) suggested that the concentration management of mineral salts is widely recommended to stimulate rooting, the formation of buds, leaves and the length of plants *in vitro*, while Piqueras (2000) observed that the concentration of mineral salts can affect the morphology of micropropagated plants through changes in osmotic pressure, mainly affecting the development of *in vitro* roots.

Jiménez et al. (2016) in their study with *Dianthus caryophyllus*, in assessing the effect of the concentration of inorganic salts of the culture medium in relation to the length of the plant did not observe differences between treatments, except with the witness, thus maybe it had the nutrients needed for its growth. However, a reduction in the growth was seen with the decrease in the concentration of mineral salts, being lower in the treatment where 25% was used. These results show that the decrease of the mineral content in the culture media favored the growth retardation, due to alterations occurring in the cellular metabolism.

Figure 2. A) Multiplication of vitroplants (MS) in a culture medium without any addition of hormones. B) Root length evaluation. C) Callus formation at the base of plants in a culture medium (MS) with the addition of BAP (0,1 $g\ l^{-1}$).

9 Conclusions

The best treatment for the disinfection phase and *in vitro* establishment of *Buddleja incana* seeds was T3 (Povidone solution 1% 60′ + Kilol 30 drops l^{-1} + distilled water 30′ + Benomil 1 $g\ l^{-1}$ + NaOCl 3% 10′), with a contamination rate of 0% and survival rate of 100%. The T3 treatment contained MS culture medium that was effective for the variable bud length, as the highest values were 1.33 ± 0.03 cm at 30 days and 1.47 ± 0.03 cm at 45 days. According to Jiménez et al. (2001), it is important to take into account the area of the tissue used to start *in vitro*, cultivation, since it has a great influence on the efficiency of disinfection.

The *in vitro* establishment phase of *Buddleja incana* buds, showed that the best treatment was T6 (Phyton 0.5 $ml\ l^{-1}$ + Carbendazim 0.5 $ml\ l^{-1}$) + Kilol 30 drops l^{-1} + Ascorbic acid $0.1g\ l^{-1}$ 20′ + NaOCl 0.5 % 60′ + NaOCl 1 % 10′ + Tween 20) with 0% contamination. The bud percentage in this treatment was 100%. The number and length of buds had an average of 1.67 ± 0.1 buds/explant and 1.55 ± 0.08 cm of length. In the work carried out by Gutiérrez (2002), in *Alnus acuminata* from nod segments and leaf, a disinfection protocol was used with very similar concentrations of NaClO and times proposed in Marulanda and Isaza (2004). The results indicated that disinfection treatment with NaClO at 1% resulted in less oxidation in the leaves than in the nodal segments.

In *in vitro* multiplication essays of *Buddleja incana*, the MS culture medium presented the best results in treatments without the addition of the hormones BAP and AG_3. The bud length had an average of 1.95 ± 0.05 cm, the number of knots had an average of 1.94 ± 0.04 knots/explant and multiplication rate of 2.60. The results obtained in the multiplication phase showed that no rooting promoter was required since the root length had an average of 2.25 ± 0.08 cm.

This research represents an initial phase of establishment and *in vitro* multiplication, where the results indicate a probability of success in the micropropagation process of this species; however, feasibility studies in the field still need to be performed.

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LIVESTOCK SYSTEMS

Integral typification of goat systems of Santa Elena province, Ecuador

Tipificación integral de sistemas caprinos de la provincia de Santa Elena, Ecuador

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Resumen

Como paso previo para proponer estrategias de desarrollo en sistemas de producción caprina (SPC) de la provincia de Santa Elena, Ecuador, se propuso tipificar los sistemas mediante indicadores socio-económicos, productivos y ambientales. Se encuestó a 172 productores sobre composición familiar y participación en el trabajo, tecnología adoptada, comunicaciones, composición del hato, existencias ganaderas, uso del suelo, ingresos, infraestructura y servicios básicos, acceso a las explotaciones, asistencia técnica y capacitación, trabajo e ingresos extraprediales, manejo e instalaciones, fuente de agua y suplemento alimenticio, aspecto reproductivo, dificultades en la producción de caprinos, comercialización de caprinos. Se redujo el número de variables mediante análisis de componentes principales (ACP) y pruebas de χ^2, para las variables cuantitativas y cualitativas, respectivamente. Con las CP que explicaron el 80% de la variabilidad se llevó a cabo un análisis de conglomerados (AC) que dividió a los establecimientos en 7 grupos. Mediante un análisis de correspondencias múltiples (ACM) se identificaron asociaciones entre grupos y modalidades de las variables cualitativas. Las variables que más aportaron a la diferenciación de los grupos de SP estaban relacionadas con la producción caprina, el tamaño de la familia, las producciones avícola, agrícola, porcina y bovina, la participación en el trabajo de la familia, los ingresos por beneficios sociales, el tamaño de la finca y la tecnología adoptada. Dos grupos eran mixtos; uno con mayor actividad porcina; dos se caracterizaron por la cría de cabras para el consumo cuyos ingresos provenían de fuentes externas y dos grupos se definieron por la edad de sus productores.

Palabras clave: Indicadores económicos, indicadores productivos, indicadores socio-culturales, sistemas de producción, cabras.

Abstract

As a preliminary step to propose development strategies in the goat productive systems (SPC) of the Santa Elena province, Ecuador, the SPC were typified by means of socio-economic, productive and environmental indicators. A sample of 172 producers was surveyed on family composition and participation in work, adopted technology, communications, composition of the herd, livestock stock, land use, income, infrastructure and basic services, access to farms, technical assistance and training, off-farm work and income, management and facilities, water sources and food supplement, reproductive aspects, difficulties in the production of goats, marketing of goats. The number of proposed variables was reduced by principal component analysis (PCA) and χ^2 tests for quantitative and qualitative variables, respectively. Based on the main components that explained 80 % of the variability, a conglomerate analysis (CA) was carried out, resulting in the division of the SPC into 7 groups. Through a multiple correspondence analysis (MCA), associations between groups and modalities of qualitative variables were identified. The variables that contributed most to the differentiation of SPC groups were related to goat production, family size, poultry, agricultural, pig and bovine production, participation in family work, income from social benefits, the size of the farm and the technology adopted. Two groups were mixed, one with a predominance of livestock activity and the other with agricultural activity; a group with greater swine activity; two groups were characterized by the breeding of goats for consumption, whose income came from external sources; and two groups were defined by the age of their producers.

Keywords: Economic indicators, productive indicators, socio-cultural indicators, production systems, goats.

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1 Introduction

Knowing the reality of production systems is necessary in order to propose solutions to critical situations, implement genetic improvement plans, assess the environmental impact of livestock, among others. The identification of strengths and weaknesses allows to propose strategies that should be specific to each type of production units if there was diversity between the systems of a region (Cabrera et al., 2004; Dufumier, 1990).

It is recommended to propose development alternatives along with the producers for productive subsistence systems (Macías, 2015), with the aim of stimulating deep personal interest in the care of animals and crops; offering more products for sale through a better use of what is available locally (biological resources, native vegetation, livestock, etc.); introducing knowledge and technology along with the assessment of skills, existing knowledge and experiences; collaborating with producers to do a better performance (Hodges et al., 2014). On the other hand, subsistence systems host valuable zoogenetic resources that need to be preserved because they have evolved in their adaptation to adverse environments and there are moments in which genetic biodiversity is being lost (Núñez-Domínguez et al., 2016; Dorji et al., 2017).

Differentiated groups can be identified and typified from the initial state analysis and system characterization. Different methods for carrying out stratification based on a wide range of environmental, productive, economic and social aspects, which meet different objectives, have been proposed. Another form of stratification of systems is the application of statistical grouping methods (Robinson et al., 2011).

The goat production systems (SPC) of the province of Santa Elena (PSE), Ecuador, are low-income subsistence systems. Santa Elena has the highest percentage of goats at the coastal level (53 %) and 7.2 % of the country's total stocks (Sistema Nacional de Información, 2018). As a pre-development step, it was proposed to test whether there is heterogeneity among the province's goat systems. A process was carried out which included: surveying establishments, survey to the producers and classification of prevailing production systems using socio-cultural, productive and economic indicators.

2 Materials and methods

Santa Elena is a province located in the coast of the Republic of Ecuador, located southwest of the Ecuadorian coast. Politically, it is divided into three parishes: La Libertad, Salinas and Santa Elena (3668.9 km^2). The latter is the largest parish of the province, with a high rural population. It has 67 communes registered at the Agricultural Provincial Directorate of Santa Elena (Ministerio de Agricultura y Ganadería, 2018).

Based on oral information received from the community and the Ministry of Agriculture and Livestock (MAG), 586 goat production systems were identified, with an estimated total of 11,977 heads, in the 3 climatic zones in which the province is divided. Within each zone, a frequency distribution of the number of goat producers was performed according to the herd size. The total number of establishments to be sampled was estimated through the application of the finite sample formula (FAO, 2012). Once the total sample size was defined, stratified sampling was performed by zone and herd size using proportional allocation.

A structured survey was designed for the producers, taking as reference those used by Falagan (1988), Bedotti (2000) and Lanari (2004), including socio-cultural, productive and economic aspects (Tables 1, 2 and 3).

It was tested whether there were differences between agro-ecological zones by means of a multivariate variance analysis (MANOVA) for the quantitative variables. In order to decrease the number of variables: the association degree between the 27 quantitative variables was estimated to define the exclusion of those variables that had the same characteristic ($r > 0{,}70$); and a main component analysis (ACP) was performed (Peña, 2002), with the components explaining at least 80 % of the total variability chosen.

With the main components selected through a cluster analysis (AC) (Dallas, 2000), production systems were classified into homogeneous groups by applying: the hierarchical method, the Euclidean

distance and the Ward method (Ward, 1963). The profiles of each group (G) were described by the standardized averages of all variables and represented on a radial chart.

For the 77 qualitative variables, the relative frequencies for each of the resulting producer groups of AC and the total frequencies were estimated. Pearson tests were performed ($p < 0,05$) with the aim of selecting the variables with significant differences between the groups. With the selected variables, a multi-match analysis (CMA) was performed to identify the most important associations between the modalities of the qualitative variables (Greenacre, 1984) and the groups of the producers. Statistical calculations were performed with the professional Infostat programme (Di Rienzo et al., 2008).

3 Results

On the basis of the information received on the total number of producers per zone, they were distributed according to the size of the herd, and the sample was estimated at 229. The proportional allocation by area and size of the herd is detailed in Table 4. Out of these, 22 % were not surveyed by different reasons: duplicate information, absence of the producer at the time of the visit, abandonment of the activity, etc. None of the producer belonging to the semi-wet zone was surveyed due to inaccessibility to the area at the time of the survey. A total of 172 establishments were not taken into account, 69 in the dry area and 103 in the semi-arid area.

Table 1. Quantitative variables related to socio-cultural, productive and economic aspects

Type of information	Variables	
Family composition and participation in the work	Age of the producer reflected in years	ED
	Number of family members living in the same house	TP
	Number of people who work exclusively in the farm	PD
	Number of people who work exclusively outside the farm	PF
	Number of people who work inside and outside the farm	PDF
Technology used	Knowledge of the reproductive cycle	GCR
	Degree of health control	GCS
Communication	Distance to the town that the producer has more communication with (km)	DL
Composition of the herd (number of heads per category))	Female goat	CHVA
	Female nanny goat	CBLA
	Female kid	CBTA
	Male goat	CHVO
	Male nanny goat	CHTO
	Male kid	CBTO
Livestock inventory	Goat	EC
	Bovine	EB
	Swine	EP
	Poultry	EA
Use of the soil	Hectares sown	HaS
	Surface of the farm in hectares	HaF
Revenue in $ the last year	By the sale of agricultural crops	$IAG
	By the bovine sale	$IB
	By the caprine sale	$IC
	By the swine sale	$IP
	By the poultry sale	$IA
	By other external sources	$IEX
	By other social benefits	$IBS

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The MANOVA results showed no significant differences between the two agroecological zones for the quantitative variables (Table 1). Therefore, from now on the analyses are presented taking the two zones together.

Out of the 27 original quantitative variables, were excluded: a) the variables related to the composition of the herd since they were highly correlated with EC; b) distance to the location that the producer has more communication with by not contributing to the total variability in the first ACP analysis. A second ACP was performed with the remaining 20 variables. The first 12 components explained 87 % of the total variability, and these were interpreted based on the coefficients with more weight of each component and their signs.

The analysis of conglomerates carried out with the first 12 main components divided the production facilities into 7 groups distributed in both agroecological zones. More than half of the establishments were in the G5 (23.8 %) and G6 (30.2 %). The lowest percentage was shared by the G3 (2.3 %) and G7 (4.07 %). The G1, G2 and G4 accounted for 14 %, 17.4 % and 8.1 %, respectively.

Table 6 shows the averages of the 20 variables for each of the 7 groups and the overall average. Figure 1 depicts the standardized averages of variables using a general radial chart. The black circle indicates the zero or average value of each standardized variable. For example, the G3 has above-average values for family work in the farm (PD) and goat income ($IC), although goat stocks are below the average it is the group with the highest poultry production.

Qualitative variables that did not provide variability within groups and had a relative frequency for a category higher than 95 % PEN, TTI, PVI, TVI, SAL, ALC, MAL, VAC, TEM, IOF, ICA, CEC, TI, SCT, CDC, DOB, VRE, LCO, FPA) were excluded from the analysis. For example, 100 % of the producers expressed electricity lighting service for SAL.

Tests χ^2 for the remaining 58 variables showed significant differences ($p < 0{,}05$) between the seven groups for 9 of them (DAP, ACT, RAT, RCA, LOC, APA, MCP, OC, MPR).

Table 2. Qualitative variables related to socio-cultural and economic aspects

Data of the producer	Infrastructure, basic services	Access to the land	Organizational	Labour
-Interviewed Person (PEN) -Gender (SEX) -Marital Status (ECI) -Level of Studies (EDU) -Social Security (SEG) -Assistant (AYU) -Successor (SUC)	-Land tenure (TTI) -Housing ownership (PVI) -Housing type (TVI) -Housing construction material (MCV) -Housing status (EVI) -Lighting service (SAL) -Drinking water supply (DAP) -Water access medium (MAA) -Use of energy to prepare food (UEN) -Sewerage service (ALC) -Sewerage medium (MAL) -Access to technology (ACT)	-Location with more communication (LCO) -Access roads (VAC) -Type of road (TCA) -State of the road (ECA) -Means of transportation (MTR)	-Participates in an organization (POrg) -Organization in which the person participates (OPA) -Receives technical assistance (RAT) -Institution from which the person receives technical assistance (IRA) -Type of institution (TIS) -Applies Techniques (ATE) -Observed Results (ORE) -Receives training (RCA) -Is interested in receiving training(IRC)	-Second employment (EEE) -Type of entity in which he works (TET) -Type of employment (TEM) -Type of work he performs (TLR) -It has other income sources (OFE) -Origin of the income from other sources (IOF)

The ACM showed that in the first dimension the use of technical assistance and training were significant (RATsi and RCAsi), and in the second the non-access to technology (ACTno). These first two dimensions explained 37% of the variability of sociocultural and management variables. The third, fourth and fifth dimensions were related to the interest of receiving or not training, and the sixth with the farm as a grazing area of the goats.

3.1 Overview of the production systems of Santa Elena Peninsula

Most goat herd owners are male, with elementary school studies (61%), with peasant social insurance, and whose helpers in the handling of goats is a family member, and it is sure that their children would continue with the breeding of goats.

Producers participate in the communal meetings and only one-third claimed to having received technical assistance, most of the beneficiaries were those producers who participated in state projects and who introduced goats from other areas in the last two decades. There is a strong interest of small producers of the PSE in improving goat production (94%), despite the highest current incomes not coming from goat production but from other external sources. They have extra income from jobs of private companies and on a temporary basis or receive the Human Development Bonus (BDH).

Table 3. Qualitative variables related to the goat production aspect

Management and infrastructure of goat systems	Water source and food supplementation	Reproductive aspect	Difficulties in the production of goats	Commercialization of goats
-Place of origin of goats (LOC) -Grazing system (SPA) -Grazing area (APA) -The goats of the neighbor graze in the same place (CVP) -Water/food implementation used in the field (ICA) -Farmyards for the goats (CEC) -Farmyard owner (PCO) -Farmyard location (UCO) -Construction material walls and floor (MCP) - Roof construction material (MCT) -Use of implements in the water/food pen (UI) -Type of water/food implements (TI) -Implement manufacturing material (MFI) -Implement origin (PI) -Goats are released every day (SCT) -Time in which goats are taken inside (HSC) -Goats are locked every day (ECT) -Time in which goats are locked (HEC) -Daily control of goats (CDC) -Maximum distance that goats walk (km) (DMC) -A dog helps with the goats (TPC) -Other paddock (OC)	-Purchase of the food supplement (CSA) -Goat water fountain (FAG)	-Parity Control (CPA) -Obtaining of Male Breeders (ORM) -Difficulty for obtaining breeders (DOB) -Interest in buying males (ICM) -Obtaining of female breeders (ORH)	-Diseases (ENF) -Problems in the production of goats (PPC) -What is needed to improve the production (MPR)	-Goat product obtained (PCO) -Breeding objective (OCR) -Reasons to sale (MVE) -Way the animal is sold (FVE) -Sale of breeders (VRE) -Places of marketing (LCO) -Payment method (FPA) -Difficulties in marketing (DCO)

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The goat establishments of the PSE are located in communal areas and with housing with low average conditions. All the farms have electric lighting, without sewerage, with access to water through the public network (57%). The province has access with unpaved roads, in poor condition, and there is no public transportation.

The handling of the goats is free grazing, without using any implements for water and food in the field. The producers have their own pens, located near the house. In general, producers release the goats and lock them when they return. In winter, animals can stay in the countryside for up to 3 months. Goats get their food in the field, if producers grow corn, goats take advantage of the stubble from the crop and in some cases they receive food scraps in the driest seasons. Supplementation is not a common practice because of the costs involved. The water consumption of goats comes from natural sources, except in the dry season, when they are forced to look for other sources of water (drinking water). Males remain with females in the herd, until they are sold once they are 8 months old.

No practices such as castration are used. Most male breeders are self-produced. Goats are detected when they are about to give birth, separate and retain in the paddock. The degree of health control (GCS) is very low, with some kind of health problem.

The product obtained is meat, and very few obtain milk or take advantage of manure as fertilizer; only 24% breed exclusively for sale, 16% sell them for the family sustenance and 84% sell their animals due to family emergencies. The sale of goats is made by walking in the houses or farms and only 5% is sold to the commercial canton of the province and other localities in cash. Only 6% of producers rarely sell some male as breeder.

Table 4. Total number of producers of goats and producers to survey by area according to the herd size

Herd size	Total number of producers			Number of producers to be surveyed		
	Semi-arid area	Dry area	Semi-wet area	Semi-arid area	Dry area	Semi-wet area
5-25	290	159	2	113	62	1
26-45	54	24		21	9	
46-65	15	9		6	4	
66-85	7	5		3	2	
86 and more	13	8		5	3	
Total of producers/area	379	205	2	148	80	1
Total goats/area	7.363	4.594	20			

Fuente: MAG, FEDECOMSE, Asociación de Capricultores, Casas Comunales, productores (Información oral)

3.2 Specific characteristics of the groups of goat production systems in the province of Santa Elena (Table 6 and Figure 1):

Group 1: it is constituted by families with lots of family members with the highest average of people working outside the farm. They have very low knowledge of the reproductive cycle (CR) and health control (CS), they received very little technical assistance and training, and have access to the use of technology. They have the lowest livestock stocks, including goats, and low numbers of planted hectares. They have farms with the lowest surface. They have other incomes by social benefits which are higher than the average.

Group 2: it is constituted by young producers with mid-sized families, their members commit at least part of their working day to work on the farm. They have the highest degree of knowledge of CR and CS, half of them received technical assistance and/or training. The farms have the largest total area and planted area. Livestock stocks are below the overall average, 71% corresponds to goat stocks. They have the highest total income and the highest

sales income from agricultural products, they are among the highest goat earners and with external incomes.

Group 3: the age of the producers is close to the average, with numerous family members working mainly in the farm. They have good knowledge of the CR and poor CS, all the establishments in the group received technical assistance and training. The poultry stocks are the highest and the number of hectares planted is one of the highest. They have high revenue from the sale of agricultural products and goats, and the highest revenue from poultry sales. They have low external incomes.

Group 4: the age of the producers is on the average; it has the smallest number of family members, and they work inside and outside the farm. The level of CR knowledge is above the average, even though they received very little technical assistance and training. They have the highest swine stocks and average goat stocks, with intermediate areas of sown hectares. They have the highest swine sales revenue and intermediate extra income.

Group 5: they are the oldest producers with the lowest number of family members who work mainly in the farm. The degree of knowledge of the CR is close to the average, but the CS is one of the lowest. They received little technical assistance and training. They are the ones with the least access to technology. Livestock stocks are low, but they have average goat stocks. The number of hectares planted is the lowest, it is the second group with the highest size of the farm. Social benefit income (BDH) is the highest and extra incomes are the lowest.

Group 6: they are the youngest producers with intermediate family size and high number of people working in the farm. The knowledge of CR and CS are close to the average, they received little technical assistance or training. They have farm with the highest surface, the area is sown and have low livestock stocks. They have the highest extra incomes and lowest incomes for social benefits.

Group 7: they are middle-aged producers, they have small families whose members work inside and outside the farm. They have a high degree of CR knowledge and very high CS; also, they have access to technology. They received technical assistance and training. It is the second group in livestock stocks, and they have the highest goat and bovine stocks, and intermediate swine stocks. They have farms with very high surface. It is the group with the highest incomes from the sale of cattle and goats, and they have one of the highest incomes by the sale of swine. It is the second group with high total incomes.

Table 5. Interpretation of the first twelve main components

Component	Interpretation		Explained variability (%)	Accumulated %
$\mathbf{Y_1}$	Goat production		14	14
$\mathbf{Y_2}$	Size of the family		12	26
$\mathbf{Y_3}$	Poultry production		10	36
$\mathbf{Y_4}$	Agricultural production *vs* swine production		9	45
$\mathbf{Y_5}$	Bovine production		8	54
$\mathbf{Y_6}$	Participation at work:	PD	6	60
$\mathbf{Y_7}$		PDF	5	65
$\mathbf{Y_8}$		PF	5	70
$\mathbf{Y_9}$	Incomes by social benefits		5	75
$\mathbf{Y_{10}}$	Size of the farm		4	79
$\mathbf{Y_{11}}$	Technology use:	GCR	4	83
$\mathbf{Y_{12}}$		GCS	3	87

Table 6. Averages by number of stablishments

Groups	1	2	3	4	5	6	7	General average
ED	55.1	49.5	57.7	57.3	71.6	46.4	55.3	56.1
TP	6.75	4.97	5.00	3.71	2.80	4.81	3.43	4.50
PD	1.68	1.65	3.13	1.52	1.43	2.12	1.57	1.87
PF	1.14	0.08	0.00	0.39	0.02	0.02	0.14	0.26
PDF	0.78	0.66	0.35	0.40	0.24	0.39	0.50	0.47
GCR	2.83	5.20	4. 25	4.50	4.02	4.12	5.00	4.27
GCS	0.88	2.90	1.50	1.71	1.49	1.90	2.57	1.85
EC	11.5	24.6	21.0	24.1	25.8	16.1	54.6	25.40
EB	1.04	4.07	0.25	1.79	1.83	0.48	31.3	5.82
EP	0.42	1.23	3.50	10.6	0.49	0.65	4.29	3.02
EA	3.13	4.57	76.2	8.07	0.61	2.23	3.86	14.10
HaS	0.96	2.89	1.63	1.00	0.51	0.80	0.71	1.210
HaF	3.54	8.48	5.00	4.54	6.76	2.01	7.71	5.43
$IAG	412	1941	1147	277	110	333	1091	759
$IB	25.4	52.7	0.00	42.9	7.32	5.00	607	105
$IC	41.2	225	355	164	175	84.4	355	199
$IP	0.00	12.3	50.0	396	0.00	12.9	146	88.2
$IA	4.08	9.33	540	22	0.00	3.85	0.00	82.7
$IEX	2316	2222	690	2039	472	2688	1522	1707
$IBS	551	481	450	429	759	404	429	500
∑ing	3350	4945	3232	3370	1524	3531	4150	3443
∑Ex	16.1	34.4	101	44.6	28.8	19.4	94	48.3

ED: age of the producer, **TP:** number of family members living in the same house, **PD:** number of people who work exclusively in the farm, **PF:** number of people who work exclusively outside the farm, **PDF:** number of people working inside and outside the farm, **GCR:** knowledge of the reproductive cycle, **GCS:** degree of health control, **EC:** goat stocks, EB: bovine stocks, **EP:** swine stocks, **EA:** poultry stocks, **HaS:** hectares planted, **HaF:** area of the farm in hectares, $**IAG:** total sale of agricultural crops, $**IB:** total bovine sale, $**IC:** total sale of goats, $**IP:** total swine sales, $**IA:** total poultry sales, $**IEX:** income from other working activities, $**IBS:** income from social benefits,∑**Ing** total income, ∑**Ex** total stocks.

4 Discussion

The classification of the PSE's goat production systems allowed defining the common characteristics of the 172 establishments, and identifying the heterogeneity present by classifying them into 7 groups, distributed homogeneously throughout the province. Two mixed groups were found, one with a predominance of livestock activity (G3) and one of agricultural activity (G2); a group committed more to swine livestock activity (G4); 2 groups defined by the rearing of goats for the consumption whose income comes from external sources (G1 and G6); and 2 other age-defined groups of their producers, whose income comes from the older social benefit group (G5) and the younger external income group (G6).

Similar variables were used in other regions to classify establishments (Coronel de Renolfi, M. and Ortuño, S., 2005; Costa et al., 2008; Garcia et al., 2010; Hernández et al., 2011; Gómez, 2013; Hernández et al., 2013; Chivangulula et al., 2014; Umunna et al., 2014; Barboza, 2018). This work also included incomes with the aim of identifying different subsistence strategies.

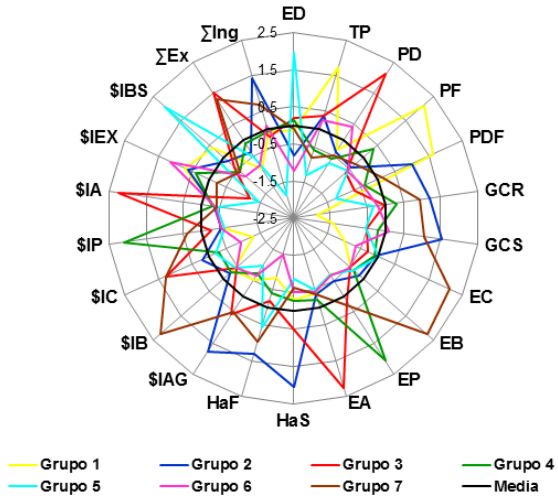

Figure 1. Profile of the seven groups belonging to the productive systems

The goat establishments of the PSE are located in the rural area, on communal land with the right of possession. In other countries, other forms of land tenure were found: property, occupants or located on tax lands (Hernández et al., 2011; Vargas et al., 2014; Torres, 2014; Bedotti, 2000). The level of education is an important aspect to highlight when carrying out specific programs that help improve the goat production. 61% of PSE producers completed elementary school. These results differ from those reported in other countries, where more than 60% of producers did not have any study (Serrano, 2010; Falagan, 1988; Luque, 2011; Bedotti et al., 2005; Gómez, 2013; Fikru and Gebeyew, 2015; Hagos et al., 2016).

The continuity in the rearing of goats by the owners' children, observed in 62% of the surveys, is another aspect to be highlighted because it allows the possibility of proposing longer-term strategies. This percentage is higher than 42% presented in Murcia, Spain (Falagan, 1988), but less than 80% or more elsewhere (Bedotti, 2000; Luque, 2011; Gómez, 2013).

97% of the PSE systems are family systems, the owner is responsible for the care of goats, without hiring any paid staff. While the majority were men (63%), only 37% were women, number which is higher than the 20% observed in Mexico and Ethiopia (Serrano, 2010; Tsegaye, 2009; Hagos et al., 2016). The presence of women in goat activities could be related to the type of animal being bred and to the management (Serrano, 2010).

According to Devendra's classification of goat systems Devendra (2015), PSE systems are categorized as extensive and free grazing. Owners were not found to lease farms or land for the exclusive use of their animals. This is similar to what was mentioned in Venezuela, Colombia, Peru and Ethiopia (Valerio et al., 2010; Guerrero et al., 2012; Gómez, 2013; Hagos et al., 2016); but different from other places where grazing grounds are leased (Carné et al., 2007).

The overall average of goats per herd in the PSE is 25. The low number could be explained by: a) theft and predation; b) the breeding target: only 25% of producers sell their animals to meet part of their basic needs, the rest raise them as a means of saving; c) low technology incorporation and low-technical assistance that generates low incomes as in other regions (Costa et al., 2008; Valerio et al., 2010; Hernández et al., 2011; Navarro et al., 2011; Gómez, 2013); d) land tenure of another person or the community (Bellido et al., 2001).

The annual income for the 7 groups is variable, with a monthly average of $287. All groups are well below meeting the basic monthly needs of producers since, according to INEC, the monthly basic foodbasket (CFB) at the time of this study was $425.

The goat systems of the PSE have characteristics of family rural systems, with a subsistence economy with less than 25 goats per herd, with monthly incomes that do not allow them to access the basic food basket; therefore, they do not cover the basic needs of the producers and their families. They are part of the the marginal category proposed by the Instituto Nacional de Estadística y Censos (2010) and the Ministerio de Agricultura y Ganadería (2018). However, they have knowledge and experience gained over time in the management and care of biodiversity. The management of this biodiversity acquired over years, as well as a set of knowledge and practices, which is not easy to describe, should not disappear (Jarvis et al., 2011). Despite the low availability of assets and low levels of productivity, family farming is a major supplier of popular staple foods (FAO/BID, 2007; Hodges et al., 2014).

5 Conclusions

The goat production systems of the province of Santa Elena have been classified for the first time. The results obtained in this research show that there is heterogeneity between the goat systems in Santa Elena, and they are a contribution to take into account when planning options to enhance their development.

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VETERINARY SCIENCES

ABYA YALA UNIVERSIDAD POLITÉCNICA SALESIANA

META-ANALYSIS OF THE EFFECT OF GLUTAMINE DIETARY INCLUSION ON PRODUCTIVE PERFORMANCE IN PIGLETS

META-ANÁLISIS DEL EFECTO DE LA INCLUSIÓN ALIMENTICIA DE GLUTAMINA SOBRE EL DESEMPEÑO PRODUCTIVO EN LECHONES

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Resumen

La glutamina no es considerada un aminoácido esencial; sin embargo, juega un rol importante en la salud y crecimiento de neonatos y adultos. En lechones el destete genera atrofia de las vellosidades intestinales y retraso en el crecimiento. Varios trabajos han demostrado que la suplementación de glutamina (0,2-2 %) disminuye los efectos adversos del estrés post-destete en lechones. El objetivo de este manuscrito fue evaluar el tamaño de efecto de la suplementación de glutamina sobre el rendimiento productivo de lechones, la consistencia de su efecto y la influencia de otros factores mediante el uso de meta-análisis. La administración de glutamina mejora la conversión alimenticia ($p < 0,001$), y los lechones que reciben glutamina convierten mejor el alimento cuando la suplementación duró entre 7 a 14 días ($p = 0,0023$), pues requieren 121,6 *g* menos de alimento en comparación con el grupo control para hacer 1 *kg* de peso vivo. Cuando la suplementación se realiza por un periodo de 15 a 30 días y 7 a 30 días, el ahorro de alimento es de 70,6 *g* ($p < 0,001$) y 87,3 *g* ($p < 0,001$) por *kg* de peso vivo respectivamente. La ganancia diaria de peso es superior en 20,3 *g/día* ($p = 0,0029$) frente al grupo control entre los 7 a 30 días de suplementación y de 28,2 *g/día* ($p = 0,0002$) entre los 15 a 30 días. La edad y peso del lechón al inicio de la suplementación, el nivel de lisina, la proteína cruda y el número de repeticiones por tratamiento influyen en el efecto de la glutamina sobre las variables evaluadas.

Palabras clave: nutrición, dieta, nutrientes, aminoácidos, cerdos

Abstract

Glutamine is not considered an essential amino acid; however, it plays an important role in the health and growth of neonates and adults. In piglets, weaning generates atrophy of the intestinal villi and growth retardation. Several

studies have shown that glutamine supplementation (0.2-2 %) decreases the adverse effects of post-weaning stress in piglets. The aim of this article was to evaluate the effect size of glutamine supplementation on the productive performance of piglets, the consistency of their effect and the influence of other factors through the use of meta-analysis. The administration of glutamine improves the feed conversion (p <0.001), the piglets that receive glutamine convert the feed better when the supplementation lasted between 7 to 14 days (p =0.0023), since they require 121.6 g less of feed in comparison with the control group to make 1 kg of body weight. When the supplementation is done for a period of 15 to 30 days and 7 to 30 days, the saving of feed is 70.6 g (p <0.001) and 87.3 g (p <0.001) per kg of body weight, respectively. The daily weight gain is higher in 20.3 g/day ($p = 0.0029$) compared to the control group between 7 to 30 days of supplementation and 28.2 g/day ($p = 0.0002$) between 15 to 30 days. Age and weight of the piglet at the beginning of the supplementation, level of lysine, crude protein and the number of repetitions per treatment influence on the effect of glutamine on the variables evaluated.
Keywords: nutrition, diet, nutrients, amino acids, pigs

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1 Introduction

Wetting, stress by transportation and thermal stress increase the incidence of diseases, especially when they occur simultaneously (Varley and Wiseman, 2001). During the weaning process, stress can be induced by separation of the mother's piglet, relocation and mixture of litters, additionally there may be sudden changes in the diet that reduce or eliminate the food consumption in the first few hours of life (Wijtten et al., 2011; Campbell et al., 2013). Stress during weaning alters the development of the barrier functions of the gastro-intestinal tract that leads to permanent harmful consequences for intestinal health during the life of the pig (Moeser et al., 2017; Pluske et al., 2018). To combat the negative effects of weaning on the health of piglet, several strategies have been implemented, including weaning age management, environmental conditioning, nutritional manipulation and antibiotic use through a diet (Lalles et al., 2007; Gresse et al., 2017; Solà-Oriol and Gasa, 2017). Antibiotics applied in the diet may allow weaned piglet to reduce pathogenic burden and promote growth (Cromwell, 2002). However, due to the possible contribution of antibiotics to the development of antibiotic-resistant bacterial lines (Smith et al., 2010), their use is being limited. Glutamine dietary supplementation decreases health problems, prevents intestinal atrophy, maintains antioxidant status, decreases incidence of diarrhea, resulting in increased weight gain and improved efficiency (Haynes et al., 2009; Zhong et al., 2011; Wang et al., 2014; Watford, 2015). Similar results have also been reported in broiler chicken (Nassiri Moghaddam and Alizadeh-Ghamsari, 2013; Jazideh et al., 2014; Nascimento et al., 2014; Manvailer et al., 2015; Muro et al., 2015; Olubodun et al., 2015; Ribeiro Jr et al., 2015; Luquetti et al., 2016; Maiorka et al., 2016; Namroud et al., 2017).

Glutamine is the preferred fuel of intestinal cells and the immune system (Horio et al., 2008; Sakiyama et al., 2009; Zhong et al., 2012), supplementation of this amino acid aims to maintain intestinal function through weaning (Curi et al., 2007). Research shows that glutamine is an amino acid that is abundant in physiological fluids and body proteins and is a regulator of the genetic expression (Wu et al., 2011; Xi et al., 2011). Glutamine serves as an energy substrate for rapidly dividing cells and the construction of polypeptides and proteins (Rhoads and Wu, 2009), as it is an essential precursor of bioactive molecules (Boza et al., 2000). Endogenous production of Gln in mammals is insufficient during adverse conditions and there is evidence that Gln supplementation may be necessary in feeding young animals during states of poor nutrition (Wu et al., 1996; Chamorro et al., 2010). Numerous studies have investigated glutamine dietary supplementation in order to improve the productive performance of the piglet. However, several have been the levels of dietary inclusion and experimental circumstances under which these studies have been developed.

The aim of this article was to evaluate the size of Gln supplementation on the productive yield of piglets, the consistency of its effect and the influence of other factors by using the statistical meta-analysis tool.

2 Materials and methods

2.1 Source of information (data)

An electronic search of scientific articles was conducted in double-blind peer-reviewed indexed journals in the following electronic bases: CAB direct, Elsevier biobase-CABS, Google Scholar, MEDLINE, PubMed, Science Direct (Journal), Scopus, Academic Search Complete, CAB Abstract, Directory of Open Access Journals. A combination of keywords was used: glutamine, amino acids, diet, food, nutrition, piglets, weaning and their English equivalents, without date restrictions.

2.2 Inclusion criteria

Items in which glutamine was administered exclusively in piglets through a diet were selected. The articles had to include information regarding the number of experimental units (replications) per treatment, and the experiments needed include at least 2 treatments (including the control group: without Gln), weight and age of the piglet at the beginning of the study period, level of glutamine included in the food and duration of supplementation, level of lysine and raw protein in the food used (covariates). In addition, it should have included at least one of the interest response variables: daily weight gain (GDP), average daily food consumption (CDPA), and food conversion (CA). They should include mean (average) and some measure of variation (standard deviation (SD), standard error (SE)).

Table 1. Descriptive statistics (covariates)

	Average	Minimum	Maximum	Mode
Inclusion level of Gln in food (%)	0.96	0.20	2.00	1.00
Duration of supplementation (days)	16.34	7.00	30.00	14.00
Living weight (kg) onset of the supplementation	6.34	4.96	9.22	5.78
Age of supplementation (kg)	22.91	17.00	28.00	21.00
Lysin level in the diet (%)	1.33	1.17	1.60	1.30
Level of RP in the diet (%)	21.32	18.08	23.74	21.09
N° replications/treatment	5.42	2.00	12.00	3.00
N° replications/control	5.39	2.00	12.00	3.00

RP= Raw protein

2.3 Statistical analysis

A total of 13 scientific articles that met the abovementioned inclusion criteria were found (Wu et al., 1996; Lee et al., 2003; Domeneghini et al., 2004; Zhou et al., 2006; Zou et al., 2006; Abreu et al., 2010; Hsu et al., 2010; Shan et al., 2012; Xiao et al., 2012; Wang et al., 2014; He et al., 2016; Duttlinger et al., 2019). The data of the variables of interest were obtained from all 13 manuscripts, and it is important to mention that the meta-analysis technique considers all articles that meet the inclusion criteria (13) and does not sample them, as one of its purposes is to have the largest n number (number of repetitions) as possible. MIX 2.0 Pro was used for statistical data processing in Microsoft Excel (Bax, 2016). The size of the effect of glutamine supplementation was determined by mean difference (MD) between the treatment group and the control, with 95% confidence intervals. Heterogeneity was assessed by the inconsistency index (I^2) (Cochran, 1954; Higgins and Thompson, 2002). If there was heterogeneity, meta-regressions were carried out in order to explain the origin of such variability (Borenstein et al., 2011).

A random effects model was used as recommended by Sauvant et al. (2008). 9 meta-analyses were carried out from the 13 scientific articles (n=1902 animals). The variables analyzed were daily weight gain, average daily food consumption and food conversion. In each variable, 3 meta-analyses were performed according to the duration of glutamine supplementation: 7 to 30 days, 7 to 14 days and 15 to 30 days. This research did not follow the protocols established by PRISMA-P (Moher et al., 2015) as these were developed for human studies. This meta-analysis study follows a methodology of animal science studies, as detailed in several published articles in which meta-analysis was used in swine nutrition (Apple et al., 2007; Kiefer and Sanches, 2009; Sales, 2011; Andretta et al., 2012; Létourneau-Montminy et al., 2012; Remus et al., 2015; Hung et al., 2017; Metzler-Zebeli et al., 2012; Torres-Pitarch et al., 2017; Zeng et al., 2017; Torres-Pitarch et al., 2019).

3 Results

Table 1 summarizes the main descriptive statistics calculated (covariates) from the 13 research articles used. The DWG, ADFC, and FC response variables in each of the evaluated periods are presented in Table 2.

The average size of the effect (expressed in mean difference) of Gln supplementation in piglets on the productive yield are presented in Table 3. The results of the inconsistency index, and heterogeneity test between studies is shown in Table 4.

The results of the meta-regressions to determine the influence of each of the covariates on the pro-

Table 2. Summary of the variables response of productive parameters

Productive parameter	**Meta-analysis**	**Variable summary response**			
		Treatment		Control	
		Mean	SD	Mean	SD
DWG *(kg/day)*	7 to 30 days	0.22	0.12	0.20	0.11
	7 to 14 days	0.16	0.10	0.15	0.09
	15 to 30 days	0.31	0.10	0.29	0.10
ADFC *(kg/day)*	7 to 30 days	0.33	0.17	0.33	0.15
	7 to 14 days	0.25	0.12	0.26	0.10
	15 to 30 days	0.46	0.16	0.44	0.14
FC *(kg/kg)*	7 to 30 days	1.81	0.89	1.94	1.12
	7 to 14 days	1.98	1.08	2.14	1.37
	15 to 30 days	1.51	0.28	1.61	0.32

SD= standard deviation

ductive variables of interest in each period are presented in Table 5 (Gln inclusion level and duration of supplementation); Table 6 (live weight and age when the supplementation started); Table 7 (lysine level and raw protein in the diet) and Table 8 (number of replications).

Table 3. Effect of glutamine supplementation

Productive parameter	Meta-analysis	Effect size			
		MD	CI		*p*
DWG *(kg/day)*	7 to 30 days	0.0203	0.0070	0.0336	0.0029
	7 to 14 days	0.0146	−0.0041	0.0332	0.1252
	15 to 30 days	0.0282	0.0133	0.0431	0.0002
ADFC *(kg/day)*	7 to 30 days	0.0058	−0.0052	0.0168	0.3023
	7 to 14 days	−0.0033	−0.0128	0.0063	0.5030
	15 to 30 days	0.0237	0.0024	0.0450	0.0295
FC *(kg/kg)*	7 to 30 days	−0.0873	−0.1271	−0.0474	0.0000
	7 to 14 days	−0.1216	−0.1999	−0.0433	0.0023
	15 to 30 days	−0.0706	−0.1120	−0.0290	0.0008

MD= mean difference
CI= confidence interval
p = probability value

Table 4. Inconsistency index

Productive parameter	Meta-analysis	I^2 (%)
DWG *(kg/day)*	7 to 30 days	81.78
	7 to 14 days	87.40
	15 to 30 days	36.87
ADFC *(kg/day)*	7 to 30 days	48.75
	7 to 14 days	20.76
	15 to 30 days	45.79
FC *(kg/kg)*	7 to 30 days	21.20
	7 to 14 days	42.33
	15 to 30 days	0.00

4 Discussion

Glutamine works as an energy source for the growth of enterocytes, thus reducing the intestinal wall atrophy and the damage of the intestinal epithelium (Wang et al., 2014, 2015). Piglets receiving Gln food supplementation improve the protection of the intestinal barrier which leads to increased resistance to pathogens (Peng et al., 2004), and increased activity of digestive enzymes (Shan et al., 2012), consequently the increased nutrient utilization and the improvement in the productive performance (Jiang et al., 2009; Johnson and Lay Jr, 2017). The above can be seen in this research paper, as it was found that the food supplementation of Gln improved (p <0.001) the food conversion into piglets in any of the periods evaluated. It emphasizes that the piglets that receive Gln convert the food better when supplementation lasted between 7 to 14 days (MD= −0.1216; p =0.0023). Supplemented piglets require 121.6 *g* less food compared to the control group to make 1 *kg* of body weight between 7 to 14 days. When supplementation was done for a longer period (15 to 30 days) the food savings are 70.6 *g* to make a *kg* of body weight and 87.3 *g* when the Gln was supplemented between 7 to 30 days. With regard to the daily weight gain, Gln causes an increase in gain of 20.3 *g/day* (p =0.0029) over the DWG of the control group between 7 and 30 days of supplementation, and 28.2 *g/day* (p =0.0002) between 15 to 30 days.

The ADFC variable only has a significant increase when Gln administration was 15 to 30 days, consuming an additional 23.7 grams per day compared to the witness group. Similarly, in broiler chickens it was found that food supplementation of glutamine allows a more efficient use of ingested nutrients, improving the productive performance (Namroud et al., 2017), associated with the effect of Gln on the length of intestinal villi (Abdulkarimi et al., 2019). This will require less food to lead pigs to the market weight or shorten the days needed to reach the market weight, representing significant savings for the pig industry. In addition, Gln supplementation has shown to favor the immune system of piglets by ensuring better health and thus preserving nutrients for growth (Johnson et al., 2006; Zhong et al., 2012).

Table 5. Meta-regression for Gln inclusion level (%) duration of supplementation (days)

Productive parameter	Meta-regression	Gln inclusion level (%)				Duration of supplementation (days)			
		Intercept		Regression Coef.		Intercept		Regression Coef.	
		Estimated	p	Estimated	p	Estimated	p	Estimated	p
DWG *(kg/day)*	7 to 30 days	0.024	0.007	0.006	0.503	0.041	<0.001	−0.001	0.002
	7 to 14 days	0.025	0.010	0.005	0.578	0.094	<0.001	−0.007	<0.001
	15 to 30 days	0.014	0.505	0.011	0.577	0.121	0.002	−0.004	0.013
ADFC *(kg/day)*	7 to 30 days	0.003	0.762	−0.003	0.737	−0.019	0.002	0.002	<0.001
	7 to 14 days	0.004	0.700	−0.010	0.372	−0.025	0.064	0.002	0.117
	15 to 30 days	0.048	0.176	−0.025	0.475	0.097	0.018	−0.003	0.069
FC *(kg/kg)*	7 to 30 days	−0.050	0.044	−0.021	0.434	−0.063	0.143	−0.0002	0.927
	7 to 14 days	−0.050	0.051	−0.025	0.456	−0.260	0.260	0.015	0.390
	15 to 30 days	−0.054	0.599	−0.016	0.873	−0.080	0.638	0.0004	0.953

p = probability value

Regarding the heterogeneity between studies, it is reported that only in DWG (7 to 30d and 7 to 14d) the variability is high (> 75%). In the other variables, heterogeneity is between low to moderate (< 50%). In order to explain the variability between studies, meta-regressions were performed between the response variable and each of the co-variables indicated above. Gln inclusion level (%) in the diet for piglets has no impact (p >0.05) on any of the productive variables evaluated. The most commonly used level is 1%; however, the values range from 0.1 a 2%. Studies in chicken report that supplementation of high levels of glutamine negatively affects the production performance because they cause a reduction in the food consumption, as shown in the results found when a 4% level was used. Ad-

Table 6. Meta-regression body weight (kg) and age (days) at the beginning of Gln supplementation

Productive parameter	Meta-regression	Live weight at the beginning of the supplementation (kg)				Age at the beginning of the supplementation (days)			
		Intercept		Coef. Regression		Intercept		Coef. Regression	
		Estimated	*p*	Estimated	*p*	Estimated	*p*	Estimated	*p*
DWG *(kg/day)*	7 to 30 days	−0.086	<0.001	0.019	<0.001	0.043	0.041	−0.001	0.528
	7 to 14 days	−0.138	<0.001	0.029	<0.001	0.020	0.629	0.001	0.797
	15 to 30 days	0.027	0.428	0.000	0.979	0.047	0.229	−0.001	0.589
ADFC *(kg/day)*	7 to 30 days	−0.020	0.118	0.004	0.114	−0.020	0.324	0.001	0.343
	7 to 14 days	−0.015	0.286	0.002	0.449	−0.014	0.586	0.000	0.716
	15 to 30 days	0.030	0.399	−0.001	0.839	0.072	0.119	−0.002	0.284
FC *(kg/kg)*	7 to 30 days	0.124	0.169	−0.030	0.032	0.066	0.386	−0.006	0.074
	7 to 14 days	0.227	0.103	−0.050	0.035	0.415	0.005	−0.025	0.001
	15 to 30 days	0.133	0.415	−0.029	0.209	−0.026	0.860	−0.002	0.767

p = probability value

ditionally, high levels of Gln create an imbalance with the other amino acids in the diet affecting their intestinal absorption, despite the evidence of increased intestinal villi size (Bartell and Batal, 2007).

The duration of supplementation (days) significantly influences (p <0.05) DWG (all evaluated periods) and ADFC (7 to 30 days). The live weight of the piglet at the beginning of the supplementation also has a significant effect (p <0.05) on DWG and FC in periods of 7 to 30 days and 7 to 14 days. It is observed that as the live weight of the piglet increases, the DWG increases and the FC decreases. The body weight values range from 4 to 16 *kg* with an average of 6.89 *kg*. The age of the piglet at the beginning of the supplementation only has an influence on FC when the supplementation lasted between 7 to 14 days. Of the studies used, it was found that the age range of the beginning of Gln supplementation is between 17 to 28 days, with 21 days being the most frequent. It is important to consider that natural Gln constitutes only 10% of the amino acid content of the total protein in a conventional pig diet, being necessary the supplementation to achieve maximum growth and facilitate normal functioning, particularly in hypercatabolic states (Wu, 2014) that occur during weaning of piglets due to social and environmental stress (Spreeuwenberg et al., 2001).

Table 7. Meta-regression for lysine level (%) and raw protein in the diet (%)

Productive parameter	Meta-regression	Lysine level in the diet (%)				RP level in diet (%)			
		Intercept		Regression Coef.		Intercept		Regression Coef.	
		Estimated	*p*	Estimated	*p*	Estimated	*p*	Estimated	*p*
DWG *(kg/day)*	7 to 30 days	−0.145	<0.001	0.126	<0.001	−0.152	<0.001	0.009	<0.001
	7 to 14 days	−0.165	<0.001	0.140	<0.001	−0.215	<0.001	0.012	<0.001
	15 to 30 days	0.185	0.019	−0.118	0.043	0.192	0.015	−0.008	0.036
ADFC *(kg/day)*	7 to 30 days	−0.041	0.335	0.033	0.337	−0.022	0.59	0.001	0.593
	7 to 14 days	−0.028	0.591	0.019	0.654	−0.021	0.643	0.001	0.715
	15 to 30 days	0.183	0.051	−0.121	0.089	0.293	0.008	−0.013	0.014
FC *(kg/kg)*	7 to 30 days	−0.137	0.495	0.052	0.725	−0.716	0.002	0.029	0.004
	7 to 14 days	−0.162	0.482	0.075	0.669	−1.370	<0.001	0.057	<0.001
	15 to 30 days	−0.219	0.721	0.106	0.809	−0.285	0.430	0.010	0.552

p = probability value
RP= Raw protein

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Table 8. Meta-regression number of replications

Productive parameter	Meta-regression	Number of replications			
		Intercept		Regression Coef.	
		Estimated	p	Estimated	p
DWG *(kg/day)*	7 to 30 days	−0.006	0.143	0.005	<0.001
	7 to 14 days	−0.010	0.022	0.006	<0.001
	15 to 30 days	0.030	0.071	−0.001	0.819
ADFC *(kg/day)*	7 to 30 days	0.008	0.209	−0.002	0.153
	7 to 14 days	0.006	0.393	−0.003	0.079
	15 to 30 days	0.064	<0.001	−0.009	0.024
FC *(kg/kg)*	7 to 30 days	−0.036	0.211	−0.007	0.245
	7 to 14 days	−0.032	0.313	−0.009	0.199
	15 to 30 days	−0.070	0.384	0.001	0.972

p = probability value

Dietary formulation factors also have significant effect on DWG, ADFC and FC after the Gln supplementation in piglets. The level of lysine (p <0.05) and raw protein (p <0.05) affects DWG over the 3 periods evaluated. It is evident that as the level of lysine and RP increases the DWG also increases for the periods 7 to 30 days and 7 to 14 days. However, in the period 15 to 30 days there is a reduction in the DWG. In addition, the RP level affects ADFC when supplementation lasted between 15 and 30 days (p =0.014) and FC when Gln administration was from 7 to 30 days (p =0.004) and 7 to 14 days (p <0.001). In this work, the RP and lysine values of the diets used in piglets were between 18 to 23% and 1.17 a 1.6%, respectively. One strategy to improve the use of protein in piglets and prevent intestinal disorders is to reduce the level of raw protein with adequate free amino acid supplementation (synthetic) (Le Floc'h et al., 2018).

Gloaguen et al. (2014) confirms the effectiveness of this strategy and the possibility of formulating diets with low RP levels (13.5%) piglets between 10 and 20 *kg* of body weight. Additionally, diets with moderate protein restriction (13-15.3%) proved to be beneficial for the health of the gut microbiota, metabolic activity in the large intestine and for the improvement in the function of the piglet intestinal barrier (Peng et al., 2017). The reduction of RP content allows to reduce the nitrogen consumption and prevents the excess of amino acids, therefore, prevents metabolic overloads.

Finally, the number of replications influenced the DWG in the periods 7 to 30 (p <0.001) and 7 to 14 days (p <0.001), as well as the ADFC when supplementation lasted between 15 and 30 days (p =0.024). The number of replications in the different works used for this study ranged from 2 to 12, 3 being the most used. This confirms the importance stated by Aaron and Hays (2004) of considering an appropriate number of individuals to be used in experimental studies in pigs. In the face of an inadequate number of replications per treatment, significant differences may not be detected, leading to a waste of time and money for the researcher.

5 Conclusion

Glutamine supplementation improves the productive performance in piglets, being an excellent option to be used in weaning diets to reduce the stress occurring in this stage.

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VETERINARY SCIENCES

Antibiotic resistance of Salmonella spp, Escherichia coli isolated from alpacas (Vicugna pacus) with and without diarrhea

Resistencia antibiótica de Salmonella spp, Escherichia coli aisladas de alpacas (Vicugna pacus) con y sin diarrea

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Resumen

A nivel mundial, el problema de resistencia a antibióticos es considerado de prioridad sanitaria pública y veterinaria, por ello el objetivo de esta investigación fue evaluar la presencia de resistencia antibiótica frente a Salmonella sp., y Escherichia coli provenientes de crías de alpacas con y sin diarrea. La investigación fue de tipo descriptivo transversal múltiple. Se recolectaron 300 muestras de heces por hisopado rectal de crías de alpacas entre 10 a 60 días nacidas con y sin cuadros diarreicos provenientes de Comunidades Campesinas de Huancavelica-Perú. La presencia de Escherichia coli y Salmonella spp. Se identificó mediante pruebas bioquímicas la susceptibilidad antibacteriana por método Kirby Bauer y se evaluaron 8 antibióticos usuales del mercado veterinaria. El 100% de muestras con diarreas fueron positivas a Escherichia coli, 40,0% Salmonella spp., 20% Escherichia coli-Salmonella spp. y muestras sin diarrea 57,0% positivas a Escherichia coli, 24,0% Salmonella spp., 19.0% E. coli-Salmonella spp. Las cepas de Escherichia coli y Salmonella spp. fueron resistentes a Ampicilina (10,4± 0,3), (9,3± 0,2); Novomicina (11,1± 0,2), (11,2± 0,1); Tetraciclina (8,2± 0,1), (9,2± 0,3); Penicilina (9,1± 0,4), (11,1± 0,3); Gentamicina (10,1± 0,4), (10,2± 0,3) provenientes de muestras con diarrea y en muestras sin diarrea resistentes a Gentamicina (10,3± 0,1), (8,2± 0,1); Tetraciclina (9,2± 0,4), (8,2± 0,4); Ampicilina (11,2± 0,1), (9,3± 0,2); Penicilina (10,2± 0,4), (10,1± 0,3). Las cepas de Salmonella spp., y Escherichia coli aisladas de crías de alpacas con y sin diarreas evidencian resistencia antibacteriana a múltiples antibióticos usados en la veterinaria.

Palabras clave: alpaca, Salmonella spp, Escherichia coli, resistencia antibiótica.

Abstract

The problem of antibiotic resistance is considered a public and veterinary sanitary priority worldwide, for that reason the aim of the study was to evaluate the presence of antibiotic resistance against Salmonella spp., Escherichia coli coming from alpaca calves with and without diarrhea. The research was cross-sectional descriptive. 300 stool samples per rectal swab were collected from alpaca calves aging from 10 and 60 days with and without diarrhea from Peasant Communities in Huancavelica – Peru. The presence of Escherichia coli and Salmonella spp. was identified by conventional biochemical test, antibacterial susceptibility by Kirby Bauer method and 8 usual antibiotics from the veterinary market were evaluated. 100% of samples with diarrhea were positive to Escherichia coli; 40.0% Salmonella spp.; 40% Escherichia coli -Salmonella spp., and samples without diarrhea 48.3% positive to Escherichia Coli; 14.0% Salmonella spp.; 9.3% Escherichia coli -Salmonella spp. The CMI in Escherichia coli and Salmonella spp. strains were resistant to Ampicillin (10.4 ± 0.3), (9.3 ± 0.2); Novomycin (11.1 ± 0.2), (11.2 ± 0.1); Tetracycline (8.2 ± 0.1), (9.2 ± 0.3); Penicillin (9.1 ± 0.4), (11.1 ± 0.3); Gentamicin (10.1 ± 0.4), (10.2 ± 0.3) from samples with diarrhea and in samples without diarrhea resistant to Gentamicin (10.3 ± 0.1), (8.2 ± 0.1); Tetracycline (9.2 ± 0.4), (8.2 ± 0.4); Ampicillin (11.2 ± 0.1), (9.3 ± 0.2); Penicillin (10.2 ± 0.4), (10.1 ± 0.3). Salmonella spp., Escherichia coli strains isolated from alpaca calves with and without diarrhea show antibacterial resistance to multiple antibiotics used in veterinary.

Keywords: alpaca, Salmonella spp., Escherichia coli, antibiotic resistance.

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1 Introduction

The breeding of alpacas for the high Andean families of Peru is an activity with socioeconomic importance, since it provides high protein meat with low cholesterol content and its fiber has a high demand in the national and global market (Rosadio et al., 2012; Siuce et al., 2015). More than 85% of Alpaca breeding in Peru is distributed in the Peasant Communities and in small producers who live in extreme poverty and with lack of technical advice, thus facing significant economic losses from pathological problems and high mortality from parasitic, bacterial and viral etiologies (Lucas et al., 2016). High mortality rates in alpaca calves reach 12 to 50%, mainly due to enteropathogenic diarrheal problems of Escherichia coli and Salmonella spp. (Ramírez, 1990; Rosadio et al., 2012), and the resistance of antibacterials to Escherichia coli and Salmonella spp. causes global concern due to the serious impact on public health and animal production, conceived by conditions of inappropriate and indiscriminate use of antibacterials (Yagui, 2018).

From the perspective of animal production, the use of antibiotics has increased really fast, generating a risk of resistant bacteria (Centeno et al., 2018), because these bacteria may have undergone genetic modifications to their residency mechanism as an enzymatic inactivation, altered receptors and transportation of the altered antibiotic which make the permanence of bacterial genotypes resistant to Escherichia coli and Salmonella spp., and there are few animal production-oriented studies that could possibly be related to what was reported in human health (Schwarz et al., 2017).

Salmonella spp. and Escherichia coli are pathogens with clinical importance in the Animal Health in the Peruvian Andes, being the causal agent of pathologies that cause intestinal dysfunction, generating the neonatal diarrheal in alpacas, which makes difficult the therapeutic treatment (Silvera et al., 2012; Rosadio et al., 2012). Several studies report an increase in antimicrobial resistance and minimal sensitivity to different antibiotics to pathogenic microorganisms of Salmonella spp. and Escherichia coli in bird, pig, guinea pigs, bovine and silvestria animals such as monkeys, generating important losses of genetic and socioeconomic value in breeders (Oha, 2012; Medina et al., 2017; S., 2018). There are no related studies on the use of antibiotics in veterinary against Salmonella spp. and Escherichia coli from alpaca calves with and without diarrheal enteropathies, even though Peru constitutes a major producer worldwide of the textile and meat market, these microns being of emerging clinical importance in the production of alpacas.

It is believed that there may be cases of strains with resistance phenotypes similar to those in chicken, pig, bovine and domestic species (Ortiz, 2011), which would make it possible for the use of different antibacterial antibiotics in the therapeutic treatment in alpaca calves with diarrheal enteropathies (salmonellosis and Escherichia coli) that are not effective in high-zone Andeans communities of Peru. Because of the latter, there is a need to evaluate the antibiotic resistance of Salmonella spp. and Escherichia coli in alpaca calves with and without diarrheal enteropathies, which will allow future actions to be implemented to reduce the use of antimicrobials to prevent the generation, dissemination of antibiotic-resistant bacteria through the application of good health practices and the good use of antimicrobials.

2 Materials and methods

According to the central limit theorem, a total of 300 samples of alpaca calves with diarrhea and 300 samples of calves without diarrhea of Huacaya breed from 10 to 60 days of birth, regardless their sex were used, since the birth population is unknown because it is progressive and temporary. Six Rural Communities of Huancavelica alpaca –Peru were taken into account, which were located above 4 200 m.a.s.l, with temperatures ranging from 5.6 to 8.5 °C, from January to March 2018. Likewise, the minutes of communal authorization and consent informed by the owners of the herds under study were also taken into account.

Samples were collected by triplicate per sampled animal by rectal swab in sterile cryovials suspended in means of Stuart transport (bufferted) at dawn (6.00 am to 7.30 am) without the presence of sun rays and with efficient biosecurity management, labeled and registered (Carhuapoma et al., 2018), transported in a refrigerant medium at a temperature 8-12 °C (technopor box with biological ice) to the Animal Health Laboratory (Microbiology

Area) of the National University of Huancavelica-Peru, for performing the bacteriological studies.

The inoculation (triplicate sample) was performed in threaded cap tubes enriched with brain heart infusion broth (BHI), making groups of 300 inocules without diarrhea and 300 inocules with diarrhea and incubated at 37 °C/24; enriched inoculs were sown independently by depletion in selective means of MacConkey Agar and Methylene Blue Eosin (EMB) for Escherichia coli and Salmonella-Shigella (SS), Xilosa lysine deoxycolate XHD for Salmonella spp. incubating it at 37 °C/ 48 h according to ISO 6579:2002.

For the optimal identification and differentiation of the strains of Salmonella spp. microscopic and macroscopic characterization of Escherichia coli (Gram coloration) were performed, such as: shape, consistency and elevation (Murcia, 2018). Suspicious colonies were inoculated in means of Triple Sugar Iron Agar (TSI), Lysine (LIA), Simmons Citrate (HS4), Sulfide-indole-Motility (SIM), Catalase and Voges-Proskauer and were incubated at 37 °C /24 hours. The results obtained were collated by the Manual of Systematic Bacteriology (Bergey's, 2008) and the Manual of Laboratory Procedures Zurita2013 for the final identification.

In relation to the isolation results, strains positive to Escherichia coli (300), Salmonella spp. (120) were selected from samples with diarrhea, and Escherichia coli (172), Salmonella spp. (72) from samples without diarrhea, from this pools of mother inoculum were prepared for Escherichia coli, Salmonella spp. and replicated to 320 independent inoculums for each study micron enriched in Brain Heart Infusion broth (BIH) and incubated at 37 °C /12 hours (Carhuapoma et al., 2018).

The antibiotic sensitivity was made using the Kirby Bauer Method, for this purpose strains of Escherichia coli, Salmonella spp. were cultured with a sterile swab homogeneously on Petri dishes with Agar Mueller Hilton, for a total of 320 strains per microorganism and distributed to 40 cultures by antibiotic as replications (observations of antibiotic sensitivity under study), in order to standardize the study, excluding following groups: with diarrheal enteropathies, Escherichia coli (n= 320 [40 replications/antibiotic]); Salmonella spp. (n= 320 [40 replications/antibiotic]); without diarrheal enteropathies, Escherichia coli (n= 320 [40 replications/antibiotic]); Salmonella spp. (n= 320 [40 replications/antibiotics]). The antimicrobial discs were placed independently infusing at 37 °C / 24 hours, most commonly used antibiotics were tested in the pharmaceutical-veterinary market (A-Gentamicin (30 μg), B-Novomycine (5 μg), C-Tetracycline (30 μg), D-Enrofloxacin (10 μg), E-Ampicillin (10 μg), F-Amikacin (30 μg), G- Ceftriaxone (30 μg) and H-Penicillin(10 μg)), subsequently, the growth inhibition halos were read and the results were interpreted in reference to the cut-off points proposed by the European Committee on Antimicrobial Susceptibility Testing manual (EUCAST, 2018).

The prevalence of microrganisms of Escherichia coli, Salmonella spp. present in alpaca calves with and without diarrhea was performed by comparing Means and Distribution Frequency (descriptive statistic) through a multiple cross-sectional descriptive level research. In order to determine antibiotic and micron-organism antibiotic sensitivity, the analysis of variance and the Tukey test was performed ($P<0.01$), using 8*2 multi-factorial design and the SPSS v. 20 program.

Table 1. Distribution percentage of Escherichia coli, Salmonella spp. and Escherichia coli-Salmonella spp. associates, isolated from samples with diarrhea (n= 300) and without diarrhea (n= 300) of alpacas calves.

Types of samples	Escherichia Coli				Salmonella spp.				E. coli-Salmonella spp.			
	P (+)	N (-)	% (+)	% (-)	P (+)	N (-)	% (+)	% (-)	P (+)	N (-)	% (+)	% (-)
With Diarrhea	300	0	100	0	120	180	40	60	60	0	20	0
Without diarrhoea	172	128	57	43	72	228	24	76	56	224	19	81

Legend: P (+) Positive, N (-) Negative.

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3 Results

Out of the 300 samples tested from alpaca calves with diarrhea, 100% (300/300) were positive to Escherichia coli, 40.0% (120/300) to Salmonella spp. and 20% (120/300) to Escherichia coli-Salmonella spp., with the highest percentage of the presence of Escherichia coli and Salmonella spp. Likewise, out of the 300 samples from alpaca calves without diarrhea, 57.0% (172/300) were positive to Escherichia coli, 24.0% (24/300) to Salmonella spp. and 19.0% (56/300) associated with E. coli-Salmonella spp. (Table 1), with lower prevalence of Escherichia coli and Salmonella spp. in samples of alpaca calves with diarrhea.

Pathogenic microorganisms of Escherichia coli and Salmonella spp. found in rectal samples of alpaca calves with diarrheal enteropathies demonstrated statistical differences (P<0.01) in halos diameters, inhibiting resistance against antibiotics Gentamicin, Novomycin, Tetracycline, Ampicillin and Penicillin; demonstrating antibiotic multibiotic resistance for both microorganisms, while antibiotic sensitivity in both microorganisms were seen in the antibiotics Enrofloxacin, Amikacin and Ceftriaxone, demonstrating diameters of halos inhibition within the sensitivity standards range (Table 2), in a total of 8 antibiotics evaluated.

Escherichia coli and Salmonella spp. isolated from samples of alpaca calf stools without diarrheal enteropathies had significant differences (p<0.01) of antibiotic resistance against Gentamicin, Tetracycline, Ampicillin, Penicillin and Novomycin, and were sensitive to Novomycin, Enrofloxacin, Amikacin and Cephaline (Table 3).

Table 2. Means and standard deviation of antibiotic resistance of Escherichia coli (n= 320) and Salmonella spp. (n= 320) from alpaca calves with diarrhea.

Antibiotic Types	N	Escherichia Coli Strains			N	Salmonella Sp strains.		
	320	S	I	R	320	S	I	R
A- GENTAMICIN	40	0.0	0.0	10.1 ± 0.4^{a}	40	0.0	16.2 ± 0.1^{a}	10.2 ± 0.3^{a}
B- NOVOMYCIN	40	0.0	0.0	11.1 ± 0.2^{b}	40	0.0	16.4 ± 0.5^{b}	11.2 ± 0.1^{b}
C- TETRACYCLINE	40	0.0	16.2 ± 0.2^{a}	$8.2\pm 0.1^{a,b}$	40	0.0	15.1 ± 0.2^{a}	$9.2\pm 0.3^{a,b}$
D- ENROFLOXACIN	40	$20.2\pm 0.3^{a,b}$	16.1 ± 0.6^{b}	0.0	40	19.3 ± 0.1^{b}	14.3 ± 0.1^{b}	0.0
E- AMPICILLIN	40	0.0	$14.1\pm 0.1^{a,c}$	10.4 ± 0.3^{c}	40	0.0	$16.2\pm 0.2^{a,c}$	9.3 ± 0.2^{c}
F- AMIKACIN	40	23.1 ± 0.4^{a}	0.0	0.0	40	19.1 ± 0.4^{a}	0.0	0.0
G- CEFTRIAXONE	40	21.3 ± 0.3^{a}	$15.2\pm 0.2^{a,c}$	0.0	40	23.3 ± 0.3^{a}	0.0	0.0
H- PENECILLIN	40	0.0	0.0	9.1 ± 0.4^{b}	40	0.0	0.0	11.1 ± 0.3^{a}

^{a}, ^{b}, ^{c} Different superscripts within columns indicate statistical difference to the Tukey test (p<0.01).
S Sensitive, I Intermediate, R Resistance.

4 Discussion

High prevalence rates of Escherichia coli (100%, 57), Salmonella spp. (40%, 19%) and Escherichia coli-Salmonella spp. (20%, 19%) in rectal samples of alpaca calves with and without diarrheal enteropathies may occur because alpaca producers may not be making the efficient and responsibly therapeutic use of the different veterinary antibacterials, and it is presumed that it could be related to the use of poor quality antibacterial products, making the pathological control difficult and increasing their incidence; this information agrees with the reported by Carhuapoma et al. (2018, 2019). In addition, the prevalence of these bacterial pathogenic microorganisms would be closely related to animals born with low immune levels and from mothers with cachetic conditions, which makes them very susceptible to Escherichia coli and Salmonella spp., being a pathology of clinical importance in the breeding of alpacas (Rosadio et al., 2012)

Lucas et al. (2016) identified Escherichia coli (8.0%), coronavirus (53.3%), rotavirus (36.6%), Salmonella spp. (18.3%) and associated bacteria and parasites 23.3%, viruses and bacteria 11.7% and triple associated 38.3% in alpaca calves with diarrhea. Morales et al. (2017), isolated Escherichia

coli 47.78% in alpacas with diarrhea and 58.33% without diarrhea, while Chuquizuta et al. (2017) detected Escherichia coli (40.84%), Salmonella spp. (39.27%) in dead guinea pigs and Carhuapoma et al. (2018) found the presence of Escherichia coli in 80% in alpaca calves with diarrhea. Carhuapoma et al. (2019) reported the presence of Escherichia coli and Salmonella spp. in 12% in males and females associated with Escherichia coli - Salmonella spp., 10% of a total of 104 calves with diarrhea; the results found in the study carried are of great importance in both microorganisms versus those reported.

Cebra et al. (2003), mentioned that salmonella in alpaca diarrhea is not a common cause, but Whitehead and Anderson (2006) detected various Salmonella species in animals with diarrhea; subsequently, Lucas et al. (2016) and Carhuapoma et al. (2019) show that the presence of salmonella in alpacas with diarrhea is very common in mixed herds (birds, pigs). This confirmation suggests that this bacterium is optional, making its cycle of epidemiological transmission very viable and its pathogenesis is common in domestic animals as well as in alpaca breeds.

Zambrano et al. (2013) identified Salmonella spp. in 23.5% and 32.4% samples of body surface and cloacal swab in broiler chicken. Salvatierra et al. (2015) identified Salmonella spp. 6.3 $\pm$ 2.4% in belly shells and 1.8% in sub-samples of head skin in swine, and Talavera Rojas et al. (2011) reported 1.34% of Salmonella group B (Typhimurium) in chicken liver samples for sale. The results found in the research are similar with some reported, but different with some others because there were more enteropathogens of Escherichia coli, Salmonella spp. and Escherichia coli-Salmonela sp in alpaca calves, thus the existence of Salmonella spp. and E. coli-Salmonela spp. would be due to causal agents of pathologies that cause intestinal dysfunction, generating the neonatal acute diarrheal in alpacas (More et al., 2011; Mancera Martínez et al., 2004; Ruiz et al., 2014), since Salmonella is an infection belonging to the enterobacteriaceae family (Tacchini A et al., 2010; Ríos, 2012) and there are very few studies of clinical cases of salmonellosis infestation in alpacas, even though this zoonotic pathology is of clinical importance in public health and it is presumed to be present in serovars Typhimurium and Enteritidis by biochemical, macromicroscopic, colonization and clinical manifestations observed in the study.

The antibiotic multi-resistance presented by Escherichia coli and Salmonella spp. against the antibacterials Gentamicin, Tetracycline, Ampicillin, Penicillin and Novomycin, show that the proven antibiotics possibly underwent modifications of its action mechanism such as: enzymatic inactivation of antibiotics, impermeability of the cell membrane or wall, expulsion by active mechanisms of the antibiotic and modification of the white site of the antibiotic in the bacteria, as reported in the literature, reducing the therapeutic options (Mancera Martínez et al., 2004; Schwarz et al., 2017; Ríos, 2012; Gatica Eguiguren and Rojas, 2018).

Table 3. Means and standard deviation of antibiotic resistance of Escherichia coli (n= 320) and Salmonella sp (n= 320) from alpaca calves without diarrhea.

Antibiotic Types	**N**	**Escherichia Coli**			**N**	**Salmonella spp.**		
	320	S	I	R	320	S	I	R
A- GENTAMICIN	40	0.0	16.2 ± 0.2^{b}	10.3 ± 0.1^{a}	40	0.0	17.2 ± 0.2^{a}	8.2 ± 0.1^{b}
B- NOVOMYCIN	40	22.1 ± 0.3^{b}	14.4 ± 0.5^{b}	0.0	40	0.0	14.4 ± 0.2^{b}	10.3 ± 0.3^{a}
C- TETRACICLINA	40	0.0	16.2 ± 0.2^{a}	$9.2 \pm 0.4^{a.b}$	40	0.0	16.2 ± 0.4^{a}	$8.2 \pm 0.4^{a.b}$
D- ENROFLOXACIN	40	$20.2 \pm 0.3^{a.b}$	15.2 ± 0.2^{b}	0.0	40	$20.1 \pm 0.2^{a.b}$	14.3 ± 0.2^{b}	0.0
E- AMPIICILLIN	40	0.0	$15.2 \pm 0.3^{a.c}$	11.2 ± 0.1^{c}	40	0.0	$17.1 \pm 0.4^{a.c}$	9.3 ± 0.3^{c}
F- AMIKACIN	40	22.1 ± 0.4^{a}	$14.2 \pm 0.3^{a.c}$	0.0	40	21.1 ± 0.4^{a}	0.0	0.0
G- CEFTRIAXONE	40	20.3 ± 0.3^{a}	$16.2 \pm 0.1^{a.c}$	0.0	40	22.3 ± 0.3^{a}	0.0	0.0
H- PENECILLIN	40	0.0	0.0	10.2 ± 0.4^{a}	40	0.0	0.0	10.1 ± 0.3^{a}

^{a}, ^{b}, ^{c} Different superscripts within columns indicate statistical difference to the Tukey test ($p<0.01$).
S Sensitive, I Intermediate, R Resistance.

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In addition, the high resistant of 8 antibiotics tested in the study would be linked to the poor application in the health management and the inappropriate use of antibiotics that have been practiced by producers in their herds, and by the irresponsibly massive distribution of drugs by national programs promoted by unskilled professionals, which makes the progressive trend of antibiotic resistance between humans and animals. Lucas et al. (2016), report that Salmonella spp. and Escherichia coli would possibly be resistant to first-line antibacterial such as Phosphomycin, Enrofloxacin, Ciprofloxacin, Gentamicin, Oxytetracycline, Penicillin, Ceftazidime and Trimethoprim-sulfamethoxazole because they are most commonly used indiscriminately by veterinarians from many years ago in cattle, chicken, sparrows and pigs; therefore, it would not be advisable to use antibiotics as first choice for the treatment of diarrhea in alpaca calves without carrying out susceptibility tests in laboratories (Pinto Jiménez et al., 2010; Siuce et al., 2015; Carhuapoma et al., 2019).

Barboza and Suarez (2012), found antibiotic resistance in 30% against Gentamicin, Norfloxacin and Tetracycline in Salmonella spp. isolated from cases of avian Tiphosis. Cordero Ruíz et al. (2002), reported multi-resistance in 84.4% to Tetracycline, Gentamicin, Ampicillin and Amikacin in gram-positive and negative bacteria of 11 proven antibiotics, and De la Fuente et al. (2015) found 91% resistance in strains of Salmonella spp. to Ampicillin, Nitrofurantoin and 55% to Cephalotin and Chloramphenicol; while Quesada et al. (2016) report multiresistance of Salmonella spp. to Nalidixic acid antibiotics, Streptomycin, Tetracycline, Chloramphenicol, Ampicillin, Trimethoprim/sulfamethoxazole, Gentamicin, Ciprofloxacin and Cephalosporin, these reports are similar to the results found in this study as they match almost all the antibiotics studied and these same resistance behaviors can be generated in hot-blooded animals (Castillo et al., 2014), for this reason, the antibiotic resistance of Salmonella spp. and Escherichia coli could be an epidemiological alarm for veterinarians (Rivera Calderón et al., 2012), caused by a lack of practice of conducting sensitivity tests in laboratories and by poor pharmacological knowledge capacity of veterinarians and operational technicians (Barboza and Suarez, 2012; Carhuapoma et al., 2018).

The antibiotic sensitivity demonstrated by the strains of Escherichia coli and Salmonella spp. isolated from rectal swab from alpaca calves with and without diarrhea versus the antibacterials of Enrofloxacin, Amikacin, Novomycin and Ceftriaxone, seem to be microbial that were not long-term used or were indiscriminate in herds (Carhuapoma et al., 2018), and may not have generated the modification of their mechanisms of antibiotic action, as well as the genes of DNA gyrase and topoisomesase IV (Romeu Álvarez et al., 2012; Lee et al., 2003; Ruiz et al., 2014; Schwarz et al., 2017). Additionally, Barrios-Arpi et al. (2016) found Escherichia coli sensitive to Trimethoxyprim (98%), Gentamicin (95%) and Phosphomycin (88%), Ciprofloxacin (85%), Ceftadizime (79%), and resistant to Nitrofurantoin (85%) from healthy and sick alpaca calves, results that differ from the ones obtained in this study with 4 antibacterial (Enrofloxacin, Amikacin, Novomiacin and Ceftriaxone) that proved to be sensitive and that would be specific for the therapeutic use of alpacas with and without diarrheal enteropathies.

5 Conclusions

The isolations of Salmonella spp. and Escherichia coli from alpaca calves with and without diarrhea were shown to be multi-resistance to the most common antibiotics in the pharmaceutical-veterinary market and only 4 antibiotics were sensitive, thus, comparative studies are required in the high Andean areas of Peru for the prevention and dissemination of antibacterial resistance.

Ethical commitments: The research team declares that strict management of animal welfare has been carried out for this research under the protocols established before and during the collection of samples for the study.

Confidentiality of data: The research team stated that a strict methodological management has been done to obtain adequate bases, as a statistical model and SPSS v. 20 program for data processing.

Reported consent: For the execution of the investigation no informed consent was required as it was a laboratory study based on protocols without the handling of animals, but they were taken into

account for greater reliability of the study.

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AGRICULTURAL PRODUCTION

Economic analysis of hydroponic lettuce under floating root system in semi-arid climate

Análisis económico de lechugas hidropónicas bajo sistema raíz flotante en clima semiárido

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Resumen

Los cultivos sin suelo se presentan como una alternativa de cultivo ante la presencia de suelos marginales con escasez hídrica característicos de la provincia de Santa Elena (PSE). La hidroponía presenta a nivel mundial una alta productividad por unidad de superficie, ahorro de agua y cosechas durante todo el año. La lechuga (*Lactuca sativa L.*), especie de estación fría, es la más representativa de un sistema hidropónico, pero no es una especie cultivada en la costa ecuatoriana. El objetivo fue analizar desde el punto de vista económico-financiero la propuesta técnica de un cultivo protegido de lechuga bajo sistema hidropónico de raíz flotante llevado a cabo en el clima semiárido de la PSE. Se realizaron cuatro siembras con el cv. Crespa, utilizando la solución nutritiva Hoagland y Arnon. El rendimiento y los costos se extrapolaron a una infraestructura de 1,000 m^2 equivalente a una superficie productiva efectiva de 240 m^2. Se asumen supuestos donde la producción se vende en jornadas laborales de acuerdo a la ley a una proyección de 5 años, con un stock de inventario para una semana, ventas al por mayor y un margen de beneficio en el año 1 de 30% y ascendente en los siguientes períodos. Bajo estos criterios, el costo de producción unitario (en dólares americanos) ascendió a USD 0,49 y el precio de venta a USD 0,70. La inversión total sumó USD 27.077,99, el VAN USD 58.581,07, con una TIR del 40% y un índice beneficio-costo de 1,26; por lo que económica y financieramente el proyecto se consideró viable.

Palabras clave: Cultivos sin suelo, costo de producción, inversión, cultivo protegido, Lactuca sativa L.

Abstract

Soilless crops are a farming alternative to marginal soils with limited water availability, which are widespread in the province of Santa Elena (PSE), Ecuadorian coast. This province has semi-arid climate. Hydroponics have worldwide high productivity per unit area, save of water and cultivation cycles throughout the year. Lettuce (*Lactuca sativa L.*) is a cold-season crop, the most representative crop in hydroponic cultivation, but it is not currently cultivated in the Ecuadorian coastal area. The aim of this study was to analyze the economic-financial viability of lettuce cultivation under hydroponic system of floating root. Four lettuce cultivation experiments were carried out with cv. Crespa, using the Hoagland and Arnon nutrient solution. Yields and costs were extrapolated to an infrastructure of $1,000\ m^2$ equivalent to an effective productive area of $240\ m^2$. The assumptions used in the analysis were: all production is sold, working days were calculated according to the law, 5-year projection, stock of inventory for a week, wholesale selling, and profit margin in Year 1 of 30% and increasing in the following periods. Under these criteria, the production cost (in US dollars) per unit amounted to USD 0.49/unit and the sale price to USD 0.70/unit. The total investment USD 27,077.99, the NPV USD 58,581.07, with an IRR of 40% and a benefit-cost ratio of 1.26; thus, the project was considered viable from an economic point of view.
Keywords: Soilless culture, production costs, investment, greenhouse crop, Lactuca sativa L.

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1 Introduction

The increase in the population and the reduction in agricultural soils have caused concern in relation to the food supply. Technology evolves in pursuit of increased productivity and sustainability through genetic improvement, including high-impact technologies on product performance and quality, agriculture, irrigation technologies and nutrition, among others. Soilless protected crops have the same objective, obtaining high yield on less surface area and throughout the year.

Hydroponics with recirculation (closed system) is the most technically, economically and environmentally efficient system by its considerable savings in water and fertilizers, and minimal discharge of residual fertilizer solution into the environment (Urrestarazu, 2015). In the protected crops, hydroponic systems are presented as an interesting option in the face of increased soil and sanitary limitations and the need for short marketing circuits. In Mexico, these already account for 50 % of protected crops (INTAGRI, 2017). In the US, in the period 2013-2018, this form of production annually obtained US $ 891 million, with a rate of 1.2 % and it generated an employment increase rate of 10.1 % (IBISWORLD, 2018). Hydroponics in the closed system has been more efficient compared to conventional cultivation (in open or greenhouse soil) (INTAGRI, 2017), resulting in better cost-benefit. However, one of the disadvantages of this system is the high initial investment in the infrastructure.

The world production of this vegetable is estimated at 26 866 557 t per year with an average yield of 21,89 $T\ ha^{-1}$ (FAO, 2019). Data from the Food Organization of the United Nations (FAO) indicate that the main producer of this species is Spain, exporting to more than 53 countries, and exceeding 900 000 tons per year. Lettuce occupies 2 % in the US in the hydroponic market, while in South America the percentage is 49 % (INTAGRI, 2017).

Ecuador's horticulture has increased due to the revival of the peasant economy and exporting agribusiness (Álvarez et al., 2014), as well as the increase in the consumption due to changes in eating habits (Espinoza, 2015). In 2000, the production of 9.770 metric tons was recorded, carried out by smallholder farmers with 58 and 63 % of production units with less than 1 ha, in cultivation and associated, respectively (INEC, 2002). Meanwhile, FAO (2019) in 2017 recorded an increase of 17 301 t produced. The Survey of Consumption and Household Expenses in Ecuador (INEC, 2013) shows that rural people invest 32 % of their income in food and non-alcoholic beverages and only 2 % is spent on vegetable purchases in this category. If the value of the Basic Family Basket in the Sierra region is considered to be $722.44 and the Costa 695.52 value, this expenditure on vegetables would correspond to USD 14 in the coastal area (INEC, 2017). According to (Zaruma, 2009), peasant family farming (AFC) for productive areas is 500 to 2000 m^2, while for small producers it is 0.25 to 1 ha and for the median 1 to 3 ha. If at the level of Latin America 80 % of farms are in the hands of the TFA, in Ecuador this figure rises to 84.5 % (Salcedo and Guzmán, 2014). The cultivation and production of vegetables depend on small producers and on peasant family farming. In Ecuador, 83 % of this production is destined for domestic consumption. Lettuce culture is traditionally grown in the open field, but also under protected cultivation in soil and hydroponic systems. It is a specied that grows during cold season with optimal temperatures of growth and daytime development between 18 and 25 $^{\circ}C$ and nightly from 10 to 15$^{\circ}C$ (Maroto, 2002; Saavedra et al., 2017).

In the province of Santa Elena, a project of agroecological family orchards has been carried out since 2014 in charge of the Ministry of Agriculture (MAG), and since 2017 it has been in charge of the peasant family farming project (AFC) covering the rural and urban sector (Prefectura Santa Elena, 2017; Mateo, 2019). Lettuce occupies an important place among the species produced there. The main problems affecting agricultural productivity in the province are low water availability, semi-arid weather conditions and degraded soils. Barbosa et al. (2015) estimated that this technique manages to increase annual productivity by 10 times with a consumption of only 8 % of water, but 87 times higher consumption of energy compared to a conventional crop in the United states (NFT, irrigation, heating, artificial lighting). Treftz and Omaye (2012) indicate positive aspects that point to the sustainability of soilless crops such as saving water, fertilizers and pesticides. They also mention that it can be carried out in arid and urban areas, bringing the product closer to the consumer, and not requiring crop rota-

tion. Soil preparation and weed control are also not required (Resh, 2013). An alternative for agricultural recovery is the hydroponic system for growing vegetables with a nutrient supply adjusted to the needs of each species, obtaining quality plants with high nutritional content. Harvesting can be carried out in a complex or simple infrastructure in small spaces and with low costs of production variables, but with a high initial investment. Resh (2013) states that one disadvantage is the easy proliferation of root diseases in a soilless system with recirculation of nutrient solution.

Lettuce is the main hydroponic crop nationally and worldwide, but due to its status as a cold season plant it is important to evaluate its technical and economic feasibility before recommending its cultivation in the province in this productive system that requires high capital investment. In the literature, there are numerous technical contributions to the topic (Khan et al., 2018; Sharma et al., 2018), but there is little information regarding the economic and financial aspect, and the information available is focused on studies in temperate climates, leading to high heating and lighting costs, which are unnecessary in this case (Barbosa et al., 2015; Quagrainie et al., 2018). The objective of the research was to mitigate this gap and perform an economic analysis to determine the profitability of the hydroponic lettuce culture under the semi-arid climate conditions in the province of Santa Elena.

2 Materials and methods

2.1 Infrastructre

The method for the development of the proposal is based on the production results of real experimental crops, which were carried out at La Libertad, Santa Elena provinces, in greenhouses belonging to the project "P06 System of hydroponic production, alternative to the change of the agroproductive matrix in Santa Elena"located in the Faculty of Agricultural Sciences of the State Santa Elena University (UPSE). The geographical location of the place is south latitude $2°13'56{,}46"$, longitud oeste $80°52'30{,}097"$, altura de 44 msnm. Dentro de la clasificación climática, la provincia de Santa Elena posee un clima semiárido, las precipitaciones anuales registran un promedio de 200 mm, humedad relativa de 81.6% y temperatura media anual de 24.5 ° (CLIRSEN-MAGAP, 2011; INAMHI, 2017). The study was carried out in a galvanized iron greenhouse of 20 m long, 10 m wide and 4 m height, with a cover of the polyethylene roof of UV/IR 6, side walls and fronts covered with a white net of 50% shaded.

The production obtained and the costs were projected for a production area equal to 1000 m^2. The growing bed at 90 cm of height had a width of 1 m, a height of 10 cm, of which 8 cm are occupied with water and 2 cm correspond to the thickness of the foam plate held by the plants. The length was 3 m and was coated with 0.2 mm thick black polyethylene in which the floating root system maintained the roots of the plants submerged in water with dissolved minerals according to the formula of Hoagland and Arnon (Beltrano and Gimenez, 2015). In the case of short-lived leaf crops such as lettuce, the fertilizer solution covers the daily requirements of the plant and does not need to be modified. In previous trials, this was identified as the best formulation among three evaluated. This requirement was subtracted from the ion inputs of the irrigation water (drinking water), resulting in the doses indicated in Table 1. The crop was supplied from a fertilizing tank (500 liters of capacity) with a system of recirculation pipes with the fertilizer solution using a submersible pump Pedrollo Top II of 0.5 HP (Figure 1).

Table 1. Nutrient content of the nutrient solution and irrigation water used for a lettuce culture, based on the Hoagland and Arnon solution.

	Chemical elements									
Macronutrient solution (mMol L^{-1})	**NO_3^-**	**$SO_4^=$**	**$H_2PO_4^-$**	**HCO_3^-**	**Cl^-**	**Ca^{++}**	**Mg^{++}**	**K^+**	**Na^+**	**NH_4^{++}**
Requirement	15	2	1			4	2	6		1
Irrigation water	0	0.05	0	0.88	1.28	1.15	0.65	0.18	0.3	0
Real supply	15	1.948	1	0.37	1.28	2.85	1.35	5.82	0.3	1
Micronutrient solution (mg L^{-1})	**Fe**	**Mn**	**Cu**	**Zn**	**B**	**Mo**				
Real contribution	2.47	0.50	0.02	0.05	0.42	0.01				

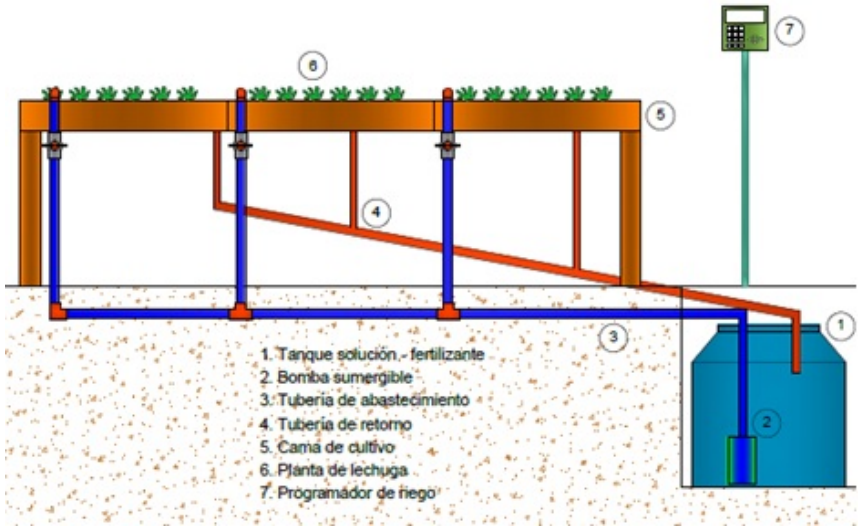

Figure 1. Diagram of bed construction and fertilizer solution supply controlled by the irrigation programmer in a protected culture of lettuce under a floating root system.

2.2 The crop

The seeds of lettuce cv. Crespa (*Lactuca sativa* var. *acephala*), a plant of loose leaves, oak type, with light green color were purchased in an agro-store in Manabí (Jipijapa, Ecuador). The seedbed was performed in situ in a growing house parallel to that of the test in PVC trays of 128 alveoli. They were watered daily and no sanitary applications were needed. From the emission of the first leaf it was fertilized with the same fertilizer solution tested at a concentration of 50 %, in volumes of 0.5 liters per tray. The transplant was performed four times during the first half of 2018: January 18, February 6, March 6 and May 17; with seedlings with 3 true leaves on the described beds, at a density of 32 m^{-2} plants.

The oxygenation of the nutrient solution was performed daily for 30 minutes, twice a day (morning and afternoon), in order to provide enough oxygen in the roots and facilitate the absorption of nutrients needed for the growth and development of the plant. This work was carried out automatically by an irrigation programmer and the feeder pipes located at the head of each experimental unit. The water consumption, mainly due to perspiration, determined the time of replenishment of the nutrient solution. This consumption was determined by measuring daily the height of the water sheet of each bed (experimental unit or 1 m^2 replica). The consumption of each repetition was averaged throughout the growing cycle and it was projected to one consumption per plant.

The health status of the crop was assessed daily for control actions. The chemical quality monitoring of the nutrient solution in the growing beds was carried out using an OAKTON ECTester11 (for salinity) and a pH-meter, Milwaukee brand Ph55 (for pH). The chemical parameters of fertilizer solutions were measured daily to establish timely correction measures in agronomic management. The fresh weight of lettuces was measured at harvest using the BOECO BWL 61 digital balance. The fresh weight of leaves and roots corresponded to yield per plant, as hydroponic lettuce is commonly marketed complete (with roots).

The harvest criterion was the size and weight of the product and the absence of floral buds. In Spain and Panama, the rules require a minimum of 100 g for the marketing of loose-leaf lettuce from protected cultivation, without specifying whether it is harvested in soil or hydroponics systems (Junta de Andalucía, 2013). Conversely, the Colombian Technical Standards (1994) require that a container of hydroponic lettuce must contain at least 150 g of product, including roots. In Ecuador, there are no standards in this regard, but on the domestic market there are packaging of hydroponic lettuce of 100

and 200 g.

For the purposes of this study, which has an economic approach, only the weight of full lettuce, averaged of the four planting dates, was considered as the only relevant agronomic variable (Table 2). The results were statistically analyzed with the Infostat v2018e program. Parametric variables were analyzed with the F test and nonparametric variables with the Kruskall Wallis test.

2.3 Economic analysis

A budget was developed based on the model proposed by Díaz et al. (2012), where the initial balance is first identified and operational processes are then determined in order to quantify them in time and currency. The calculation of the investment of the galvanized iron and polyethylene cover was for an area corresponding to 5 sheds covering 1000 m^2 of surface, each shed was 30 m long, 7 m wide and 3.5 m height. This area had an efficiency of 24%, i.e., it implies 240 m^2 of effective productive area. The number of annual harvests was estimated according to the duration of the plantings carried out in the project. 32 plants were obtained per m^2 and a product loss of 20% was assumed. Whereas the production is sold in its entirety and a week's inventory is planned with 1 418 lettuces, relative to the annual production of 73 728 units per year.

The costing process was carried out *in site* by quantifying everything related to the project. For the cost projection, an average inflation of 2.40% Banco Central del Ecuador (2018) and a discount rate of 15.40% were considered. As a comparison point for expected prices, a value was determined based on the analysis at 9 outlets of the province of Santa Elena, which sell a wide variety of vegetables. A token was applied for each sale point selected in Salinas, La Libertad and Santa Elena provinces (Ecuador). The instrument allows to obtain product information regarding quality, specifications according to the brand, quantities, prices and grams per pack. The budget was done using Excel.

3 Results and discussion

3.1 Yield of hydroponic lettuces

The duration of transplant to harvest at the different dates of establishment varied from 21 to 25 days (Table 2), the longer period corresponded to the time with lower temperatures (Ecuador). This allowed to project 12 harvests annually, considering the staggered elaboration of seedlings to avoid downtime. Therefore, no matter the time of the year (winter or summer), the duration of the growing cycle is similar due to the small variation in the climatic conditions of the Ecuadorian coast. Unlike a country with a temperate climate such as Spain, where the same variety has growing cycles between 31 (hot season) and 81 days (cold season) (Sábada et al., 2007).

Table 2. Dates of establishment of four planting cycles of hydroponic lettuce cv. Crespa cultivated in floating root system during 2018 in the province of Santa Elena and the associated climate.

Transplant	Harvest	Duration of the Cycle (days)	Average of temperatures Max. - Min. (°C)	Average of relative humidity Max. - Min.
18-Jan	06-Feb	22	37.31 - 24.34	88.20 - 25.35
06-Feb	27-Feb	21	35.94 - 24.10	91.59 - 29.19
06-Mar	25-Mar	22	36.59 - 23.43	84.90 - 27.46
17-May	08-Jun	25	32.01 - 21.55	83.74 - 36.00

Hydroponic lettuce can be marketed with roots, which demonstrate the production method used and the freshness of the product. Based on the four crops carried out between January and May 2018, harvest weights (parametric variable) were obtained between 115.8 and 150,1 $g\ plant^{-1}$, with an average of 139.0 g (Figure 2).

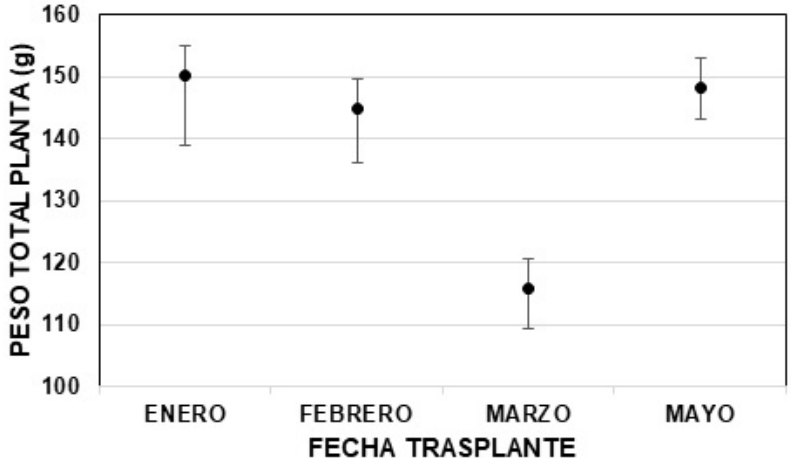

Figure 2. Full plant weight to lettuce cv. Crespa on four transplant dates (2018), cultivated in the province of Santa Elena under floating root system and nutritive formula of Hoagland and Arnon.

The variation coefficient in the data did not exceed 11.7 %, so the plant weights obtained were assumed to be highly reliable. The harvest weights obtained in January, February and May crops were similar. Only the March harvest was significantly smaller, moving away from an optimal commercial weight.

These weights were associated with an average for the four 22-leaf crops per plant. Solís (2017) working in the province of El Oro reported four weeks of hydroponic culture lettuces with 10.5 leaves, and a total unit weight of 64.32 g, probably due to adverse weather conditions. In other studies, plant weights with ranges from 80.10 to 271.02 g were reported with hydroponic lettuce in other latitudes (Defilipis et al., 2006; Barrientos, 2011, 2014; Tarqui et al., 2017). Maboko and Du Plooy (2009) propose planting density increases of up to 50 $units/m^2$ to improve performance, depending on climate and variety. Mandizvidza (2017) suggests modifying, depending on the variety, the cation ratios in the nutrient solution to improve the performance and quality of postharvest in lettuces.

3.2 Water resource

The consumption of nutrient solution in recirculation (water + fertilizer) was estimated at 7,7 m^3 per growing cycle in 1000 m^2 dof greenhouse, which would amount to 92,4 m^3 for the 12 annual growing cycles. At the end of the crop, the remaining fertilizer solution was applied to an ornamental garden. This represents a marginal cost of production and considerable savings of the water resource if the values reported in the literature between 52 to 125 m^3 of irrigation water consumption are considered in 1000 m^2 for each cycle of lettuce growing in low soil greenhouse conditions (Defilipis et al., 2006). The consumption represented 8,7 % of water consumption under a protected crop. In open field, the water requirement can rise up to 411 $mm\ ha^{-1}$, depending on the weather, the time of the year, the variety and irrigation system; which is equal to 411 L 1000 m^2 (Tarqui et al., 2017). In this case, water savings would be even more evident, representing hydroponic consumption by only 1.9 % compared to an open field crop.

4 Economic analysis

The cost of unit production consisting of direct labor costs (four operators), direct raw material (seedlings, fertilizers and agrochemicals) and the indirect costs of direct labor (four operators), direct raw material (seedlings, fertilizers and agrochemicals) and the indirect costs of production (field supervisor, beds and plastic covers), were determined for 1000 m^2 of infrastructure, without considering outsourcing costs. The initial investment amounted to USD 27 027.99, of which 17,00 % was for working capital and the remainder for fixed asset investment. This consisted of: (a) the galvanized iron greenhou-

se shed, which was estimated at the market price of Quito companies that offered it a price of USD 10 m^{-2}; b) the infrastructure of wooden beds with a height of 90 cm (Table 3); c) the irrigation system (input and recirculation pipes, programmer and its installation) (Table 4); d) additional ones such as irrigation pump, beds, balance and plastic bags.

Table 3. Construction costs of 240 m^2 wooden beds for the floating root system for 1000 m^2 of greenhouse (in US dollars, values as of September 2018).

Detail	Quantity	Measured Unit	Unit cost (USD)	Total Cost (USD)
Wooden bed construction				
Boards of 20 cm × 4m, semi-hard	580	Unit	4.00	2 320.00
Black Plastic Sleeve 1.5 m wide	m^2	1.19	297.50	
Foam of 2.5 cm thickness (1×1m)	270	Unit	5.29	1 428.30
6×6 cm sticks	90	Unit	3.00	270.00
Accessories (nails, cardboard, foam, pond outlet, etc.)		Unit		372,30
Flex hose	10	Unit	0.80	8.00
TOTAL (USD)				**4 696.10**

Table 4. Construction costs of a recirculating irrigation system for the floating root system for 1,000 m^2 of greenhouse (in US dollars, values as of September 2018).

Detail	Quantity	U. Unit	Cost Measure (USD)	Total Cost (USD)
Pipe 3/4 inches (6 meters)	90	Unit	3.34	300.60
Pipe 1 inch (6 meters c/u)	20	Unit	6.76	135.20
63 mm 0.8 mpa (6 m)	100	Unit	15.81	1581.00
Flex hose 1"	120	M	0.80	96.00
1 000-litre tank	10	Unit	250.00	2500.00
Irrigation accessories (T, elbows,etc.)		Unit		580.88
Electrovalves	5	Unit	52.00	260.00
Mesh filter	5	Unit	12.80	64.00
TOTAL (USD)				**3438.73**

Under these conditions, the cost per unit produced amounted to USD $0.49 (Table 5). The units to be produced for the second year decreased by the inventory that has been considered at the end of the first year (2019). The unit cost of production decreased in the second year because the installation cost of irrigation equipment only applies for the first year.

The price paid to the hydroponic lettuce producer in the domestic market (supermarket chains) is approximately USD 0.50. The final sale price on the same chains is around USD 1.00 per bag, based on a survey conducted on supermarkets in the province of Santa Elena (Figure 3). The unit cost obtained would only be sustainable with a direct sale to the consumer, without intermediaries. According to the same survey, weekly sales of these supermarkets in the province of Santa Elena increase in approximately 2 000 units. The weekly sales (1 418) would correspond to 70% of that value, exceeding the capacity of the local market. The product is commercialized in surrounding provinces such as Guayas, Manabí and Los Ríos. Currently the market is co-produced by the provinces of the Ecuadorian mountains, mainly three companies, but there is plenty of place to grow (own data).

Table 5. Total and unit production cost budget for 1000 m^2 of greenhouse (in US dollars, as of September 2018) of hydroponic lettuce cv. Crespa cultivated in floating root system in the province of Santa Elena.

Detail	2019	2020	2021	2022	2023
Cost Materials	3 628.50	3 645.40	3 732.80	3 822.30	3 914.00
Costs work	24 003.60	25 503.20	25 548.40	25 593.60	26 731.90
Costs CIF	9 073.00	2 856.80	2 856.80	2 856.80	28 566.80
Total Cost (USD)	36 705.10	32 005.40	32 138.00	32 272.70	33 502.70
Units produced	75 146.00	73 728.00	73 728.00	73 728.00	73 728.00
Unit Cost	0.49	0.43	0.44	0.44	0.45

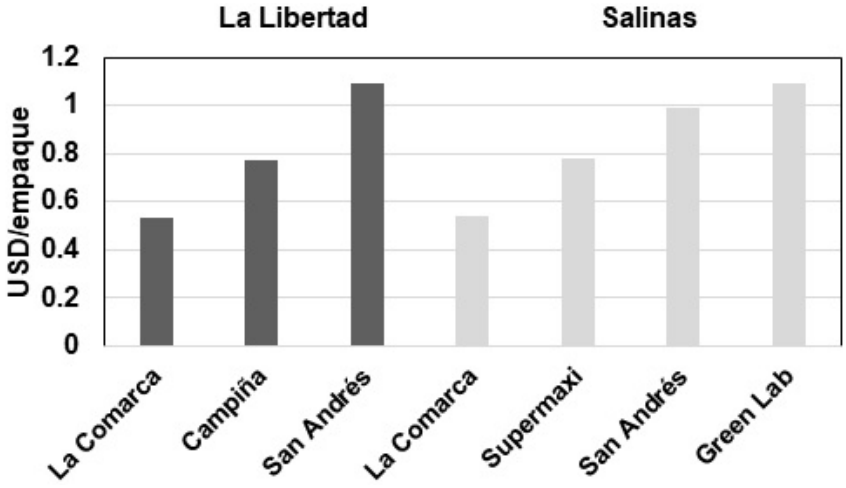

Figure 3. Comparison of unit sales price between brands of hydroponic lettuce in bags of 100 to 200 grams, in supermarkets in the province of Santa Elena (black bars= La Libertad province; gray bars = Salinas province). U.S. Dollar Values as of September 2018.

To be competitive in this scenario, an initial profit margin of 30 % was considered, allowing a sale price of USD \$0.70 per marketing package. From the second year onwards, a profit margin of 45 % was established for the package with one unit. According to Álvarez et al. (2014) in Ecuador the marketing of tomato (similar for all vegetables) presents three modalities (channels): a) collector - wholesaler- retail, b) supplier -wholesaler - supermarket and c) producer - supermarket. The third model is aimed to be without intermediaries, but considering the costs and existing market players for the purchase of the product (Zaruma, 2009) super/hypermarkets would be excluded, leaving as potential customers families (who buy in specialized local markets), restaurants, hotels and casinos (HORECA). This decision would allow the producer to move from the category of self-consumption and temporary to that of permanent producers, obtaining a position in the market.

Projected revenue and production costs allowed calculating the net value (VAN), the internal rate of return (TIR), and the profit-cost index (B/C). A discount rate of 15.40 % has been determined, taking into account that there is financing and market risks by being a new project. Therefore, the risk has been estimated at 25 % and the average inflation in recent years is 2.40 % per year. The VAN obtained was USD 31 101.62 with a TIR of 40 % and a B/C of 1.26; therefore, economically and financially the project was considered viable (Table 6). This index indicates that for every dollar invested, USD 0.26 of net gain is obtained.

Ríos (2013) by doing a financial analysis for a 700 m^2 hydroponic greenhouse with hydroponic lettuces in NFT system achieved a TIR of 50.91 % and a profit/cost ratio of 0.7, indicating the project as unviable. Ortega et al. (2016)reported in tomato hy-

droponic variations in the benefit/cost ratio (C/B) between 0.7 (coconut fiber) and 1.8 (tezontle). Therefore, the hydroponic system with inert substrate (coconut fiber) was not profitable, while in agricultural soil the B/C ratio was 1.5. Quagrainie et al. (2018) determined in their inert substrate lettuce assay VAN, TIR and B/C values of USD 73 872, 48.7% and 1.3, respectively. The return on investment was achieved over the third year. In hydroponic tomato the return on investment is also achieved in the second or third year, according to the Red Agrícola (2017).

At the national level, family farming has a smallholding structure with an average of 3.48 ha per farm, which supports food in general and contributes to 9.9% of the agricultural production and 43% to the value of sectoral production (Salcedo and Guzmán, 2014). FAO y CAF (2009) offer a credit contribution of around 70% to high-investment projects presented by chambers of agriculture, trade union associations, agricultural centers, peasant organizations. It emphasizes that credit must be reimbursable for projects that offer the best guarantees and signs of sustainability from three criteria: economically profitable, environmentally functional and socially viable. They also suggest that working capital, marketing and training requirements should not be excluded from the investment.

Conventional crops require large areas to become profitable, while horticulture and even more protected crops, including hydroponics, operate efficiently on small surfaces. Therefore, it is more effective to contribute to sector policies that stimulate investment in high-efficiency areas of soil and water resources. Hydroponics meets this criterion and in a closed system (with recirculation) no polluting drains are emitted into the environment, complying with the criterion of environmental functionality.

On the other hand, the profits with a hydroponic project can go beyond the economic. Castiblanco (2016) evaluated a 10-year hydroponic project in a women's prison, estimating a social discount rate (TSD by its acronym in Spanish) of 12%. While, as in this case, annual operating costs exceeded the initial investment, and despite the high costs of investment, operation and maintenance, the economic net flow was positive by making this proposal economically and socially profitable.

Table 6. Cash flow with funding for a hydroponic project with cv lettuces. Crespa under floating root system, in the province of Santa Elena (values as of September 2018).

Detail	**2018**	**2019**	**2020**	**2021**	**2022**	**2023**
Initial Balance		5 003.70	1 594.81	9 066.99	16 461.70	31 603.36
Sales Revenue		54 249.69	58 191.56	58 432.68	58 677.62	60 913.97
Loan	20 202.93					
Total Income	20 202.93	59 253.40	59 786.38	67 499.68	75 139.32	92 517.33
Investment	28 861.33					
Payment providers		3 628.47	3 645.37	3 732.78	3 822.30	3 915.39
MOD Payment		24 003.62	25 503.15	25 548.35	25 593.55	26 731.89
CIF Payment		8 427.99	2 211.84	2 211.84	2 211.84	2 211.84
Payment Expenses Sales		10 160.90	10 891.32	11 077.30	11 263.27	11 449.25
Payment of fee		7 822.70	7 822.70	7 822.70		0.00
Outflow total	28 861.33	54 688.68	50 719.38	51 037.97	43 535.96	44 953.37
Cash Flow	-8 658.40	1 594.81	9 066.99	16 461.70	31 603.36	47 563.97
Capital Contribution	8 658.40					
Accumulated Cash Flow	0.00	1 594.81	10 661.81	27 123.51	58 726.87	106 290.84

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Likewise, these productive systems can be activated in urban or rural community orchards, with training and strengthening of the society. In the province of Santa Elena, people work massively in these orchards (Prefectura Santa Elena, 2017), but hydroponic systems have not yet been implemented. With regard to social viability in this low-scale proposal, four direct and indirect jobs associated with the transportation and marketing of the product were achieved, thus improving the living conditions of those involved. Additionally, it would enrich the basket and the family diet, generate employment and savings in the purchase of fresh products.

5 Conclusions

The total weight of lettuces obtained on four transplant dates under the agroecological conditions of the province of Santa Elena, meets the minimum commercial weight, is relevant to the current offer in shopping centers and it is competitive in the market as long as it is sold without intermediaries.

The consumption of nutrient solution (fertilizing water) was estimated at 7.7 m^3 per growing cycle in 1000 m^2 of greenhouse, representing 8.7 % of the consumption of water under protected crop, and only 1.9% compared to an open field crop.

With the established assumptions and the technical-economic evaluation, a unit production cost of USD 0.49 was obtained. A VAN of USD $ 31 101.62, was obtained with a sale price of each package of USD 0.70 (higher than zero), a TIR of 40%. This value was higher than the discount rate. The profit-cost ratio obtained was 1.26.

The sustainability of the proposal was evident in the economic and financial viability, the considerable savings of the water resource, the non-pollution of the environment as a closed system and the social viability shown in the generation of employment and improvement of quality of life of those involved, as well as being a contribution to the local diet.

It will be a future task to evaluate other genetic materials of lettuce that have a higher tolerance to high temperatures, with the aim of obtaining better yields and exploring with other horticultural, aromatic or medicinal species, baby vegetables or fourth-range products that have an economic activity. On the other hand, an increase in productivity can occur by increasing plant density or by applying biostimulants that allow lettuce to cope better with abiotic stress.

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Scientific paper/ Artículo científico
AGRICULTURAL PRODUCTION

Effect of the roof (Low tunnel) on the productivity of two varieties of strawberry (Fragaria vesca) in Cajanuma, Loja

Efecto de la cubierta (microtúnel) en la productividad de dos variedades de fresa (Fragaria vesca) en el sector Cajanuma cantón Loja

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Resumen

La fresa (*Fragaria vesca*) es un cultivo de gran aceptación a nivel mundial, no siendo la excepción en Ecuador. La mayor parte de la producción se realiza a campo abierto, haciendo que existan limitaciones por el ataque de factores bióticos y la influencia de factores abióticos, generando pérdidas en la producción. El presente trabajo se planteó en la provincia de Loja al no existir información precisa de este cultivo e investigaciones sobre el uso de sistemas protegidos (microtúnel). El objetivo principal fue evaluar el efecto de la producción de fresa en los dos sistemas de producción (Campo abierto y microtúnel), con variedades Albion y Monterey; además analizar el costo y rentabilidad de la producción. El sistema a campo abierto fue considerado como un tratamiento testigo para su evaluación frente al de microtunel. El experimento se realizó en la Estación Agropecuaria de la Universidad Técnica Particular de Loja, bajo condiciones de microtúnel y campo abierto, en la cuales se comparó el desarrollo fenológico y productivo de la fresa. Los resultados encontrados en el estudio en los dos sistemas a campo abierto y microtúnel no presentaron una diferencia estadística en las variables fisiológicas evaluadas, concluyendo que la cubierta con microtúnel al cultivo no influyó en el desarrollo en las dos variedades. En relación al análisis de costos-beneficio de la producción, este fue superior en el sistema microtúnel, la inversión fue más fuerte al inicio, sin embargo, se debe indicar que existen varias ventajas productivas en relación con campo abierto, generando beneficios al productor de fresa.
Palabras clave: Fresa, Microtúnel, producción, rendimiento, rentabilidad.

Abstract

The strawberry (*Fragaria vesca*) is a crop of great acceptance worldwide. In Ecuador, most of the production is done in open field, presenting limitations by the attack of biotic factors and abiotic factors that generate losses in the production. This research was carried out in the province of Loja, since there was no precise information on this crop, and research on the use of protected systems (low tunnels). The main objective of the study was to evaluate the effect of strawberry production on the two production systems (open field and low tunnels), with Albion and Monterey varieties, as well as to analyze the costs and profitability of production. The experiment was conducted at the Agricultural Station of Universidad Técnica Particular de Loja, under low tunnels and open field conditions, in which the phenological and productive development of the strawberry was compared. The results found in the study in the two open field and low tunnel systems did not present a statistical difference in the physiological variables evaluated, which allow to conclude that the low tunnel cover on the crop did not influence the development of the two varieties. In relation to the cost-benefit analysis of production, it was higher in the low tunnel system, the investment was stronger at the beginning, but it must be indicated that there are several productive advantages in relation to the open field that generate benefits for the strawberry producer.
Keywords: Low tunnels, production, profitability, strawberry yield.

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1 Introduction

The crop of strawberry by agricultural producers and its consumption has a very good acceptance worldwide (Khoshnevisan et al., 2013), the same happens in Ecuador, where it has started to be part of the family consumption (Vizcaino, 2011). The fruit is quite attractive to the consumer for its aroma and exquisite taste; it also has a lot of vitamins, minerals and organic acids, acting as disinfectants and anti-inflammatory drugs that decreases cholesterol. In addition, essential oils can be extracted, and their consumption can be fresh or processed (Karim et al., 2011; Giampieri et al., 2012; Pradas et al., 2015; Bernarda and Bawab, 2017; Estrada et al., 2017). Strawberry is characterized by being herbaceous and perennial, it is very sensitive to environmental conditions and its development is subjected to the climatic characteristics, chemical, physical and structural properties of the soil (Ferriol, 2010; López et al., 2011; Pineda, 2017). There are short-day varieties subjected to photoperiod, fewer hours of light for the development of flower buds; and neutral-day varieties that are not subjected to photoperiod for the development of flower buds (Undurraga and Vargas, 2013; Pérez et al., 2017).

The mostly used production systems are open field and under protection (Rubio et al., 2014). Open field production is highly susceptible to biotic and abiotic factors such as pests, diseases, high temperatures, precipitation, winds and frosts (Dominí, 2012; Pineda, 2017), which can affect productivity. Lamont and William (2009) mention that technology helps to have better control of the climatic conditions for its cultivation. Environmental, genetic and variety factors are important in the plant growth, productivity and fruit quality (Rodríguez et al., 2012). The cultivation of strawberry in protected systems such as the low tunnel system can be an alternative in the production of this crop, because these systems have some advantages such as low cost, small size and practicality of installation (Juárez et al., 2011; Pernuzz et al., 2016), compared to other protected systems such as greenhouses.

In Ecuador an area of 108 ha is harvested, reaching a yield of 16.27 T/ha, being very low compared to other strawberry producing countries, such as the United States with 66.90 T/ha of production; Spain (47.6 T/ha); Egypt 46.6 T/ha; Israel with 43.5 T/ha, and Colombia with 36.5 T/ha of strawberry production per year (Corrêa and Peres, 2013; FAOSTAT, 2016). However, in the last decade it has had an interesting growth, either by adopting new technology or new varieties.

The search and application of new innovations in agricultural production to improve production systems are very important, since each area dedicated to strawberry cultivation has its common features. In the province of Loja, it is necessary to carry out research on the subject in the absence of accurate information or research on the use of protected systems (low tunnel) in this crop. Therefore, this study aims to assess the effect of the use of protection (low tunnel) on the development and productivity of strawberry cultivation in the Albion and Monterey varieties, which are the most commercialized in this area, in order to analyze the most feasible alternatives, so that smallholder farmers at the local level increase yield and economic benefits.

2 Materials and Methods

2.1 Study area

This study was carried out in the province of Loja – Ecuador, at the Agricultural Station of the Technical University of Loja, located in the Cajanuma sector, 9 km away from the city, with the X coordinates: −4,0887 Y: −79,2082 (Google Maps, 2018). at an altitude of approximately 2230 m.a.s.l; an average temperature of 16 $^{\circ}C$ and an average rainfall of 780 mm/m^2 according to data from Estación meteorológica «La Argelia» (2017).

2.2 Experimental design

The study area is a place with homogeneous topography and edaphoclimatic characteristics. Four plots (treatments) were established under an experimental design of complete blocks. The established treatments were: two low cover (low tunnel) in which the Monterey and Albion varieties were grown (one variety in each low tunnel), and the same treatments were established in open field (without cover). Each treatment had a total of 54 plants (F1) and each was considered as an experimental unit (Pernuzz et al., 2016).

The symbols established for the treatments were: **CAV1**= Open field treatment monterey variety, **CAV2** = Open field treatment albion variety, **MTV1** = low tunnel system treatment monterey variety, **MTV2** = low tunnel system treatment albion variety. The size of the low tunnels was 8 m long $\times$ 1 m wide and 0.90 m high, with polyethylene plastic cover Nro 8. A planting density of 0.30 $\times$ 0.40 m between row and plant (54 plants per treatment) was used in each treatment.

The fertilization was similar in all treatments, using organic fertilizer (BioCompost) at the time of transplantation (250 g/plant), and mineral fertilizers based on $N - P_2O_5 - K_2O - MgO - CaO - S$ ($200 - 200 - 300 - 40 - 100 - 40$ Kg/ha, respectively), all based on soil analyses and crop needs (Patiño et al., 2013; Avitia et al., 2014).

The irrigation system was installed by low flow drip, with vortex emitters inserted and flow output 2 to 4 Lt/hour, and was watered based on the weather conditions, as commonly handled by farmers in the area. The integrated management of pests and diseases (MIP) was thorough, each plant was monitored, preemptively performing sanitation pruning to avoid diseases such as *Botrytis cinerea*, *Colletotrichun* sp. y de *Trips* sp. (Santoyo and Martinez, 2010).

2.3 Variables evaluated

The evaluation variables are detailed below: **Detachment percentage:** it was performed by counting the total of plants (experimental unit) of each treatment that was successful in its detachment, indicating that the results were expressed as percentage (Ibadango, 2017).
Number of flowers: The number of flowers on each plant of the four treatments was counted; this data was recorded throughout the project evaluation (Yaselga, 2015).
Number of fruits: The amount of fruits in the plants of each treatment was determined for a period of 6 months, the data were recorded daily in order to have more detail of the productivity by production system and variety of cultivated strawberries (Ibadango, 2017).
Fruit diameter: The data was taken from the fruits harvested with a Vernier calibrator, the measurement was done from the central part of the fruit (unfructescence), expressing the values in centimeters (Verdugo, 2011).
Fruit weight: The weight of each harvested fruit was considered. It should be mentioned that strawberry fruits were harvested when they were ripe, just as the registration of the previous variables, this was done daily once the production started (Verdugo, 2011).
Economic analysis of strawberry production: The production of the crop was calculated taking into account the total harvested (without considering the losses), and the final production of the treatments was transferred and expressed in kg/ha.

The net income (benefit) of these production systems in this study was estimated with the difference between the input and costs of strawberry production (Cost/benefit (C/B) = Input/expenses) (Infante Villarreal, 1984), calculating inputs from sales of the fruit at average MAG (2018). Total costs were estimated from the average planting costs and its management: fertilization, weed control, irrigation system, pest control, diseases, pruning, among others.

2.4 Statistical analysis

The normality or abnormality of the data was checked with KS tests to establish statistical differences in the measured parameters (variables), then one-way ANOVA was applied with homogeneous Tukey tests at a significance level $p < 0,05$, for this the statistical software SPSS 24.0 was used.

3 Results and discussion

Table 1 details the results obtained in the different treatments. It is observed that there are not significant statistical differences between the physiological variables evaluated, indicating that the cover of low tunnel did not influence the development of strawberry cultivation in the two varieties, probably because there were no heavy periods of rain.

Table 1. Percentage of detachment, number of flowers, number of fruits, fruit diameter and weight of *Fragaria vesca* fruit in the evaluated treatments.

	CAV1	CAV2	MTV1	MTV2
Parameters				
%Detachment	94.45 ± 0.23 a	94.45 ± 0.23 a	100.00 ± 0.00 a	98.15 ± 0.13 a
Number of flowers	37.92 ± 7.20 a	34.85 ± 6.70 a	37.35 ± 8.83 a	36.57 ± 7.53 a
Number of fruits	36.20 ± 7.87 a	34.57 ± 6.78 a	36.62 ± 6.63 a	35.07 ± 9.11 a
Fruit diameter (cm)	3.26 ± 0.39 a	3.29 ± 1.11 a	3.26 ± 0.32 a	3.30 ± 1.09 a
Fruit weight (g)	23.74 ± 6.60 a	23.79 ± 6.74 a	23,94 ± 5,59 a	23,95 ± 7,06 a

Table 1 shows the mean data and standard deviation of the percentage of the detachment, number of flowers, number of fruits, fruit diameter (cm/fruit) and fruit weight (g/fruit). Different lowercase letters indicate significant statistical difference between treatments. CAV1 = Open field treatment variety monterey, CAV2 = Open field treatment variety albion, MTV1 = low tunnel system treatment variety monterey, MTV2 = low tunnel system treatment variety albion.

The high percentages of the detachment in this study is possibly due to the good agrotechnical management in the crop, thus complying with all the standards of good agricultural practices. This work reinforces what is indicated by Montero (2016) y Yaselga (2015), who say that a proper irrigation treatment, a substrate enriched with abundant organic matter, adequate pest and disease control and proper soil disinfection facilitated seedlings' detachment; hence, their adaptability, which is directly related to the percentage of seedling detachment. In addition to this, Nin et al. (2018) mentioned that it is recommended to adjust the crop date according to light/temperature conditions for a better seedling survival, in order to control the interactions between the plant and the environment. The percentage of the crop's production played an important role in the fruit production by the number of live plants in each treatment.

Moreover, the number of flowers/plant in all installed treatments showed no significant statistical differences between treatments. If comparing this study with that of Juárez et al. (2007), the number of flowers was high, since the study carried out by the aforementioned authors obtained a smaller number (five flowers/plant), attributing this low number of flowers to climate factors that were not right for the crop, indicating that plants are sensitive to the effect of the photoperiod and high temperatures, which could have influenced these differences.

Likewise, León et al. (2014), mention that nutritional requirements in strawberry crop are essential for its floral development; thus, a good nutrition plan made according to the crop requirements and the soil quality also supported that the number of flowers are high in this research, regardless the treatment applied. Authors such as Caruso et al. (2011) indicate that photosynthesis influences the number of flowers, which would be influencing to obtain these results. In addition, being located in the center of the earth with approximately 12 hours of light exposure has also played an essential role in this study, which contributed to the good flowering of the crop in the two production systems.

The variable numbers of fruit/plant in this study (Table 1), for the evaluation of the first productions have been considered acceptable. It should be noted that these are directly related to the number of flowers. The results obtained are similar to those presented by Montero (2016) y Radin et al. (2011) who found 29 and 37 fruits/plant, respectively. It is worth mentioning that Montero's research was carried out at Sierra del Ecuador, with conditions similar to the province of Loja. The aforementioned authors suggest that it should be taken into account the influence of environmental factors such as water deficit, temperature, solar radiation, among others with the aim of obtaining a good number of fruits. This means that relatively low and high temperature changes, relative to the ideal temperature range which is between 18 and 25 $^{\circ}C$, negatively affect the fruits set (Ledesma et al., 2008). Another very important factor to consider for a better production is the health and nutrition of plants, in addition to what is indicated by Poveda et al. (2018) who mentioned that wind and the presence of pollinating insects also have significant influence

to obtain a greater number of fruits with good quality whether in the open field or under protection, as it is in this case.

The average diameter of the fruit in each of the treatments (Table 1), similar to the other parameters evaluated, has shown no significant differences. The diameter has a direct relationship to the weight of the fruit. Hollender et al. (2012) mention that the diameter varies depending on the number of carpels and the number of achenes present in the fruit; the achenes splash the surface of the receptacle and produce auxins to stimulate growth. Similarly, Ledesma et al. (2008) indicate that when achenes are not properly fertilized, the development of the area in the fruit around the achenes is inhibited, which is observed in the malformation of the whole fruit and is evidenced in the diameter of the fruit. This is consistent with this study, as the diameter of the fruit did not vary because of the same fertility doses in all treatments. On the other hand, Radin et al. (2011), mention that insufficient pollination and damage caused by insects produces the malformation of the strawberrys fruit, which was not presented in this study due to strict monitoring and control of the crop in all treatments. However, this data should be taken into account for plantations of commercial areas or bigger plantations.

As regards the fruit weight variable, no difference was found between the treatments (Table 1). These results are similar to those found by Ibadango (2017) which also shows that there are no differences in the average weight of strawberry fruit grown under a protected system and under an open field. In another study they state that the weight of the fruits will mainly depend on the nutritional status of the plants and the total soluble solids, which are characteristics dependent on the variety (Ortiz et al., 2016); This is consistent with this study, while having the same treatments in fertilization, has shown no difference in the weights of strawberry fruits produced under low tunnel and open field. This is further corroborated by the study conducted by Vignolo et al. (2011), who found significant differences in the average weight of the fruit, testing different doses of manure and fertilization. Another cause that can attribute more or less weight to the fruits is what Radin et al. (2011), indicated when saying that weight is related to the variety grown, and that the difference between the average weight per fruit may be associated with genetic factors, without neglecting the conditions of the environment.

3.1 Productive and economic analysis of the crop

Figure 1 shows the total production of the different strawberry treatments evaluated in this study, expressed in Kg/ha/year. Higher net production was obtained in the low tunnel system in the two varieties, with a significant statistical difference compared to open field production. These results are in agreement with the studies conducted in Colombia by Grijalba et al. (2015), who obtained higher productions in low tunnel systems (1260 g/plant) than in open field (1197 g/plant), being the ratio for the same time period as that of this research (840 g/plant open field system and 860 g/plant low tunnel system). Grijalba et al. (2015) say that this increased production occurred since there was lower incidence of pest and diseases in their protected crops.

The growing system (open field or under cover) can play an important role in the yield, such as in the fruit quality (Nin et al., 2018). In addition, productivity is related to two characteristics of the crop, the average weight of fruits and the number of fruits produced per plant throughout the cycle (Otto et al., 2009), a figure that matches the fruit weights obtained in this work. While there were no significant differences between treatments (Table 1), the weight of the fruits produced under the greenhouse was higher, which has been expressed in better total production.

According to Pernuzz et al. (2016) in its comparison study in micro and macrotunnel production systems, the low tunnel system has a higher net production compared to that of this study, which reached 45950 kg/ha; however, it continues to confirm the hypothesis of good production results when growing under low tunnels or under cover. In addition, they indicate that low production in the open field system may be due to the impact of weather conditions during the harvest, making the crop to be more attacked by pests and diseases.

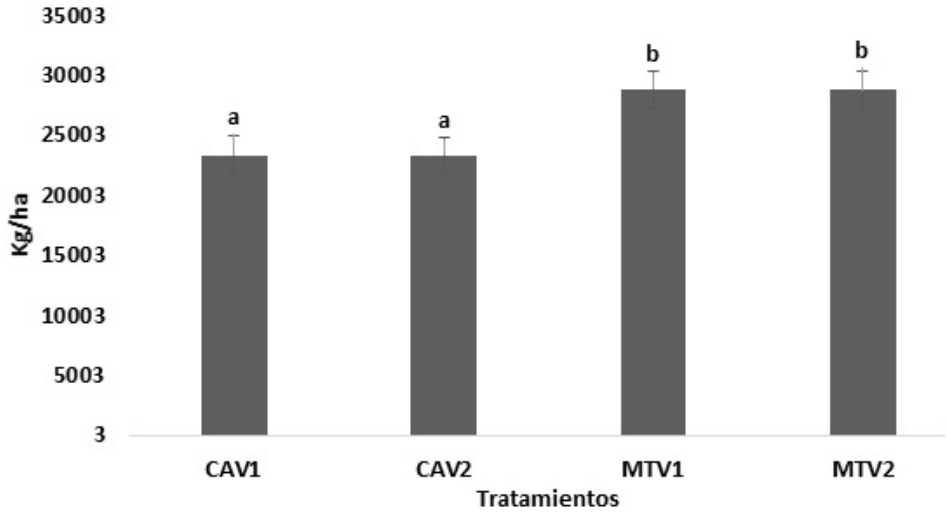

Figure 1. Net production (kg/ha/year) of *Fragaria vesca* in the evaluated treatments.
Note: Final production expressed in one kg/ha is presented. The bars represent the mean of each treatment with their corresponding standard deviation. Different lowercase letters mean significant statistical difference. CAV1 = Open field treatment variety monterey, CAV2 = Open field treatment variety albion, MTV1 = low tunnel system treatment variety monterey, MTV2 = low tunnel system treatment variety albion.

In the study conducted by Rubio et al. (2014),there is a higher number of disease attacks in the open field system, being mainly affected by *Botrytis cinerea*, *Sphaerotheca macularis*, which directly affects net production, versus a protected and least attacked system; this because the humidity is lower in the protected system, and the production losses are lower. Similarly, the attack by lepidoptera and mites affected the protected system, because in that study the net production was lower than in this study, since they obtained 15365 kg/ha in open field and 20070 kg/ha in protected system, being one of the possible causes even to the productive reduction in both systems. The net yields in this study, when compared to the data of the aforementioned authors, are good and acceptable, being up to 10% higher than the production obtained Rubio et al. (2014), and about 30% higher over Ecuador's average net production. However, these values are lower than those of other countries such as Colombia, which has a production of around 40000 Kg/ha.

3.2 Cost-benefit (C/B).

Table 2 shows the cost-benefit per ha/year of production for all treatments. It is evident that the open field system and protected low tunnel system are very similar in the first year of production in its C/B ratio: 1.57 versus 1.59, respectively. In either system the investment would be profitable within this first year. However, it must be considered that the investment costs in low tunnel systems have been higher because of the initial infrastructure required; but, given the good production obtained, its cost-benefit ratio is positive as shown in Table 2. Following this year 1 production pattern in the protected production system, it is assumed that the potential economic benefits in subsequent years should be higher in this production system (low tunnel), as infrastructure investments would reduce considerably, so its use would be advisable. In addition, these systems are an option to crop in areas with adverse weather conditions to the climatic requirements of the crop, such as constant frosts, low temperatures or heavy rainfall (Rowley et al., 2010).

The crop production is directly related to economic benefit, which is an important factor for a better profitability. On the other hand, Caruso et al. (2011) mention that the yield is one of the basic factors for improving revenue, although they indicate that product quality is also another factor, as in many markets quality is a desired value. However, in many cases producers do not have a satisfactory benefit, their profitability for the production of this crop is low or zero, which is mainly attributed to the high production costs (Pineda, 2017), and even

more in the initial part of a protected system. However, as indicated by Pernuzz et al. (2016), it should be considered that the protected system has a lifespan of about 10 years, except for plastic casings that could be useful on an average of 5 years, being a benefit in crop production, which will be reflected in long-term net gain. On the other hand, it is also necessary to consider what is stated by Rubio et al. (2014), who mention, from the investment point of view, that the value of open field losses in each cycle is about one-fifth of what it costs to make the investment to build the protected system; thus, many producers as not to risk capital decide to opt for a traditional production system and make lower profits.

Table 2. Relationship of the cost-benefit of *Fragaria vesca* in treatments, expressed in ha/year of production.

Treatments	Income (USD)	Expenses (USD)	Benefit (USD)	Cost Benefit Relationship
CAV1	276777,77	175638,88	101138,89	1,57
CAV2	276740,74	175638,88	101101,86	1,57
MTV1	342324,07	215055,55	127268,52	1,59
MTV2	342101,85	215055,55	127046,30	1,59

Table 2 shows the income, income, benefit and cost-benefit data from the evaluated treatments. CAV1 = Open field treatment variety monterey, CAV2 = Open field treatment variety albion, MTV1 = low tunnel system treatment variety monterey, MTV2 = low tunnel system treatment variety albion.

4 Conclusions

In this case, in the two production systems (traditional-open field and under cover), there was no statistically significant difference in the physiological variables evaluated ($p < 0,05$). However, it should be noted that as the weight of the fruits of the system under low tunnel is greater, this has been reflected in higher final net production per ha, being this production higher than in the open field.

In the production cost-benefit analysis, the investment in the low tunnel system was higher compared to the open field system. However, it should be noted that the benefits of low tunnels, in terms of their improved productivity, ease of handling and even durability of infrastructure, generate greater economic benefits to the producer in the medium and long term.

The use of low tunnels in strawberry production is a viable option for smallholder farmers, especially when weather conditions are not recommended to the climatic requirements of the crop, as well as the possibility of achieving up to 30% better production versus the national average.

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