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La GranjA:
Revista de
Ciencias de la Vida

Editorial

Dear reader:

This special edition of La Granja titled "Understanding dairy cows for a sustainable production", seeks to generate awareness and reflection on primary milk production, and for this to happen we must first understand, support and respect producers. In general, human solves problems in the short term according to the needs, but without considering the integrative look that would allow a continuous analysis, emphasizing the sustainability of the systems from economic, productive, environment and social aspects.

In this regard, Dr. Darwin Yánez-Avalos and other researchers from Universidad de Córdoba, and Universidad del Rosario in Argentina as well as the Ministry of Agriculture and Livestock of Ecuador, highlight the results of artificial insemination protocols adapted to the reality of the Ecuadorian Amazon, providing alternatives for improved conception and reproductive efficiency.

Likewise, in relation to the postpartum period of the cow, Dr. Javier Huerta-Peña, Dr. Miguel Lammoglia-Villagómez and Dr. Pablo Marini, demonstrate that the origin of infertility in dairy cows in the Mexican highland is associated with postpartum pathologies which have increased in recent years. Thus, they recommend to maximize the care of cows in this period to reduce the presence of pathologies and to avoid problems in cows.

Dr. Francisco de la Cueva, Dr. Alexandra Naranjo, Dr. Byron Puga Torres and Dr. Eduardo Aragón of the Veterinary and Zoology Faculty of Universidad Central del Ecuador, demonstrate the need to control the vehicle of heavy metals in milk in the productive systems close to cities. In order to determine the presence of these metals, they propose a continuous monitoring in milk, specially to identify the causes of its contamination.

To finish this second special issue, and continuing with the quality of the product, Dr. Rocío Contero-Callay and her team at the Milk Quality Laboratory of Universidad Politécnica Salesiana analyze whether the parameters set out in the law for the payment of raw bovine milk in Ecuador are met. The author points out that these rules are fulfilled and stresses the need to concentrate efforts on health and good milking practices.

In addition, in the miscellaneous section of the journal, six articles with different topics are presented. The first one, about soil science, conducted at the Polytechnic School of Chimborazo and the Ministry of Agriculture and Livestock, Jorge Silva and his team, presents a literature review of the environmental, medical, pharmacological and industrial potential of andisol nanoparticles found in Ecuador. Meanwhile, the research group of Universidad Técnica and la Universidad Laica Eloy Alfaro de Manabí, led by Rubén Darío Fernández, show the effects of the use of hydrogel and the moistening volume in different types of soils.

On the subject of Agriculture and Climate Change, Ingrid Betancourt and Lenin Ramirez, from Universidad Andina and Yachan Tech, respectively, assess the efficiency of hard maize production under different models, analyzing its effect on the climate and its changes. Likewise, Sebastian Cedillo and his group of researchers from Universidad de Cuenca carry out a descriptive roughness model in two dimensions in Mountain rivers.

Finally, in order to continue with the issue of sustainability, Fiorella Cáceres and José Iannacone, from Universidad Científica del Sur and Universidad Nacional Federico Villarreal del Perú, show the environmental effects of insecticides in the production of river shrimp. Also, Bécquer Camayo and his team from Universidad Nacional del Centro de Perú and Alma Global Network of Spain, present more ecological strategies for solar dryers of Aguamayto.

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It is evident that the research in the region, represented by this issue of the 33 Journal of Life Sciences, La Granja, points to a more sustainable form of consumption and production, which seeks not only the respect for the environment, but also its care and understanding.

Sincerely,

Dr. Pablo Marini
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Scientific paper/ Artículo científico

The dairy cow: Understanding the sustainable production

Protocols J-Synch with and without eCG in Brown Swiss and crosses whith Bos indicus cows in the Ecuadorian Amazons

Protocolo J-Synch con y sin eCG en vacas Brown Swiss y sus cruzas con Bos Indicus en la amazonía ecuatoriana

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Abstract

The incorporation of protocols with prolonged proestrus in extreme conditions has allowed to improve pregnancy. The objective was to evaluate the results of the protocol with prolonged proestrus called J-Synch with and without eCG, on follicular dynamics and pregnancy percentage in dual-purpose cows in the Ecuadorian Amazon. The project was developed of the Province of Pastaza - Ecuador, from October 2018 to October 2019. The study used 448 multiparous dual purpose Brown Swiss cows with calves and their crosses (*Bos indicus*). Four treatments were used, the cows were artificially inseminated at a fixed time on day nine T1: J-Synch+ eCG+ estrus FTAI 60 hours (n=120); T2: J-Synch+ eCG+ without estrus+ GnRH FTAI 72 hours (n=118); T3: J-Synch+ estrus FTAI 60 hours (n=103); T4: J-Synch+ without estrus + GnRH + FTAI 72 hours (n=107). The pregnancy rate was 55% (T1), 49% (T2), 51% (T3) and 50% (T4) without showing significant differences. Follicular development and corpus luteum diameter showed significant differences ($p \leq 0.05$). It is concluded that the J-Synch treatments plus eCG at 60 hours and 72 hours had different behavior in follicular development and in the size of the corpus luteum. The pregnancy percentage did not differ between treatments. Although, there was a 44.5 % more probability of getting a cow pregnant with T1 compared to T2. Furthermore, pregnancy would increase by 22.5 % applying T1 compared to T3, and pregnancy with T3 would be 18 % higher if compared to T2.

Keywords: Follicular dynamics, pregnancy, progesterone, Fixed Time Artificial Insemination

Resumen

La incorporación de protocolos con proestro prolongado en condiciones extremas ha permitido mejorar la concepción. Por lo tanto, el objetivo de esta investigación fue evaluar los resultados del protocolo con proestro prolongado denominado J-Synch con y sin eCG, sobre la dinámica folicular y porcentaje de preñez en vacas doble propósito en la Amazonía Ecuatoriana. El trabajo se efectuó en la provincia de Pastaza - Ecuador, desde octubre del 2018 a octubre del 2019. Se utilizaron 448 vacas multíparas en producción con cría al pie de las razas Brown Swiss y sus cruzas (*Bos indicus*). Se trabajó con cuatro tratamientos, las vacas fueron inseminadas artificialmente a tiempo fijo en el día nueve T1: J-Synch + eCG +Celo IATF 60 horas (n=120); T2: J-Synch+eCG +Sin Celo+ GnRH IATF 72 horas (n=118); T3: J-Synch+ Celo IATF 60 horas (n=103); T4: J-Synch+ Sin Celo+ GnRH +IATF 72 Horas (n=107). La tasa de preñez fue del 55 % (T1), el 49 % (T2), el 51 % (T3) y el 50 % (T4) sin mostrar diferencias significativas. El desarrollo folicular y el diámetro del cuerpo lúteo mostraron diferencias significativas ($p \leq 0{,}05$). Se concluye que los tratamientos J-Synch más eCG a las 60 horas y las 72 horas tuvieron diferente comportamiento en el desarrollo folicular y en el tamaño del cuerpo lúteo. El porcentaje de preñez no difirió entre los tratamientos, aunque existió un 44,5 % más probabilidad de quedar preñada una vaca con el T1 en comparación con el T2. Además, que aumentaría un 22,5 % la preñez aplicando el T1 en comparación con el T3, y se lograría un 18 % mayor de preñez con el T3 si se compara con el T2.

Palabras clave: Dinámica follicular, preñez, progesterone, inseminación artificial a tiempo fijo

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1 Introduction

Brown Swiss cow production systems and their crossings with *Bos indicus* are common in the humid tropical region, with an average daily milk production per cow of 3 to 9 liters, the duration of lactation from 120 to 180 days and a delivery interval of 18 to 24 months (Rodriguez et al., 2015).

These reproductive parameters are generated by multiple factors, among them environmental (high temperatures and humidity), physiological and zootechnical management. In the Amazon region, producers need to improve the reproductive outcomes in cows for meat and milk (Moyano et al., 2015).

J–Synch protocol has suffered a number of variations, without altering its initial form as: an intramuscular dose of Estradiol Benzoate and the placement of progesterone through an intravaginal device to synchronize the beginning of a new follicular wave with lower insertion time of the device with progesterone for 6 days and no longer than 7 or 8 days, applying a GnRH as ovulation initiator at 72 hours after the removal of the device, along with the FTAI, to ensure a more durable proestrus (De la Mata and Bó, 2012).

Yánez et al. (2017), showed the pregnancy results of two FTAI protocols by inseminating double purpose cows from the Ecuadorian Amazon at 60 and 72 hours of having removed the intravaginal device impregnated with progesterone, where the J-Synch and eCG 60 hour protocol had the best pregnancy rate compared to the J-Synch and eCG 72 hour protocol. Cedeño et al. (2019) compared the follicular and luteal dynamics of an alternative J-Synch proestrus prolongation treatment of 7 days of progesterone in *Bos indicus* heifers that received fixed time artificial insemination in Manabí Province-San Vincente, concluding that the extension of one more day of progesterone in the 7 day J-Synch treatment does not alter the characteristics of the dominant ovulation follicle and the time of ovulation in relation to the 6 day J-Synch protocol, although in both prolonged proestrus protocols, the interval to ovulation is higher than in animals treated with the conventional protocol with Estradiol Cypionate as an ovulation inductor. Castellanos et al. (2019) conducted a study in Etzatlán, Jalisco, Mexico, with 211 cows (90 Brahman cows and 121 F1 Brahman × Swiss Brown cows) without breeding with more than 180 postpartum days and a body condition of 3 to 4; on Day 6, 105 cows were randomly assigned to the J-Synch Group and the remaining cows (106) were assigned to the Traditional Group, the two groups with eCG, finding no significant differences ($p \geq 0.05$) in the pregnancy percentage among the groups. In addition, it showed no significant differences ($p \geq 0.05$) in the pregnancy percentage of the breed or the bull used.

Based on the findings of the application of J-Synch, (Bó et al., 2018a) proposed that heifers that manifest an earlier estrus could be inseminated earlier without affecting the pregnancy percentage, but it would be necessary to determine the optimal time to perform FTAI in those that do not show estrus. A research was carried out, in which 1,283 Angus and Hereford cross heifers were treated with the J-Synch protocol and heifers were painted at the base of the tail when the progesterone device was removed. Heifers received GnRH/FTAI at 60 or 72 h, regardless the paint removal. Pregnancy was higher in those who showed estrus before FTAI than in those who did not, no matter the time of insemination (53.6%, 542/1012 vs. 45.0%, 122/271, respectively, $P \leq 0.05$).

The practical implication of this result is that when large herds are synchronized, the device could be removed in the afternoon of the 6th day and the FTAI begins at 60 h (day 9 a.m.) on all heifers with altered paint; and those with the paint intact could be separated to receive GnRH at the time of artificial insemination on a fixed time on the afternoon of the 9th (i.e. around or after 72 hours).

The incorporation of protocols with prolonged proestrus has allowed to increase the pregnancy rates in cows with anestrus, with elevated open days, with foot breeding in tropical climates. However, the J-Synch protocol is still required to continue to be validated under conditions in the Ecuadorian Amazon. The objective was to evaluate the results of the protocol with prolonged proestrus called J-Synch, with and without eCG, in follicular behavior and the pregnancy percentage in Brown Swiss cows and their crosses with *Bos indicus* in the Ecuadorian Amazon.

2 Materials and Methods

The research was carried out in Pastaza, province of Ecuador, starting from October 2018 to October 2019. In the Amazon, the precipitation is 4000 – 5000 mm/year, high humidity and heat with temperatures ranging between 18 and 24 °C. Producers own farms of 50 ha average on uneven land, using *Axonopusscoparius* (Gramalote) (González-Marcillo et al., 1997).

448 cows of the Brown Swiss breeds and their *Bos indicus* crosses were used, which were fed by a grazing system (Carrera Durazno et al., 2015), from 90 to 132 days of postpartum, from 34 to 65 months old, with a body condition from 2.5 to 3 (scale 1 to 5, Ben et al. (2002)), and a weight between 350 and 380 kg, and were selected by the presence of a follicle > 8 mm in diameter in their ovaries by means of ultrasonography. Finally, 0.50 ml brown Swiss semen straws previously analyzed in FTAIs were used.

2.1 Treatments

The long term proestrus resistant synchronization protocol called J-Synch (De la Mata and Bó, 2012) was used, which is modified by adding eCG and performing the FTAI at 60 and 72 hours of having removed the intravaginal device. The total cows were randomly divided into two groups: Group 1 with the addition of eCG and Group 2 without eCG. In turn, these two groups were subdivided into two more groups, one that is performed with FTAI at 60 hours and another with FTAI at 72 hours after the intravaginal device had been removed. Schematically represented.

T1: J-Synch + eCG + *Estrus* - IA 60 hours (n=120)
T2: J-Synch + eCG + *Without estrus* + GnRH - FTAI 72 hours (n=118)
T3: J-Synch + *Estrus* – IA 60 hours (n=103)
T4: J-Synch+ *Without estrus* + GnRH - FTAI 72 Hours (n=107)

The distribution of treatments (T) was completely at random, each of which carried out four repetitions (*T1: 30, 30, 30 and 30 (120), T2: 29, 29, 30 and 30 (118), T3: 25, 26, 26 and 26 (103) T4: 26, 27, 27 and 27 (107)* cows with each of the treatments).

For the conduction of the research, on day 0 a first ultrasound reproductive evaluation was carried out on each of the 306 cows to analyze ovarian status and observe if animals meet the proposed parameters to be included in this research; additionally, an intravaginal device with progesterone (DIB 0.5 gr) was placed and 2 mg of BE per IM was applied; on day 6 cows were divided into groups according to their treatments as follows:

2.1.1 Treatment 1

References: On day 0, the first ultrasound evaluation was performed to analyze the ovarian status and BE (2 mg of Estradiol Benzoate) was applied via IM together with an intravaginal DIB device 0.5 g. On day 6 the device was removed with the administration of $PGF_{2\alpha}$ (500 μg of Cloprostenol) plus the application of eCG (400UI of equine chorionic Gonadotropin) and divided into two groups: *T1* (120 cows) and *T2* (118 cows) were measured with ultrasonography of follicular development at the onset of the proestrus, a paint marker was applied at the base of the tail to observe the presence of preFTAI (Artificial insemination at a fixed time) (Figure 1). The animals that presented estrus within 60 hours were inseminated at 60 hours *(T1)* and those that did not, at 72 hours with the addition of GnRH (100 μg gonadorelin acetate) *(T2)*. On day 9 or end of the proestrus, the ultrasonogram was performed to see the behavior of the follicles. Following this, 60 hours after having removed the intravaginal device, the time of ovulation was determined by ultrasonogram every 12 hours in the two treatments. On day 7 post insemination, the size of the luteal body was measured by ultrasound and quality was measured through a serum sample, and progesterone levels (ng/ml) were analyzed. The gestation diagnosis was performed 35 to 40 days after FTAI by ultrasound (Veterinary ultrasound Ibex-pro with linear probe 5 MH in V mode).

2.1.2 Treatment 2

References: On day 0, the first ultrasound evaluation was performed to analyze the ovarian status and BE (2 mg of Estradiol Benzoate) was applied via IM along with an intravaginal 0.5 g DIB device. On day 6 the device was removed with the administra-

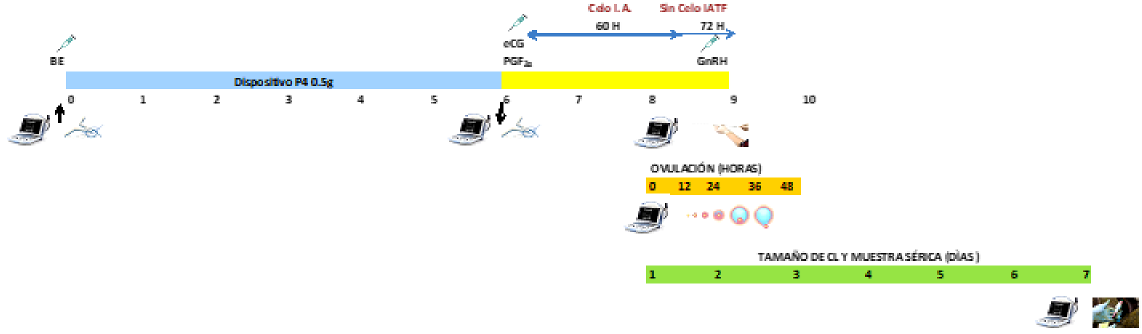


Figure 1. T1: J-Synch +eCG+ Estrus – I.A. 60 hours. Without estrus I.A.T.F. 72 Hours + GnRH

tion of $PGF_{2\alpha}$ (500 μg of Cloprostenol) and divided into two groups: *T3* (103 cows) and *T4* (107 cows) a measurement with ultrasonography of follicular development was performed at the beginning of the proestrus, a paint marker was applied at the base of the tail to observe the presence of preFTAI (Fixed time artificial insemination). The animals that showed estrus before 60 hours were inseminated at 60 hours *(T3)* and those that did not, at 72 hours plus the addition of GnRH (100 μg gonadorelin acetate) *(T4)*. On day 9 or end of the proestrus, the ultrasonogram was performed to see the behavior of the follicles. Following this, 60 hours after having removed the intravaginal device, the time of ovulation was determined by ultrasonogram every 12 hours in the two treatments. On day 7 post insemination, the size of the luteal body was measured by ultrasound and quality was measured through a serum sample, and progesterone levels (ng/ml) were analyzed. The gestation diagnosis was performed 35 to 40 days after FTAI by ultrasound (Veterinary ultrasound Ibex-pro with linear probe 5 MH in V mode) (Figure 2).

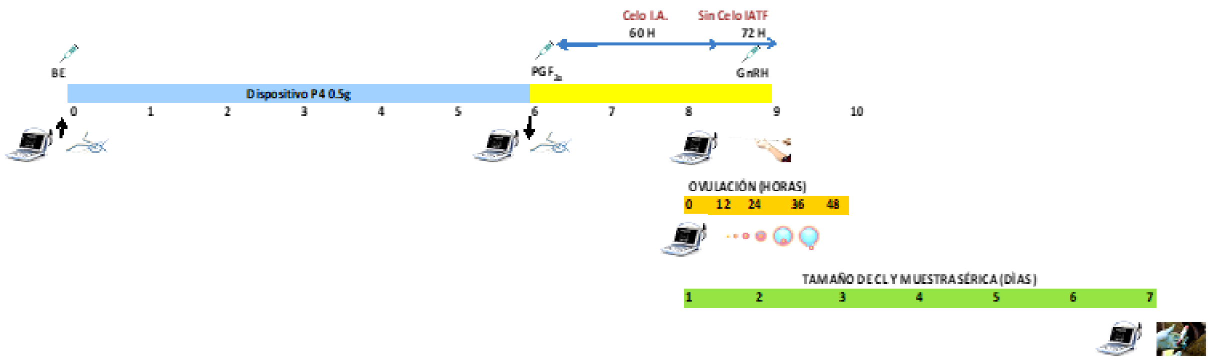


Figure 2. T2: J-Synch+ Estrus – I.A. 60 hours. Without estrus I.A.T.F. 72 Hours + GnRH

Frozen/thawed semen was used for the FTAI using the technique described by Bernardi et al. (2011), of a single bull of proven fertility, also before using it an analysis was done in the Biotechnology Laboratory of the Center for Research, Postgraduate and Amazonian Conservation and were inseminated by the same technician.

Estruses were observed through two behaviors: depainted in the bases of the tail and vaginal mucus (egg white), this observation was made before the ultrasound and FTAI started. In addition, estrus detection started 24 hours after the device was removed, twice a day (morning and afternoon) on days 7, 8 and 9.

2.2 Follicular and luteal dynamics

The follicular development was evaluated with an ultrasound on day six when the intravaginal device of progesterone was removed, also at the time of conducting the FTAI (60 and 72 hours of having removed the intravaginal progesterone device) with the aim of monitoring the ovary behavior and at the end of the estrus. In addition, on day seven after the insemination the measurements of the size of the luteal body are measured with the ultrasound, using what is recommended by Kastelic et al. (1990), on two measures (height-width in mm) of each structure (luteal body or follicle) to obtain the average of both variables.

In order to determine the timing of ovulation (disappearance of the larger diameter follicle), the follicle behavior was evaluated ultrasonically, observing the follicular development. It was performed in a smaller group of cows T1 and T2 = 24; T3 and T4 = 18 due to the difficulties in accessing or staying at different times in the establishments used; for this purpose, an ultrasound was performed 12 hours after the FTAI, then the animals that had not yet registered ovulation had another ultrasound at 24 hours, and the animals that had not yet ovulated until this moment were subjectively determined to ovulate > 24 hours after the FTAI, which was reconfirmed with the presence of CL 7 days after the FTAI.

Gestation was determined through ultrasonography between 40 to 45 days of FTAI (Ibex Pro 2011 and Lyte, USA, L6.2 transducer with a linear probe of 5.0 MHz)

2.3 Variables analyzed

- Estrus detection (presence or absence)
- Follicular development in mm
- Time of ovulation (hours)
- Luteal body size post-ovulation in mm
- Pregnancy: presence or absence
- Time: day 6 and day 8

2.4 Statistical analysis.

A Multiple Logistic Regression model was used for the adjustment to study the pregnancy (presence or absence) depending on the different protocols, days, follicular development in mm, size of the post-ovulation luteal body in mm. This model allows to take into account the binary nature of the response variable. The model interpretation was performed by analyzing the estimated Odds Reasons, along with their confidence intervals. For statistical analyses the JMP version 5.0 program for Windows was used.

3 Results

3.1 Pregnancy percentage

Out of the 448 inseminated cows, 230 (51.3 %) cows got pregnant and 218 (48.6 %) cows did not get pregnant. The pregnancy percentage of the T1, T2, T3 and T4 protocols showed no significant differences (P≥0.7989) (Table 1).

Table 1. The percentage of pregnancies in Brown Swiss cows and their crosses subjected to J-Synch treatments with and without eCG at 60 and 72 hours of FTAI.

Treatments	N° animals	Pregnant animals	% pregnancy
T1	120	(66/120)	55
T2	118	(58/118)	49
T3	103	(53/103)	51
T4	107	(53/107)	50
Total	448	(230/448)	51.3

3.2 Estrus manifestation

There was 51 % (230/448) of estrus in the total of animals for both treatments (Table 2). The percentage of animals with a presence of estrus in the J-Synch treatment with eCG (T1 and T2) was 52.1 % (124/238) and in the J-Synch treatment without eCG (T3 and T4) was 50 % (106/210) without significant differences (P≥0.05).

In the cows that presented estrus, the percentage of pregnancy in the J-Synch protocol with eCG + I.A. 60 hours was 55 % (66/120) *T1* and J-Synch without eCG + I.A., at 60 hours *(T3)* it was 54 % (39/72), which showed no significant difference, as well as cows without the presence of estrus + GnRH + FTAI 72 hours that were 49 % (58/118) *T2* and 49 % (67/138) *T4*, respectively (P≥0.05).

Table 2. Estrus in Brown Swiss cows and their crosses subjected to J-Synch treatment with eCG and without eCG.

Treatments	N° animals	With estrus	Without estrus
J- Synch con eCG	238	124 (52.1 %)	114 (47.8 %)
J-Synch sin eCG	210	106 (50.5 %)	104 (49.5 %)
TOTAL	448	230 (51.3 %)	218 (48.6 %)

3.3 Follicular dynamics at different times

The follicular size when the device was removed showed significant differences (p≤0.05) with a larger diameter for *T2* (9.7 ± 0.1 mm); in the same way, the size of the follicle initiation of artificial insemination showed significant differences (P≤0.05) with a larger diameter for *T4* (13.2 ± 0.1 mm) and when the estrus ended, the size of the follicle showed significant differences (P≤0.05) with a larger diameter for *T3* (13.9 ± 0.2 mm).

Table 3. Follicular dynamics (mm) in the four protocols used in Brown Swiss cows and their crosses. Standard ave-rages and errors.

Follicle size in mm			
Treatment Moment	**Once removed the device**	**Artificial insemination**	**At the end of the estrus**
T1	9.3 ± 0.1 ab	12.8 ± 0.1 a	13.0 ± 0.2 b
T2	9.7 ± 0.1 a	12.1 ± 0.1 b	12.8 ± 0.2 b
T3	9.3 ± 0.1 ab	12.2 ± 0.1 b	13.9 ± 0.2 a
T4	9.1 ± 0.1 b	13.2 ± 0.1 a	12.5 ± 0.8 ab

Note: different letters in each column indicate significant differences (P≤0.05).

3.4 Moment of ovulation

Ovulation, since the removal of the device, was higher (F=18.2; P≤0,001) or the treatment J-Synch with eCG (T1 and T2) (94.5 ± 0.9) hours than for the J-Synch group without eCG (88.0 ± 1.1) hours. Figure 3 shows the ovulation response and pregnancy rate registered in percentage at 84, 96 and 108 hours of FTAI in Brown Swiss cows and their crosses under the J-Synch treatment with eCG. Figure 4 shows the ovulation response and pregnancy rate registered in percentage at 84-96 and 108 hours of FTAI in Brown Swiss cows and their crosses with the J-Synch treatment without eCG.

Table 4. Ovulation after 24 hours of FTAI in Brown Swiss cows and their crosses according to J-Synch treatments with and without eCG.

Treatments	N° animals	Hours of ovulation	% ovulation
J-Synch with eCG	24	94.5 ± 0.9	51.0
J-Synch without eCG	18	88.0 ± 1.1	48.5
TOTAL	42	91.25 ± 1.0	50.0

Figure 3. Ovulation response and pregnancy rate with J-Synch treatment with eCG.

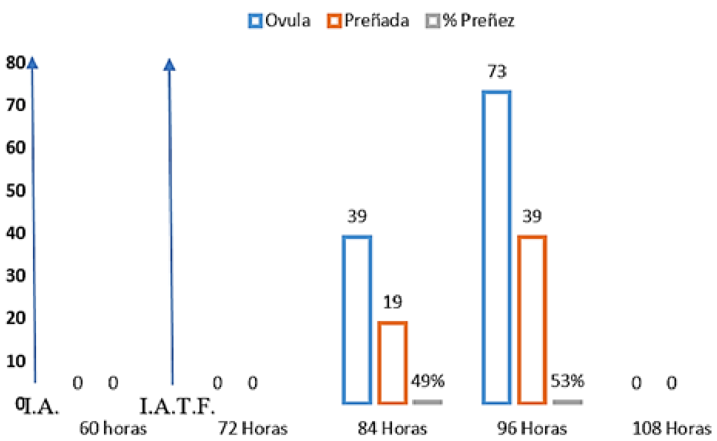
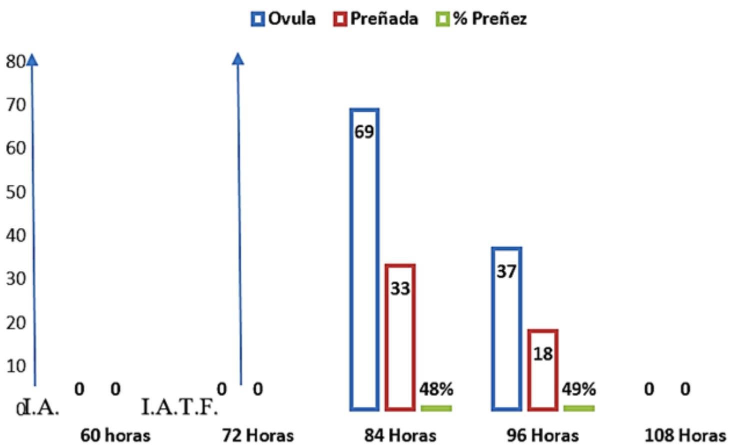


Figure 4. Ovulation response and pregnancy rate with J-Synch treatment without eCG.



3.5 Luteal body size

The luteal body was measured (its diameter in mm) at day 7 post insemination, differing significantly between treatments (P≤0.05) (T1: 21.6 ± 0.2b mm; T2: 22.0 ± 0.2b mm; T3: 22.1 ± 0.2b mm y T4: 25.0 ± 0.2a mm). In Table 5 can be observed that variables contribute significantly to the model. It means that the pregnancy percentage is significantly affected by the protocol used, the size of the dominant follicle and the size of the luteal body.

There is 44.5 % more chance of getting pregnant with A1 (with eCG 60 hs) compared to T2 (with eCG 72 hs). The conception rate would increase to 22.5 % by applying T1 (with eCG 60 hs) compared to T3 (without eCG 60hs). Pregnancy would be 18 % higher with T3 (without eCG 60hs) compared to T2 (with eCG 72hs). In all of these cases, the estimated RO confidence intervals include a value of 1. In other words, there might be cases where the chances of pregnancy in both groups are equal, and the difference is caused at random (since a RO=1 means that the chance of getting pregnant is the same for the two categories being compared).

The probability of getting pregnant is four times more in T1 (with eCG 60hs) when compared to T4 (without eCG 72 hs). It is almost three times more likely to get pregnant with T2 (with eCG 72hs) in comparison with T4 (without eCG 72hs). It is 3.5 times more likely on the T3 (without eCG 60hs) when compared to T4 (without eCG 72hs). At eight days it is almost four times likely to pregnant a cow than the same chance at six days. There is a 50% greater chance of pregnancy when the follicle size increases by 1mm and 35.2% higher when the luteal body size increases by 1mm.

Table 5. Interaction of variables and pregnancy rate.

Effect	DF	Wald's χ^2	Pr > χ^2
Protocol	3	372.609	<.0001
Day	1	298.451	<.0001
TF mm	1	461.369	<.0001
TCL mm	1	739.256	<.0001

4 Discussion

Brown Swiss cows and their crosses with *Bos indicus* were the most used for the production in the Ecuadorian Amazon, although there is a great need to improve reproductive indicators (Moyano et al., 2015). Within a set of problems that surround, among the most important is the delay in the restart of ovarian activity after the delivery (Guáqueta et al., 2014). Currently, there are several protocols available in Fixed Time Artificial Insemination (FTAI) to be used in cows for meat and milk (Colazo et al., 2009; Bó et al., 2018b; Uslenghi et al., 2014), but they need to be revalidated in the Amazon conditions and in Brown Swiss cows and their crosses with *Bos indicus*.

The early results obtained in this work with J-Synch with and without eCG in 448 Brown Swiss cows and their inseminated crosses show that 230 cows were pregnant, representing 51.3%, which agrees with the results reported by Baruselli et al. (2005) and López (2001) in the tropics, and also coincide with those found by López et al. (2014) and Quinteros (2009) who conducted the research in the Ecuadorian Amazon. In addition, the pregnancy percentages are consistent with those obtained by other researchers using similar protocols of prolonged proestrus J-Synch in the Amazon, when eCG was administered and they were inseminated at 60 hours of having removed the device with 61% (70/115) of pregnancy, compared to the inseminated at 72 hours adding eCG, showing 47% (52/111) of pregnancy (López, 2017; Yánez-Avalos et al., 2018).

Table 6. Estimated coefficients and maximum likelihood method

Parameter	DF	Estimate	Standard Error	Wald's χ^2	Pr > χ^2
Intercept	1	-133.388	11.689	1.302.273	<.0001
Protocol	1	14.499	0.2523	330.160	<.0001
Protocol	1	10.820	0.2369	208.638	<.0001
Protocol	1	12.468	0.2417	266.001	<.0001
Day	1	13.733	0.2514	298.451	<.0001
TF mm	1	0.4171	0.0614	461.369	<.0001
TCL mm	1	0.3165	0.0368	739.256	<.0001

All variables are statistically significant in the model (P≤0.001 in all cases).

Artificial insemination programs to detect estrus are affected and have a low efficiency in the tropics because the estrus has a short duration during the day and a tendency to express at night (López, 2014). The expression of estrus was shown in 51% of the total of animals, below 90.4% reported by López (2017) and De La Mata et al. (2015). In turn, these data contrast with those reported by López (2017) who showed that 78.2% of mixed Swiss Brown cows presented estrus when treated with the J-Synch protocol in the Amazon with a free grazing diet. One of the explanations of the low expression percentage of estrus in cows in this work could be that they are individually tied in confined spaces, they cannot express the previous symptoms of the onset of estrus (sexually active group) such as the friction and game between the treated cows, thus they cannot be mounted between them; therefore, the detection of estrus is reduced to subjective observation by the person in charge of other less expressive symptoms.

The results of the diameters of the ovulatory dominant follicles found in this research agree with those quoted above by López (2017), who showed that the J-Synch protocol had the lowest value of 8.9 ± 0.1 mm, when the device was removed, but it showed larger size of 13.5 ± 0.1 mm, at the time of the FTAI, compared to the treatment Cypriot Cypionate of Estradiol which had a larger size of 9.7 ± 0.1 mm when the device was removed and 12.4 ± 0.1 mm at the time of FTAI. The Benzoate treatment of Estradiol had a size 9.5 ± 0.1 mm when the device was removed and 12.9 ± 0.1 mm to the FTAI, being smaller than the one obtained by the dominant ovulation follicle of the J-Synch treatment.

An experiment in Uruguay with heifers for meat and in another environment, showed that the J-Synch group had an average ovulation schedule of 93.7 ± 12.9 hours (De La Mata et al., 2015). In this work, the average ovulation schedule for J-Synch with eCG at 60 and 72 hours was 94.5 ± 0.9 hours and for J-Synch without eCG at 60 and 72 hours was 88.0 ± 1.1 hours, which agrees with the results found by De La Mata et al. (2015), who by using a J-Synch protocol obtained an ovulation average of 93.7 ± 12.9 hours and those reported by López (2017) who obtained an ovulation interval of 87.7 ± 0.6 hours when using Brown Swiss in the same environment.

The results of the size of the ovulatory dominant follicles found in this work showed that when the progesterone device was removed, there were significant differences (p≤0.05) that indicated a larger size for *T2* (9.7 ± 0.1 mm); in the same way, the size of the follicle at the time of the artificial insemination expressed significant differences (p≤0.05) with a larger size for *T4* (13.2 ± 0.1 mm) and at the end of the estrus the size of the follicle showed significant differences (p≤0.05) with a larger size for *T3* (13.9 ± 0.2 mm). *Lopez2017* reported in the moment of the removal a size 8.9 ± 0.1 mm and 13.5 ± 0.1 mm at the time of artificial insemination, using Brown Swiss mixed cows in the Amazon with a free grazing regimen for J-Synch treatment. Yánez-Avalos et al. (2018), also using Brown Swiss cows in the Amazon found that the follicle size corresponded to J-Synch with eCG 60 hours of 9.53 ± 0.1 mm at the time of removing the progesterone device and to J-Synch with eCG 72 h of 9.87 ± 0.1 mm whereas, at the time of insemination for J-Synch with eCG 60 hours it was 12.3 ± 0.1 mm and for J-Synch with eCG 72 h it was 11.9 ± 0.1 mm, without significant differences. De La Mata et al. (2015), found that the incorporation of eCG into the J-Synch protocol causes a better moment to perform insemination, making the insemination easier in larger groups of animals, where the J-Synch group inseminated at 60 hours had pregnancy percentages of 60.6 % (P≤0.05) than those in the same group that were inseminated at 72 hours.

Table 7. Maximum Likelihood Estimate Analysis

Odds ratio	Estimate	Confidence limits	
Proto with eCG 60 vs with eCG 72	1.445	0.962	2.169
Proto with eCG 60 vs without eCG 60	1.225	0.805	1.865
Proto with eCG 60 vs without eCG 72	4.263	2.600	6.990
Proto with eCG 72 vs without eCG 60	0.848	0.562	1.279
Proto with eCG 72 vs without eCG 72	2.950	1.855	4.694
Proto without eCG 60 vs without eCG 72	3.479	2.166	5.588
day 6 vs 8	3.949	2.412	6.463
TFmm	1.518	1.345	1.712
TCLmm	1.372	1.277	1.475

Núñez-Olivera et al. (2014) assessed the effect on the prolongation of the proestrus and how it affected the development of the ovulator follicle when a dose of eCG, and GnRH was applied to them at 48 and 72 hours after the device was removed, showing that at 48 hours the diameter was 8.8 ± 0.4

mm and the FTAI of 12.3 ± 0.5 mm; at 72 hours of having removed the device, the diameter was 8.2 ± 0.3 mm and the FTAI was 13.5 ± 0.3 mm without significant differences.

Van-Eerdenburg et al. (2002) found that cows with larger follicle size had a higher chance of ovulation. In accordance with the above, Yánez et al. (2016) found that cows with a larger follicular size at the time of artificial insemination were fourteen times more likely to get pregnant (pregnant: 13.4 ± 1.1 mm; empty: 10.4 ± 1.3 mm).

In the work, significant differences ($p \leq 0.05$) were found in the size of the luteal body on day 7 post insemination between the treatments used. De La Mata et al. (2015) found that the size of the luteal body between days 4 and 13 after ovulation tended to be higher ($P \leq 0.074$) in the J-Synch group compared to the conventional protocol. The size of the luteal body would be related to the higher or lower serum concentrations of progesterone that would affect fertility (Vasconcelos et al., 2001; Busch et al., 2008; Mann, 2009).

Previous cited work indicated that the incorporation of the J-Synch protocol in extreme environmental conditions allowed to improve the rates of pregnancies in cows with breeding, with anestrus and with extensive open period, in double purpose cattle and for meat in tropical climates. However, it is necessary to continue to add a larger number of Brown Swiss cows and their crosses, which are the most frequently used cow in production systems in the Amazon, where the J-Synch protocol is used to consolidate the results.

5 Conclusions

J-Synch treatments plus eCG at 60 hours and 72 hours had different behavior in the follicular development and the size of the Luteal Body. The percentage of pregnancies did not differ between the treatments. Although, there was a 44.5 % more chance of getting pregnant with T1 compared to T2. In addition, pregnancy would increase by 22.5 % by applying T1 compared to T3, and pregnancy would be 18 % higher with T3 when compared to T2.

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Scientific paper/ Artículo científico

The dairy cow: Understanding the sustainable production

Presence of heavy metals in raw bovine milk from Machachi, Ecuador

Presencia de metales pesados en leche cruda bovina de Machachi, Ecuador

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Abstract

The presence of heavy metals in milk is an indicator of local environmental contamination. The objective of this investigation was to test raw milk from Machachi, Pichincha Province, Ecuador for the presence of lead, mercury, and arsenic. Fifty eight samples were collected from twenty nine dairy farms with extensive grazing systems located up to one kilometer from the Municipality of Machachi. Machachi is a site of industrial activity and is located near the Panamericana Sur highway. The samples were analyzed using atomic absorption spectrophotometry with a hydride generator (mercury and arsenic) and a graphite furnace (lead). All analyzed samples indicated the presence of lead, with an average abundance of 0.208 mg kg^{-1} (range between 0.0016 to 0.719 mg kg^{-1}). Of these samples, 98.28% (57/58) contained lead at levels higher than the maximum allowed by the NTE INEN 9, 0.02 mg kg^{-1}. Mercury was detected in four samples with a mean abundance of 0.00009 mg kg^{-1} (range between 0.00 to 0.002 mg kg^{-1}); and arsenic was detected in two samples with a mean abundance of 0.00003 mg kg^{-1} (0.00 to 0.001 mg kg^{-1}). Although these levels are very low, they remain worrisome because these carcinogenic elements are capable of accumulating. Based on these results, it can be concluded that lead contamination has occurred in the studied area. Furthermore, the detection of arsenic and mercury, two highly toxic substances, warrants continuous monitoring of the regional milk supply and a search for possible sources of contamination.

Keywords: Lead, Arsenic, Mercury, raw milk, Machachi

Resumen

La evaluación de metales pesados en la leche puede considerarse como indicador de contaminación ambiental de un lugar, por lo que el objetivo de la investigación fue determinar la presencia de plomo, mercurio y arsénico en leche cruda de Machachi, Provincia de Pichincha-Ecuador. Se recolectaron 58 muestras provenientes de 29 fincas lecheras con sistema de pastoreo extensivo y ubicadas hasta máximo un kilómetro a la redonda del Municipio de Machachi, donde existe actividad industrial y está cerca de la Panamericana Sur. Las muestras fueron analizadas mediante la técnica de espectrofotometría de absorción atómica por generador de hidruros (mercurio y arsénico) y con horno de grafito (plomo). Todas las muestras analizadas mostraron niveles de plomo, con una media de 0,208 mg kg^{-1} (rango entre 0,0016 a 0,719 mg kg^{-1}), de las cuales el 98,28% (57/58) contienen niveles superiores a los máximos permitidos por la NTE INEN 9 de 0,02 mg kg^{-1}. También se detectó mercurio y arsénico en cuatro y dos muestras de leche, respectivamente, encontrándose en el primer caso en una media de 0,00009 mg kg^{-1} (rango entre 0,00 a 0,002 mg kg^{-1}) y en el segundo caso un promedio de 0,00003 mg kg^{-1} (0,00 a 0,001 mg kg^{-1}), y aunque los niveles son muy bajos, los mismos son bastante preocupantes ya que son capaces de acumularse y ser potencialmente cancerígenos. Con los resultados obtenidos, se concluye que se ha detectado contaminación por plomo en el área estudiada, pero sobre todo por dos sustancias altamente tóxicas (arsénico y mercurio), para lo cual es necesario un monitoreo continuo en la leche, y sobre todo para buscar posibles fuentes de contaminación.

Palabras clave: Plomo, Arsénico, Mercurio, leche cruda, Machachi

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1 Introduction

In 2018, Ecuadorian milk production reached ∼5 million liters per day, with more than 70% of that being produced in the inter-Andean alley. Pichincha is the largest milk-producing province, with its milk accounting for approximately 16% of the total Ecuadorian milk supply (INEC, 2019). The Canton Mejía has historically been the largest dairy in Pichincha Province, and in all of Ecuador, largely due to its climatic and soil conditions, which are very favorable for livestock development (CIL, 2015). Ecuadorian Technical Standard (NTE) INEN 9 (INEN, zC5C) and Codex Alimentarius (Codex-Alimentarius, 1995) set environmental standards for raw milk, and set the maximum permitted level of lead (Pb) at 0.02 mg/kg. NTE INEN 9 does not indicate the maximum permitted levels for mercury (Hg) or arsenic (As); but NTE INEN 1108, which deals with drinking water, sets a maximum limit of 0.006 mg/L for Hg and 0.01 mg/L for As (INEN, 2011).

The presence of heavy metals in food is a critical public health concern since these elements cause a number of health problems in humans, animals, and agricultural crops (Anastasio et al., 2006). In humans, heavy metals disrupt the functions of the nervous system, the liver, and the kidney; while also promoting mutagenesis and carcinogenesis. In animals, heavy metals can cause a loss of appetite, anemia, reproductive disorders, cancer, and teratogenesis. All of these effects decrease production yield in the long run (González-Montaña, 2009). The primary source of heavy metals is environmental pollution due to the presence of various industrial activities (Zhou et al., 2019). These industrial activities pollute the soil (Ashraf et al., 2019), the water, and the air; and eventually reach people and animals through the food chain. Thus, this pollution greatly harms the health of consumers (Karasakal, 2020). Currently, this contamination is measured through the use of biomonitoring, within which the surveillance of animal products plays an important role (Scaramozzino et al., 2019). In the case of dairy farming, heavy metals can contaminate the water and food of dairy cows, eventually being transferred to their milk (Zhou et al., 2019). This constitutes a major public health problem, especially for vulnerable populations like children (Chirinos-Peinado and Castro-Bedriñana, 2020), because even low levels of heavy metals incorporated into a diet can lead to chronic illness (Miclean et al., 2019). Thus, monitoring locally-produced raw milk for the presence of heavy metals is a useful indication of environmental contamination in the region (Miclean et al., 2019).

Lead (Pb) is frequently produced by the radioactive decomposition of uranium and actinium, and can be found in the soil (Silva et al., 2010). However, lead can also be introduced into the environment through other avenues such as the use of lead-containing equipment, chemical fertilizers and pesticides, or lead-contaminated water (Litter et al., Jl6s). Lead is one of the most frequent intoxicants of cattle; even more so in young calves since these animals lack a functional rumen microflora (Perrin et al., 1990). In the case of arsenic, it can be distributed in water, air, and soil. Arsenic is very toxic in its inorganic form, and can cause skin changes and even cancer citep{WHO2018. The presence of mercury is also a matter of importance due to its ability to contaminate and damage the trophic chain. Chronic exposure to this metal can result in asymptomatic toxicity (Rodríguez et al., 2010). Mercury is the only volatile metal, so it is easily absorbed through the skin and lungs. Its presence in the environment is mainly due to human activity, and is commonly introduced by heating systems, mining, and other industrial processes (WHO, 2019).

Air pollution is composed of fine particulate matter that can combine and transport other toxic substances, such as heavy metals. This enables transportation of these metals through the air and allows them to be deposited in irrigation water, agricultural soils, and grass (Dergham et al., 2012; Alloway, 2013; Yilmaz et al., 2009). In the context of dairy cows, if the animal forages on heavy metal-contaminated materials, those toxic metals are introduced to the animal and eventually are passed into its milk (Miclean et al., 2019). It is essential to study this process (de Oliveira et al., 2017) in order to estimate toxicological effects (Samiee et al., 2019) and safeguard public health (Miclean et al., 2019). Thus far, few studies have investigated the presence of heavy metals in raw milk from Ecuador, despite the utmost importance of this monitoring process in order to prevent food contamination (Kim et al., 2016) and the transfer of heavy metals to consumers (Hashemi, 2018). The objective of this study was to quantify the levels of lead, arsenic, and mercury in raw milk produced in the largest dairy basin in Ecuador.

2 Materials and Methods

2.1 Study area location

The study was carried out within the parish of Machachi, Mejía Canton, Pichincha Province, Ecuador. 58 raw milk samples were randomly collected from 29 dairy farming production units (UPAs) with extensive grazing systems located within one kilometer of the city of Machachi. Machachi is a center of industrial activity with a particular emphasis on steel production, and lies close to the Panamericana Sur highway. The UPAs are all located near -0.510110, -78.567123, and occur at an altitude of approximately 2900 meters above sea level (GeoDatos, 2019). The region experiences temperatures from 12 to 20°C, and is generally characterized as having a cold-temperate climate. The geographical location given was provided using georeferencing based on the global positioning system (GPS), as shown in Figure 1.

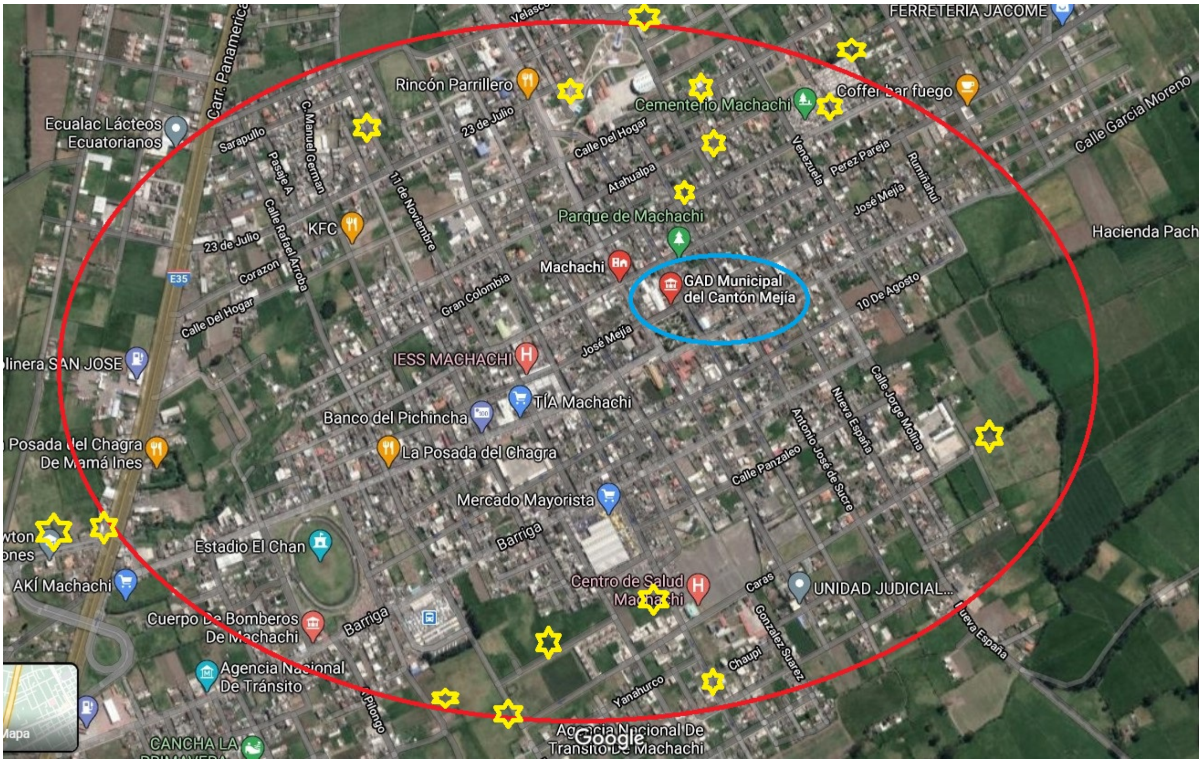


Figure 1. UPAs sampled in the Machachi Parish.

2.2 Sampling

Approximately 250 ml of raw milk were taken directly from the refrigeration tanks, buckets, or collection containers at each UPA. This sampling operation was repeated again within the span of eight days and was carried out in accordance with NTE INEN-ISO 707: "Milk and dairy products. Guidelines for sampling" (INEN, VdOC). The samples were transported in thermoses containing frozen gel refrigerants between 2 and 5 oC. Upon arrival at the Laboratory of the Department of Petroleum, Energy, and Contamination (DPEC) of the Faculty of Chemical Engineering of the Central University of Ecuador, the samples were stored at 20°C until analysis.

2.3 Analytical procedure

For the determination of Hg and As levels, a hydride generator atomic absorption spectrophotometry technique was used (Rocha, 2011). The determination of Pb levels was carried out using the same atomic absorption spectrophotometry approach, but with a graphite furnace. Technical operations were performed in accordance with Ecuadorian Technical Specification INEN-ISO / TS 6733 (INEN, 2014).

A UNICAM Solar model 9626 spectrophotometer was used. Hollow cathode lamps were used for each element analyzed, with aliquot vaporization being accomplished using an air-acetylene flame. Deionized water was utilized for all analytical processes. Calibration curves were created using certified standards, and blank solutions were prepared and treated identically as samples. The limit of detection (LOD) and the limit of quantification (LOQ) for Pb were 0.1 mg/L and 0.8 mg/L, respectively. For As, the LOD was 0.39 μg/L and the LOQ was 1.19 μg/L. The LOD for mercury was 0.14 μg/L and the LOQ was 0.42 μg/L. All samples were analyzed in triplicate. Respective calibration curves were prepared using the following concentrations: Pb = 0.0, 0.5, 1.5, 3.0, and 5.0 mg/L; Hg = 1.0, 2.5, 5.0, 10.0, 25.0 and 50.0 μg/L; As = 0, 1, 2, 5, and 10 μg/L.

2.4 Statistical analysis

The results are expressed as the mean, the minimum, and the maximum observed concentration of Pb, As, or Hg. A Shapiro-Wilk test was performed to check the data for normality. The results of these tests indicated that the data were not normally distributed ($p \leq 0{,}05$). For this reason, non-parametric statistical tests were used (Celis De La Rosa and Labrada, 2014). A Kruskal-Wallis test was used to compare the heavy metal levels in raw milk between suppliers. A Wilconox test was used to compare data from the first and second samples taken from a given supplier. The open source statistical software RStudio version 1.2.5019 [RStudio Inc. Boston, MA, USA] was utilized to conduct all statistical tests. For all analyses, a significance threshold of $p = 0{,}05$ was adopted.

3 Results

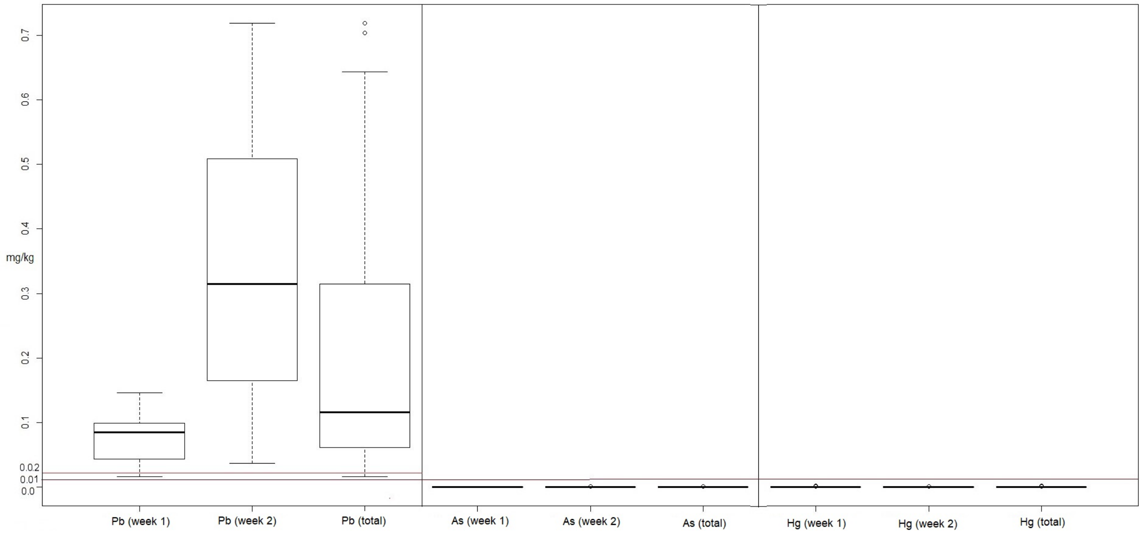
The minimum, mean, and maximum observed abundances of Pb, Hg, and As in sampled raw milk from Macachi is shown in Table 1 and Figure 2. In the case of Pb, 100 % (58/58) of the raw milk samples had levels of lead above the limit of detection for this heavy metal. Of these, 98.28 % (57/58) contained lead at levels higher than 0.02 mg/kg, the maximum limit established by NTE INEN 9 and the Codex Alimentarius. The mean value of lead observed in sampled raw milk was 0.208 mg/kg, with a minimum value of 0.0016 mg/kg (found in the first sample), and a maximum value of 0.719 mg/kg (from the second sample). There were no significant differences ($p \geq 0{,}05$) observed comparing raw milk from the 29 suppliers sampled, but there were significant differences ($p \leq 0{,}05$) between raw milk from the first sampling and raw milk from the second sampling (Table 1).

Regarding the abundance of Hg, 6.9 % (4/58) of the raw milk samples contained detectable levels. The mean level of mercury was 0.00009 mg/kg, with a minimum value of 0.00 mg/kg, and a maximum value of 0.002 mg/kg (found in the first sampling). No significant differences in mercury abundance were detected between suppliers or between samplings (Table 1). Also, 3.44 % (2/58) of analyzed raw milk samples contained detectable levels of As, with a mean value of 0.00003 mg/kg, a minimum value of 0.00 mg/kg, and a maximum value of 0.001 mg/kg. No significant differences in mercury abundance were detected between suppliers or between samplings (Table 1).

4 Discussion

All raw milk samples indicate the presence of Pb and 98.28 % of them had lead levels higher than 0.02 mg/kg, the maximum allowed by NTE INEN 9 and the Codex Alimentarius. However, of graver concern is the presence of As and Hg in milk. Although the vast majority of samples did not contain either of these heavy metals, the fact that they were detected at all is alarming since even minute amounts are toxic to human health. Mercury, which can occur naturally (Bernhoft, 2012), has been released into the environment for centuries as the result of anthropogenic activities. Mercury has been shown to have harmful effects on various human tissues and

Figure 2. Box diagram of Pb, Hg, and As abundances in analyzed raw milk.



organs (Rocha et al., 2012), and also possesses genotoxic potential. As a result of this, mercury may be involved in carcinogenesis, but further studies are needed (Yang et al., 2020). In humans, arsenic exposure increases the risk of lung, skin, and bladder cancer, particularly when said exposure is chronic (Khairul et al., 2017). Arsenic is also linked to numerous other pathological conditions, which are collectively known as arsenicosis (Bjørklund et al., 2018).

The presence of heavy metals in raw milk could be the result of the dairy farms being located in an area with industrial activity that is also near an important highway (González-Montaña et al., 2019). Heavy metal levels are known to be higher is such areas (Zhou et al., 2019), and indeed heavy metal accumulation has been previously reported in alfalfa from industrially active regions (Rezaeian et al., 2020). Contamination can be introduced through various avenues including drinking water, pastures, and/or the soil itself. Determining the source of heavy metal contamination is critical to its containment and other studies have noted contamination in both the silage (Pb) and the drinking water (As and Hg) consumed by dairy cows (Zhou et al., 2017, 2019).

In Ecuador, very few studies have investigated the presence of heavy metals in milk. In one study using 20 samples of raw milk from the Arenillas Canton, El Oro Province (Ayala and Romero, 2013), Hg and As were detected (mean abundance of 0.01035 mg/kg) in 100% of the samples. While this does not coincide with the results from the present study, this contrast can perhaps be explained due to the fact that Arenillas is a mining sector unlike Machachi. With regards to Pb contamination, El Oro data demonstrated a mean abundance of 0.011 mg/kg (range of 0.006 to 0.018 mg/kg). This level of lead contamination is less than that found in the data presented herein. One possible explanation could be the presence of lead in river water that supplies the irrigation channels used by the herds. Furthermore, a study from the city of Guayaquil, found high levels of Pb contamination in powdered milk (5.450 ± 2.474 mg/kg), but no Pb was detected in pasteurized and ultrapasteurized milks from the same study.

Variability in such data is common worldwide. A meta-analysis of 72 investigations from 37 countries demonstrated that heavy metal levels varied greatly. Some of the highest Pb concentrations were observed in Brazil, Croatia, Egypt, Mexico, Nigeria, Palestine, Serbia, and Turkey. Likewise, higher levels of As and Pb were observed in milk produced using traditional systems (such as that employed in Machachi) compared to milk produced using organic systems. In the case of Hg, all levels were observed to be below the minimum risk threshold regardless of production system (Zwierzchowski and Ametaj, 2018).

Table 1. Minimum, mean, and maximum observed abundances of Pb, Hg, and As in sampled raw milk.

Value	Lead (mg/kg)			Mercury (mg/kg)			Arsenic (mg/kg)		
	First sampling	Second sampling	Total	First sampling	Second sampling	Total	First sampling	Second sampling	Total
Minimum	0.16	0.037	0.016	0	0	0	0	0	0
Mean	0.07631	0.339	0.20764	0.0001	3E-05	9E-05	0	0.0001	3E-05
Maximum	0.146	0.719	0.719	0.002	0.001	0.002	0	0.001	0.001
P value	1.302e-06*	0.979*	0.3045*	0.052**	0.1609*	0.492**	0.1609*		0.492**

* Kruskal Wallis test between the 29 providers
** Wilconox test between the samplings

If the results of this study are compared with other investigations from across Latin America, the variability remains large even within the same country. In the case of Brazil, Pb contamination was found exceeding the set limits in both raw milk and milk derivatives (Silva et al., 2010; de Oliveira et al., 2017; de Vasconcelos et al., 2019; Gomes et al., 2013). In Puebla, Mexico, the analysis of raw milk samples revealed the presence of Pb at a mean abundance of 0.03 mg/kg, and As at a mean abundance of 0.12 mg/kg. In both cases, heavy metal contamination exceeded the maximum allowed under the Codex Alimentarius, but occurred below the limit set by Mexican law. Samples of dairy derivatives analyzed in the same study also contained Pb and As. Therefore, both raw milk and dairy derivatives from this study may represent a health risk for consumers (Castro-González et al., 2018a).

In another study, carried out on 160 raw milk samples from Puebla and Tlaxcala, Mexico; Pb levels were found to range from 0.039 $\pm$ 0.02 to 0.059 $\pm$ 0.05 mg/kg, while As levels ranged from 0.029 to 0.039 mg/kg. In this region, the soil receives industrial wastewater, and pastures are mainly composed of alfalfa. These Pb levels exceed the European and FAO limits, but both the Pb and As abundances comply with Mexican regulations (Pb 0.1 mg/kg and 0.2 mg/kg for As). It is possible that these quantities were observed due to the fact that alfalfa may facilitate bioaccumulation of the heavy metals, ultimately enabling their transfer to milk (Castro-González et al., 2018b).

In a recent Peruvian study, it was determined that heavy metals can be transferred from blood to cow's milk, with Pb values in milk being 54% higher compared to levels in blood. The Pb levels observed in this study were 29 times higher than those allowed by the Codex Alimentarius, and can be attributed to mining wastes that pollute the environment (Chirinos-Peinado and Castro-Bedriñana, 2020).

When comparing data from studies at other latitudes, results continue to vary depending on the locale. For example, in raw milk from nine regions of Korea, Cd and Pb concentrations did not exceed the limits set by Korean standards (Kim et al., 2016). In a study carried out in Asturias, Spain, raw milk from 7 dairy farms (36 samples in total) was analyzed. Arsenic was detected in only 4 samples with a mean value of 0.01845 $\pm$ 0.00689 mg/kg. No Hg was found in any sample (González-Montaña et al., 2019). In milk from six Indonesian goat farms, Pb was found at levels between 50 and 80 mg/kg, while As was found at levels between 70 and 110 mg/kg (Wanniatie et al., 2019). In raw milk from Turkey, 70% of analyzed samples had Pb at levels 2.5 times higher than that allowed by European standards. In addition, 100% of the samples exhibited As contamination at levels above the maximum allowed threshold (Koyuncu and Alwazeer, 2019).

In a different study from Turkey, 90 milk samples were analyzed and failed to demonstrate any detectable levels of Pb. However, As was detected in 94.45% of the samples, albeit at levels below established standards (Totan and Filazi, 2020). A study from Romania sought to evaluate the risk of heavy metal transmission from forage to milk, but did not report the risk to public health (Miclean et al., 2019). Both Hg and As were found in raw milk from Arak, Iran; but at levels lower than the standards suggested by the Codex Alimentarius. In that study, the mean concentration of Hg was significantly higher ($p < 0{,}05$) in milk produced on traditional farms as compared to milk produced on industrial farms

(Arianejad et al., 2015).

Even at levels below the maximum thresholds set by regulations, both Hg and As have the potential to be carcinogenic, and have the capability to accumulate further. Based on the data presented herein, heavy metal contamination remains a distinct possibility and continuous monitoring is recommended to protect public health. In order to fully address the possibility of contamination in the surveyed area, future efforts should investigate possible sources of these heavy metals, such as drinking water, irrigation water, forages, and food received by dairy cows in the area.

5 Conclusions

In the present investigation, 100% of analyzed raw milk samples were positive for the presence of Pb; with 98.28% of samples having Pb levels above the maximum limit set by NTE INEN 9 and the Codex Alimentarius (0.02 mg/kg). With regards to Hg and As, 6.9% and 3.44% of samples contained this heavy metal, respectively and while the vast majority of analyzed samples did not contain any Hg or As, or only showed low levels of these heavy metals, these data remain very worrisome. Based on the data presented, heavy metal contamination remains a distinct possibility and continuous monitoring is recommended to protect public health. Future efforts should be aimed at investigating possible sources of these heavy metals, such as drinking water, irrigation water, forages, and the feed that dairy cows in the area receive.

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THE DAIRY COW: UNDERSTANDING
THE SUSTAINABLE PRODUCTION

QUALITY OF RAW MILK AND PAYMENT SYSTEM FOR QUALITY IN ECUADOR

CALIDAD DE LA LECHE CRUDA Y SISTEMA DE PAGO POR CALIDAD EN EL ECUADOR

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Abstract

States legislate quality to maintain the health of the population, safety and nutritional properties of food, regulations that apply on the production and industrialization, thus Ecuador implemented a policy to improve the quality of raw milk through price. This study aimed to determine the compliance of the parameters established in the regulation of payment for quality of raw bovine milk (RPQM) in Ecuador during the period 2009-2018. Total data (n= 103204) were obtained by the milk quality laboratory of the Salesian Polytechnic University from 3 regions of the country. The parameters of fat, protein, total solids, total bacteria count (TBC) and somatic cell count (SCC), and the Ecuadorian regulations that promoted the quality and productivity of milk in this study period were analyzed. The general averages were: fat $3{,}80 \pm 0{,}05\,\%$; protein $3{,}12 \pm 0{,}10\,\%$; total solids $12{,}36 \pm 0{,}16\,\%$. SCC and TBC decreased between 2009 and 2018: SCC from 460×10^3 to 447×10^3 cells/mL and TBC from 1 million CFU/mL to averages around 200×10^3 CFU/mL, respectively. In conclusion the RPQM was positive for the chemical parameters with values above the limits established. The hygienic quality (TBC and SSC) showing improvements over time although, the SCC does not within the maximum permissible because it implies a multifactorial action for improvement being necessary to concentrate efforts on health and GMP.

Keywords: Milk composition, milk quality, payment system, bacteria count, somatic cells

Resumen

Para mantener la salud de la población los Estados legislan la calidad, inocuidad y propiedades nutricionales de los alimentos, regulaciones que se aplican en la producción e industrialización, así, Ecuador implementó una política de mejora de la calidad de la leche cruda a través del precio. .El objetivo del estudio fue determinar el cumplimiento de los parámetros establecidos en el reglamento de pago por calidad de leche (RPCL) cruda bovina vigente en el Ecuador durante el periodo 2009-2018. Se utilizaron (n =103 204) datos obtenidos por el Laboratorio de Calidad de Leche de la Universidad Politécnica Salesiana provenientes de 3 regiones del país. Fueron analizados los parámetros de: grasa, proteína, sólidos totales, contaje de bacterias totales (CBT), conteo de células somáticas (CCS) y se revisaron las diferentes regulaciones ecuatorianas que impulsaron la calidad y productividad de la leche en el periodo de estudio. Los promedios generales fueron: $3,80 \pm 0,05\,\%$; proteína $3,12 \pm 0,10\,\%$; sólidos totales $12,36 \pm 0,16\,\%$. CCS y CBT disminuyeron entre el 2009 y 2018: CCS de 460×10^3 a 447×10^3 células/mL y el CBT de 1 millón UFC/mL a promedios entorno a los 200×10^3 UFC/mL, respectivamente. Se concluye que el RPCL fue positivo para los parámetros de composición con valores sobre los límites establecidos. La calidad higiénica y sanitaria muestran mejoras en el tiempo, aunque CCS no entra en los límites máximos permisibles debido a que implica un abordaje multifactorial de acciones para la mejora.

Palabras clave: Composición de leche, calidad de leche, sistema de pago, contaje de bacterias, células somáticas

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1 Introduction

Because of the importance of nurturing the body and maintaining a healthy life, states have created laws to guarantee the quality, safety and nutritional properties of food (Hoyos and D'Agostini, 2017). Since the industrial revolution, there is the need to control products and services, but from 1960 the Food Law emerged in Europe. This law along with regulations have guided the construction of methods to improve national and international trade relations. These regulations were imposed for the production, industrialization and collection of innocuous food with consumer health protection.

Agricultural policies in Ecuador began with agrarian reforms (1950-1978) by peasant struggles, which modified the structure of land ownership and distribution. The Agricultural Development Act of 1979 promoted Integral Rural Development, where the first policies were generated to carry out agricultural research, technical assistance, supply of inputs and regulations for processed products (García et al., 2019; Madrid, 2019).

Since 2000, policies focused on promoting small and medium-sized industries, and standardized protocols for the packaging and labeling of agricultural products. In post-neoliberal period, the concept of food security and food sovereignty, arose the inclusive business of traditional and non-traditional products, was promoted the export, requiring compliance the regulations in whole production chain (Madrid, 2019). From 2007, policies and regulations were aimed at guaranteeing the consumers rights, quality and competitiveness in production areas.

Under the 2008 Ecuadorian constitution of 2015-2025 agricultural policy was generated, promoting sustainable rural development, eradicating hunger and malnutrition. At the same time, other related laws were generated, and the institutional component necessary for the implementation (Lasso and Clark, 2016).

The milk production in Ecuador is the most important livestock activities, since its production represents around 5.1 million L/day from the three regions of the country: Costa, Sierra and Amazonia (INEC, 2018). A high-quality milk is defined as a food with an enjoyable taste, without odor, with adequate chemical composition, without pathogens or contaminants (Pereira, 2014; Sah et al., 2018). The milk parameters for determining the quality are evaluated through physical, chemical and microbiological analyzes. These parameters are regulated in all countries with slight variations in compliance limits and methods used (Dürr et al., 2004).

Until 2008, the price of milk in Ecuador did not influence on the composition or other parameters, was it determined directly between the industries and the producers (MAGAP, 2008). Know the quality of milk is important for allows to make decisions on the management of industrial production. Under the quality policy in 2013, the Ministry of Agriculture of Ecuador (MAGAP) issued the milk quality payment regulation (RPCL), the Regulation 394, defined the payment per liter on farm, demanding compliance of quality parameters, hygienic and sanitary quality. Other related regulations and changes in state institutions were established for their implementation.

In this context, the objective of this research was to determine the compliance with the requirements established in the RPCL in the parameters of fat, protein, total solids, total bacterial count and somatic cell count during the period 2009-2018, comparing the information available for each region. Besides to analyze the regulations issued around the RPCL their impact on producers, production and productivity.

2 Materials and methods

2.1 Study area

Ecuador is divided into 3 regions: Coast, Sierra and Amazonia due to factors environmental such as the presence of the Andes mountain, the vegetation of the Amazon, and the currents of El Niño and Humboldt (IGM, 2017). In each region the climate varies according to the altitude, humidity, precipitation and temperature. According to the National Institute of Statistics and Census (INEC, 2018), these factors also affect the volume production, reporting 23 %, 72 % and 5 % for the Coast, Sierra and Amazonia, respectively.

2.2 Data, sample collection and analysis methodology of quality parameters

The data was provided from the milk quality laboratory database of the Salesian Polytechnic University (UPS). There was a total of 99271 (n) analyzes requested between 2009 and 2018 by milk storage facilities (CA) of associated producers, community farms and farms with a frequency of 1-2 times/month. The data correspond to 12 provinces in the 3 regions of Ecuador. The Sierra region was divided into 2 by its importance in the production, North Sierra: Carchi, Imbabura, Pichincha with 78.9% (n = 81431) and Central Sierra: Chimborazo and Tungurahua with 4.7% (n= 4835). The Coast: El Oro, Esmeraldas, Guayas, Manabí and Santo Domingo de los Tsatchílas with 9.8% (n= 10066). The Amazon region: Napo with 6.7% (n= 6872).

The samples were collected followed standard procedures (Cassoli et al., 2007, 2010), were used 40 mL sterile bottles containing Bronopol for the composition and somatic cells, and Azidiol for TBC. The milk samples were stored at 4-7°C. The composition minimum limit allows fat, protein and total solids of: 3.0%, 2.9%, and 11.2%, respectively (INEN, 2012). Both for total bacteria count (TBC) allows an upper limit of300 $\times 10^3$ UFC/mL (MAGAP, 2013) and for somatic cell count (SCC) an upper limit of 400 $\times 10^3$ cells/mL (Cerón-Muñoz et al., 2007).

Fat, protein and solids were analyzed by Fourier transformed infrared spectrophotometry (FTIR) with the MilkoScan FT 6000 (Foss Analytical Instruments, Denmark) and the results were expressed as percentage values (g/mL) (Takahashi, 2011). The Fossomatic minor FC (Foss- Analytical, Denmark), an electronic counter based on cytometry, was used for SCC, where a colored milk sample emits light pulses that are expanded by a photomultiplier (Ramos, 2019).

The flow cytometry method was used to determine TBC with the BactoScan FC 50H (Foss Analytical Instruments, Denmark), an analytical principle based on injecting a colored sample through a flow chamber, the optical system detects fluorescence-stained particles. The results are expressed as CFU/mL and represent the amount of mesophilic aerobic bacteria (Cassoli et al., 2007; Numthuam et al., 2017).

All analysis were subjected to quality controls in accordance with ISO-17025, 2017. The equipment and methods remained unchanged during the study period.

2.3 Statistical analysis

A descriptive analysis was analyzed using the statistical software Infostat version 2018, for variables according to limits allows and the information between the regions.

2.4 Review of Regulations

According to the information available in MAGAP, the regulations, instructions and procedures, and other documents that supported the execution of RPCL.

2.5 Analysis of producers, production and productivity

Milk producers required modifications to their production practices and infrastructure to comply with the indications of RPCL. Small producers (<200 L/day) were grouped in CA and farms, and between 2009-2018 many had agreement with the UPS for milk analysis, monitoring and training in good milking practices (GMP), and good agricultural practices (GAP). Production and productivity information was analyzed according to INEC data for the same years.

3 Results and discussion

3.1 Evolution of compositional quality

Composition parameters are influenced by different factors such as diet, genetics, breed, lactation period, season, frequency and milking moment (Belage et al., 2017). When milk has higher concentration of total solids, essentially protein and fat, more nutrients are provided, the yield and quality of dairy products are improved (Barbano et al., 2006; Pereira, 2014). However, the relationship between its components is stable and variation can be used as indicator. Continuous monitoring of chemical and microbiological factors is essential for the establishment of quality control program ((Dürr et al., 2004; Johnson, 2017; Takahashi, 2011).

3.1.1 Fat

This parameter tends to vary more than other milk components. The main influencing factors are breed, age, diet, lactation, bacterial activity in the rumen, mastitis and environmental effects (Ramírez-Rivera et al., 2019). In addition to dietary concentrates, type, quantity and size of the fiber particle also contribute to the variation of fat percentages in milk.

Fat is composed of 98 % triglycerides and 2 % diglycerides, cholesterol, phospholipids and free fatty acids. Bovine milk fat is one of the most complex, having more than 400 different fatty acids that form triglycerides, of which 70 % are saturated fatty acids and 30 % unsaturated fatty acids (García et al., 2014; Pereira, 2014; Sah et al., 2018).

The overall average fat content was 3.80 $\pm$ 0.05 %, higher than the limit for all years analyzed (Figure 1a). The highest values were reported for the Coast and Amazon region (3.82 $\pm$ 0.10 %) where there are dual-purpose cows (meat and milk) (INEN, 2012; Oñate, 2018). These values correspond to those reported in similar works in Hidalgo- Mexico, where 1416 samples were analyzed, obtaining 3.46 $\pm$ 0.26 % of butyric fat by classifying milk as type A, indicating excellent quality in this parameter (Cervantes et al., 2013). Guevara-Freire et al. (2019), working with producers in the Ecuadorian Sierra, reported that feeding of herds based on grains and forage legumes, as well as the use of concentrates increased the percentages of fat. Another influential factor was the breed, mainly of Holstein and creole dairy cows (Creole x Holstein), which maintained fat values between 3.4 % and 3.7 %.

3.1.2 Protein

The protein content in dairy cows is important in the human diet as it provides between 30-32 g/L. Soluble proteins are serum proteins such as lactoalbumin, lactoglobulin and other non-nitrogenous proteins such as urea that represent 20 %, and insoluble proteins known as casein that represent 78-80 % (Pereira, 2014). Since casein forms a stable colloidal system with calcium, phosphorus and magnesium, it helps to digestibility and transportation of minerals. Due to the high biological quality of bioactive peptides, milk proteins contribute to the human health by favoring the absorption of other nutrients (García et al., 2014; Sah et al., 2018).

In the industry the protein percentage influences the yield of processed products, mainly cheese and yogurt, and in Ecuador it accounts for 41 % of the most consumed dairies (Alvarado, 2017; Oñate, 2018). Several factors that influence fat content also influence the protein composition of milk such as nutrition, disease management, lactation, and cow´s age, as well as climate (García et al., 2014; Johnson, 2017). The pasturelands used in the Coast region and improved pasturelands in the Sierra region (Requelme and Bonifaz, 2012) allow protein to be maintained at values from 3.01 to 3.25 %, for 70 % of producers reported by Valladares (2016).

The overall average for this parameter (Figure 1b) was between 3.12 $\pm$ 0.10 % and it was higher than the allowable limit in all regions. The lowest values in the Amazon region (3.04 $\pm$ 0.09 %) are due to the different food patterns, energy levels and animal breeds that determine the milk composition in this parameter (García et al., 2014). Another study carried out to evaluate the physical-chemical, microbiological and toxicological properties in seven dairy farms in the Puno region of Peru that produce 7.5% of the country's production found values of 2.81-3.20 %, which could vary by the type of food provided, showing higher percentages by the use of protein concentrates derived from fishmeal, soybeans, alfalfa and corn versus grazing (Brousett-Minaya et al., 2015).

In countries that produce yogurt, cheese and powdered milk, the payment system prefers the protein content, as Spain and Costa Rica (Dürr et al., 2004; Martinez and Gomez, 2013).

3.1.3 Total Solids

In food where water is the dominant component (83-84 %), total solids extract (EST) corresponding to the total of fat, true protein, lactose and minerals (Ramírez-Rivera et al., 2019). In the international market, the EST is the most important benchmarks of dairy quality, it represents lower costs of dehydration and greater conversion of final product

(Barbano et al., 2006). Production systems, instead of increasing the volume, should to obtain milk with more total solids. The criteria for maximizing the EST should be based on efficient balance of nutrients, maximization of food consumption, monitoring diet and periodic corrections for quantitative and qualitative changes in the resources used. For some authors, the increase in milk production is observed in the concentration of total solids, fats, proteins and non-fatty solids (Ramírez-Rivera et al., 2019).

EST values in all regions were 12.36 $\pm$ 0.16 % above the limit corresponding to good quality milk (Figure 1c). The Central Sierra, compared to North Sierra, the lowest averages (12.27 $\pm$ 0.22 %) are highlighted, because the northern region initiated technical training programs promoted by the government and private institutions (Vinueza, 2015).

These values are similar to those presented in Sucre state- Colombia in 12 municipalities, where the compositional and hygienic quality of the milk was evaluated during summer and winter, obtaining average values of 12.79 and 13.11 %, respectively and indicating a high-quality milk. This trend can be explained by the predominant presence of Zebu breeds with crosses between *Bos taurus* and *Bos indicus*, concentrate a lot more the solids present (Martinez and Gomez, 2013). Zebu cattle are also reported on the Coast of Ecuador, but there are also crosses with Brahman, Charolaise and Holstein. In Sierra, most of the cattle have been improved with Holstein, Brown Swiss and Jersey (Requelme and Bonifaz, 2012).

3.2 Evolution of sanitary and hygienic quality

3.2.1 Somatic cell count

Somatic cells (leukocytes, especially neutrophils and epithelial cells) indicate the health of the mammary gland, therefore, the quality of the milk. An increase in somatic cells may be an indicator of inflammatory processes due to the response of phagocytosis. Factors that increase CCS may be postpartum periods, the number of lactations, physical agents or irritant chemicals in udders, among others, therefore early detection of the increase in these values is essential for the control (Carloni et al., 2016; Gonçalves et al., 2018).

CCS values in cases of absence of breast infection range from $200 - 300 \times 10^3$ cells/mL, while counts $> 800 \times 10^3$ cells/mL are usually associated with persistent inflammatory processes. Although CCS is not included in the current RPCL of Ecuador, a limit of 400×10^3 cells/mL will be considered for this study, since it is a standardized value in several countries, especially in Europe and Brazil, associated with herds containing GAP (Cerón-Muñoz et al., 2007; MAPA, 2011).

The results of this study (Figure 2a) show a reduction continual of SCC in all regions based on an average value of $460 \times 10^3 \pm 118$ to $447 \times 10^3 \pm 32$ cells/mL for 2009 and 2018, respectively. North Sierra, Central Sierra and the Amazon region showed values <400 x 103 cells/mL and the Coast region showed a value higher than 500×10^3 cells/mL.

A study carried out in 7 provinces of Ecuador, 90 % of producers reported the importance of conducting udder cleaning prior to milking and drying, but only 65 % of respondents applied a routine test for mastitis control (Bonifaz and Requelme, 2011; Guevara-Freire et al., 2019). It was necessary to define the conditions of management, production and nutrition of livestock for the compliance of RPCL in Ecuador.

3.2.2 Total bacteria count

Milk quality is essential to obtain quality products, with TBC being the most frequently used parameter as a reference (Martinez and Gomez, 2013). Ecuador as in other Latin American countries, the titratable acidity and reductase (methylene blue) tests were used until 2008 for the indirect determination of bacterial. For this reason, it was necessary to incorporate faster systems with modern technologies.

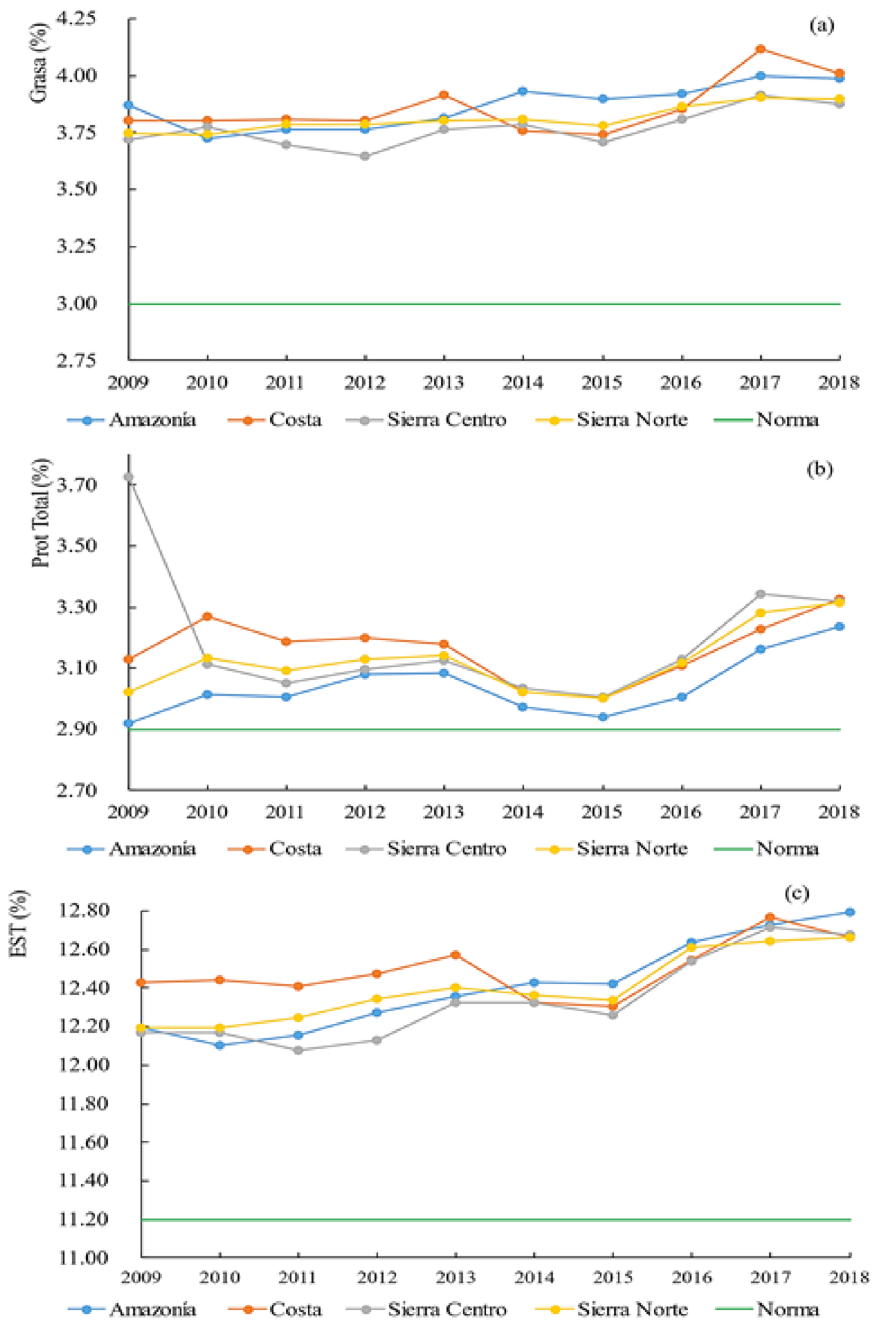

Figure 1. Milk composition parameters presented in percentages during the study period: (A) fat (b) protein (c) total solids.

International requirements are increasing, for CBT Europe and USA accept a maximum value of 1100×10^3 UFC/mL and 300×10^3 UFC/mL, respectively. The RPCL for 2008 defined a limit of 600×10^3 UFC/mL, which was likely to influence producers in the reduction of averages, from about 1 million UFC/mL to 800×10^3 UFC/mL for 2011 and 2012. The Central Sierra maintained average values of 1.5 million UFC/mL until 2013, qualifying milk as poor-quality milk; but from 2014 the averages decreased and from 2015 they were $< 400 \times 10^3$ UFC/mL. Although in 2016 and 2017, the CBT increased in the overall average ($> 500 \times 10^3$ UFC/mL), in 2018 they decreased again to 200×10^3 CFU/mL, qualifying it as of good quality milk. Is important to mention that these data correspond to formal producers (98.6%) with contractual commitments of industries (Figure 2b).

These results are similar to those reported when analyzing 10 704 milk samples collected between 1993 and 2009 in the northwestern area of Santa Fe and southern Santiago del Estero in Argentina, showing TBC averages $< 100 \times 10^3$ UFC/mL, noting a significant improvement by the training of the producer and management processes included in the program for the integral improvement of milk quality among producers, entrepreneurs and the government (Revelli et al., 2011).

3.3 Public regulations around the RPCL

When establishing a quality payment system, it is important to have a prior diagnosis (Dürr et al., 2004). However, the first RPCL issued in Ecuador by Regulation 1042 (MAGAP, 2008) was defined on the basis of adapted international standards. The initiative to regulate the marketing of milk production with a focus on quality was a strategy to establish it. In this decree, the price of the liter of milk was increased and set at USD 0.35 if meeting the limits described in INEN 009 for the physical–chemical and microbiological requirements of raw milk. In addition, bonus criteria for meeting livestock health requirements (vaccination and GMP) were also incorporated.

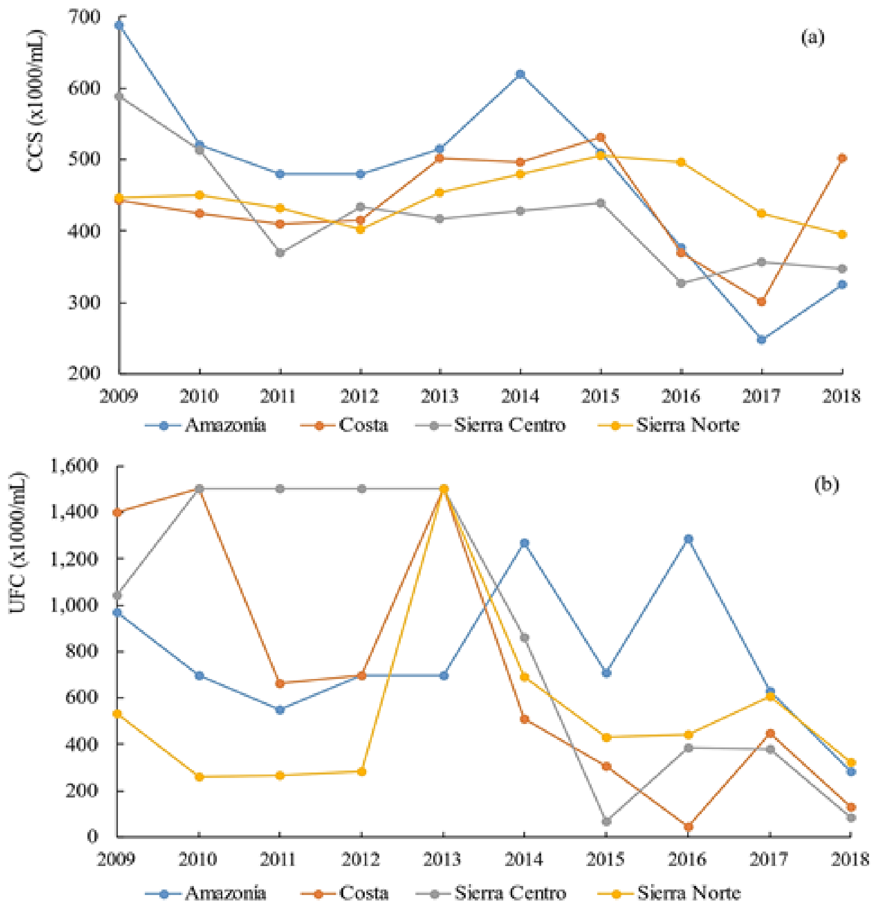

Figure 2. Microbiological milk parameters during the study period: (A) somatic cell counts (cells/10^3 cells/mL); (b) Total bacteria count ($\times 10^3$ UFC/mL).

In 2010 (Table 1), an amendment is made to the Ministerial Agreement 394 (MAGAP, 2010), which includes the price of liter of milk on farm to 52.4% on the price of UHT milk. In 2013, the current RPCL (MAGAP, 2013) set the base price of a liter of milk (USD 0.42) and included the health-quality on BPA.

The implementation of the RPCL was accompanied by the reorganization of government institutions, like Agrocalidad along with MAGAP, was responsible for monitoring and evaluating the system of agricultural production chains (Agrocalidad, 2017). As the nutritional management of livestock and the health of the udders are influential factors in quality indicators, the BPP Guide and health surveillance procedures were established. These resolutions were disseminated between 2010-2013 in training processes such as the Agricultural Revolution Schools (ERA's) and other processes promoted by private entities such as universities and agricultural input distributors (Derks et al., 2014; FAO, 2011; Vinueza, 2015).

Another important contribution to establishing the RPCL was the certification of laboratories for the verification of quality parameters, which until 2008 were performed by the same industries (Alvarado, 2017; MAGAP, 2008). Having specialized laboratories for these services has several advantages because it centralizes investment in the personnel, infrastructure and technology, and it optimizes the use of equipment capable of performing a high number of samples (Dürr et al., 2004).

Table 1. Regulations supporting the establishment of the RPCL in Ecuador.

Date of issued	Description	Bases on
23/04/2008	Agreement *N*°077-MAG. Executive decree 1042	Law to rule the payment for milk quality and animal health
21/04/2010	Ministerial agreement MAGAP*N*° 136	Minimum price for the liter of milk to the producer, incorporated to 52.4% at the retail price (PVP) of UHT of liquid milk in a bag
04/09/2013	Ministerial agreement MAGAP *N*° 394	Regulate and control the price of the liter of raw milk paid on farm and/or collection center and promote the quality and safety of raw milk
30/06/2015	Regulation *N*° 16. It modifies the regulation *N*° DAJ-2013461-0201.0213	Technical specifications for the collection and transportation of milk
29/02/2016	Regulation *N*° 19. It modifies the regulation *N*° 217	Guidelines for milk production. An annual health management plan is required
15/04/2016	Regulation *N*° 71	Instructions for the registration and control of raw milk analysis laboratories
15/08/2016	Regulation *N*° 154. It modifies the regulation *N*° DAJ-2013461-0201.0213	Guidelines for the monitoring and control of milk safety
30/01/2017	Regulation *N*° 276	Guidelines to good livestock practices in milk farming for small producers. It establishes monitoring and evaluation systems in the agricultural milk production chain
30/08/2017	Regulation *N*° 95. It modifies the regulation *N*° DAJ-2013461-0201.0213	Guidelines of procedures for the monitoring and control of milk safety

3.4 Impact on producers, production and productivity

From 2001, the CA formation was an important event in Ecuador, since it marked the beginning of the commercialization with cooling milk by small producers and the reduction of milk sale by informal sellers (Oñate, 2018). Public and private entities channeled offers of credit and technical assistance programs, investment in infrastructure for the pur-chase of cooling equipment, fertilizers, milking implements, vaccines, among others (Valladares, 2016). Since 2013, several programs and projects have been implemented to support CA, reduce production costs and promote a cleaner pro-duction (Valladares, 2016).

In 2009 milk production in Ecuador was 6 249 785.0 L/day (INEC, 2019). There were increments and reductions in the following years, and a negative trend was observed from 2013 to 2018 (Figure 3a). However, productivity increased at the national level (6.12 to 7.03 L/cow/day), with the Sierra region being the highest with 7.2 to 8.3 L/cow/day (Figure 3b). It can be assumed that production decreased due to difficulties of small producers when implementing and complying with RPCL. In 2017, the number of members in the CA decreased (36%) from those registered in 2008, fact can be explained because some producers abandoned the CA and others started working again with an intermediary to avoid the relationship with the CA and quality requirements (Alvarado, 2017).

The analysis should mention that 18% of the producers were people >61 years old, meaning a negative scenario for the adoption of "new" procedures such as the use of disinfectants for pre- and post-milking, use of paper to clean udders, and others (Nuñez, 2017; Oñate, 2018; Valladares, 2016).

To improve productivity during the study period, there were strong investments in the installation of irrigation systems, improving the efficient use of water increasing dry matter in livestock feeding (Cachipuendo et al., 2017), as well as the use of improved seeds adapted to local technology and livestock genetic improvement (Requelme and Bonifaz, 2012).

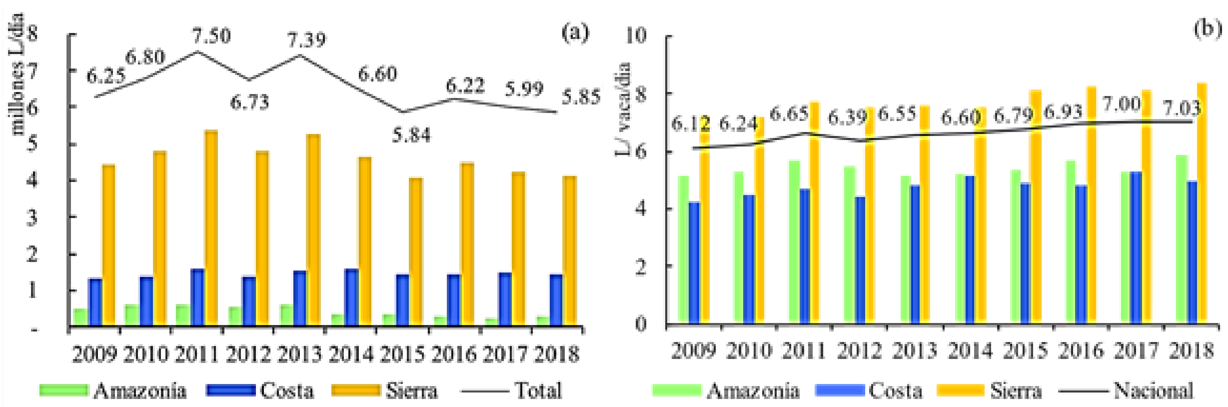

Figure 3. Production and productivity of milk by regions of Ecuador: (a) production in millions of L/ day and (b) productivity L x cow x day (INEC, 2019).

4 Conclusions

The establishment of the RPCL and the policies applied during 2009-2018 had a positive effect on the quality parameters, which meet the established limits not so on production that has a downward tendency.

The total fat, protein, and solid composition parameters are remained on limits with trend to improvement over the years. Sanitary and hygienic parameters (SCC and TBC) also show improvements over time, with SCC still not falling within the maximum allowable limits, it involves a multifactorial action for its improvement; therefore, it is necessary to concentrate efforts on the health and application of GMP dairy farms.

The establishment of milk quality payment systems should be accompanied by training and management programs between the producers, industries and the government.

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The dairy cow: Understanding
the sustainable production

Postpartum Pathologies and Origin of Infertile Cows in Dairy Cattle in the Mexican Highlands

Patologías Posparto y Origen de Vacas Infértiles en Ganado Lechero en el Altiplano Mexicano

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Abstract

Infertility in dairy cows under intensive productive systems is a growing problem and is one of the main causes of culling. The objective was to determine the origin of infertility in dairy cows under intensive productive systems from the Mexican highlands with postpartum pathologies. In this experiment 1,110 housed Holstein cows were studied. Cows were divided by calving number: First (n=389); Second (n=296) and three or more (n=425). Statistical analysis: comparison of means (Anova model) and univariate analysis. High percentage of the cows remained healthy after calving (80.5%); however, 15.6% of these cows were infertile. The prevalence of pathologies was 19.5%, and 26.3% of these cows were infertile. Cows with reproductive pathologies had a higher percentage (p <0.05) of infertile cows (25.0%). The infertility increased with the number of calvings, cows with three or more calvings and with reproductive pathologies had the highest percentage of infertile cows compared with those of second and first calving (39.0%, 30.0% and 14.0%, respectively). Second calving cows (OR: 2.24 95% CI: 1.06-4.95) and cows of three or more calvings and that presented reproductive pathologies (OR: 1.95 95% CI: 1.03-3.71) were identified as risk factors. In conclusion, cows that presented reproductive pathologies had the highest percentage of infertile cows and the risk of remaining infertile increased up to 4 times, especially if they presented more than 2 calvings. The percentage of infertile cows with postpartum pathologies could not be fully explained, leaving 15.6% of infertile cows with different origins.

Keywords: Infertility, metabolic problems, dairy cows, housed cows, highlands.

Resumen

La infertilidad en vacas lecheras estabuladas es un problema creciente y una de las principales causas de desecho. El objetivo fue determinar el origen de infertilidad en vacas lecheras estabuladas del altiplano mexicano con patologías posparto. Se estudiaron 1,110 vacas Holstein estabuladas y divididas por número de parto: primero (n=389); segundo, (n=296) y tres o más (n=425). El análisis estadístico se realizó haciendo una comparación de medias (modelo ANOVA) y factores de riesgo (Odds Ratio). El 80.5% de las vacas permanecieron sanas después del parto, 15,6% resultaron infértiles y el 3,9% no presentó cambios. La prevalencia de patologías fue del 19,5% encontrando que el grupo de vacas con patologías reproductivas tuvieron mayor porcentaje (p<0,05) de infertilidad (25,0%). Se incrementó el problema de infertilidad con el número de partos, siendo el grupo de vacas de tres o más partos y con patologías reproductivas las de mayor porcentaje de vacas infértiles comparadas con las de segundo y primer parto (39,0%, 30,0% y 14,0%; p<0,05, respectivamente). Se identificaron como factores de riesgo vacas de segundo parto (OR: 2,24 IC95%: 1,06-4,95) y aquellas con más de tres partos que presentaron patologías reproductivas (OR: 1,95 IC95%: 1,03-3,71). En conclusión, las vacas que presentaron patologías reproductivas tuvieron el mayor porcentaje de infertilidad, el cual incrementó hasta cuatro veces el riesgo de quedar vacías, especialmente si presentaban más de dos partos. No se pudo explicar por completo el porcentaje de vacas infértiles con las patologías posparto, quedando un 15.6% de vacas infértiles con diferente origen.

Palabras clave: Infertilidad, problemas metabólicos, vacas lecheras, vacas estabuladas, altiplano.

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1 Introduction

Over the past 70 years, the high yields of milk production specialized in dairy cows have been the result of an intense genetic selection (Oltenacu and Algers, 2005; Miglior et al., 2017), and specially of the genomic evaluation of young bulls. However, genetic selection has had problems since highly producing cows have drastically reduced their fertility (Diskin et al., 2006; Sheldon et al., 2009a,b); thus, health problems have increased during the first postpartum weeks (LeBlanc, 2010). However, despite progress in dairy cattle, there are still health problems (Esposito et al., 2014). LeBlanc (2010) reported a high prevalence of postpartum conditions concluding that one out of two dairy cows had some pathology, so the increase in diseases will have a negative impact on the reproduction (Barker et al., 1998; Lammoglia et al., 2015; Sheldon, 2020). Cows with some postpartum pathology, whether metabolic (ketosis, hypocalcemia, abomasum displacement, etc), or immunological (placenta retention, metritis, mastitis, etc) had a lower production as well as a lower fertility rate, increasing their risk of being discarded from the herd (Walsh et al., 2007; Dubuc et al., 2012; Hudson et al., 2012; Vieira-Neto et al., 2014; Denis-Robichaud and Dubuc, 2015; Vallejo-Timarán et al., 2017).

It has been mentioned that the consequences of reproductive pathologies during postpartum are also medium-term, as they affect fertility in the first calving and increase the open and rest period between calving; but these aspects are very expensive for any production system, mainly intensive livestock farming. Sheldon (2020) found that some of the infertility originated due to the infection of the uterus, since the bacteria, toxins and lipopolysaccharides could reach the ovary by using the circulation of the uterine-ovarian vascular pedicle system as transport. Once in the ovary, it causes an inflammatory process that can last for weeks or even months, causing oophoritis (damage to the corpus luteum) to the oocyte and ovarian dysfunction. The aim of the research was to determine the origin of infertility in dairy cows in the Mexican highlands with postpartum pathologies.

2 Materials and methods

The study was carried out in a specialized dairy cattle farm, located in the Mexican highland, in Hidalgo, Mexico (24°N 103°W, 24°N 103°W) at an altitude of 1980 masl, with a semi-dry temperate climate, with average temperature of 14.5°C, maximum temperature 24.4°C and minimum temperature of 5.3°C, average relative humidity of 65.6%, (maximum relative humidity of 93.5% and minimum relative humidity of 30.4%).

2.1 Catalytic preparation

The livestock production unit has 1 058 Holstein dairy cows, with an average of 36.5 ± 1.5 L/cow/day. Cows are in a poultry and are milked three times a day, and an average of 110 ± 15 calving occur monthly.

The management of cows consisted on: cows after calving were milked three times a day and were kept 21 days in a poultry under comfortable conditions (fresh vessels, individual and wide hatcheries) (±7 m^2), clean and abundant sand, fresh water with free access, exercise area with ground floor (±8 m^2), and a low number of cows per poultry (±25)) in a transition phase that consisted on the same prepared food ration (Alfalfa 87%, corn 88%, silo oat 44%), without anion salts, later cows were introduced again into the high production paddocks.

For 10 consecutive days after calving, rectal temperature was measured daily and records were taken as an indicator of an ideal health status. Cows were grouped according to the conditions they presented from day 0 to 45 postpartum. The diagnoses and treatments of each condition were carried out by a clinical specialist in dairy cattle.

Information was obtained from 1 110 cows under the Dairy-Com 305® administrative software program for dairy farms, distributed in cows with one, two and three or more calving; they were also classified as healthy, with reproductive, food, metabolic, locomotive and sanitary problems (Table 1).

Because of the low number of animals in the different categories, except reproductive problems, cows were located in a category called "other" so that the statistical analysis had a higher number of cows within this variable; however, some characteristics such as body condition, post-partum health condition or level of dairy production were not evaluated during the investigation because it was not part of this research.

Table 1. Categorization of the animal by number of calving and health status.

Categories	First Calving	Second Calving	Three or more Calving	Total Cows
Healthy	309	254	330	893
Reproductive	64	20	44	128
Food	4	5	8	17
Metabolic	3	1	10	14
Locomotive	3	6	10	19
Sanitary	6	10	23	39
Total	389	296	425	1110

The statistical analysis consisted of comparing means using ANOVA with Tukey test and multivariate analysis using the Statistical software version 10®. The risk determination was calculated using Odds Ratio (OR) with the Win Episcope program 2.0® (Thrusfield et al., 2001) where the interpretation of risk characteristics according to the number of calving was considered, as well as categories with 95% confidence intervals (IC95%).

3 Results and Discussion

3.1 Results

A prevalence of 19.5% was found from a total of 1 110 cows with health problems after calving. Females with pathologies after calving are those that showed lower fertility percentages ($p < 0{,}05$) compared to cows that did not get sick (Table 2). Cows that presented reproductive pathologies after calving, such as retention of fetal membranes and/or metritis, showed lower fertility ($p < 0{,}05$) compared to other conditions (Table 2). The number of calving affected (p<0.05) the fertility of healthy cows. Primaparous dairy cows had the best fertility rate, and cows with three or more calving had the lowest fertility rate of all groups (Figure 1).

Reproductive pathologies affected the fertility of cows ($p < 0{,}05$). Primaporous cows with postpartum reproductive pathologies had 6.2% less fertility rate ($p < 0{,}05$) compared to healthy cows; likewise, cows with two calving and cows with three or more calving with reproductive postpartum pathologies had 15% less fertility rate than cows that remained healthy. However, reproductive pathologies had a minor effect ($p < 0{,}05$) on the fertility of primaporous cows compared with cows with two and three or more calving (Figure 2).

The univariate analysis to determine risk factors showed that cows with three or more calving with reproductive pathologies had up to four times more risk (OR 1.95%; $IC_{95\%}$ 4) to remain empty (Table 3), and cows with more than two calving had up to five times less chance of becoming pregnant (OR 2.24%; $IC_{95\%}$ 5; Table 3). It was found that the conditions in the first 30 postpartum days increase infertility in primarous cows, and in cows with two, three or more calving, although this does not fully explain the causes of infertility.

Table 2. Fertility percentage of cows ($p < 0{,}05$) affected by postpartum pathologies and by the number of calving.

Condition of cows	Number of calving			
	One	**Two**	**Three or more**	$p < 0{,}05$
Healthy	92.2^a	85.8^b	76.0^c	0.05
Reproductive pathologies	86.0^a	70.0^b	61.0^c	0.05
Others	87.5^a	59.0^b	72.5^c	0.05

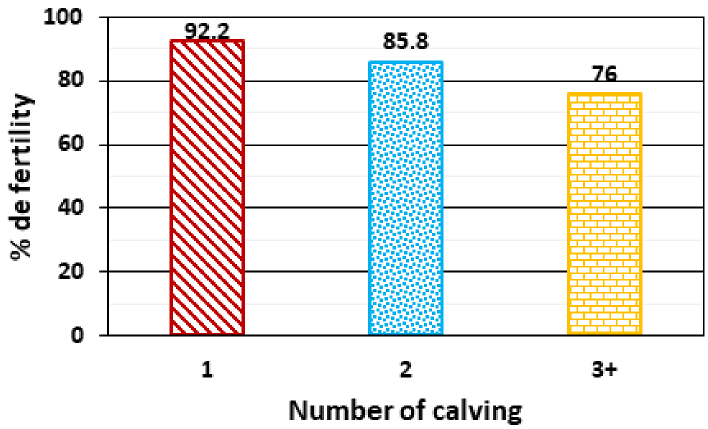

Figure 1. Number of calving and its effect on the fertility of dairy cows.

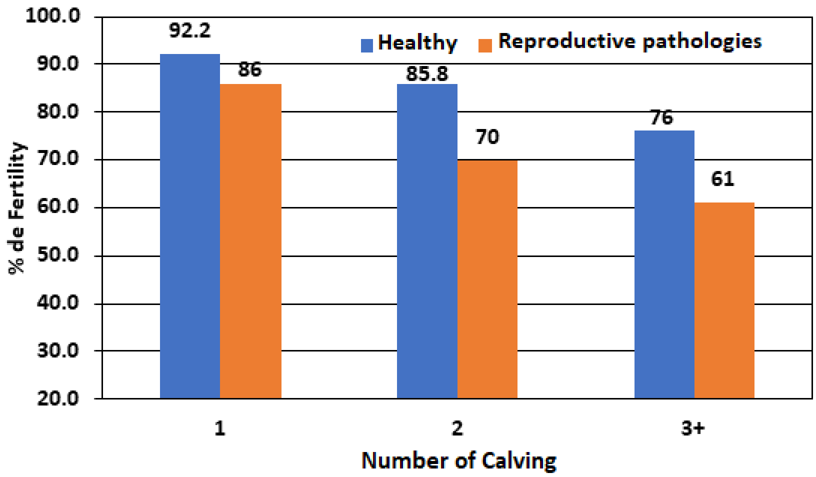

Figure 2. Effect of postpartum reproductive pathologies on the fertility of dairy cows in the Mexican highland.

3.2 Discussion

Based on the results, it can be inferred that the prevalence of postpartum pathologies of the dairy herds analyzed in the Mexican highland was 19.5 %, i.e., one in five cows suffered some pathology during postpartum. These results are lower than the reported by LeBlanc (2010) where the prevalence of postpartum disease was one in two dairy cows with some pathology. Dubuc and Denis-Robichaud (2017) found in 126 dairy cows a prevalence of postpartum diseases from 0 to 80 %, raising the need to continue researching due to the significant difference in the prevalence of postpartum disease between herds.

The prevalence of postpartum pathologies found in this study was also lower than the one reported by Santos et al. (2010)in a study with 5 179 cows, where they found 44.2 % of cows with pathologies. Postpartum pathologies are caused by multiple factors, such as the dry period to the fresh period (Chebel et al., 2018). For example, a loss of body condition during the dry period may have a marked health effect during postpartum (Chebel et al., 2018). It should be noted that the estrus in dairy cows is longer in winter, while in summer it is usually lower, making it difficult to detect the estrus correctly. This characteristic is triggered by the thermal stress suffered by dairy cows, especially Holstein cows (Castaño et al., 2014).

Table 3. Risk of infertility by categories of postpartum pathologies associated with the number of calving.

Primarous cows	**OR**	$IC_{95\%}$
Healthy pregnant	1.98	0.93-4.20
Healthy empty	0.51	0.24-1.08
Reproducive Problems (Pregnant)	0.51	0.23-1.14
Reproductive Problems (Empty)	1.96	0.88-4.37
Other problems (Pregnant)	0.66	0.14-3.00
Other problems (Empty)	1.52	0.33-6.92
Cows with two calving	**OR**	$IC_{95\%}$
Healthy pregnant	**2.24**	**1.06-4.95**
Healthy empty	0.45	0.20-0.98
Reproducive Problems (Pregnant)	0.40	0.15-1.08
Reproductive Problems (Empty)	2.48	0.92-6.64
Other problems (Pregnant)	0.59	0.18-1.87
Other problems (Empty)	1.70	0.53-5.41
Cows with three claving	**OR**	$IC_{95\%}$
Healthy pregnant	1.54	0.94-2.53
Healthy empty	0.65	0.40-1.07
Reproducive Problems (Pregnant)	0.51	0.27-0.97
Reproductive Problems (Empty)	**1.95**	**1.03-3.71**
Other problems (Pregnant)	0.91	0.47-1.76
Other problems (Empty)	1.10	0.57-2.12

The prevalence of postpartum pathologies may have been lower than the results published by other authors due to the difference in the management of cows that started from the dry period to the cool period.

The results of the research indicate that the number of calving affected the prevalence. Primarous cows had a lower prevalence. These results are corroborated by Wittrock et al. (2011) who reported a lower incidence of postpartum pathologies in primarous cows than in multiparous. The low prevalence of postpartum pathologies in these cows may be due to the fact that primarous cows have better metabolic adaptation and less loss of body condition than cows with two or more calving (Adrien et al., 2012; Wathes et al., 2007). Among the main causes of infertility in cattle are genetic disorders, including chromosomal abnormalities, congenital malformations, or freemartinism; similarly, hormonal factors result in the cyclic disability of females (Córdova et al., 2002).

Reproductive problems caused by retention of fetal membranes, metritis or the combination of both pathologies, affected the percentage of pregnant cows regardless the number of calving. These results are confirmed by Melendez et al. (2009) and Deori and Phookan (2015) who mention that postpartum reproductive pathologies reduced fertility in dairy cows. Also, Santos et al. (2010) found that females with postpartum metritis or endometritis had lower fertility rate than healthy cows. Sheldon (2020) found a possible explanation of the physiological cause by mentioning that because cows have a unique circulation between the uterus and ovaries (uterine-ovarian vascular pedicle system), it has a very important structural relationship of arterial and venous irrigation in the establishment of coordinated physiological mechanisms of the uterus and ovaries. In the apposition area, there is a decrease in the thickness of the walls between the ovarian artery and the uterine vein, although no direct vascular connection between the artery and the vein has been demonstrated, and this is where the exchange of molecules such as prostaglandin $F2\alpha$ occurs, passing from the uterine venous system to the ovarian arterial system.

There are two mechanisms for exchanging molecules; the first is the diffusion through the uterine-ovarian lymph vessels (the lymph vessels attach closely to the venous and arterial vessels in the ligament of the ovary) and the second by the transportation proteins. In this way, the bacteria, their toxins and lipopolysaccharides reach the ovary, producing an inflammatory reaction that interferes with the health of the oocytes and the production of hormones, both belonging the corpus luteum and the follicles, thus affecting the fertility of cows (Sheldon, 2020; Fabian et al., 2010; Chebel, 2007).

The percentage of pregnant cows was also affected by the number of calving, where cows with the highest number of calving were more likely to become empty, regardless whether they had health problems during postpartum. These results are corroborated by other studies published by Lucy et al. (2014), who relate dairy production in females with more calving; in other words, when producing more milk, it is common to find a greater number of these cows with negative energy balance and with high concentrations of both β-hydroxybutyrate and non-esterified fatty acids. Negative energy balance during postpartum in dairy cow is associated with a reduction in GnRH and LH, affecting follicle growth, maturation and ovulation, resulting in a low fertility rate (Crowe et al., 2014), which would explain why not all cows that remained healthy during postpartum became pregnant.

Similarly, the explanation for the results of healthy cows that were empty may be due to causes other than health problems such as metabolic problems (negative energy balance, increased concentrations of β-hydroxybutyrate and nonesterified fatty acids) that contribute to the infertility of healthy cows. Trevisi et al. (2011) also concluded that subclinical proinflammatory processes postpartum in dairy cows have long-term effects, including reducing or increasing the risk of infertility rate in dairy cows.

4 Conclusions

Reproductive pathologies had the greatest impact on fertility and increased the risk of empty cows, especially those with more than two calving. Although the number of calving affected fertility and increased the risk of being empty, infertility due to postpartum pathologies could not be fully determined. It is recommended to maximize cow´s care in the transition period in order to reduce the presence of diseases and decrease the percentage of cow problems.

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SOIL SCIENCE

ALLOPHANE, A NATURAL NANOPARTICLE PRESENT IN ANDISOLES OF ECUADOR, PROPERTIES AND APPLICATIONS

ALOFÁN, UNA NANOPARTÍCULA NATURAL PRESENTE EN ANDISOLES DEL ECUADOR, PROPIEDADES Y APLICACIONES

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Abstract

The allophane is a natural nanoparticle present in soils from volcanic origin such as andisols, which are distributed worldwide, especially in countries that have active volcanoes. In Ecuador, andisols are in high and humid areas from the Highland/North region, constituting 30% of the territory. The allophane can be obtained from andisols through physical and chemical processes or it can be also synthesized. This nanomaterial has multiple properties for various applications in different areas; and there are studies about these nanoparticles and this kind of soil, but they have not yet been conducted in Ecuador. This article presents a review of structural characteristics, properties, formation, isolation, synthesis and uses of allophane to extend knowledge and encourage the conduction of research in these soils, which are the source of the aforementioned nanoparticle. The literature review was performed on Science Direct and Google Scholar databases using high impact articles related to natural or synthetic allophane. Allophane has characteristics that allow it to be used as an environmental remediator, bactericidal, anti-inflammatory, flame retardant, enzyme support and also in catalysis, photocatalysis and electrocatalysis. Considering the availability and the large area covered by andisols in Ecuador, research based on international investigations can be perform to take advantage of it.

Keywords: natural nanoparticle, andosol, volcanic soils, halloysite, imogolite.

Resumen

El alofán es una nanopartícula natural presente en suelos de origen volcánico como los andisoles, que se encuentran distribuidos alrededor de todo el mundo en países con actividad volcánica. En Ecuador, los andisoles constituyen el 30% del territorio en zonas altas y húmedas de la región sierra-norte. El alofán se puede obtener de los andisoles a través de procesos físicos y químicos, o a su vez se puede sintetizar. Este nanomaterial posee múltiples propiedades para varias aplicaciones en diferentes áreas. Existen muchas investigaciones de estas nanopartículas y de este tipo de suelos, pero no se han estudiado aún en el Ecuador. En este artículo se presentan las características estructurales, propiedades, la formación, aislamiento, síntesis y usos del alofán, con el fin de generar conocimiento e incentivar la investigación de estos suelos que son fuente de la mencionada nanopartícula. La búsqueda de literatura se realizó en bases de datos de Science Direct y Google Académico, y se utilizaron artículos de alto impacto relacionados con investigaciones de alofán natural o sintético. Las características particulares que tiene el alofán le permite ser usado como remediador ambiental, bactericida, antiinflamatorio, ignífugo, soporte de enzimas, pero además se ha estudiado en catálisis, fotocatálisis y electrocatálisis. Al considerar el área cubierta por el alofán en el territorio ecuatoriano y su disponibilidad, este se puede aprovechar para realizar investigaciones basadas en los estudios internacionales que se han desarrollado para aprovechar en el área ambiental y médica.

Palabras clave: nanopartícula natural, andisol, suelos de origen volcánico, halloysita, imogolita.

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1 Introduction

Allophane is a natural nanoparticle Nishikiori2012 that is present in volcanic soils called andosols according to the global reference of the soil resource Vistoso2012 or andisols according to the Soil Taxonomy NRCS/USDA. Andosols or andisols are soils formed from volcanic materials such as ashes, through weathering processes under acidic conditions in humid climates (Cervini-Silva et al., 2015; Saeki et al., 2010), and are distributed around the world in regions with increased volcanic activity.

Allophane particle has a porous sphere shape, with a diameter between 3 and 5 nm (Henmi and Wada, 1976), and is structured by an outer layer consisting of aluminum octahedrons (Al) and an inner layer consisting of silicon tetrahedrons (Si). It has a large surface area, high porosity and variable load, allowing it to have potential applications as a cation adsorbent (Silva-Yumi et al., 2018), anions (Nishikiori et al., 2017), benzene-derived compounds, fatty acids, detergents, organochlorine compounds (Arakawa et al., 2014; Baldermann et al., 2018; Garrido-Ramírez et al., 2013), DNA and amino acids (Saeki et al., 2010), enzymes (Yu-Huang et al., 2016), in the catalysis area (Garrido-Ramírez et al., 2013), in the preparation of electrodes (Nishikiori et al., 2014), as flame-retardant (Iyoda et al., 2012; Shukla et al., 2013) as ink element for printers (Calabi-Floody et al., 2012), as anti-inflammatory and bactericidal (Calabi-Floody et al., 2012), for the purification of biodiesel (Yu-Huang et al., 2016), etc.

In Ecuador, andisoles constitute approximately 30 % of the territorial area, and these are distributed in the north-central highlands in the high and humid zones, although they extend toward the coast and the east (Calvache, 2014). Research on soils of Ecuadorian volcanic origin has focused on pedogenesis (Zehetner et al., 2003), carbon stabilization and storage (C), the effects of overgrazing on vegetation (Podwojewski et al., 2002), the short-term management effects on soil structure of hardened volcanic origin in deep layers, the variability of soils of volcanic origin and their relationship with parental material, climate and their use (Podwojewski and Germain, 2005), chemical weathering (Poncelet and Jouhannaud, 2013) and the presence of n-methyl ketones as products of n-alkane degradation (Jansen and Nierop, 2009); however, none of these articles mention or have focused on the study of allophane nanoparticles present in this type of soil, only one article published in 2007 studies the influence of allophane content and organic matter on soil properties of volcanic origin (Buytaert et al., 2007).

In 2009, an article was published about the existence of a massive allophane deposit located in the community of San José de Achotillo, in the province of Santo Domingo de los Tsatchila. Its quantification was carried out in 2010 and the presence of a high allophane content was found ($> 60\,\%$), as well as halloysite which is another natural nanoparticle with similar characteristics; iron oxides and a low organic matter content (Kaufhold et al., 2010). Since this year, several researches have emerged on allophane present in the deposit and focused on the adsorption of anions as fluoride and its comparison with other similar adsorbents (Kaufhold et al., 2010), adsorption of cations: barium, cobalt, strontium and zinc (Baldermann et al., 2018), the anti-inflammatory activity of allophane present in this deposit (Cervini-Silva et al., 2015), as well as its cytotoxicity (Cervini-Silva et al., 2014), and activation to be used as a catalyst (Vaca and Lalangui, 2018).

Studies on Ecuadorian andisols and allophane nanoparticles are limited, and research on allophane has been conducted from institutions located outside the country as seen in the literature. Although Ecuador's scientific production has increased in recent years (Araujo-Bilmonte et al., 2020), trained personnel have returned from abroad to research in this area and with higher education institutions to conduct research in nanoscience and nanotechnology (Gutiérrez Coronado, 2018). Perhaps one of the limitations is the lack of knowledge on the existence of this nanomaterial, reason for which the objective of this article is to present the characteristics, properties, formation, isolation, synthesis and uses of allophane in order to highlight the potential of this type of soil, which covers a large area of the Ecuadorian territory.

2 Methodology

The search and compilation of the articles used in the literature review was carried out between January 2018 and January 2019 through the databases: Scopus, Sciencedirect and Google Academia. The criteria were: (1) Keywords: Allophane, alofán, andisol Ecuador, andosol Ecuador, allophane Ecuador, alofán Ecuador, with boolean algorithms "and" and "with". (2) Type of articles: Research and review, (3) Inclusion and Exclusion Criteria: Articles focused on the study of allophane, articles in quartile 1 according to Scimago Journal & Country Rank for documents in English, (4) documents published in the last 10 years. 59 documents have been taken into consideration between articles and congress works, two reference documents and a patent, from which the main ideas shared in this document have been extracted. The articles have been considered based on their information about the origin, synthesis, properties and applications of allophane.

3 Andisols around the world and in Ecuador

Andisols are soils that are formed from volcanic materials such as ash, through weathering processes under acidic conditions in humid climates (FAO, 2014, 2015), and are distributed around the world in regions with significant volcanic activity. In Asia and the Pacific, these are found in Japan, Korea, the Philippines, Indonesia, Papua New Guinea and New Zealand. In Europe they are in Italy and France; in Africa and Oceania they are in Kenya, Rwanda, Tanzania, Ethiopia and the Canary Islands. In America they are located in countries around the Pacific Fire Belt: Alaska, United States, Mexico (Pérez et al., 2016), Guatemala, El Salvador, Honduras, Nicaragua, Costa Rica (Alvarado et al., 2014), Panama, Las Antilles, Colombia, Ecuador, Chile and Argentina.

Although there are several types of soils in Ecuador, andisols constitute 60593 km^2 of the Ecuadorian territory (Fig. 1), and these are distributed around the central-north highland region in high and humid zones, though they also extend to the coast and the east (Calvache, 2015; Gonzáles, 2015; González, 2010). They are the result of weathering of pyroclastic material resulting from the eruptions of numerous volcanoes located in the so-called "avenue of volcanoes", with more than 100 volcanoes, five of them active. They are soils with a low apparent density, usually with high organic matter content, low resistance to tangential cutting, good drainage, but in turn good moisture retention (Calvache, 2015; FAO, 2015; Gonzáles, 2015).

4 Formation

Allophane is formed by the rapid weathering of volcanic glass. When the pH of the soil is 5, silicon and aluminum are released from the volcanic material, thus reacting and forming allophane, imogolite and halloysite, which are other minerals present in this type of soil and which can also be formed from the hydrolysis of primary minerals. The formation and persistence of allophane, imogolite or halloysite is influenced by the organic matter content and precipitation. Allophane predominates in soils that are subjected to high precipitation, while halloysite excels in soils with a low precipitation regime. These minerals are present in soil with low organic matter content where aluminum does not exist as complexes with organic acids or humic substances but in the form of inorganic complexes (Yuan and Wada, 2012).

Allophane can be extracted from soils of volcanic origin following a process that generally involves the removal of organic matter and iron oxides, and the separation of nanometric fractions from the sand, lime and clay fractions. This nanoparticle can also be synthesized by the co-precipitation method or by the sun-gel method. The first method uses orthosilicic acid (H_4SiO_4) or sodium orthosilicate (Na_4SiO_4) and aluminum chloride ($AlCl_3$) or aluminum perchlorate ($Al(ClO_4)_3$) as precursors. The SOL-GEL method that involves hydrolysis of reagents, and condensation of the obtained products uses tetraethyl orthosilicate ($TEOS$) ($Si(OC_2H_5)_4$) and aluminum chloride ($AlCl_3$).

5 Structural characteristics

Allophane particle is shaped as a porous sphere (Fig. 2) with an external diameter between 3.5 and 5.0 nm, an internal radius between 1.0-2.0 nm and a wall with a thickness between 0.7 and 1.0 nm. It has pores with a diameter of around 0.3-0.4 nm and has a specific surface area determined by the EG-

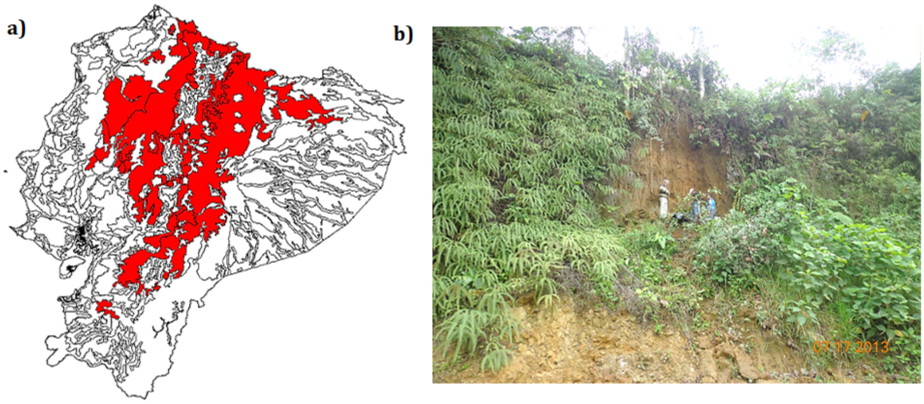

Figure 1. a) Surface area of Ecuador covered by volcanic soils taken from Calvache (2015). b) An example of area rich in allophane and located in Santo Domingo de los Tsatachilas (own image).

ME (ethylene glycol monomethyl ether) method, ranging from 400-900 m^2g^{-1}. The outer wall is composed of aluminum octahedrons, while the inner wall by silicon tetrahedrons (Fig. 2), although it is possible to find aluminum in both tetrahedrons and octahedrons.

The chemical composition of allophane changes according to the predominance of Al or Si. It is possible to find allophane rich in aluminum ($Al/Si = 2$) and allophane rich in silicon ($Al/Si = 1$), thus its chemical formula can be represented as $1-2SiO_2.Al_2O_3,5-6H_2O$. The presence of silanol groups ($Si-OH$) on the surface of the inner wall and aluminum groups ($Al-OH$) located on the outer wall allow allophane to acquire negative or positive charge (Fig. 3), depending on the conditions of the medium with the permanent or structural load it may possess, due to the isomorph replacement of Al^{3+} by Si^{4+}.

6 Application

6.1 As an absorbent for the environmental remediation

The high porosity of allophane, its large surface area and the charge it can take depending on the environment make allophane to be a potential adsorbent of positive species (cations) and negative species (anions), with internal (silanol) and external (aluminol) sites being the responsible of the process (Reinert et al., 2011). Adsorption processes can be used for treating drinking water and wastewater, as they allow contaminants (organic or inorganic species) to be retained on the surface of the material and be removed from the aqueous environment.

One of the most frequently studied processes is phosphate adsorption due to its particular fixation on allophane, although there are studies on the adsorption of molybdate (Elhadi and Henmi, 2000), arsenate, silicic acid, boric acid, chromate (Opiso et al., 2009), selenate, sulphate, oxalate, nitrate, orthylicic acid, fluoride (Kaufhold et al., 2010), citrate, among other anionic species. Adsorption of cationic species has been less studied in relation to anion species; the few species studied include zinc, cesium, copper, cobalt, cadmium, barium and strontium (Baldermann et al., 2018; Silva-Yumi et al., 2018).

Adsorption of organic species has also been of interest to scientists, including 2.4-dichlorophenol, pentachlorophenol, humic acid, benzene, benzoic acid, phthalic acid, benzaldehyde, ethyl benzoate, diethyl phthalate, acetic acid, oxalic acid, citric acid, polymers such as xanthan, detergents and fatty acids (**?**). Figure 4 shows some of the structures that correspond to the above species and how they are adsorbed on the surface of allophane. Gas adsorption has not been too studied, and only the adsorption of ammonia can be mentioned (Zaenal et al., 2013).

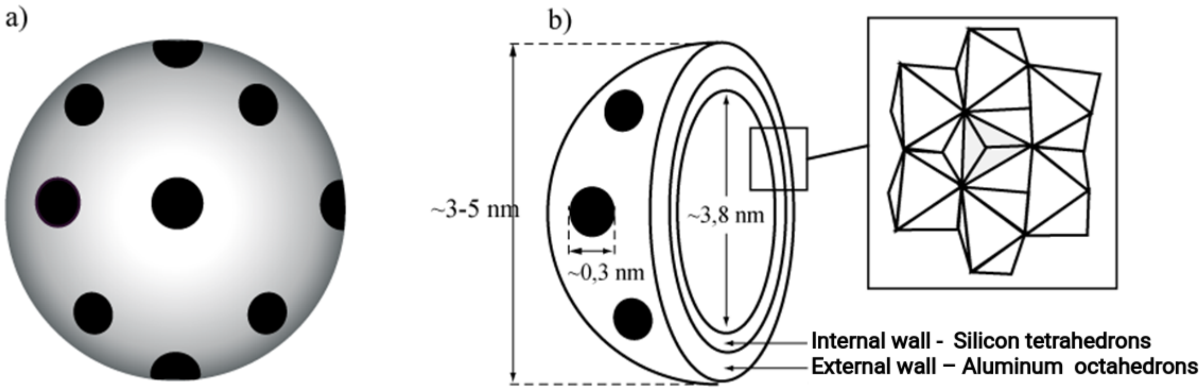

Figure 2. a) External morphology of an allophane nanoparticle. b) Detail of the internal structure.

6.2 Adsorption of essential molecules and origin of life

Understanding the processes that occur in this type of soil is possible by studies carried out, such as those of Hashizume et al. (2002; 2007) where allophane has been determined to have high affinity for nucleotides, which implies its possible role in the abiotic formation of RNA-type polynucleotides, although immobilization of these nucleotides by complexation may make it difficult to oligomerize RNA. The persistence and survival of amino acids in soils has been attributed to the adsorption and protection by clays and other minerals. For example, it has been observed that the adsorption of DL-alanine is higher on suspended allophane and it depends on the pH. A preference for L-alanyl-L-alanine is observed in the adsorption of D- and L-alanine and their dimers on allophane with different relation to Al/Si, suggesting that the size, the separation of intramolecular load and the surface orientation are the responsible factors.

In addition, the adsorption of DNA on allophane has been studied by several researchers in order to reconstruct and study past environments as well as microbial communities in soil and carbon storage (Huang and Rawlence, 2014; Matsuura et al., 2013; Yu-Huang et al., 2016). The results show that DNA adsorption is facilitated by the interaction of phosphate groups with allophane $Al-OH$ groups, although it is lower than the adsorption of adenosine-5′-monophosphate (5′-AMP), a molecule used as a reference (Matsuura et al., 2013), reason for which there is more affinity for 5′-AMP than adenine, adenosine or ribose, also due to the presence of phosphate groups that facilitate interaction with $Al-OH$ groups.

DNA adsorption is almost unaffected by the ionic strength and it decreases the presence of phosphate when the pH increases, due to the deprotonation of the $Al-OH$ groups (Matsuura et al., 2013; Saeki et al., 2010) as a result of the competition for active sites. The presence of humic acid also causes a decrease in the adsorption due to its active sites in allophane (Yu-Huang et al., 2016). DNA adsorption is higher on allophane than on silica and montmorillonite but lower on gibbbsite and goethite. The interaction of DNA and 5′-AMP with $(OH)Al(OH_2)$ groups present in the ultramicropores of allophane has also been studied through computer simulation, and has shown that DNA is elongated and that the main phosphate chain is altered after binding to allophane (Matsuura et al., 2014).

6.3 Sequestration, carbon stabilization and greenhouse control

As soils are the largest reservoir of carbon and organic matter, it is necessary to study their dynamics

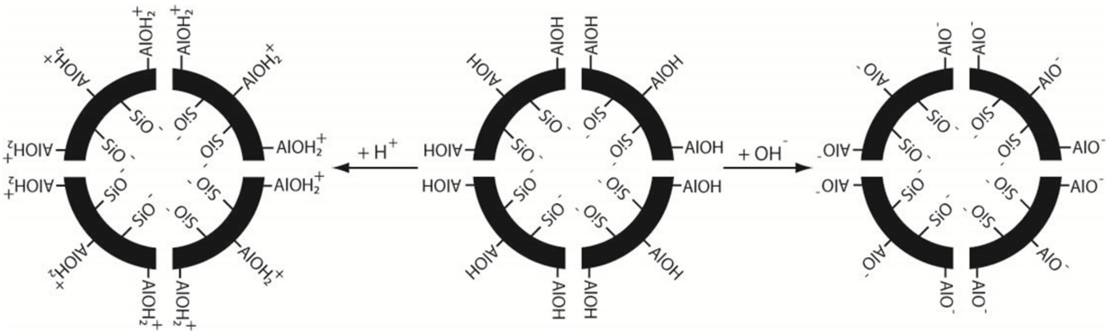

Figure 3. Superficial charge of allophane subjected to pH.

as well as their physical-chemical interactions with the main minerals present in them, due to the role as a CO_2 storage potential and key element to the greenhouse effect control (Huang and Rawlence, 2014; Triomphe and Livermore, 2005), as well as for the protection against mineralization and microbial degradation.

Carbon stabilization in allophane soils is difficult, as on the one hand it is attributed to Al^{3+} and sesquioxides, and on the other, there is a relationship between carbon and allophane content (Garrido-Ramírez et al., 2012), observing that the amount of linked organic matter is higher on imogolite material (proto imogolite, proto imogolite allophane) than on gibbbsite or non-weathered feldspar. This shows that non-crystalline (non-long range) minerals such as allophane and imogolite control the storage and movement of organic carbon in the soil (Yu et al., 2012). Stabilization of organic matter is produced by the adsorption on the specific surface area, protecting it from microbial disintegration.

In addition to surface adsorption, the entrapment of organic carbon into the fractal structure of allophane would make organic matter less accessible for microbial degradation and enzyme attack (Chevallier et al., 2010). This phenomenon is due to the mesoporous structure that occurs by the addition of allophane particles. The carbon variation in allophane is not explained by the loam and clay content; however, allophane, soil pH in water and aluminum content explains the greater carbon variation of the soil, observing an inverse relationship between the pH of the soil in water and Al (also Fe) with organic matter of the soil (Garrido-Ramírez et al., 2012).

6.4 Catalysis

6.4.1 Catalysis by Fenton-type reactions

Clays and iron oxide are an alternative to catalysts used in reactions (Fenton-type) for the decontamination of soils, water, sediment, and industrial effluents, because they are low cost, abundant and harmless. The processes of Fenton-type involve the reaction of Fe^{2+} with hydrogen peroxide, originating the formation of radical hydroxylones and Fe^{3+}. The Fe^{3+} reacts with peroxide forming Fe^{2+}, which generates more hydroxyl radicals that are highly oxidizing and capable of breaking down a wide spectrum of organic compounds. On this basis, methylene blue degradation has been studied using allophane coated with iron oxides, where allophane adsorbs methylene blue, while the interaction of the allophane Fe with hydrogen peroxide results in the formation of radicals, which break down methylene blue into smaller organic molecules (Abidin et al., 2011).

Iron oxide-coated allophane has also been evaluated for the oxidation of atrazine (1-chlorine-3-ethyl amino-5-isopropyl amino-2,4,6-triazine) in a heterogeneous electro-Fenton system, using vitreous carbon electrodes and showing greater efficiency than a heterogeneous Fenton process. Atrazine is an organic herbicide found as a pollutant in groundwater sources and drinking water supplies (Garrido-Ramírez et al., 2013). Garrido-Ramírez et al. (2012, 2016) have also evaluated the catalytic activity of iron oxides and copper oxides supported on allophane, as well as nanoparticles of Fe, Cu and bimetallic Fe-Cu for the oxidation of phenol by heterogeneous electro-Fenton reactions. In the first case, an influence of the Si/Al relationship and its structure was observed, while in the second case, a greater efficiency was evident when bimetallic nanoparticles were used compared to nanoparticles of Cu and Fe, due to a synergistic effect.

6.4.2 Photocatalysis

Photocatalysis is a remediation technique involving the adsorption of UV-visible radiation, which allows the degradation of organochlorine compounds. The degradation of trichloroethylene, a dangerous organic pollutant, and acetaldehyde in allophane-titanium composites has been studied (Nishikiori et al., 2010, 2015, 2017). The presence of allophane results in an increase in the adsorption of titanium and it inhibits the emission of phosgene ($COCl_2$) and dichloroacetyl chloride, which are intermediate products for the decomposition of trichloroethylene, which once adsorbed in the allophane are gradually degraded after moving in titanium. The photocatalytic activity of the composite improves with the treatment in the acid medium, which can be observed in the photocatalytic decomposition of gaseous acetaldehyde (Ono and Katsumata, 2014).

Hojamberdiev et al. (2014), used allophane-wakefieldite-(Ce) composites obtained by mechanical mixture and hydrothermal synthesis for the photocatalytic degradation of gaseous acetaldehyde, and found that both showed high photocatalytic activity compared to allophane or wakefieldite ($CeVO_4$)-(Ce) individually, because in the composites allophane produces an increase in the adsorption. The composite obtained by hydrothermal synthesis shows a greater photocatalytic activity than that obtained by mechanical mixture, due to a more homogeneous distribution of wakefieldite-(Ce) and allophane.

In another study, Hojamberdiev et al. (2014) use Bi_2WO_6–allophane and $BiOI$-allophane that were also obtained by mechanical mixing and hydrothermal synthesis for the photodegradation of gaseous acetaldehyde. BiOI and BiOI-allophane break down acetaldehyde completely within 5 to 7 hours, while Bi_2WO_6 and the composites obtained by mixture and synthesis break down 75.5%, 100% and 85.6% in 8 hours, respectively. Allophane also contributes to these composites due to the significant adsorption of acetaldehyde.

6.4.3 Heterogenous catalysis

The search for energy materials such as biomass is an option to fossil fuels. Polysaccharide hydrolysis is a process for obtaining monosaccharides, from which ethanol can be obtained. Okagi et al. (2011) studied hydrolysis of bamboo, silk and rice husks using sulfonated allophane. In bamboo, hemicellulose was decomposed to xylose and xylo-oligosaccharides; in silk the main products were xylose and mannose, although there was also a production of glucose, galactose and arabinose; in rice husk the main product was arabinose, although xylose and galactose were also obtained.

The degradation of compounds such as silicones or poly (dimethylsiloxane) in the presence of clays, as well as the effect of clay on the adsorption of degradation products has also been studied. In a study in which 12 different clays were used, allophane showed a lower effect on the catalytic activity compared to kaolinite, beidellite and nontronite, while degradation products were bound stronger to goethite and esmectite. The use of synthetic allophane also promotes the reduction of K_2PtCl to platinum (Pt^0) and it acts as a support for the Pt^0 nanoparticles of 2 nm, thus obtaining a composite with potential use in heterogeneous catalysis (Arakawa et al., 2014).

6.5 Photofuel cells

The use of photofuel cells to generate electricity by oxidizing combustible materials during UV irradiation is a trend in recent years. Electrodes in photofuel cells act as the phase that interacts with the combustible material. In these systems, the concentration of combustible material on the surface of the photocatalyst used is essential to improving energy conversion efficiency. One method for increasing concentration is the use of adsorbent materials. Taking advantage that allophane has a large surface area, Nishikiori et al. (2012, 2014) studied fuel cells using electrodes made from the allophane-titanium composite, and glucose and starch as combustible materials. In the first case, allophane adsorbs glucose transporting it to titanium, in which its oxidation induces the electrogeneration, in the case of starch it also improves the generation of electricity.

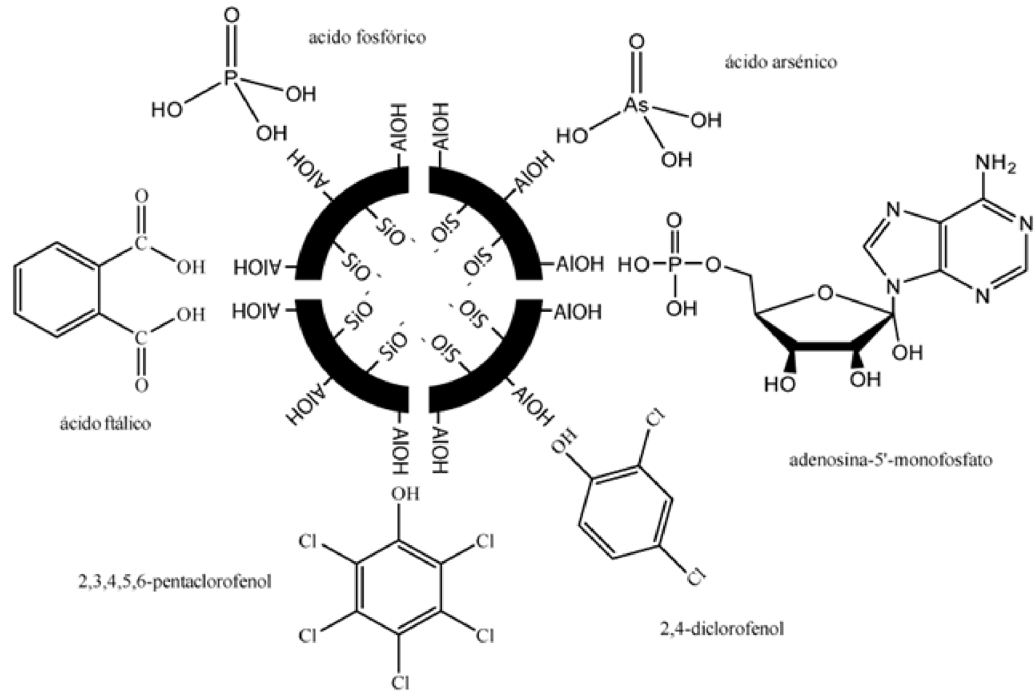

Figure 4. Diverse species adsorbed on allophane. The presence of OH groups in most of the studied species allows the exchange of ligands and the retention of allophane in the surface.

6.6 Farmacología

Clays have been used in cosmetics and industrial products, but their application is now expanding continuously in the pharmaceutical industry, tissue engineering and medical area. Since allophane is a non-toxic and biocompatible material, it is a good candidate for medical applications, as it has bactericidal properties that can be enhanced by the formation of nano-composites based on the immobilization of copper and silver nanoparticles, also with bactericidal properties. In this context, allophane has been used to support antibacterial agents such as silver nanoparticles, with strong bactericidal activity toward Escherichia coli and Staphylococcus aureus (Cervini-Silva et al., 2015). This effect is because silver exhibits strong bactericidal activity against a wide spectrum of fungal and bacterial species, in addition to low toxicity, high thermal stability and low volatility.

Allophane, and other clays such as halloysite, has anti-inflammatory properties because its application inhibits edema formation up to 39 to 60%. Even though Fe present in the allophane structure may have some role in this effect, the mechanisms have not yet been identified, hence its use for healing purposes can be recommended (Cervini-Silva et al., 2015, 2016). Natural allophane induces lipid peroxidation, oxidative degradation of lipids in cell membranes, and cytotoxicity of murine monocytes, which may be due to the presence of Fe in the surface and which may generate reactive oxygen species (Cervini-Silva et al., 2014, 2016; Toyota et al., 2017). These results have promoted studies of the cytotoxicity of natural and synthetic allophane nanoparticles against human cancer cells, with the intention of using allophane nanoparticles as a nanopower for drugs´ administration.

Kawachi et al. (2013), carried out a study of hydrogels based on DNA molecules and natural allophane, where DNA molecules adsorb on the surface, wrapping around the allophane particles and forming the hydrogel. Adsorption is facilitated by the interaction between the phosphate groups of DNAs and the groups present in the perforations of the allophane wall. The study of this type of hydrogel could be useful for the generation of new forms of drug release at specific doses.

6.7 Immobilization of enzymes

Due to its large surface area, allophane is a material that is a useful support for multiple species, including enzymes. In this way, allophane has been evaluated as a supporting material to immobilize acid phosphatase and evaluate the mineralization of organic phosphorus from decomposed cattle manure with clay complexes and nanoclay acid phosphatase. It has been observed that immobilization increases both the specific enzymatic activity and the kinetics of organic phosphorus mineralization (Calabi-Floody et al., 2012). Synthetic complexes have been obtained by the interaction between acid phosphatase, tannic acid and natural allophane in order to have a better understanding of organic phosphorus mineralization. Immobilization of phosphatase in tannic acid decreases enzymatic activity and affects kinetics, while immobilization in allophane increases the activity of the enzyme compared to the free enzyme, which would indicate that it has a protective effect on the enzyme's structure. In the case of Mn, the presence of Mn and Mo in the catalytic activity of immobilized acid phosphatase decreases the velocity compared with free phosphatase when added at the same time as the enzyme. However, no effects are observed when added after the interaction, not so for Mo, although the effect is minor when added after the clay enzyme interaction (Rosas et al., 2008).

Stabilization of the activity of two commercial microbial phytases after immobilization in synthetic allophane, iron oxide-covered allophane and natural montmorillonite have also been studied. Immobilization improves thermal stability and resistance to proteolysis, and the residual activity of both phytases was higher under acidic conditions (Menezes-Blackburn et al., 2011).

Nanomaterials have become ubiquitous materials, as there are commercial products containing some sort of nanoparticle (Heiligtag and Niederberger, 2013; Nguyen and S., 2020). In Ecuador, products containing nanomaterials are consumed, and in most of these cases these products are not generated, despite having higher education institutions capable of conducting research in this area and with a source that can provide these materials.

There is growing interest in the development of natural nanoproducts, for example, in phyto and nanotechnology, medicine, nutrition, cosmetics and agriculture (Griffin et al., 2018). In this context, allophane can be used as a reinforcement material for the design of degradable packaging; in agriculture for a controlled release of fertilizers; in medicine for the release of drugs; in the oil area as a nanofluid for the drilling of wells; as nanocatalysts in the refining of crude oil (Rashidi et al., 2018), and in the environmental area for the reduction of pollution (Wilson, 2018).

Multidisciplinary groups, lines, programs and research projects in the area of nanomaterials are needed, as well as the support of the corresponding agencies to conduct studies of this type of soil and of deposits with a significant concentration of natural nanoparticles.

7 Conclusions

National research on Ecuadorian volcanic soils has focused on issues that are not related to the allophane nanoparticle, and research on allophane has been observed in recent years. However, investigations are conducted by institutions located outside the country. Allophane has unique and versatile properties such as a big surface area, variable load, high moisture retention and high porosity. It can be extracted from natural sources, but can also be obtained by the different synthesis methods mentioned. It has application potentials for environmental remediation, oil extraction, catalysis, photocatalysis, electrocatalysis, smart packaging, nano sensors, enzyme support, drugs and fertilizers.

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SOIL SCIENCE

EFFECTS OF HYDROGEL ON MOISTURE VOLUME IN SOILS WITH DIFFERENT TEXTURES

VOLUMEN DE HUMEDECIMIENTO POR LA APLICACIÓN DE HIDROGEL EN SUELOS DE DIFERENTES TEXTURAS

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Abstract

The objective of the study was to determine the wetting volume (wet bulb) of the hydrogel applied to three types of soil. Soils used were: a) clay (clay 52%; silt 32%; sand 16%); b) silty clay loam (36% clay; 56% silt; 6% sand) c) sandy loam (12% clay; 32% silt; 56% sand), to which 1% potassium hydrogel was applied. The application was made with previously hydrated hydrogel, in three diameters that were: 4.7, 7.0 and 10.5 cm with a length of 10 cm where the following initial volumes were obtained 173.5, 384.8 and 866 cm^3 occupied by the hydrogel. The wetting volume (cm^3), moisture percent, and the hydration of the hydrogel in the soil were measured. The results indicate that the humidification volume depends on the initial volume, so the higher the initial volume, the greater the humidification volume regardless of the type of soil; however, it presents a greater volume of humidification in the sandy loam soil, probably due to mobility of the water in it. The moistened area increases its humidity by 14% regardless of the type of soil. The hydration of the hydrogel in the soil only reaches 42% compared to hydrating it in free water. Determining the volume of wetting allows estimating the amount and location of hydrogel to be applied to a crop based on the bulb that needs to be formed in the soil.

Keywords: Soil hydration, wet bulb, increased humidity, sandy loam.

Resumen

El estudio tuvo como objetivo determinar el volumen de humedecimiento (bulbo húmedo) del hidrogel aplicado en tres tipos de suelo. Se utilizaron suelos: a) arcilloso (arcilla 52 %; limo 32 %; arena 16 %); b) franco arcilloso limoso (arcilla 36 %; limo 56 %; arena 6 %) c) franco arenoso (arcilla 12 %; limo 32 %; arena 56 %), a los cuales se aplicó hidrogel al 1 % de potasio. La aplicación se realizó con hidrogel previamente hidratado, en tres diámetros que fueron: 4.7, 7.0 y 10.5 cm con una longitud de 10 cm donde se obtuvieron los siguientes volúmenes iniciales 173.5, 384.8 y 866 cm^3 que ocupaba el hidrogel. Se midió el volumen de humedecimiento (cm^3), porcentaje de humedad y la hidratación del hidrogel en el suelo. Los resultados indican que el volumen de humedecimiento depende del volumen inicial, de manera que a mayor volumen inicial se tendrá mayor volumen de humedecimiento indiferente del tipo de suelo; sin embargo, el suelo franco arenoso presenta un mayor volumen de humedecimiento, seguramente por la movilidad del agua en el mismo. La zona humedecida incrementa su humedad en un 14 % indiferentemente del tipo de suelo. La hidratación del hidrogel en el suelo solo alcanza un 42 % en comparación con la hidratación en agua libre. La determinación del volumen de humedecimiento permite estimar la cantidad y ubicación de hidrogel que se debe aplicar en un cultivo en función del bulbo que se requiere formar en el suelo.

Palabras clave: Hidratación en suelo, bulbo húmedo, incremento de humedad, franco arenoso.

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1 Introduction

In agriculture, hydrogel is used for economic and agronomic benefits (Cisneros et al., 2020), in vegetables (Pereira et al., 2019) and perennial crops (M'barki et al., 2019). Hydrogel is a hydrophilic, soft, elastic polymer that expands with water, increasing its weight but without losing its structure, and it looks like crystals when dehydrated (Ahmed, 2015). Its goodness has allowed hydrogel to be used in areas such as biomedicine (Nicolson and Vogt, 2001; Amin et al., 2009) and in agriculture it has been used as an element to avoid water stress in crops (Dragusin et al., 1996), since water retention is its main characteristic (Satriani et al., 2018). The first studies in this area date back to the 1970s in the germination and growth of seeds (Palacios et al., 2016).

At the moment, its application is known and its varies depending on the crop and substrate or soil to be used. Agaba et al. (2011) recommend 0.4% of hydrogel in Agrostis stolonifera, and Montesano et al. (2015) recommend 2% in sandy soils. Jankowski et al. (2011) applied 50 gm^2 to different substrates and cultures. Rivera et al. (2018) achieved the highest yields when applying from 2 to 2.5 g/plant in pepper. However, it is worth mentioning that studies that show a high influence of hydrogel on crops have used high amounts of hydrogel. Maldonado-Benitez et al. (2011) recommend $4gL^{-1}$ or higher, and Chirino et al. (2011) mention that a 1.5% dose of hydrogel is effective.

On the other hand, the behavior of hydrogel is related to the physical characteristics of the soil, especially its texture (Rivera and Mesías, 2018), showing the dynamics of the behavior of the soil. Hydrogel has been widely used in soils with low water retention (Lopes et al., 2013; Rojas et al., 2004; Idrobo et al., 2010), and in agricultural areas with lack of rainfall or drought (Santelices, 2005), with several types of soil texture. In addition, most of the experiments carried out have been in pots and in mixed substrates or disturbed soil (Maldonado-Benitez et al., 2011; Idrobo et al., 2010; Najafi et al., 2013; Jankowski et al., 2011) which may have different behavior when applied in the field directly. Fonteno and Bilderback (1993) state that the effectiveness of hydrogel is determined by the type of soil or substrate, basically by its porosity. Barón et al. (2007) mention that hydrogel modifies the hydraulic dynamics of the soil. Orikiriza et al. (2013) identified that hydrogel caused increased survival of *Picea abies*, *Pinus syllivestris* and *Fagus sylvatica* in sandy soils.

Similarly, hydrogel is frequently associated with the management of water resources in crops, with regard to the irrigation interval (Wadas et al., 2010; Yazdani et al., 2007), particularly in forest crops (Hüttermann et al., 1999; Al-Humaid and Moftah, 2007; Agaba et al., 2010). However, studies also show that the presence of hydrogel does not have a significant influence. Wang (1989), studying the application of hydrogel in *Codiaeum variegatum*, found that the growth is the same compared to the control; however, it avoids its wilting. Geesing and Schmidhalter (2004) indicate that the application of hydrogel shows no benefit to the survival or growth of *Triticum aestivum*. This variation leads to the belief that there are aspects that are not considered at the moment of using hydrogel in crops. These include this area or volume of moistening (wet bulb) in the soil caused by hydrogel, which would be necessary to know the location of the product on the soil. Plant roots can expand their root system with a higher moisture content in the soil, allowing it to be more efficient even when the product is placed locally in the crops. Because of the latter, the aim of the research is to determine the volume of hydrogel moistening in different diameters and soil types.

2 Materials and Methods

2.1 Experimental material

The study was conducted at the Soil and Water Laboratory of the Lay University Eloy Alfaro de Manabí, Chone-Ecuador. NewGel G, distributed by Marketing of Ecuador and manufactured by Whidden Industrial Park was used as experimental material, which has the following composition: Potassium acrylamine polymer 99.9% and potassium 0.01%. Soils of fluvial origin (*Mollic Udifluvent.*) and coluvial origin (V*Vertic Hapluodoll*) were used. A texture analysis was performed by the pipetting method to separate the sand and clay particles, and the texture classification was done using the texture triangle (Moorberg and Crouse, 2017). Three textural classes were used for the study: A) clay (clay 52%; silt 32%; sand 16%); b) clay, silty and loamy (clay 36%; silt 56%; sand 6%) c) loamy sandy (clay 12%; silt 32%; sand 56%).

Table 1. Averages of the final parameters of the hole where hydrogel was placed in the different soils.

Sandy loamy soils			
Final diameter (*cm*)*	9.3±0.9	12.8±1.1	17.5±1.6
Final lenght (*cm*)*	13.2±0.2	13.7±0.1	1.7±0.3
Moistened volume (cm^3)*	723.2±24.2	902±35.2	1539.4±56.3
Silty clay loam soil			
Final diameter (*cm*)*	9.02±0.8	13±0.7	14.22±1.1
Final lenght (*cm*)*	13±1.8	12.3±1.3	12.2±1.2
Moistened volume (cm^3)*	657.2±22.4	1247.8±54.2	1071.6±48.6
Clay soil			
Final diameter (*cm*)*	7.1±0.6	10.6±1.1	14.06±1.7
Final lenght (*cm*)*	12.4±1.8	12.3±2.0	12.5±1.9
Moistened volume (cm^3)*	317.4±23.6	700.6±31.1	1074.8±49.3

*Initial parameters: Diameter (*cm*): 4.7; 7; 10.5; Length: 10 *cm*.
Volume: 173.5; 384.8; 865.9 cm^3; values for each column respectively.

2.2 Experimental management

A transparent container (glass) was used to observe the moisture caused by hydrogel in the soil. It had a capacity of 0.027 m^3 with dimensions of 0.30 m on all sides, in which the samples of the different soil types were placed. Soil samples were taken and dried once they were placed in the containers.

2.3 Moistening volume

Previously hydrated hydrogel was used as recommended by Rivera and Mesías (2018), who suggest 100 mL of water per gram of hydrogel. It was then placed in holes with the following diameters 4.7; 7.0 and 10.5 cm at a depth of 15 cm of which 10 cm were occupied, leaving 5 cm of soil covering the product. It was set aside for 24 hours without any sun protection. Moistening was measured from the edges of the product to the changes of dark tone caused by the moisture of the hydrogel in the soil, obtaining the diameter (*cm*), length (*cm*) and volume (cm^3); it was calculated by adjusting the values to the equation of a cylinder (1), obtaining an initial volume of 173.5; 384.8 and 866 cm^3.

$$V = \pi * r^2 * h \quad (1)$$

Where, V is the volume in cm^3, r is the ratio of the hole (*cm*) and h is the lenght (*cm*) of the hole with hidrogel.

2.4 Soil moisture

The moisture percentage was measured before and after (24 hours) hydrogel was applied. The soil section moistened by hydrogel was sampled and the moisture percentage was determined by gravimetry. The increase in humidity was determined, taking into account the initial soil moisture and the final moisture. This procedure was performed on each soil type and by triplicate.

2.5 Moisture of the hydrogel in the soil

To estimate the hydrogel hydration in the soil, 2 g of unhydrated hydrogel was placed in a sieve #60, which was placed in dry soil of the different soil types under study. This procedure prevented hydrogel from spreading at the time of hydration in order to measure its hydration. Sufficient water was then applied simulating surface and drip irrigation, until the soil was achieved at field capacity. After one hour of irrigation, the strainer was removed with hydrogel and the hydrated hydrogel was separated from the soil, the percentage of hydration was weighed and was estimated compared to free water hydration.

3 Results and discussion

3.1 Volume of soil moistening

Table 1 shows the final parameters of the moistened area in the different soils. It is observed that the final diameter of moistening is related to the initial diameter, with some tendency to increase as the initial diameter increases. The length varied due to an increase in the moisture of the lower part of the initial bulb; there was no significant increase in the upper part. The increase in the lower part had less variation with respect to the diameter, increasing to 4.7 cm in sandy-loamy soil. These parameters show the movement of the vertical and horizontal water in the soil, which is directly related to the texture of the soil and was observed when evaluating this variable in the different soils.

On the other hand, the final volume had a significant increase according to the type of soil, being the sandy loamy soil the one with the highest moisture content as stated by Rivera and Mesías (2018), who relate the behavior of the hydrogel to the texture of the soil and in turn to the specified surface of the soil (Ruiz et al., 2016). Narjary et al. (2012), mentions that hydrogel is usually more efficient in sandy soils than in heavy soils.

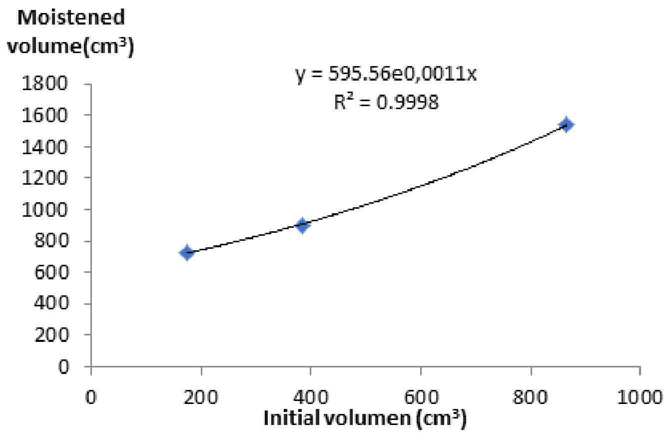

Figure 1. Ratio between initial volume and final moistening volume in sandy soil.

Sandy soils inflitrate a lot more water compared to heavy soils, so it can be deduced that water contained in the hydrogel capsule, when passing to the soil, has a similar behavior when applying irrigation water, with the only difference that the amount of water contained in the hydrogel is small compared to traditional irrigation, and this delivery of water to the soil occurs when the soil exerts a high matrix pressure and when the soil has less moisture content, which is consistent because the lower initial moisture content (sandy-loamy) was presented by the soil with higher moisture volume. However, the latter should be corroborated in experiments, since it was not studied in this research. On the other hand, the initial volume is directly proportional to the moistening volume, although this condition was only met in sandy, clay loamy soil (Figure 1 and 3), while the clay-loamy soil did not present any tendency (Figure 2). The determination of the wet bulb formed by hydrogel is important to decide where to place the product according to the root system, in order to estimate the amount of hydrogel to be applied and the diameter and depth of the hole where the hydrogel will be placed, so that the roots can catch water for their development.

3.2 Soil moisture

The moisture percentage of the soil moistening is shown in Table 2, where moisture has increased 14 % regardless the type of soil. These values are similar to those found by Rivera and Mesías (2018) although they differ in loamy soils, where the increase was 17.4 %. This could suggest a 14 % increase of soil moisture in dry soils. It is posible that the soil with low humidity present soil moisture because of hydrogel; therefore, it is necessay to conduct studies with different soil moisture levels.

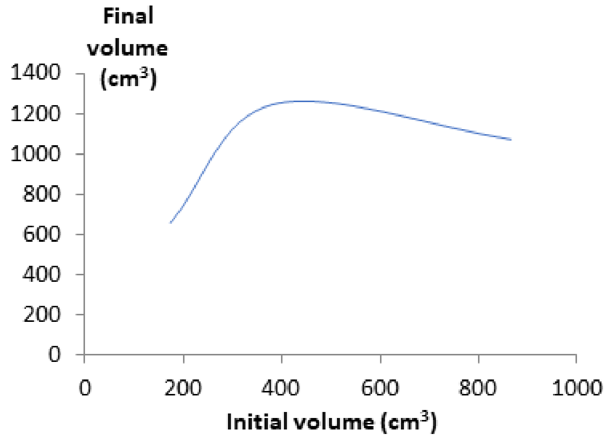

Figure 2. Initial and final volume in loamy soils.

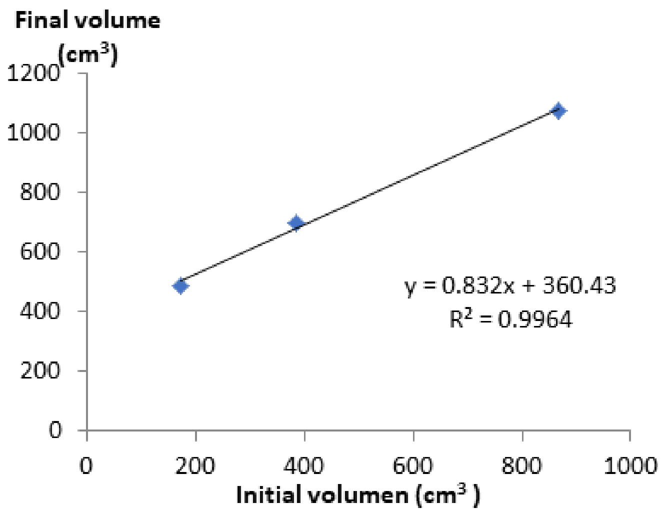

Figure 3. Relationship between the initial and final volume in clay soils.

3.3 Hydration of hydrogel in the soil

The hydration of the hydrogel in the soil is lower than the hydration percentage in water, only as high as 42 % with respect to the hydration in water (Figure 4). Hence, if using hydrogel on the soil without prior hydration, it must be taken into account its ability to hydrate the soil and thus the plant. Information on this hydrogel hydration in soil is not defined in the scientific literature, although it is widely recommended in crops, mainly in forestry crops. The fact that it is not hydrated in equal proportion to the hydration that occurs in free water may indicate the soil will resist and the hydrogel will not expand normally, and therefore will not absorb water fully.

4 Conclusions

The volume of hydrogel moistening occurs depending on the type of soil and the initial volume of hydrogel in the soil. Sandy-loamy soil finds it easier to form a larger wet bulb unlike other soils. The area moistened by the hydrogel has increased humidity in about 14 % with respect to the initial content and regardless the type of soil, with the possibility of preparing the soil to the field capacity.

Table 2. Increase of soil moisture applying hydrogel in all three soils.

Type of soil	Initial moisture % (Dry soil)	Moistening percentage (%)
Sandy	4.7	18.8±0.8
Loamy	6.5	20.8±1.2
Clayey	8.6	22.8±1.5

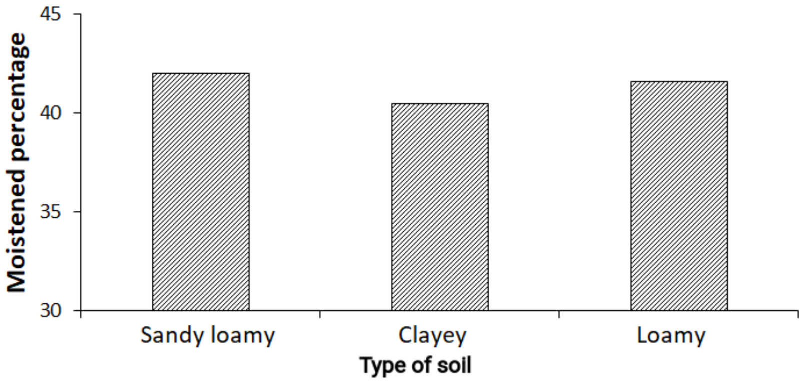

Figure 4. Percentage of hydrogel hydration in the soil with respect to hydration in free water.

When applying unhydrated hydrogel to the soil, it cannot be hydrated as equal as with free water, because the soil is pressurized and makes it difficult for the hydrogel particle to be hydrated normally. In all soils, 42 % of hydration was achieved with respect to free water hydration. It is important to consider that knowing the behavior of hydrogel in soil will optimize its use; therefore, studies should be conducted on its stability, rehydration and soil duration in crops under production.

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Scientific paper/ Artículo científico
Agriculture and Climate Change

Eco-efficiency of the models of agricultural production of hard corn and its influence on climate change in Shushufindi Ecuador

Ecoeficiencia de los modelos de producción agrícola de maíz duro y su influencia al cambio climático en Shushufindi Ecuador

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Abstract

Eco-efficiency is defined as the quantity or economic value of a product by its environmental influence, and it identifies the sustainability of a system in socio-economic and environmental aspects. The objective of this research was to evaluate the ecoefficiency of three models of agricultural production of hard corn present in the Shushufindi canton, Ecuador. The models identified in the study were the conventional model (MC), semi-conventional (MS) and traditional (MT). The environmental influence was determined through two impact indicators contemplated in the Life Cycle Analysis, such as GHG emissions, according to the IPCC guidelines (IPCC, 2006a), and the water footprint (HH) through the components stated by Hoekstra et al., (2011). For the evaluation of ecoefficiency, the guidelines of Ribal et al. (2009) were considered, applying a non-linear programming optimization (LPG) model. GHG emissions per MC were 2926.92 kgCO_2eq ha^{-1} year^{-1} and an HH of 1157.86 m^3 ton^{-1}, MS contributed 1209.45 kg CO_2eq ha^{-1} year^{-1} and a HH of 1201.85 m^3 ton^{-1}, while the resulting MT emissions were 570 kg CO_2eq ha^{-1} year^{-1} and a HH of 1008.16 m^3 ton^{-1}, and it was determined that the MT is the most eco-efficient model with a value of 0.99. The results allowed to know the impacts associated to the models of agricultural production of maize, its contribution to the Climate Change (CC) in sensitive ecosystems like those of the Ecuadorian Amazon, so that in this way sustainable agricultural practices are implemented.

Keywords: Climate Change, greenhouse gases, water footprint, eco-efficiency, corn.

Resumen

La ecoeficiencia se define como la cantidad o valor económico de un producto por su influencia ambiental e identifica en términos socioeconómicos y ambientales la sostenibilidad de un sistema. El objetivo de esta investigación fue evaluar la ecoeficiencia de tres modelos de producción agrícola de maíz duro presentes en el cantón Shushufindi, Ecuador. Los modelos identificados en el estudio fueron el modelo convencional (MC), semi-convencional (MS) y tradicional (MT). La influencia ambiental se determinó mediante dos indicadores de impacto contemplados en el Análisis de Ciclo de Vida, como son las emisiones de GEIs, según las directrices del IPCC (2006a) y la huella hídrica (HH), a través de los componentes dados por Hoekstra et al., (2011). Para la evaluación de la ecoeficiencia se consideraron los lineamientos de Ribal et al. (2009), aplicando un modelo de optimización por programación no lineal (GLP). Las emisiones de GEIs del MC fueron de 2926,92 kgCO_2eq ha^{-1}año^{-1} y una HH de 1157,86 m^3 ton^{-1}, el MS contribuyó con 1209,45 kgCO_2eq ha^{-1}año^{-1} y una HH de 1201,85 m^3 ton^{-1}, mientras que las emisiones del MT fueron de 570 kgCO_2eq ha^{-1} año^{-1} y una HH de 1008,16 m^3 ton^{-1}. Se determinó que el MT es el modelo más ecoeficiente con un valor de 0,99. Los resultados permitieron conocer los impactos asociados a los modelos de producción agrícola de maíz y su contribución al Cambio Climático (CC) en ecosistemas sensibles como los que alberga la Amazonía ecuatoriana, para que de esta manera se implementen prácticas agrícolas sostenibles.

Palabras clave: Ecoeficiencia, GEIs, huella hídrica, Cambio Climático, maíz.

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1 Introduction

Among the agricultural production problems that have arisen are the environmental impact associated with the type and dose of fertilizers and plant protection, the use of machinery, irrigation systems, certified seeds, among others, which impact the water, soil and air resources. However, various options arise for establishing better agricultural practices that integrate environmental and economic aspects, promoting sustainability in agricultural production. In this sense, the concept of ecoefficiency originates, which is defined by Masuda (2016) as the quantity or value of the product by the environmental influence and "economic value/environmental impacts". In the case of eco-efficiency in agriculture, (Rodríguez, 2018) conceptualizes eco-efficiency as the capacity of a land-use system to be sustainable in economic, social and environmental aspects. In this context, greenhouse gases (GHG) are important factors of climate change (CC) because of their global warming potential (GWP) (IPCC, 2013). The increase in the GHG is associated with activities carried out by the economic sectors, such as the agricultural which has contributed to 24% of global emissions (IPCC, 2014). The IPCC (2015) reported that emissions from the global agricultural sector were 11.7 Gt CO_2eq. In the case of Ecuador, emissions in 2012 were 14 512.88 Gg of CO_2eq, corresponding to gases such as CO_2, CH_4 and N_2O from agricultural soils (46.37%), enteric fermentation (43.43%), rice cultivation (7.48%), manure management (2.34%) and the burning of agricultural waste (0.39%) (MAE, 2017). The increase in these GHG can cause serious ecological and economic changes, as well as unpredictable changes in climate systems (OMM, 2017).

Considering that the agriculture is one of the most demanding areas of GHG, in 2018 temporary crops accounted for 15.1% of Ecuador's total agricultural area, corresponding to 5.3 million hectares (INEC, 2019). Temporary crops with the greatest cropped areas are dry hard maize (40.7%), rice (32.1%) and potato (2.5%) (INEC, 2019). The agriculture present in the Ecuadorian territory has been replacing native ecosystems such as the moors and the forests, as evidenced between 2008 and 2014, where the expansion of the maize crop caused most of the change in land use with 42%, followed by cocoa (15.32%), African palm (14.5%) and coffee (11,18%) (Lasso, 2017). Besides, 80% of maize is used as raw matter in the industry for elaborating food for animals such as birds and pigs (Baca, 2016).

In Ecuador, Los Ríos is the province with the highest production of dry hard maize with 38.8% of the national total, corresponding to 602 thousand Tm and a planted area of 383 399 ha (INEC, 2019). According to data from the Continuous Agricultural Production and Surface Survey (ESPAC), maize production in Los Ríos in 2017 decreased by 4.88%, and an increase in maize production was observed in provinces that were not producers of this grain on a large scale, such as in the province of Sucumbíos, which had a total sown area of 1.99% of the national area equivalent to 7732 ha (INEC, 2017). In Shushufindi, agricultural maize production is made up of small and medium producers distributed throughout the territory. In parishes such as Central Shushufindi, Siete Julio and San Roque approximately 1018 ha of maize are planted (GAD Shushufindi, 2015).

Maize is traditionally grown under a family farming dynamic with crop rotation and association (GADP Limoncocha, 2015). On the other hand, the community production model is being replaced by a mechanized and industrial production model (Maza, 2015), and it is accompanied by technology containing certified seeds and inputs such as fertilizers, herbicides, insecticides, as well as machinery such as threshing, maize harvesters and tractors (GAD Provincial Sucumbíos, 2015; MAG, 2017; GADPR Siete de Julio, 2018). For all of the above, the objectives of the research are to characterize the agricultural models of hard maize present in Shushufindi; to estimate the emissions of GHG and freshwater consumption; and to determine the eco-efficiency of maize production models.

2 Methods

The investigation was based on the guidelines proposed by Ribal et al. (2009). The first step was to specify and characterize the scenarios or models to be studied. An environmental evaluation of the models was carried out using the Life Cycle Analysis (LCA), which includes two impact categories: Global Warming (GHG emissions) and Freshwater Consumption (water footprint). Additionally, an economic evaluation of these models was carried out through the K&K model developed by Kuosmanen and Kortelainen. Finally, the two previous evaluations were integrated using a non-linear GLP (Graphic Linear Optimizer) programming model to determine in this way which traditional, semi-conventional or conventional model is more eco-efficient in socio-economic and environmental terms.

2.1 Characterization and identification of models

Three agricultural maize models present in the study area were identified, the conventional model (CM), the semi-conventional model (SC) and the traditional model (TT). These models were characterized taking into account Martinez's attributes (Martínez, 2008) and other attributes suitable for the study as presented in Table 1.

In addition, CM, SC and TM models were georeferenced with the help of an unmanned aerial vehicle (UAV). The information was then processed in a GIS (ArcGIS ®), in which the location and area of the plots identified for each model was identified (Table 2). The geographical distribution of maize cultivation in Shushufindi was carried out using data provided by the SIPA inventory (Agricultural Public Information System) (Figure 1).

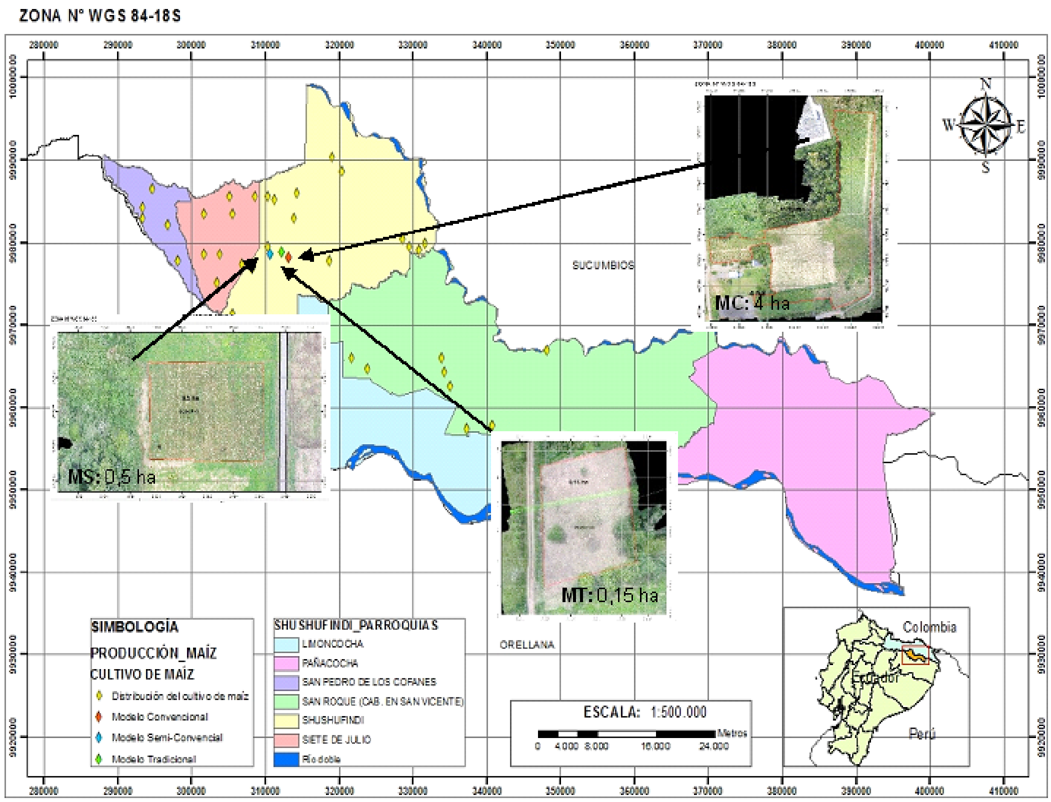


Figure 1. Geographical distribution of maize crops in the parishes of San Pedro de los Cofanes, Siete de Julio, San Roque, Shushufindi, and the location of study plots for each model CM, SM and TM. Source: Essential GPS data, georeferencing of drone Phantom 4 plots, National Information System (SNI), (GAD Shushufindi, 2015)

Once the models were identified, semi-structured interviews were conducted with the maize producers in order to collect information for socioeconomic and environmental factors, performing a convenience and consecutive sampling (non-probabilistic sampling).

Table 1. Characterization of the maize production models in Shushufindi

	Scenario 1	**Scenario 2**	**Scenario 3**
Characterization Attributes	**Conventional model (CM)**	**Semi-conventional model (SC)**	**Traditional model (TM)**
Energy	Predominates the use of fossil energy (gas and oil)	Predominates the use of fossil energy (gas and oil) – or alternatives (sun)	Use of renewable energy (sun). Less use of fossil fuel
Scale	Production area >1 ha	Plot of 0.5 ha area of 0.71 ha $\leq$ 1 ha	Plot as a production area
Objetive	Commerce	Self-consumption commerce	Self-consumption (Little or very Little to the commerce)
Workforce	Wage	Wage-family	Familiar - community
Diversity	Monocrop Low diversity	Monocrop – Associated Low diversity	Multicrop – High diversity
Productivity	"Irregular in the time, with high working productivity; low ecological and energetic productivity" (Martínez, 2008)	"Irregular in the time, with high working productivity; low ecological and energetic productivity" (Martínez, 2008)	"Regular in the time. High ecological-energetic productivity; low productivity at work" (Martínez, 2008)
Seed	Modified, hybrid	Cured	Creole
Machinery and tools	Large size agricultural machinery (destemmer, harvester, scythe)	Artisan agricultural machinery (artisan destemmer, scythe)	-There is not any use of agricultural machinery. -In this research, the producer used a scythe
Inputs	-Phytosanitary -Synthetic fertilizers	-Phytosanitary -Synthetic fertilizers	-Organic manure -Compost
Agricultural practice	-Without crop rotation	-There may or may not be crop rotation	-Crop rotation
Presence of pests	Yes	Yes	Yes-No
Pest control	Agro-chemical	Agro-chemical	Natural control
Wastes	-Incorporation of wastes, burning -Agro-chemical wastes	-Incorporation of wastes, burning -Agro-chemical wastes	-Incorporation of wastes -Production of organic wastes
Knowledge	Specialized, conventional science, standardized	Local-conventional	Local, traditional based in limited beliefs and knowledge and permacultural knowledge
Cosmovision	Market-based: "Nature is a separate system of society, whose richness must be exploited through science and technology" (Martínez, 2008)	Market-based	Eco-based: "Nature is a living and sacral identity. Nature is embodied in deity with whom the producer must dialogue during appropriation" (Martínez, 2008)

Source: (Martínez, 2008) Observations in the field and semi-structured interviews

Table 2. Description of the area and location of the research plots for each model studied

Type of production model	Sampled area		Location		
			X*	Y*	Place
(CM)	4	ha	310599	9978558	Atahualpa Route
(SC)	0.5	ha	310662	9978571	Los Ríos-Land Nº 11 Area belonging to the employee association of Shushufindi.
(TM)	0.15	ha	312274	9978832	Shushufindi Route-RICAAMA farm**

* Coordinates of the plots under study in UTM WSG 84 18S
** RICAAMA: Richness of the Amazon field – permacultural farm.

Soil-root and biol samples were collected in the field for further analysis in the laboratory. The edaphological parameters analyzed in the samples were organic matter (OM), organic carbon (OC), pH, texture and humidity; and nitrogen (N), phosphorus (P) and potassium (K) were analyzed in biol. All samples were analyzed in certified laboratories (LABSU and AGROCALIDAD).

2.2 Environmental evaluation

The environmental influence was determined by the Life Cycle Analysis for Agriculture "LCA Agriculture" following the methodology of Arango et al. (2014) and the recommendations of Ramírez-Cando and Spugnoli (2016), Oliveral et al. (2016), Ramírez-Cando et al. (2017) and IHOBE (2009).

2.2.1 Limitations of the system to be evaluated

The system evaluated was limited from the agricultural production phase to the maize distribution phase. Inputs (resources, raw materials, inputs, transport, energy, etc.) and outputs (air emissions (GGG-GWP), water and soil, waste and by-products) are included, as observed in Figure 2.

2.2.2 Impact categories

The study considered two impact categories: global warming (kg CO_2 equivalents) and water consumption (m^3/ton), estimated through the water footprint.

a) **GHG emissions**

The IPCC 2006 guidelines for the AFOLU sector (IPCC, 2006a,b) were considered for estimating GHG emissions. In addition, the recommendations of the GHG protocol (WRI & WBCSD, 2011) and the guidelines taken from Agri-footprint (Durlinger et al., 2017a,b). were followed. It should be noted that the study considered sources of GHG emissions (E) with an impact of more than 1 % according to the "cut-off" IPCC criterion, as follows:

- E for the use of fuels and lubricants.
- E for fertilizers (organic-synthetic) N,P,K.
- E of CO_2 by the application of urea.
- E of N_2O by N applied on managed soils.
- E for the application of plant protection.
- E for maize seed.
- E because of the burning of agricultural waste.

GHG emissions were estimated using the general Equation given by (WRI & WBCSD, 2011) (Equation 1). Where DA represents the magnitude-amount of an input used in a place over a period of time and in a certain area, e.g. the amount of fuel used by the tractor. FE refers to the coefficients that quantify emissions or removals of a gas depending on activity data. The emission factors for the study were those determined by the IPCC and BioGrace (2011). GWP is the Global Warming Potential for CO_2 (1), CH_4 (25) and N_2O (298) gases for a 100-year time horizon (IPCC, 2007). It is important to mention that for comparative purposes, the activity data of the TM and SM models were extrapolated to one hectare.

$$kgCO_2eq/ha = DA * FE * GWP \quad (1)$$

b) **Consumption of fresh water (water footprint WF)**

The components of Hoekstra et al., (2011) were used to measure green water footprint WF_{green} (precipitation) and gray water footprint WF_{gray} (freshwater pollution), thus allowing to know the total volume of fresh water used by maize crops in Shushufindi. It is important to mention that irrigation is not applied at the area under study due to the significant rainfall, so the blue water footprint WF_{blue} (WF component) associated with precipitation was not evaluated. Using Equation 2 (Pérez, 2012), the WF of the maize crop was calculated for each agricultural production model studied.

$$WF_{crop} = WF_{green} + WF_{Gray} \left(\frac{m^3}{ton}\right) \quad (2)$$

Finally, CROPWAT 8.0 ® program developed by the Food and Agriculture Organization of the United Nations (FAO) and tabulations in EXCEL were used to calculate WF, followed by the Water Footprint Network (WFN) and FAO Water Footprint Assessment Manuals by Franke et al. (2013).

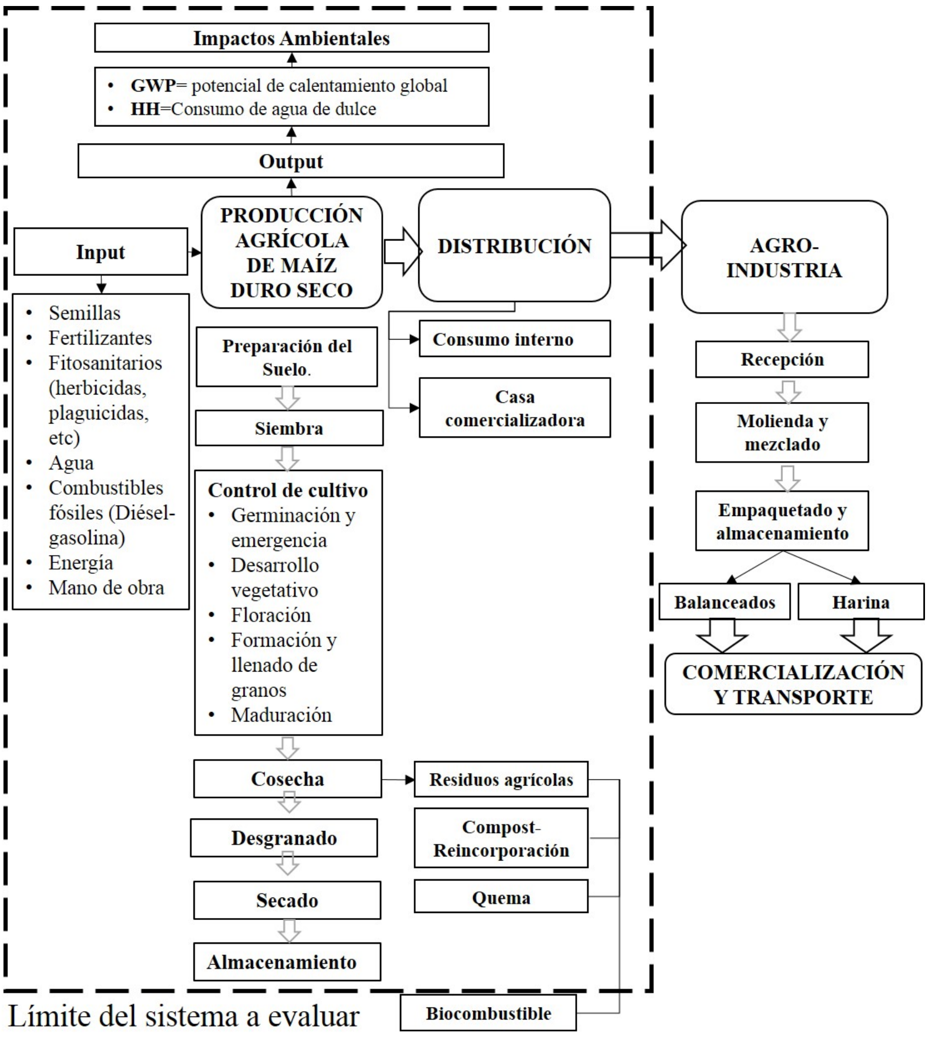


Figure 2. Limitations of the evaluated system - agricultural LCA of hard maize. The system evaluated ranges from maize production to distribution, excluding the agro-industry, marketing and transport phases.

2.3 Economic evaluation

K&K model developed by Kuosmanen and Kortelainen and described by Ribal et al. (2009) was applied. The partial accumulation of costs throughout the agricultural maize production process (seed, fuels, fertilizers, plant protection, wages, inputs, rental of machinery) was considered for each model analyzed ($ha/year). In addition, the profitability of maize production was quantified using Equations 3, 4 and 5, raised by Ayala-Garay et al. (2013).

$$\text{Profitability} = IT - CT \tag{3}$$

$$IT = P_y Y \tag{4}$$

$$CT = P_x X \tag{5}$$

Where IT is the total income $\left(\text{ha}^{-1}\right)$, CT is the total production cost, P_y is the market price of the crop Y ($\$/ton$), Y is the crop yield (ton ha^{-1}), P_x represent the price of the input or activity X (ton ha^{-1}) and X is the activity or input.

2.4 Ecoefficiency

Ecoefficiency was assessed using Rincón and Wellens (2011), as presented in Equation 6.

$$Ecoefficiency = \frac{Value\ of\ the\ product\ or\ service}{Environmental\ influence} \tag{6}$$

From Equation 6, (Ribal et al., 2009), propose a nonlinear programming model for m scenarios (CM, SC, TM models) and n impact categories (GHG-WF emissions) (Equations 7, 8, 9). This calculation was conducted in Microsoft Excel using the Solver application.

$$\underset{x}{maw} EE\ i = \frac{V_i}{w_1 \cdot z_{i1} + w_2 \cdot z_{i2} + \cdots + w_n \cdot z_{in}} \tag{7}$$

Subjected to:

$$\frac{V_1}{w_1 \cdot z_{i1} + w_2 \cdot z_{i2} + \cdots + w_n \cdot z_{in}} \leq 1 \tag{8}$$

$$\frac{V_m}{w_1 \cdot z_{m1} + w_2 \cdot z_{m2} + \cdots + w_n \cdot z_{mn}} \leq 1 \tag{9}$$

And $w_1, w_2, \ldots, w_n \geq 0$. Where V_i is the economic value added to scenary $i = 1\ldots, m$ $/ton, w_i is the weight of the environmental impact $j = 1,\ldots n$, z_{ij} is the environmental impact (gray footprint, green footprint and GHG) with $j = 1,\ldots,n$ by functional unit for the scenario $i = 1,\ldots,m$. The eco-efficiency index varies between 0 and 1, where value 1 will indicate that the scenario is eco-efficient (Ribal et al., 2009).

3 Results and discussion

3.1 Inputs y outputs

Table 3 presents the inputs and outputs of the models evaluated by taking into account laboratory results, interviews with producers and field observations.

3.2 Category of global warming

3.2.1 GHG Estimation of maize production models

The agricultural maize production model with the greatest contribution of GHG was CM with an estimated emission of approximately 2926.92 kg CO_2eq ha^{-1}year^{-1}, followed by SC emissions that emitted 1209.45 kg CO_2eq ha^{-1}year^{-1}, while TM emissions were lower with 570 kg CO_2eq ha^{-1}year^{-1} (Table 4).

This study showed that TM emissions are 80% lower than those of CM and 57% lower than those of SC. Similarly, a study conducted by Eranki et al. (2019) reported that were 41% lower in an organic farming scenario emission than emissions from conventional agriculture.
Values of 145.32 (TM), 561.21 (SC) and 460.91 (CM) kgCO_2eq/ton (Figure 4-Table 6) were also reported, compared to the study conducted by Altuna et al. (2012) where the carbon footprint of maize was determined to be 514.76 kg CO_2 eq/ton of product, which is higher than other cereals such as wheat (380.87 kg CO_2eq/ton) and barley (297.75 kg CO_2 eq/ton). It is important to mention that regardless the model, SC and CM emissions are higher than those reported in Peru by mechanized maize production with 224 kg CO_2eq ton^{-1} (Morales et al., 2018).

3.2.2 Emissions from the use of fertilizers

Emissions from the application of fertilizers with NPK inputs were 54.26 (TM) and 4.49 (SC) kg of CO_2 eq ha^{-1} year^{-1} and 1032 (CM), kg of CO_2eq ha^{-1}year^{-1}. In addition, CM contributed to 133 kg of CO_2eq year/ha by fertilization with urea. Abrahão et al. (2016) reported that the main source of GHG emissions were the use of liquid fertilizers (69 %) and 18 % by the use of compound fertilizers, providing a carbon footprint for maize production of 1700 kg of CO_2eq year/ha.

Table 3. Inputs and outputs of the evaluated models

DESCRIPTION		QUANTITY TM	QUANTITY SC	QUANTITY CM	UNIT
Inputs*		**TM**	**SC**	**CM**	
Synthetic fertilizers	**10-30-10**	-	2	1088.62	kg/ha $year^{-1}$
	N	-	0.2	108.86	
	P	-	0.6	326.59	
	K	-	0.2	108.86	
	13-40-13	-	-	2	
	N	-	-	0.26	
	P	-	-	0.80	
	K	-	-	0.26	
	Thickener	-	4	-	
	N		0.33	-	
	P		0	-	
	K		1.16	-	
Biol	**Leachate of compost + urine**	16	-	-	L/ha $year^{-1}$
	N	8.96	-	-	kg/ha $year^{-1}$
	P	0.35	-	-	
	K	2.13	-	-	
Urea		-	-	181.82	kg/ha $year^{-1}$
				83.64	
Plant production		-	0.43	1.25	kg/ha $year^{-1}$
Fuels	Gasoline	6.28	22.75	9,46	gal/ha $year^{-1}$
	Diesel	-	-	29	
	Oil 2T	0.35	0.53	-	
Seed		48.38	45.36	40	kg/ha $year^{-1}$
Water	Irrigation	0	0	0	m^3/ha $year^{-1}$
Work		1366.67	552	121.68	h/ha $year^{-1}$
Outputs**					
Hard maize		3.92	2.40	6.35	ton/ha $year^{-1}$
Agriculture waste		4.49	3.16	7.28	

* **inputs:** Quantity of inputs, resources and energy employed by maize producers for the production of a maize hectare
** **outputs:** Quantity of by-products (maize) and agricultural wastes obtained in the production of a maize hectare
\- Inputs not used by maize producers

3.2.3 N_2O Emissions

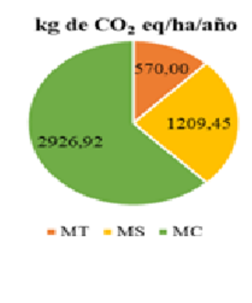
The use of synthetic, organic fertilizers and the breakdown of stubble are responsible for significant N_2O emissions due to nitrification, denitrification, leaching-volatilization and runoff processes in the soil.

These emissions were 443.93 (TM), 234.72 (SC) and 1279.81 (CM) kg of CO_2 eq ha^{-1} $year^{-1}$; CM is the model with the greatest contribution of emissions emitted to the atmosphere.

3.2.4 Emissions from the use of fossil fuels

Emissions from the use of fossil fuels (gasoline, diesel and lubricants) by agricultural machinery such as tractor, combine, threshing and transportation) had an emission contribution to the atmosphere of 406.49, 196.30 and 54.88 kg CO_2eq $ha^{-1}year^{-1}$ for CM, SC and TM models, respectively. Figure 5 shows the percentage and kg of CO_2eq ha^{-1} $year^{-1}$ emitted by the machinery used in each model studied.

Table 4. Total emissions from the models studied.

TOTAL OF GHG EMISSIONS	Agricultural production models of hard maize			
	TM	SC	CM	
kg of CO_2 eq/ha/year	570.00	1209.45	2926.92	
Contribution % of GHG	13	27	60	
$kgCO_2eq/ton$	145.32	561.21	460.91	

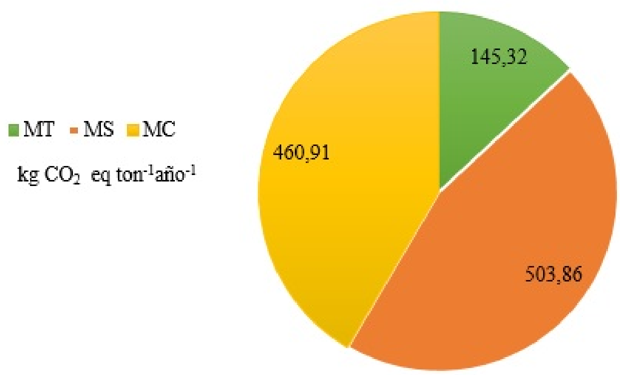


Figure 3. GHG emissions by the studied models (kg CO_2eq ton^{-1} $year^{-1}$)

3.2.5 Emissions from phytosanitary

Agricultural production models reported emissions from phytosanitary applications of 9.48 kg of CO_2 eq ha^{-1} (SC) and 60.34 kg of CO_2 eq ha^{-1} $year^{-1}$ for the CM model, while TM did not report emissions from this source, since the producer does not apply any type of plant protection.

According to research conducted by Morales et al. (2018), the use of pesticides in mechanized maize crops contributed to 205 $kgCO_2$eq ha^{-1}, being higher than those estimated in the present study. Table 5 presents the contribution of GHG emissions from each phytosanitary system used by CM and SC models.

3.2.6 Emissions from the seed input

The use of seeds contributed to 2.97%, 1.18% and 0.48% for TM, SC and CM models, respectively, corresponding to emissions of 16.93 (TM), 15.88 (SC) and 14 (CM) kg of CO_2eq ha^{-1} $year^{-1}$, and similar to those reported by Abrahão et al. (2016), that contributed to 3% to emissions.

3.3 Water footprint

3.3.1 Water footprint of the crop (WFcrop)

Maize WFcultivation was 1008.16 (TM), 1201.85 (SC) and 1157.86 (CM) m^3/ton (Figure 6), showing a higher impact on CM and SC models, as the volume of fresh water used directly or indirectly to produce maize is very high compared to TM. In SC, 1153.75 m^3/ton of WFgreen was obtained, higher than TM with 1 008.16 m^3/ton of WFgreen. On the other hand, the conventional model had a green footprint of 599.69 m^3/ton, which depended on the performance (ton/ha) presented by the conventional model compared between the SC and TM models.

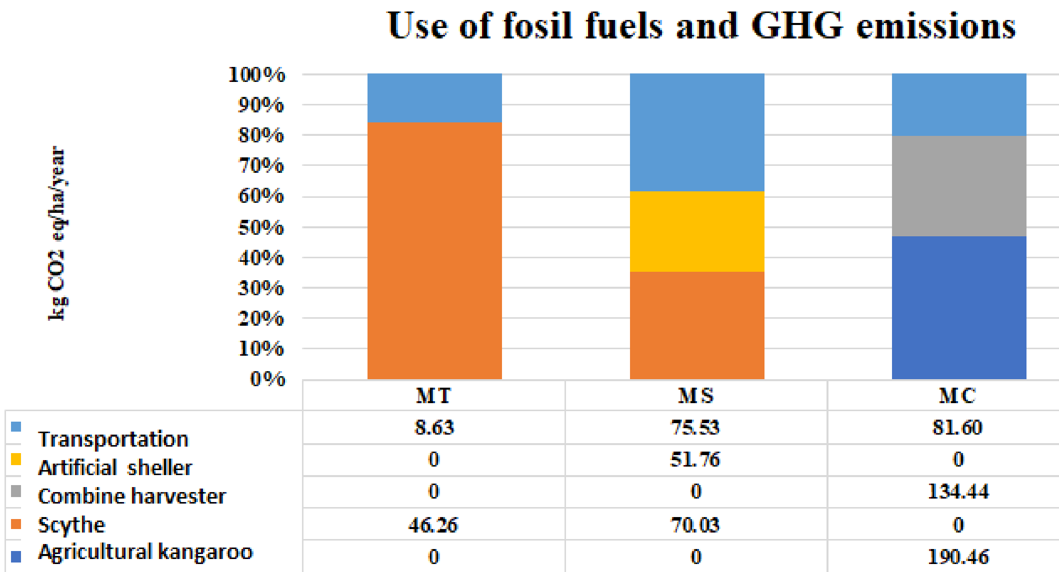


Figure 4. Participation of GHG by machinery and transport.

Table 5. GHG emissions by phytosanitary

Phytosanitary		**Quemante (Herbicide)**	**TEJO (insecticide)**	**NOSTOC (Herbicide)**	**Total of emissions**
		kg de CO_2eq ha^{-1} $year^{-1}$			
Model	CM	27.425	32.91	-	60.34
	SC	-	8.78	0.7	9.48

The WFgreen of the maize crop in Sucumbíos was 2073 m^3/ton according to Pérez (2012); this footprint is higher than the estimated in the three models analyzed in the present study. In addition, Romero et al. (2016) reported that the average green footprint represents 60 % of the total agricultural footprint (820.24 m^3/ton) in maize crops in Colombia, and green WF represents 52 % compared to CM.

As for the gray footprint, this was 48.10 and 558.17 m^3/ton for SC and CM, respectively. For its part, TM did not report gray WF values because no synthetic or phytosanitary fertilizer was used. In the province of Sucumbios, Pérez (2012) reported a gray footprint of 330 m^3/ton for the cultivation of maize, which is smaller than the gray footprint of the CM of this study, because this model has a high consumption of agricultural inputs such as fertilizers and plant protection.

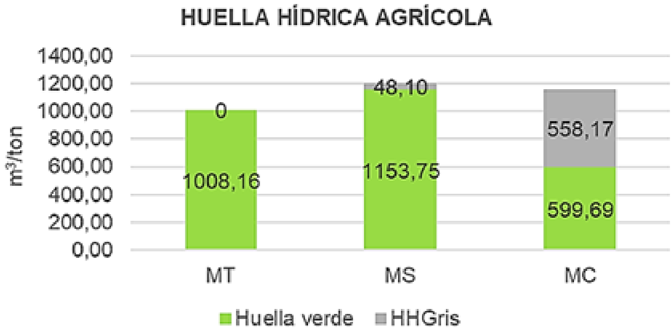


Figure 5. WFcrops of the maize production models in Shushufindi.

3.3.2 Economic evaluation

Table 6 presents the cost balance for the maize production by model studied, and it shows that maize production is unprofitable due to the high costs of agricultural inputs, with very low profits.

3.4 Ecoefficiency

The weights for each impact category were 1 for gray footprint, 0.41 for green footprint, and 0.92 for GHG emissions (Table 7). The traditional model is the most eco-efficient model in environmental and economic terms with 0.99 eco-efficiency.

Table 6. Cost balance for the maize production in the studied model

Model	TM	SC	CM
	($/ha year^{-1})		
Incomes	863.00	756.00	2100.00
Costs	312.95	521.21	1712.85
Seed	0.00	100.00	176
Fuels	23.45	27.71	12.35
Fertilizers	0.00	8.00	286.00
Phytosanitary	0.00	64.00	84.00
Wages	270.00	240.00	540
Inputs	9.50	12.50	34.50
Rent of machinery	10.00	69.00	580.00
Profitability	550.05	234.79	387.15

Eco-efficiency is influenced by the hotspots or critical points that each impact category has (green footprint, gray and GHG emissions); for this reason, the WFgray category has greater weight, due to the high use of synthetic and phytosanitary fertilizers used by SC and CM models. For the GHG emissions category, the hotspot is influenced by the high consumption of nitrogen fertilizers, which generates significant N_2O emissions from managed soils. These two components double their weight (importance for a more efficient management of the process), with respect to WFgreen, considering that the GHG and WFgray have their greatest influence from the use of fertilizers.

This hotspot is the first to be taken into account to improve the ecoefficiency of conventional crops or combined maize production systems. It is important to mention that ecoefficiency is relative to the models studied, i.e., there may be other agricultural practices that make the models more environmental and socio-economic efficient (Ribal et al., 2009).

Table 7. Ecoeficiencia de los modelos de producción de maíz

Impact categories (Z)	**WFgray**	**WFgreen**	**GHG Emissions**
Weight (W)	W_1	W_2	W_3
	1	0.41	0.92
max. EE	0.999999617		
Ecoefficiency of agricultural production models			
$\mathbf{EE}_{SC}$	0.24		
$\mathbf{EE}_{CM}$	0.32		
$\mathbf{EE}_{TM}$	0.99		

4 Conclusions

Using the eco-efficiency index, it was possible to determine that the most economic and environment sustainable model of agricultural maize production is the traditional TM model, since it showed an eco-efficiency of 0.99. This high ecoefficiency is due to the fact that this model does not depend on inputs such as plant protection and synthetic fertilizers which are expensive. In addition, the use of these agrochemical inputs has a significant environmental impact on the environment, as evidenced by the different categories of impact.

Although the TM model is more eco-efficient, it is very little used by the producers, because it demands more working hours and low yield (Table 3) compared to a conventional model (Pinzón and Ramírez, 2019); which does not satisfy the economic demand. However, this type of TM model is more environmentally friendly, since it does not require technology but instead it uses unmodified creole seeds from the previous crop and do not need any plant protection or chemical fertilizers. Finally, this model preserves family farming, and sustainable agricultural practices such as crop rotation for pest control, as well as a vision for permaculture (Pinzón and Ramírez, 2019).

Finally, the results made it possible to know the impacts associated with agricultural maize production models and their contribution to Climate Change (CC) in sensitive ecosystems such as those of the Ecuadorian Amazon, so that sustainable agricultural practices can be implemented.

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HYDRAULIC

ON THE EFFECT OF THE REFINEMENT OF THE ROUGHNESS DESCRIPTION IN A 2D APPROACH FOR A MOUNTAIN RIVER: A CASE STUDY

EFECTO DEL REFINAMIENTO DE LA DESCRIPCIÓN DE LA RUGOSIDAD EN UNA APROXIMACIÓN 2D PARA UN RÍO DE MONTAÑA: UN CASO DE ESTUDIO

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Abstract

The prediction of water levels in rivers is important to prevent economical as well as human losses caused by flooding. Hydraulic models are commonly used to predict those water levels and take actions to mitigate flooding damage. In this research, a 2D approach to solve the depth average Reynolds Average Navier Stokes (RANS) equations, called Conveyance Estimation System (CES), is analyzed to explore its capabilities for prediction. This article presents an extension of the study performed in Knight et al. (2009). More specifically, in this study, a more detailed characterization of the roughness parameter and the number of roughness zones is explored producing additional scenarios. The performance of each scenario is evaluated by means of different fitting functions using rating curves for comparison. The research shows that the use of an adequate roughness description, such as a roughness factor calibrated for the whole cross section or a boulder roughness model calibrated for the channel bed plus roughness values from the CES roughness advisor for banks, leads to optimal model results in a mountain river.

Keywords: Conveyance Estimation System, Mountain Rivers, roughness coefficient

Resumen

La predicción de niveles de agua en ríos es importante para prevenir pérdidas económicas así como de vidas humanas causadas por inundaciones. Los modelos hidráulicos son comúnmente usados para predecir estos niveles de agua y tomar acciones para mitigar el daño debido a inundaciones. En la presente investigación, se analizó una aproximación 2D para resolver las ecuaciones promediadas en profundidad de Reynolds Average Navier Stokes (RANS), llamado Conveyance Estimation System (CES), para explorar sus capacidades predictivas. Este artículo presenta una ampliación del estudio realizado por Knight et al. (2009). De igual forma, en esta investigación se explora una caracterización más detallada del parámetro de rugosidad y del número de zonas de rugosidad produciendo diversos escenarios. Se evaluó el desempeño de cada escenario mediante diferentes funciones de ajuste usando curvas de descarga para comparación. La investigación muestra que el uso de una adecuada descripción de la rugosidad, como un factor de rugosidad calibrado para toda la sección transversal o un modelo de rugosidad para cantos rodados calibrado para el lecho junto con valores de rugosidad obtenidos en valores sugeridos por el CES para los bancos, produce resultados del modelo óptimos en un río de montaña.

Palabras clave: Sistema de estimación de capacidad de trasporte, Ríos de montaña, coeficientes de rugosidad.

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1 Introduction

Flooding is one of the most dangerous natural disasters producing human as well as economic losses (Douben, 2006; Camp et al., 2016). From the modeling point of view, the mitigation of flooding effects requires the computation of water levels. When 1-D hydrodynamic models are used, the Saint-Venant equations are solved (Papanicolaou et al., 2004). These equations contain one parameter for resistance, usually the Manning resistance parameter (n). However, this is sometimes not enough to have a useful tool for decision making. Motivated by this limitation, in this research, we focus on a 2-D approach called CES (Conveyance Estimation System) to estimate the water level at a specific cross-section of a mountain river.

There are different inputs for this 2D approach, such as the geometry of the river or the parameters that appear in different terms of the modelling equations. However, we focus on the roughness factor f. This roughness factor can be estimated in different ways (Marcus et al., 1992). In the methodology, empirical equations are considered. These equations require variables that are easily measurable, that consider at-a-site as well as between-site effects on resistance, and that are reliable (Jarrett, 1985; Bathurst, 2002; Ferguson, 2007). Usually, available formulas to estimate f may have errors of around 30% because they were developed through the average of variations in multiple sites (Bathurst, 2002).

Additionally, formulations derived “from local conditions to a single formulation” for mountain rivers are found under special conditions, in which skin friction is the only or main component of resistance (Bathurst, 2002; Romero et al., 2010). Hence, their application is limited to only certain type of reaches (straight, no vegetation, and no air intrusion). Moreover, the roughness factor comprises different elements depending on the model structure: 1D, 2D, or 3D. In 1D models, the parameter contains an incorrect representation of turbulence (Bhola et al., 2019), while in some 2D models the representation of roughness does not include turbulence (Morvan et al., 2008). In this research, the performance of three empirical equations is tested with data collected in a mountain river. Two of them are semilogarithmic expressions found by Knight et al. (2009) and Romero et al. (2010) and the remaining one is an exponential expression obtained in Bathurst (2002). These equations are comprehensively explained in the Materials and Methods section.

There are some characteristics of mountain rivers that influence the roughness factor, f. The water depth (d) is comparable to the bed material with a relative submergence d/D84 ranging between 4 to 10 (Bathurst, 2002). Consequently, the bed material contributes more to resistance than in flat rivers (Jarrett, 1984). The velocity distribution in mountain rivers has an S-shape instead of the semilogarithmic profile used in low gradient rivers (Bathurst, 1985) due to the presence of boulders, which have a mean diameter bigger than 256 mm, and less than 4000 mm (Bunte and Abt, 2001). There is low flow velocity below the boulder level and between boulders, as well as high velocities above the boulders. The velocity pattern is important since there is a relationship between velocity and resistance (Wohl, 2000). There are additional effects of boulders in resistance due to the impact of flowing water in its protruding surface as well as the formation of eddies behind it (Jarrett, 1984). In addition, Pagliara et al. (2008) demonstrate that the interaction of the water surface with the boulders increases as the boulder concentration increases.

The Conveyance Estimation System (CES) is a two-dimensional model solving the depth-averaged Reynolds Average Navier Stokes (RANS) equations across a cross section. These equations include a term to consider the boundary friction using a unitary roughness factor (nl), representative of a segment of the cross-section boundary. Furthermore, the factor nl is a resistance parameter obtained in a wide reach where the roughness elements are small relative to the water depth. Other resistance contributors such as transverse currents or secondary circulations are considered in different equation terms (Knight et al., 2009). CES was developed by different organizations in the UK such as the Environmental Agency, Northern Ireland Rivers Agency, Flooding Policy Team, and HR Wallingford/ JBA Project Team. Knight et al. (2009) performed several study cases around the world: in rivers located in the UK, Argentina, United States, and so on. In particular, there is an example of the application of CES in two cross-sections in mountainous rivers in Ecuador and New Zealand (Knight et al., 2009).

The main goal of this article is to explore the possibility of a better cross-sectional description of roughness in the application example given by Knight et al. (2009) for the Tomebamba river in Ecuador. In this study, the inclusion of additional roughness zones and additional empirical equations to predict the roughness parameter is tested. The roughness zones are varied according to the bed material. Some scenarios contain only one roughness zone and others contain three roughness zones: left bank, right bank, and channel bed. In banks, values from the roughness advisor in CES are used. This advisor has a database of roughness values from several references ((Fisher and Dawson, 2003) as cited in (Wallingford, 2013)). However, for channel bed, a constant calibrated value by Knight as well as empirical equations whose roughness values vary with water depth are used due to the presence of boulders, which affects resistance due to their interaction with water.

2 Materials and Methods

2.1 Site Description

The cross-section and the discharge curve data were taken from Knight et al. (2009). The cross section to be evaluated belongs to the Tomebamba river at Monay in Cuenca, Ecuador (Figure 1). The bed material is composed of boulders and gravel, giving a D_{90} =1.3 meters. The reach has a slope of 0.0176 and a width of 25 m. Information about the bed material sampling is available in Bunte and Abt (2001) and Wolman (1954).

2.2 Conveyance Estimation System (CES)

CES discretizes the width of a cross section at constant intervals. The intersection of the water level analyzed with the cross section is taken as the first and last element. In Figure 2a, those elements are y_1 and y_{100}. The number of elements taken by default is 100. Then, CES solves the depth-averaged RANS equations through the Finite Element Method (FEM), where the unitary flow (q) is obtained for each element ($y_1, y_2 \ldots, y_{100}$). The selection of q is due to its continuity properties (Knight et al., 2009). The boundary conditions at the extreme elements are unitary flows equal to zero. The unitary flow is then transformed into depth-averaged flow velocity (Ud in Figure 2b) for each element. However, the cross velocity distribution is not always available for model validation but a rating curve that relates flow with water depth. This data is obtained by CES since there is an integration of depth-averaged velocities to get an average flow for the whole section. All the previous process is repeated by CES for 25 depths automatically, so a rating curve is obtained.

The roughness input parameter in CES is through a coefficient called unitary roughness (nl), which has the same units as the Manning coefficient. This coefficient is representative of rivers in the UK, where there is high relative submergence and a 1-meter water depth. Thus, this parameter is related to boundary friction dissipation only. Depending on the variation of bed material, vegetation, or irregularities across the cross-section, it is possible to determine roughness zones where a certain unitary roughness value is assigned.

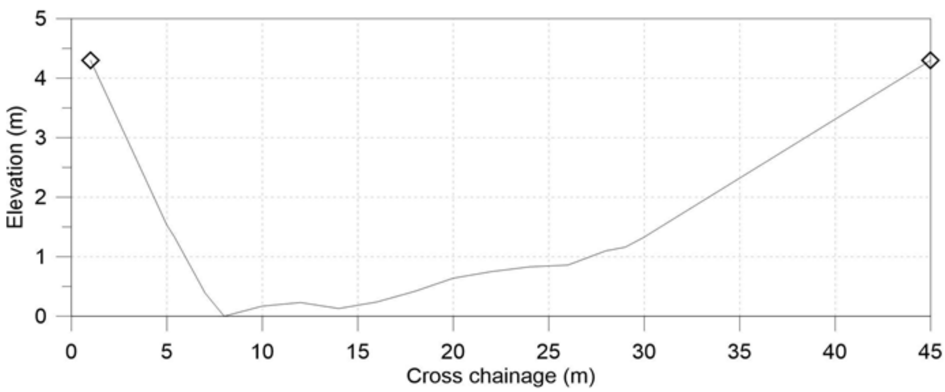

Figure 1. Cross section of Tomebamba river. Cross sectional data adapted from (Knight et al., 2009)

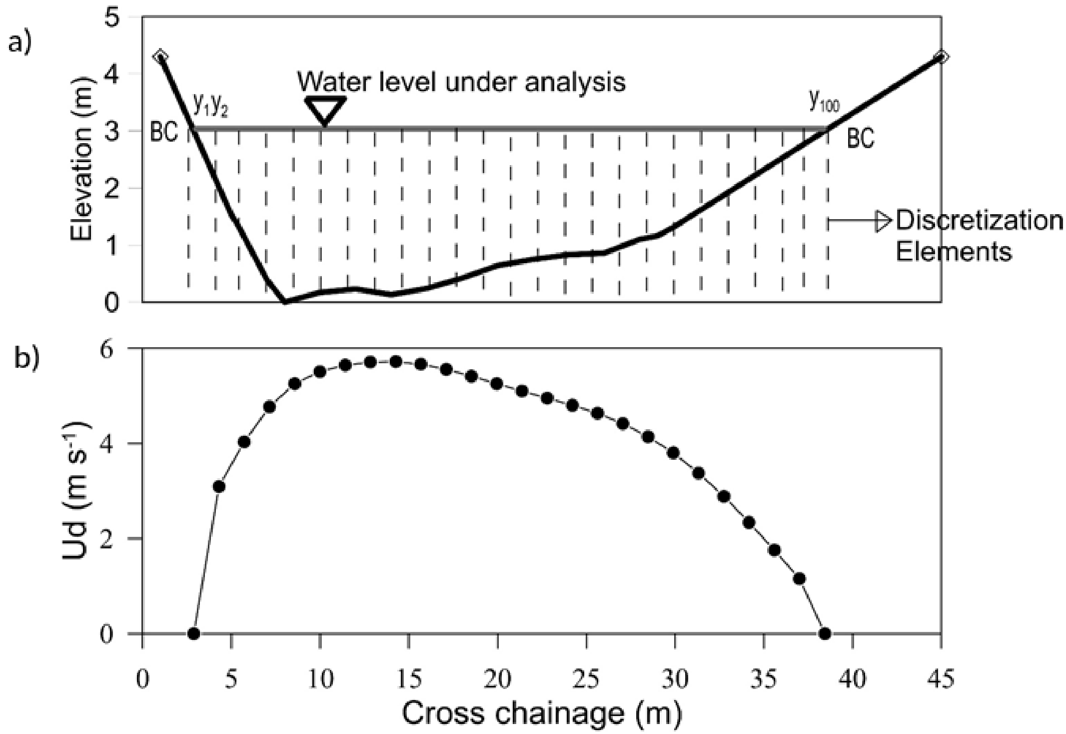

Figure 2. a) Cross section discretization. Cross section data adapted from (Wallingford, 2013) b) Example of cross sectional velocity distribution corresponding to cross section illustrated in a)

For example, in Figure 3 the red lines indicate the roughness zones limits. Left and right bank for example has a bed material of rock covered by vegetation, while channel bed has boulders without vegetation. CES software has a Roughness Advisor, which has typical values of unitary resistance for surface material, vegetation, and irregularities. Each term is described individually and combined with a root sum of squares.

2.3 Scenarios

In this work, we take as our point of departure the case study of the Tomebamba river presented in Knight et al. (2009). It consisted on applying CES to a mountain river whose channel bed is covered by boulders. Knight et al. (2009) analyzed three cases with a single roughness zone. First, they use the roughness advisor values. Second, they use a calibrated unitary roughness value. Finally, they consider a boulder model (see Equation 1). The validation data consisted of points of the rating curve. In our research, we expand those scenarios by adding two roughness zones and additional boulder models in order to explore the possibility to improve the model prediction by enhancing the cross-sectional roughness description. The boulder models are used to predict channel bed roughness values. On the other hand, the left bank and right bank roughness values were based on the Roughness Advisor in CES.

The boulder models used are based on exponential and semilogarithmic expressions considered as traditional approaches for resistance prediction (Zimmermann, 2010). The boulder model of Knight et al. (2009) is a calibrated model based on data from the Tomebamba and Cuenca rivers:

$$f_{mc} = 8 \cdot \left[5{,}75 \cdot log\left(\frac{12 \cdot d_{mc}}{3 \cdot D_{90}}\right)\right]^{-2} \qquad (1)$$

Where *dmc* is the maximum local cross section depth [m], D90 is the 90 percentile of the material size distribution [m]. Details of this model can be found in Abril and Knight (2004).

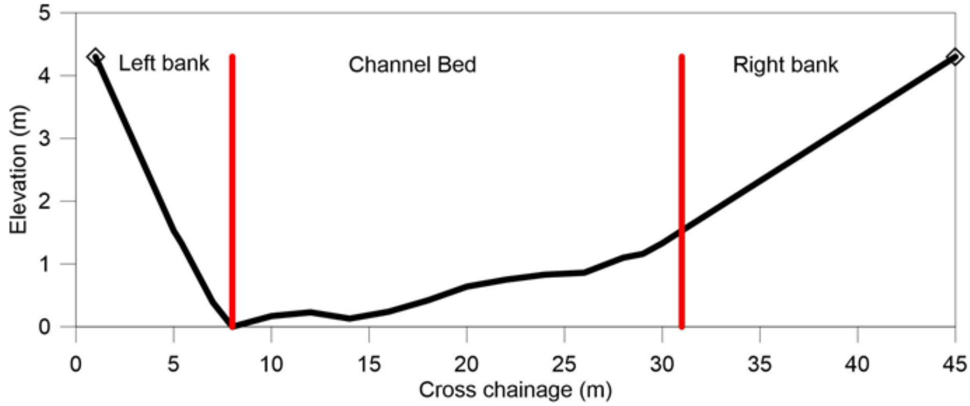

Figure 3. Division of roughness zones

The boulder model of Romero et al. (2010) (Equation 2), obtained through the regression of data from five mountain rivers in Bolivia, results in the following equation with S_O the bed slope:

$$f = 1{,}21 \cdot ln(S_O) + 6{,}259 \qquad (2)$$

The boulder model of Bathurst (2002) was obtained with the analysis of twenty-seven data sets. In that article, the author focuses on the effects of at-a-site and between-site variations on resistance. Equation 3 is used for bed slopes higher than 0.8% and d is the mean water depth average [m].

$$\left(\frac{8}{f}\right)^{1/2} = 3{,}1 \cdot \left(\frac{d}{D_{84}}\right)^{0{,}93} \qquad (3)$$

The reason behind the use of D84 is that it depicts a 3-D view of bed material. However, when there are morphologies such as step-pools or cascades this roughness height seems not to be appropriate (Lee and Ferguson, 2002; Maxwell and Papanicolaou, 2001; Aberle and Smart, 2003). However, there are other studies where D84 was successfully used for these morphologies (Comiti et al., 2007). If, in a scenario, one of the boulder models is used, the value of f will be calculated and transformed into a unitary roughness with Equation 4. Where g is the gravitational acceleration [m s^{-1}], nl unitary roughness [s m$^{-1/3}$], and d is water depth [m]. This equation is the rough-turbulent component of the Colebrook-White law.

$$f = \frac{8 \cdot g \cdot nl^2}{d^{\frac{1}{3}}} \qquad (4)$$

The CES package calculates a rating curve (Flow-Water level) for each scenario described in Table 1. The results are compared against the measured rating curves points through different metrics. Each scenario is described in Table 1.

Table 1. Scenarios for the analysis of rating curves using the CES package.

Scenario	Description	Channel Bed	Banks
0	One roughness zone	Calibrated unitary roughness found in Knight et al. (2009)	
1	Two roughness zones	Calibrated unitary roughness found in Knight et al. (2009)	Height Varying grass +Fine Gravel
2	Two roughness zones	Calibrated unitary roughness found in Knight et al. (2009)	Height Varying grass +Cobbles
3	One roughness zone	Boulder model of Knight et al. (2009)	
4	Two roughness zones	Boulder model of Knight et al. (2009)	Height Varying grass+Fine gravel
5	Two roughness zones	Boulder model of Knight et al. (2009)	Height Varying grass+Cobbles
6	Two roughness zones	Boulder model of Bathurst (2002)	Height Varying grass+Cobbles
7	Two roughness zones	Boulder model of Romero et al. (2010)	Height Varying grass+Cobbles

2.4 Statistical Fitting Metrics

The quantification of the performance of each scenario was done through statistical indices. These statistical indices encompass in a single number the prediction quality of a model in comparison with validation data. Nevertheless, each metric shows a specific projection of the model accuracy relative to measured data (Chai and Draxler, 2014). In the following paragraphs, an explanation of the used metrics can be found.

RMSE (Root-mean-square error) is a widely used metric in meteorological and environmental studies such as air quality or climate research (Willmott and Matsuura, 2005; Nayak et al., 2013; Ritter and Muñoz-Carpena, 2013; Chai and Draxler, 2014). It is a qualitative methodology where larger errors of a model have more weight than smaller ones (Willmott and Matsuura, 2005; Chai and Draxler, 2014). Moreover, Ferguson (2007) states that this metric is useful to estimate the model performance of high values. It has the same units as the variable under analysis.

This metric is sensible towards outliers, so it is considered reasonable to remove observed values with several orders of magnitude larger than the other values in the sample (Chai and Draxler, 2014). The equation of RMSE is given by Equation 5. Where S mean simulated values, O is observed data, and N is the number of data. A perfect fit model will have a RMSE value of 0.

$$RMSE = \left(\frac{\sum_{i=1}^{n} S_i - O_i}{N} \right)^{0,5} \tag{5}$$

EF (Efficiency coefficient) is a statistical parameter that measures the capacity of estimation of the 1:1 line between observed and measured data (Nash and Sutcliffe, 1970). This metric is widely used to compute the goodness of fit of models due to its flexibility and reliability (McCuen and Cutter, 2006; Merz and Doppmann, 2006; Nayak et al., 2013). However, it is sensible towards bias, outliers (McCuen and Cutter, 2006; Ritter and Muñoz-Carpena, 2013) and overemphasizes large differences between observed and predicted values as RMSE does.

When Equation 6 is analyzed, the numerator represents the unexplained variation of the data by the model, while the denominator is the difference of the observed data with respect to the mean (McCuen and Cutter, 2006). Ritter and Muñoz-Carpena (2013) provides Table 2 with range of values for EF and its implications for the model performance. An EF of 1 means that the model and measured data fit perfectly; where $\bar{O}_i$ is the mean of the observations.

$$EF = 1 - \left(\frac{\sum_{i=1}^{n} (O_i - S_i)^2}{\sum_{i=1}^{n} (O_i - \bar{O}_i)^2} \right) \tag{6}$$

Table 2. Model performance based on EF values.

Performance	EF
Very good	≥0.9
Good	0.8-0.9
Acceptable	0.65-0.8
Unsatisfactory	<0.65

Source: Ritter and Muñoz-Carpena (2013)

MAE (Mean Absolute Error) is the average of the errors (Equation 7). RMSE is bigger than MAE, so the magnitude of the difference indicates model goodness (Alvarado, 2001). This metric is widely used for model evaluations (Chai and Draxler, 2014). Willmott and Matsuura (2005) considers this metric a better indicator of the average error than RMSE. A model with a perfect fit has a MAE of 0.

$$MAE = \frac{\sum_{i=1}^{n} |S_i - O_i|}{N} \tag{7}$$

Relative error (RE) (Equation 8), this metric represents the relative error of the prediction taking as a base the measured data.

$$RE = \frac{S_i - O_i}{O_i} \cdot 100\,\% \tag{8}$$

3 Results

3.1 Rating Curve

Figure 4a depicts the rating curves when a calibrated unitary roughness is used. This figure shows that the inclusion of additional roughness zones for banks leads to a better approximation to measured data for flows higher than 20 m^3s^{-1}.

Figure 4b illustrates the sensibility of the model towards the selection of the boulder roughness model. For example, in models 6 and 7 new boulder roughness models plus additional roughness zones do not improve the rating curve prediction. Instead, it leads to lower values of water height. According to Figure 4b, the best scenario is number 3, which uses one roughness zone with the roughness boulder model of Knight et al. (2009).

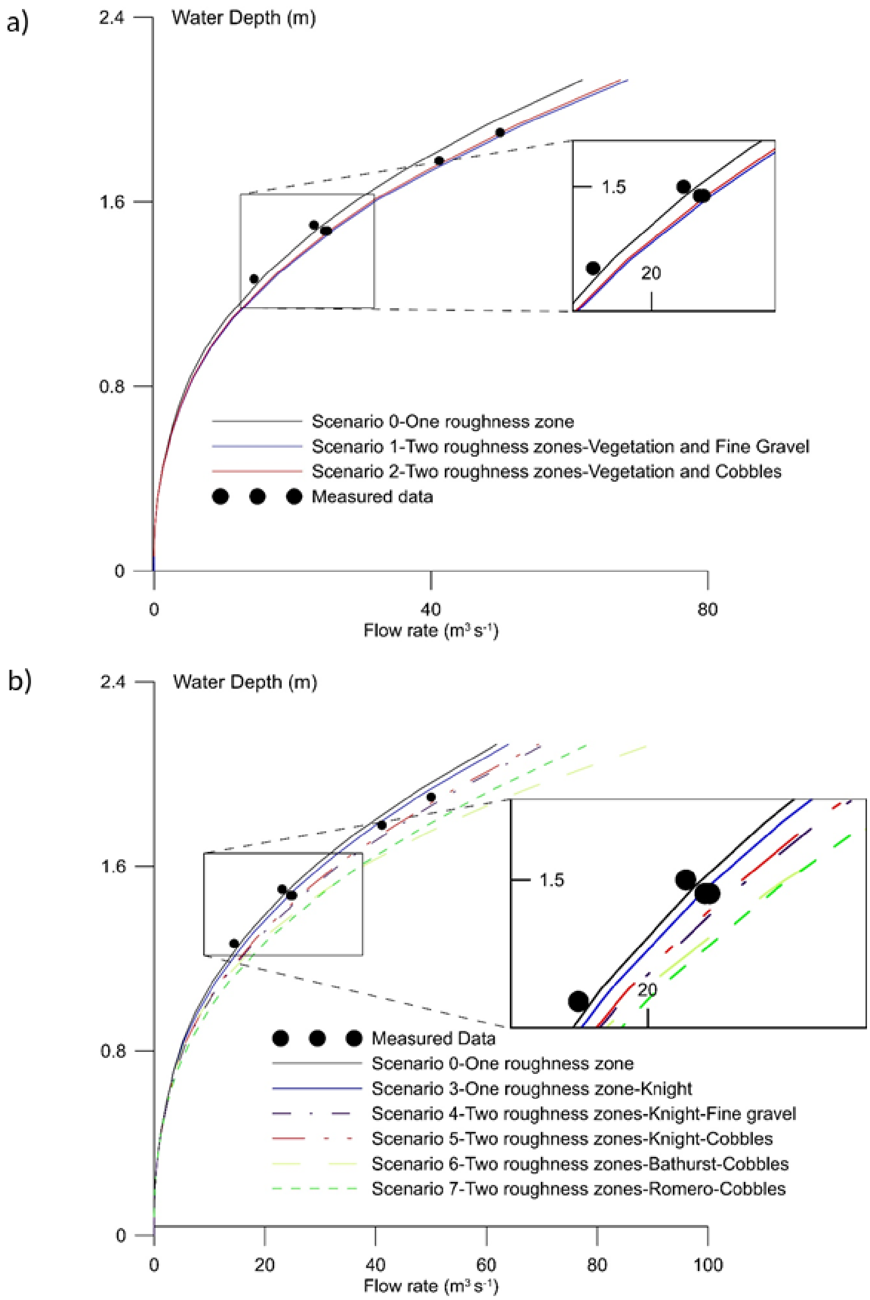

Figure 4. Rating curves for different scenario. a) Improvement of banks coverage description. b) Boulder model and improvement of bank coverage description.

3.2 Statistical Indices

Table 3 presents the results of statistical indices, and bold cells show the model with the closest match to the optimum value for each metric. RMSE shows that Scenario 3 (One roughness zone, Boulder roughness model of Knight et al. (2009)) is the best model. Moreover, the scenario with the smallest mean average error (MAE) was Scenario 2 (Two roughness zones, banks description: cobbles and varying height grass). The magnitude of the difference between these two indices shows that Scenario 3 is the best to predict the rating curve in the Tomebamba river. EF values confirm that Scenario 2 and Scenario 3 are the best, having a very good performance (see Table 2). Furthermore, Table 4 presents the relative error with respect to measured data for each flow and for each scenario. This table shows that for low flow values, Scenario 0 has less difference relative to the measured data. Also, at this flow magnitudes Table 4 depicts the highest relative error for all scenarios. However, for higher values of flow, Scenario 2 has the lowest relative difference for most data. This aspect is in accordance with the best MAE value seen in Table 3 for Scenario 2.

Table 3. Statistical Indices values for evaluated scenarios

	Scenario 0	Scenario 1	Scenario 2	Scenario 3	Scenario 4	Scenario 5	Scenario 6	Scenario 7
RMSE	4.94	4.31	2.92	**2.38**	10.22	7.33	70.92	47.82
MAE	1.77	1.54	**1.1**	1.25	2.62	2.16	6.63	5.8
RMSE-MAE	3.16	2.77	1.81	**1.13**	7.6	5.17	64.3	42.03
EF	0.96	0.97	**0.98**	**0.98**	0.92	0.94	0.43	0.61

Table 4. Relative error related with flow magnitude

Q	Scenario 0	Scenario 1	Scenario 2	Scenario 3	Scenario 4	Scenario 5	Scenario 6	Scenario 7
14.42	**7.12**	18.63	16.62	12.67	24.18	22.10	29.35	37.68
23.08	**5.47**	16.65	14.79	10.46	21.64	19.69	34.64	35.72
24.62	-5.67	4.57	2.86	**-1.28**	9.04	7.21	19.84	21.55
25.00	-7.12	2.96	**1.28**	-2.80	7.36	5.56	18.00	19.68
41.15	-6.67	3.83	**2.13**	-2.76	7.72	5.94	26.87	19.80
50.00	-8.38	1.62	**-0.02**	-4.74	5.18	3.52	27.46	17.06

4 Discussion

Two scenarios have shown to improve the CES predictability capacity. The best one according to the metrics, Scenario 3, uses a boulder model with a single roughness zone. This method had the best performance in Knight et al. (2009) as well. The model performance may be due to a better description of the resistance pattern in mountain rivers, where resistance values change with depth. In contrast, the original model had a fixed calibrated value of resistance for all the water depths. The model ranked as the second best is the one with three roughness zones: channel bed has a calibrated roughness parameter and banks a refinement of the bank description. This model is an improvement compared to the original scenario since there is a more realistic description of the resistance across the cross-section. This model has a better MAE than Scenario 3. MAE assigns the same weight across different error values, while RMSE assigns a higher weight to larger errors. The difference between both can be seen in Scenario 3.

Table 4 illustrates that the inclusion of a boulder model and/or roughness zones decrease the relative error as flow increases. At low flow, water and boulders interact generating jetting flows and waves behind particles which increase resistance to flow, increasing the roughness parameter (Jarrett, 1984; Bathurst, 2002). As flow increases, this effect is reduced, and the model seems to be more accurate to predict the points in the rating curve.

The boulder roughness of Bathurst (2002) and Romero et al. (2010) have the worst results according to the applied metrics. The data from which both models were derived made them, in principle, good candidates to represent boulder roughness in this river. Bathurst (2002) used an important amount of data from literature, and Romero et al. (2010) used data of rivers from the same Andean region than the Tomebamba river. Knight et al. (2009) boulder model was calibrated with data from this river and another from the same city, but the crucial aspect influencing its predictability may reside in its logarithmic relation to reflect the boulder roughness in comparison with the exponential relation obtained in Bathurst (2002). The model of Romero et al. (2010) may fail in the excessive simplicity of the relationship, since it does not have a term that represents the at-a-site resistance variation, such as relative submergence. Hence, the use of an inadequate boulder roughness description can imply important errors in the predictions. Furthermore, the results show that a calibrated roughness factor for the channel bed and the use of the roughness advisor for banks can potentially provide good modeling results when an appropriate boulder roughness boulder is not available.

5 Conclusions

In this research, an extension of the Tomebamba case study presented in Knight et al. (2009) is shown. We consider new scenarios where the boulder model and/or the roughness zones are varied. The predictability is quantified through different metrics to verify different aspects of the errors. The data used for validation was taken from a measured rating curve, which was compared with the output of the model.

The majority of the metrics depict that boulder roughness description of Knight et al. (2009) provides the best results to match the measured rating curve in this section of the Tomebamba river. Furthermore, the scenario where a calibrated roughness value and two additional roughness zones were added provides the best MAE value and the lowest relative error. Thus, CES is able to predict the rating curve in a mountain river, but it is limited to predict good results when the roughness description takes into account local conditions, as done by Knight et al. (2009). On the other hand, the use of a boulder roughness description obtained with a data set which did not include Tomebamba river ((Bathurst, 2002; Romero et al., 2010) in this study) underestimate the water levels having a potentially negative effect during flood prediction. Hence; the local knowledge of roughness values seems necessary to have appropriate modelling results, and the use of any empirical equation requires a previous test before using in a model.

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Environmental Risk Assessment by Fipronil and Imidacloprid Insecticides in River Shrimp (*Cryphiops caementarius*)

Evaluación del Riesgo Ambiental por los insecticidas Fipronil e Imidacloprid en el Camarón de río (*Cryphiops caementarius*)

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Abstract

Fipronil and imidacloprid currently represent approximately one third of the global insecticide market. In the present study, the environmental risk (ERA) of fipronil and imidacloprid in the postlarvae of the river shrimp (*Cryphiops caementarius*, Molina 1782) was evaluated. Short-term toxicity bioassays were performed based on LC_{50} (mean lethal concentration) (mortality) and EC_{50} (mean effective concentration) (swimming hypoactivity). PNEC (Predicted Concentration with No Known Effect) and available environmental standards for PEC (Expected Environmental Concentration) were calculated for fipronil and imidacloprid to determine risk quotient (RQ). Imidacloprid was more at risk for the aquatic environment than fipronil for the lethal response (mortality) and sublethal response (swimming hypoactivity). The observed risk difference between the two insecticides could be due to their different modes of action. *C. caementarius* should be considered as a sensitive species when defining an environmental quality standard for the conservation of the aquatic environment. Therefore, it is recommended to continue monitoring the presence of these insecticides in coastal freshwater bodies, and to reduce the use of fipronil and imidacloprid in the agricultural crops that use them.

Keywords: Environmental quality, *Cryphiops caementarius*, Aquatic ecosystem, Fipronil, swimming hypoactivity, Imidacloprid

Resumen

En la actualidad, el fipronil y el imidacloprid representan aproximadamente un tercio del mercado mundial de insecticidas. En el presente estudio se evaluó el riesgo ambiental (ERA) del fipronil e imidacloprid en las postlarvas del camarón de río (*Cryphiops caementarius*, Molina 1782). Se realizaron bioensayos de toxicidad de corta duración en base a la CL_{50} (Concentración letal media) (mortalidad) y CE_{50} (Concentración efectiva media) (hipoactividad natatoria). Se calculó la PNEC (Concentración prevista sin efecto conocido) y los estándares ambientales disponibles para la PEC (Concentración ambiental esperada) para el fipronil y el imidacloprid para determinar los cocientes de riesgo (CR). El imidacloprid resultó con mayor riesgo para el ambiente acuático que el fipronil para la respuesta letal (mortalidad) y subletal (hipoactividad natatoria). La diferencia del riesgo observada entre ambos insecticidas pudiera deberse a sus diferentes modos de acción. *C. caementarius* debería ser considerado como una especie sensible al momento de definir un estándar de calidad ambiental para la conservación del ambiente acuático. Por ende, es recomendable continuar el monitoreo para observar la presencia de estos insecticidas en los ecosistemas dulceacuícolas costeros, y reducir el uso del fipronil y del imidacloprid en los cultivos agrícolas que los emplean.

Palabras clave: Calidad ambiental, *Cryphiops caementarius*, Ecosistema acuático, Fipronil, Hipoactividad natatoria, Imidacloprid.

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1 Introduction

Imports of insecticides in Peru increased from 3481 tn to 5037 tn between 2007 and 2017 (INEI, 2018), and its use is regulated by the National Agrarian Health Service (MINAGRI, 2020) for the protection of health and the environment, because of the ecological impact insecticides have on water bodies and the impact on aquatic biodiversity (Escobar-Chávez et al., 2019; Sotelo-Vásquez and Iannacone, 2019). Currently, fipronil and imidacloprid account for approximately one third of the world's insecticide market (MINAGRI, 2020), and they work by blocking transmission into the central nervous system, but each chemical has a different mode of action (Al-Badran et al., 2018, 2019). Fipronil interferes with the passage of chloride ions by binding to a specific site within the gamma-aminobutyric acid receptor (GABA), while Imidacloprid binds to postsynaptic nicotinic acetylcholine receptors (nAChR) (Al-Badran et al., 2018).

Fipronil (phenylpirazole) and imidacloprid (neonicotinoids) are used on agricultural pests, domestic pests and ectoparasites of domestic animals (Al-Badran et al., 2018, 2019; Escobar-Chávez et al., 2019). In Peru, fipronil and imidacloprid insecticides are widely used for the pest control in agricultural crops of rice and onions (Gangwar et al., 2016; Pathak et al., 2018). Compared to other types of insecticides, fipronil and imidacloprid are considered safer due to their low toxicity in fish and mammals. Fipronil and Imidacloprid in small concentrations are very effective on arthropods (Al-Badran et al., 2018). Increased use in recent decades, moderate to high solubility and persistence in water raise serious concerns about the potential negative effects on aquatic invertebrates that are not the target of control (Al-Badran et al., 2019).

River shrimp (*Cryphiops caementarius*, Molina 1782), is a species with a biological, commercial and economic relevance in Peru, and is one of the endemic hydrobiological components maintained by the commercial research of the southern coast of Peru and northern Chile (Campos et al., 2017). Its highest populations have been observed in the rivers of the department of Arequipa (Peru), because these bodies of water have a higher flow, in addition to the predominance in this area over the cultivation of rice and onions. *Cryphiops caementarius* is used in aquaculture for food (Reyes, 2011, 2018; Romero-Camarena et al., 2013; Campos et al., 2017), subjected to a high hydrobiological exploitation in the natural environment, with impacts on habitat destruction due to natural drought and anthropic processes such as water use, agricultural and mining activities (Wasiw, 2017).

There is no research assessing the environmental risk of insecticides when using *C. caementarius* as a bioindicator species (Smit et al., 2015; Al-Badran et al., 2018, 2019). Therefore, the objective of this article was to assess the environmental risk of fipronil and Imidacloprid insecticides in *C. caementarius*.

2 Materials and methods

2.1 *Cryphiops caementarius* River Shrimp (Molina 1782)

Postlarvae of river shrimp (*C. caementarius*) Postlarvae of river shrimp (12°31′35″S 76°32′38″W) by an authorized fisherman belonging to the fishermen's guild of the area (Resolution N°83-2007-PRODUCE), Peru. Postlarvae were obtained in the closure period of this species (Baltazar and Colón, 2014; Wasiw and Yépez, 2015).

Postlarvae of *C. caementarius* were acclimatized at the Laboratory of Larviculture of the Southern Scientific University (UCSUR), Lima, Peru, two weeks before starting bioassays and following this scheme: about 1000 postlarvae were kept in a container of 750 L capacity with constant aeration at an average temperature of 21±2 °C, supplying precoated chicken liver as food every 24 hours to prevent the death of organisms. Water changes were carried out daily, and it was previously dechlorinated using sodium thiosulfate by siphon (Rice et al., 2017). Postlarvae of *C. caementarius* with an average size of 15 mm were used for bioassays (Baltazar and Colón, 2014). A calibrator (± 0.1 mm) was used to measure the total length of each postlarvae of *C. caementarius* by carefully straightening the shrimp body onto the table and measuring the total length from the tip of the head to the end of the tail (Al-Badran et al., 2019).

2.2 Insecticides

Fipronil of the Regent SC® was used at a concentration of 250 g L^{-1} and seven nominal concentrations were established (0.10 μg L^{-1}; 0.26 μg L^{-1}; 0.64 μg L^{-1}; 1.6 μg L^{-1}; 4 μg L^{-1}; 0.02 μg L^{-1} and 0.04 μg L^{-1}). Imidamin® brand was used as imidacloprid at a concentration of 350 g L^{-1} with five nominal concentrations (28.8 μg L^{-1}; 71.9 μg L^{-1}; 179.8 μg L^{-1}; 449.6 μg L^{-1} and 1124 μg L^{-1}).

2.3 Bioassays

Four replications were used for each insecticide and one control. 2 L with dilution water was placed in containers with a capacity of 3 L, which were connected to a system with constant aeration in series for each concentration and repetition. Later, 10 postlarvae specimens of *C. caementarius* were placed in each of the containers (Escobar-Chávez et al., 2019). The water used in the containers was conditioned with Nutrafin Aqua Plus® (Hagen, USA).

River shrimp postlarvae were fed every 48 hours with cooked rice to prevent their death by cannibalism. Postlarvae mortality and chronic hypoactivity (hNPL) measurements were performed at 3h, 8h, 24h, 48h, 72h and 96 h of exposure. The organisms were considered dead in the total absence of movement during 2 minutes after being gently touched with a stick. hNPL was listed as lack of displacement, lack of struggle, lack of reaction to mechanical stimuli, and lethargy. Normal postlarvae swimming activity was considered as the search for food, movement throughout the water column and rapid reaction to mechanical stimuli.

2.4 Data analysis and environmental risk assessment

Mortality and hNPL percentages of *C. caementarius*. were determined. The mean lethal concentration (LC_{50}) for mortality and mean effective concentration (EC_{50}) for hNPL was calculated with the Probit version 1.5 program with a 95% confidence level, and the regression model was verified with the Chi-square (χ^2) statistic (Rice et al., 2017). The LOEC (lowest concentration where effect is observed) and NOEC (non-observed effect concentration) parameters were calculated with Past 3.2 statistical program, using Krustal-Wallis test based on significant differences between mortality and hNPLs for the fipronil and imidacloprid concentrations used.

2.4.1 Expected concentration with no known effect (PNEC)

PNEC was found from $LC(E)_{50}$, LOEC y NOEC derived from the results of short-term toxicity tests. These parameters were given the valuation or safety factor (FV) established for toxicity tests, which was 1000 (UNEP/IPCS, 1999). With the relationship PNEC= Toxicity Parameters/Valuation Factor.

2.4.2 Expected Environmental Concentration (PEC)

The environmental quality standards (EQS) of The Netherlands legislation for fipronil (Tennekes, 2018) and imidacloprid (Smit et al., 2015) were used, in which EQS of imidacloprid presented two scenarios. Scenario 1: Short-term EQS, maximum acceptable concentration (MAC-EQS) based on acute toxicity data. Scenario 2: Long-term EQS, expressed as an average annual concentration (AA-EQS) based on chronic toxicity data with the aim of protecting the ecosystem against adverse effects resulting from long-term exposure (EC, 2011).

2.4.3 Risk characterization (PEC/PNEC)

The risk coefficient (RC) was calculated as RC= PEC/PNEC. It states that if the PEC/PNEC is <1 the fipronil and Imidacloprid evaluated are considered to have low risk; while when PEC/PNEC is >1, it is considered to have high risk (De la Torre et al., 2004).

3 Results

Mortality and hNPL increase from 3 h to 96 h of exposure in postlarvae of *C. caementarius* that presented a higher effect on fipronil at 96 h of exposure, reaching 100% of mortality and hNPL. For Imidacloprid, 87.5% and 100% were reached for mortality and hNPL, respectively. The LC_{50} and EC_{50} values for fipronil insecticides with their upper and lower limits of 95% were obtained from 3 h to 96 h of exposure, and their respective determination coefficients (R^2) (Tables 1 and 2). Similarly, LOEC and NOEC parameters for fipronil and imidacloprid are observed from 3 h to 96 h of exposure in the postlarvae of *C. caementarius* (Table 3 and 4).

Table 5 shows the values that establish the relationship between PEC and PNEC to determine the existing risk of insecticides, based on the PNEC-LC_{50}-$_{mortality}$, PNEC-EC_{50}-$_{hNPL}$, PNEC-LOEC-$_{mortality}$ and PNEC-LOEC-$_{hNPL}$ parameters. Values of RCs higher than one were obtained with fipronil and imidacloprid in all cases (Table 5). Imidacloprid presented a higher risk to the aquatic environment compared to fipronil for lethal (mortality) and sublethal response (postlarvae swimming hypoactivity).

Table 1. Mean lethal concentration (LC_{50}) and Mean effective concentration (EC_{50}) and upper and lower limits for lethal and sublethal parameters based on hNPL (swimming hypoactivity) in *Cryphiops caementarius* with fipronil at six different times of exposure.

Exposure time (h)	Mortality LC_{50} (μg·L^{-1})	R^2	hNPL EC_{50} (μg·L^{-1})	R^2
3 h	0.901 (0.506- 1.601)	0.98	0.252 (0.175- 0.364)	0.99
8 h	0.679 (0.354- 1.303)	0.97	0.074 (0.042- 0.131)	0.96
24 h	0.035 (0.007- 0.171)	0.87	0.003 (0.001- 0.007)	0.99
48 h	< 0.02	ND	< 0.02	ND
72 h	< 0.02	ND	< 0.02	ND
96 h	< 0.02	ND	< 0.02	ND

( ): Upper and lower limits (95 %). R^2: determination coefficient.

Table 2. Mean lethal concentration (LC_{50}) and mean effective concentration (EC_{50}) and upper and lower limits for lethal (mortality) and sublethal parameters based on hNPL (swimming hypoactivity) in *Cryphiops caementarius* with Imidacloprid at six different times of exposure.

Exposure time (h)	Mortality LC_{50} (μg·L^{-1})	R^2	hNPL EC_{50} (μg·L^{-1})	R^2
3 h	ND	ND	260.5 (82.97- 817.92)	0.83
8 h	ND	ND	246.7 (152.42- 399.42)	0.94
24 h	5353.7 (1832.17- 15643.56)	1	28.4 (6.62- 122.17)	0.97
48 h	53540.8 (1032.89- 2775350.23)	0.52	5.3 (1.11- 25.66)	0.46
72 h	13.68 (1.72- 109.11)	0.97	1.2 (0.14- 9.83)	0.93
96 h	0.23 (0.01- 6.37)	0.91	0.002 (0.00- 0.16)	0.79

( ): Upper and lower limits of 95 %. R^2: determination coefficient.

4 Discussion

No toxicity bioassays have been conducted with fipronil and imidacloprid in *C. caementarius*, but research is observed with other aquatic crustacean species (Goff et al., 2017; Al-Badran et al., 2019). *C. caementarius* had effects on mortality and swimming hypoactivity (hNPL) for both insecticides. There was a lack of reaction in hNPL to provided stimuli, reduction of movement and different swimming. The results obtained varied with concentrations and exposure times in both insecticides (Mendoza-Rodríguez, 2009).

Shan et al. (2003) when working with *Macrobrachium rosenbergii* (De Man, 1879) and *Macrobrachium niponnensis* (De Haan, 1849), found values of LC_{50} (24 h) de 6.41 μg·L^{-1} and $>$ 25.70 μg·L^{-1}, respectively with fipronil. *C. caementarius* was more sensitive to fipronil than the two *Macrobrachium* species with a LC_{50} value of 0.035 μg·L^{-1} at 24 h of exposure. The differences obtained in LC_{50} may be due to the different protocols used in bioassays, where Shan et al. (2003) conducted a simulation of rice fields under laboratory conditions. These differences can also be explained by specific biodistribution patterns, metabolization rates, or even to the specific sensitivity of each target taxon.

Fipronil and Imidacloprid in *Palaemonetes pugio* Holthuis, 1949 at 96 h of exposure obtained a LC_{50} of 0.68 $\mu g \cdot L^{-1}$ for larvae and a LC_{50} of 0.32 $\mu g \cdot L^{-1}$ for adults with fipronil; it was significantly more toxic in the larvae with Imidacloprids (LC_{50} of 308 $\mu g \cdot L^{-1}$) than in adults (LC_{50} of 563.5 $\mu g \cdot L^{-1}$) (Key et al., 2007). In the case of *C. caementarius*, exposure to fipronil and Imidacloprid at 96 h showed mortality in shrimp postlarvae, which differs from Key et al. (2007). The LOEC and NOEC parameters for mortality were also found, which were 0.02 $\mu g \cdot L^{-1}$ and <0.02 $\mu g \cdot L^{-1}$ at 96 h of exposure with fipronil, and 28.8 $\mu g \cdot L^{-1}$ and <28.8 $\mu g \cdot L^{-1}$ (96 h) with Imidacloprid, respectively. Key et al. (2007), found lower toxicity values for *P. pugio* than those obtained in this research.

Fipronil had higher lethal toxicity than Imidacloprid based on LC_{50} *C. caementarius* at 96 h of exposure. Omar et al. (2016), in *Marsupenaeus japonicus* (Spence Bate 1888), found variable effects according to the development stage tested. The increased lethal toxicity of Fipronil compared to Imidacloprid has been observed in other species of decapod crustaceans such as *Farfantepenaeus aztecus* (Al-Badran et al., 2019). *Penaeus monodon* Fabricius, 1798 was subjected to Fipronil and Imidacloprid in the postlarvae stage at 48 h of exposure, finding LC_{50} of 0.2 $\mu g \cdot L^{-1}$ and 175 $\mu g \cdot L^{-1}$, respectively (Hook et al., 2018).

Table 3. Lower concentration where effect (LOEC) and non-observed concentration effect (NOEC) are observed for lethal (mortality) and sublethal parameters based on hNPL (swimming hypoactivity) in *Cryphiops caementarius* with fipronil at six different times of exposure.

Exposure time (h)	Mortality ($\mu g \cdot L^{-1}$)		hNPL ($\mu g \cdot L^{-1}$)	
	LOEC	NOEC	LOEC	NOEC
3 h	1.60	0.64	0.26	0.10
8 h	0.26	0.10	0.04	0.02
24 h	0.02	< 0.02	0.02	< 0.02
48 h	0.02	< 0.02	0.02	< 0.02
72 h	0.02	< 0.02	0.02	< 0.02
96 h	0.02	< 0.02	0.02	< 0.02

LOEC: Lowest concentration where effect is observed.
NOEC: Concentration of non-observed effect.

Table 4. Lower concentration where effect (LOEC) and non-observed concentration effect (NOEC) are observed for lethal (mortality) and sublethal parameters based on hNPL (swimming hypoactivity) in *Cryphiops caementarius* with imidacloprid at different exposure times.

Exposure time (h)	Mortality ($\mu g \cdot L^{-1}$)		hNPL ($\mu g \cdot L^{-1}$)	
	LOEC	NOEC	LOEC	NOEC
3 h	28.8	< 28.8	179.8	71.9
8 h	28.8	< 28.8	179.8	71.9
24 h	28.8	< 28.8	28.8	< 28.8
48 h	28.8	< 28.8	28.8	< 28.8
72 h	28.8	< 28.8	28.8	< 28.8
96 h	28.8	< 28.8	28.8	< 28.8

LOEC: Lowest concentration where effect is observed.
NOEC: Concentration of non-observed effect.

Table 5. PEC (Exposure Assessment) values, PNEC (expected concentration with no known effect) to determine the RC (Risk Coefficient) of fipronil and Imidacloprid insecticides using *Cryphiops caementarius* river shrimp.

Parameters	Fipronil	Imidacloprid - Scenario 1	Imidacloprid - Scenario 2
PEC	0.00007 (EQS)	0.2 (MAC-EQS)	0.0083 (AA-EQS)
PNEC (LC_{50}-$_{mortality}$)	0.00002	0.00023	0.00023
PNEC (EC_{50}-$_{hNPL}$)	0.00002	0.000002	0.000002
PNEC (LOEC-$_{mortality}$)	0.00002	0.028	0.028
PNEC (LOEC-$_{hNPL}$)	0.00002	0.028	0.028
RC (LC_{50}-$_{mortality}$)	3.5	869.56	36.08
RC (EC_{50}-$_{hNPL}$)	3.5	100.000	4.150
RC (LOEC-$_{mortality}$)	3.5	7.14	2.96
RC (LOEC-$_{hNPL}$)	3.5	7.14	2.96

EQS= Environmental Quality Standard. LC_{50} = Average lethal concentration at 96 h of exposure. EC_{50} = Average effective concentration at 96 h of exposure. LOEC= lowest concentration where effect is observed at 96 h of exposure. MAC-EQS= Maximum permissible concentration for a short-term Environmental Quality Standard. Long-term AA-EQS, expressed as an average annual concentration.

Arthropods are among the most fipronil-sensitive taxa, and related species may have very varied sensitivities to this insecticide (Stevens et al., 2011), because fipronil may be more toxic once metabolized, since fipronil sulfide and fipronil sulfone are generally two to three times more toxic than the original compound. In addition, there is a very wide range in the sensitivity of crustaceans to Imidacloprid, with LC_{50} values ranging from 1 to 52.500 $\mu g \cdot L^{-1}$ (Smit et al., 2015).

Sublethal effects of fipronil and imidacloprid on the behavior, physiology, reproduction and development of non-target aquatic invertebrates have been observed (Al-Badran et al., 2018; Sohn et al., 2018). A LC_{50} was found for the hNPL sublethal parameter, in a range from 260.5 $\mu g \cdot L^{-1}$ to 0.002 $\mu g \cdot L^{-1}$, between 3 h and 96 h with Imidacloprid, and a range of 0.252 $\mu g \cdot L^{-1}$ and <0.02 $\mu g \cdot L^{-1}$ obtained between 3 h and 96 h of exposure with fipronil. Fipronil, unlike Imidacloprid, caused erratic swimming in all directions or seizures and immediate reaction to movement stimulus, while imidacloprid caused lethargy in larvae, decreased swimming, and provoked late reaction to stimulation of movement.

For both insecticides there was a struggle for food, total absence of movement before their death, despite the attempted swimming that was observed in the mobility of the locomotive appendages. Al-Badran et al. (2019) found changes in the behavior of *F. aztecus* by the action of fipronil and imidacloprid under different exposure times depending on concentrations. Imidacloprid reduced the defense behavior of the crustacean *Orconecres rusticus* (Sohn et al., 2018). In this work imidacloprid had higher sublethal effects than fipronil. The different effects of postlarvae on both insecticides are due to action of each. fipronil is a GABA antagonist that causes hyperexcitement and seizures; while Imidacloprid is an nAChR antagonist that causes a variety of symptoms from hyperexcitation to lethargy and paralysis (Cox et al., 1998; Al-Badran et al., 2019).

In relation to the active ingredients of fipronil and imidacloprid, the results obtained with short-term RC show the existence of an environmental risk, and these are consistent with studies conducted by Van der Sluijs et al. (2015), which show the risks to biodiversity and ecosystem functioning by the widespread use of neonicotinoids such as imidacloprid and fipronil. Samples taken in groundwater and surface water have been found to exceed limits based on ecological thresholds established in different countries of North America and Europe, indicating that they exist in soils, waterways and plants in agricultural, urban and drainage areas that are contaminated with mixtures of fipronil, neonicotinoids or their metabolites (Bonmatin et al., 2015). Van der Sluijs et al. (2015) show evidence that these insecticides pose a high risk for a wide range of non-target invertebrate taxa, which would have an impact on aquatic food chains.

Pesticides can be leached in ditches and rivers by rains, and surface water can be contaminated with direct spraying by runoff and leaching of agricultural fields (Vijver and Van den Brink, 2014). The emission of fipronil and Imidacloprid to the surface waters are caused by many factors, such as the distance from the crop to the trench, mode of application, climatic conditions, etc. This is a problem if certain protocols of application are not applied or the potential effects on aquatic ecosystems are unknown (Stoorvogel et al., 2003; Pisa et al., 2015), such is the case of the river shrimp, which is often found in rivers near the rice fields, where fipronil and imidacloprid are widely use (Wasiw, 2017).

Several laboratory studies have been published on the toxicity of Imidacloprid in a variety of aquatic invertebrates and the standard test organism, *Daphnia magna* Straus, 1820, which is less toxic to neonicotinoids (Imidacloprid) compared to other invertebrates (Beketov and Liess, 2008; Escobar-Chávez et al., 2019). An acute LC_{50} of about 7 000 μg·L^{-1} represents more magnitude compared to the effective concentrations found for other invertebrates. This implies that *D. magna* cannot always be used as a sensitive and protective test organism for the entire aquatic species, unlike *C. caementarius* which showed greater sensitivity (Ngim and Crosby, 2001).

Although no information is available on the toxicity of these insecticides in *C. caementarius*, there are studies such as the one conducted by Van Dijk et al. (2013), where the abundance of aquatic macroinvertebrates and Imidacloprid concentrations in surface waters were evaluated. The abundance of macroinvertebrates was observed to decrease as the concentration of Imidacloprid in the aquatic environment increased (Beketov et al., 2013). According to the level of risk obtained from the literature (i.e. $RQ \geq 1$ high risk, $0.1 \leq RQ < 1$ medium risk, $0.01 \leq RQ < 0.1$ low risk) (Sánchez-Bayo et al., 2002), the two insecticides presented a high risk to the aquatic ecosystem based on the lethal and sub-lethal effects of *C. caementarius.*

In general terms, the results of this research and published literature indicate that both insecticides have the potential to cause significant damage to aquatic ecosystems by provoking negative effects on individuals and populations of aquatic invertebrates at very low concentrations (Chaton et al., 2002). There would be an increased risk in *C. caementarius* from December to March, and according to Peruvian regulations this species is in a closure season because during this stage there is more presence of eggs and female carrying eggs (Baltazar and Colón, 2014).

5 Conclusions

The bioindicator *C. caementarius* allows the environmental risk of fipronil and imidacloprid to be assessed in the aquatic ecosystem by using the 96 h risk ratio of exposure based on mortality in the lethal response, as well as mortality and sub-lethality based on swimming hypoactivity. Imidacloprid presented a higher risk to the aquatic environment than fipronil for the lethal (mortality) and sublethal response (postlarvae swimming hypoactivity). The observed toxic difference between the two insecticides could be due to their different modes of action. *C. caementarius*, an invertebrate from Peru, should be considered as a sensitive species when defining an EQS for the conservation of the aquatic environment, especially from January to March, in which according to Peruvian regulations this species is in a closure period. It is advisable to continue monitoring the presence of these insecticides in coastal freshwater bodies, and to reduce the use of fipronil and imidacloprid in the agricultural crops that use them.

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SUSTAINABLE AGROTECHNICS

AUTONOMOUS SOLAR THERMAL SYSTEM DESIGN FOR INDIRECT DEHYDRATION OF AGUAYMANTO (*Physalis Peruviana L.*), JUNIN

DISEÑO AUTÓNOMO DEL SISTEMA SOLAR TÉRMICO PARA LA DESHIDRATACIÓN INDIRECTA DE AGUAYMANTO (*Physalis Peruviana L.*), JUNÍN

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Abstract

This paper aimed to design an autonomous indirect solar dryer, which can dehydrate the aguaymanto in a cost-effective manner, yielding a quality product suitable for export from the central part highland of Peru. To complete this task, it was proposed to design a prototype of autonomous solar dryer of 100 kg per batch of aguaymanto, equipped with flat reflectors and forced air feed, and powered with photovoltaic energy. This system allows to dry aguaymanto fruit at the requirements needed for its exportation. The fryer has the following dimensions: inner dimensions of the drying chamber: bottom 0.60 m, width 1.40 m, and height 1.10 m, with additional 0.05 m for insulation. Hence, the outer measures are bottom 0.70 m, width 1.50 m, and height 1.20 m. Two solar collectors are proposed with the dimensions of each: 1.50 m wide, 2.40 m long, and 0.15 m height; 2 flat mirror reflectors are required. A 80 Wp photovoltaic panel was selected for the forced air system and process control. This solar dryer is expected to cope with the problem of post-harvest deterioration. Also, it will facilitate the export by improving product quality and providing a cost-effective technology.

Keywords: Agro-industrial, dehydration, solar collector, solar thermal system, aguaymanto.

Resumen

Con el propósito de tener un diseño de secador solar indirecto autónomo para deshidratar el aguaymanto, que sea económico y de calidad y que permita exportar de la parte alta central de nuestro país, se propuso el objetivo general: diseñar, un prototipo de autónomo secador solar de 100 kg por lote de aguaymanto, con reflectores planos y aire forzado alimentado con energía fotovoltaica que permita secar esta fruta con los requisitos para su exportación. Las dimensiones de la secadora son las siguientes: dimensiones interiores de la cámara de secado: fondo 0,60 m, ancho 1,40 m, altura 1,10 m, y 0,05 m para el aislamiento, por lo que las medidas exteriores son fondo 0,70 m, ancho 1,50 m, altura 1,20 m. Se proponen dos colectores solares con dimensiones cada una de: 1,50 m de ancho, 2,40 m de largo y 0,15 m de alto, de los cuales se requerirán 2 reflectores de espejo plano. Se seleccionó un panel fotovoltaico de 80 Wp para el sistema de aire forzado y el control del procesado. Este secador solar permitirá abordar el problema del deterioro posterior a la cosecha y facilitará la exportación, porque mejorará la calidad y el costo económico.

Palabras clave: Agroindustrial, deshidratación, colector solar, sistema solar térmico, aguaymanto.

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1 Introduction

Governmental institutions, organizations, international companies and academics all around the world are becoming more and more aware of the importance of natural resource management (Solangi et al., 2011; Sándor et al., 2020; Jakab, 2020) and the renewable energy transition (Reid et al., 2010; Solangi et al., 2011; Marcucci and Turton, 2015). The main objective of global energy sustainability is to replace the fossil fuel- generated energy by renewable energy (Lachuriya and Kulkarni, 2017; Lowy and Mátyás, 2020). With respect to policies on the planet, many North-American (USDE, 2010a,b; Liming et al., 2008; Smitherman, 2009; OPA, 2009; Branker and Pearce, 2010), European (BMU, 2008; Bhandari and Stadler, 2009; Dusonchet and Telaretti, 2010; Campoccia et al., 2009) and Asian (Wang and Qiu, 2009; Wang, 2010; Chaudhry et al., 2009) countries have passed solar energy policies in order not to depend on diesel and to increase national production of solar energy, as it has less impact on the environment (Solangi et al., 2011; Espinoza, 1991; Sommerfeld and Buys, 2014). As stated by Gamio, "Peru is an ideal place for renewable energy projects, because it has more resources and sources of green energy: such as sun, water, geothermal energy, wind or biomass" (Gamio, 2014).

In Latin America, there are proposals for the use of solar thermal energy in food processing, nonmetallic, textile, construction, chemical, and even business-related industries (Mekhilef et al., 2011). Despite the enormous technical potential, the large-scale development and deployment of solar energy technologies around the world must yet overcome a number of technical, financial, regulatory, and institutional barriers. Therefore, it is necessary to expand the incentives of the Kyoto Protocol, which are insufficient (Timilsina et al., 2012). A practical, economic and environmental option is the use of solar thermal systems to preserve vegetables and other products. Heating systems using solar thermal energy can improve product quality, as well as reduce waste and the use of traditional fuels, improving people's quality of life (Roche-Delgado et al., 2017). With respect to food conservation techniques, one problem is the implementation of dehydration facilities for national export institutions.

According to Benavides (2014), one of the constraints in the industry of dehydrated fruit and vegetables is the limited availability of dehydration machinery, therefore, plans are made to implement pilot dehydration plants in Ancash and Tarma. The basic objective is to use solar dryers with improved thermal performance and reduced environmental impact (AOAC International, 2000). In present, 10 to 40 % of the harvested products do not get to the final consumer. Because intermediaries pay less than the cost of production, producers often leave their products on their farm. As a detrimental result, in developing countries decomposition and contamination of the products is frequent, particularly in the rural regions of Peru. In the agricultural practice several post-harvest technologies can be observed, with the goal of food preservation. Nevertheless, the oldest method used is solar drying, given that it maintains physical, chemical, and nutritional properties.

For drying food, farmers believe that 35 % and 40 % of the total cost of the processing is currently due to industrial machines of high cost. Such devices are difficult to use in small farms; therefore, technological alternatives are needed, such as the solar dryer, which is less expensive and does not pollute the environment. In present, in Peru solar drying outdoors are an alternative solution, but they do not offer the quality of products needed for export. Considering that most of the farmers are either in places of difficult access or at the borders of the country, solar dehydrator is an option for enabling to export their products and get out of poverty. In other countries, this technology is being used in tropical and subtropical areas. Unfortunately, solar drying will not be adopted by farmers if they cannot evidence significant differences relative to open air drying, impacting the quality of the products.

Therefore, it is important to improve the drying of products specifically aguyamanto, which can reduce losses, increase quality, efficiency of the processing, and can provide greater acceptance by the agricultural community. Hence, this investigation has the following goals: (a) to design an autonomous indirect solar dryer for dehydrating aguaymanto, in a cost-effective manner and at an outcome, which meets the export quality; (b) to equip with advanced technology producers from the central part of Peru's highland area, zone in which this procedure is not yet available; (c) to build a prototype of autonomous solar dryer of 100 kg capacity by batch of aguaymanto, equipped with flat reflectors and forced air feed secured by photovoltaic energy; (d) this device should comply with dry fruit quality requirements for export.

To achieve the proposed objective, the area of the flat solar collector was reduced. Aguaymanto was chosen as the raw material to be dried, its morphology and drying rate were considered. Expected results were to design of a prototype for the indirect autonomous drying of a high quality aguaymanto, and devising plans for the local construction of the solar dryer according with the technological development of the local metal processing and machine construction companies. As recommendation, direct users of this innovative solar drying system should be small and medium farmers and agroindustrial plants that produce aguaymanto in Peru. So far, most of these facilities do not possess a cheap and and efficient drying technology. To a significant extent, this system will solve the problem of postharvest deterioration and will facilitate the exports. Climate change can also be mitigated.

2 Materials and methods

The physical-chemical characteristics (pH, acidity and soluble solids), proximal chemical (humidity, fat, protein, fiber, ash, carbohydrates) and the drying parameters of aguaymanto were determined according to the standards established by AOAC International (2000). An autonomous solar drying system was designed by the authors considering the followings: (I) Morphology and drying conditions of the aguaymanto shall be considered. Calculation of drying chamber dimensions are based on quantity of raw material to be dried; area of the transversal section of the hot air flow; manual unloading of the products to be dried. (II) Design of flat solar collectors: Meteorological variables are considered: wind velocity, intensity of solar radiation, latitude, altitude, relative humidity and air temperature (information will be obtained from the test site). It will be proceeded with the followings: analysis of the thermal circuit in a flat plate collector; energy balance accounting for absorbed heat, useful heat, and losses at the top and bottom of the solar collector of flat plates; theoretical calculation of the efficiency of solar collectors equipped with flat plates, determining the optimum collection area and the section area of air flow; in this process it is relied on the reflectors and photovoltaic system for forced flow, which can be optimized for dimensions of (length, width, and height the solar collector). (III) Design of forced air systems: control of temperatures inside the drying chamber will be implemented and the humidity of aguaymanto measured. (IV) Design of the control systems: stabilization and monitoring of temperatures, humidity and speed in the solar drying.

Considerations for the calculation and design of the solar drying system were encompassed into three categories: (1) Thermal, where the Sun was chosen as the source of energy. (2) Mechanical, which evaluated materials and construction details of the local technology. (3) Economic considerations referred to the cost of the system and was addressed for both the economic and cultural conditions of the farmers living in the area. Collecting the above data allowed to calculate the dimensions of the solar dryer and to select the right materials for making it. Also, in some cases, experimental data was used to figure some of the design parameters.

Characteristics of the forced-air-dryer's location: altitude: 3000 m.a.s.l.; typical climatic conditions of the highland; within cities, electric power is available, but in the field no electricity is accessible, therefore, both thermal and photovoltaic solar energy should be used.

3 Results and discussion

3.1 Chemical characteristics of dehydrated aguaymanto

In this experimental trial performed with an indirect dehydrator, results were obtained on the immediate chemical composition. Out of the measured parameters, listed in Table 1, humidity of aguaymanto fruit is of greatest interest. It has an average value of 15 %.

Table 1. Approximate chemical composition of the dehydrated aguaymanto (100 g) obtained in an indirect dehydrator.

Analysis	Results
Humidity	15.06 ± 0.03
Fat	1.32 ± 0.18
Protein	5.58 ± 0.32
Fiber	4.36 ± 0.28
Ash	1.72 ± 0.03
Carbohydrates	71.96 ± 0.35

3.2 Physical-chemical composition of dehydrated aguaymanto

In experimental trials conducted in an indirect dehydrator, results listed in Table 2 were obtained on the physicochemical composition of dehydrated aguaymanto, where the percentage of soluble solids represents the greatest interest. Its value averaged at 85%.

Table 2. Physical-chemical composition of dehydrated aquaymanto, obtained in an indirect dehydrator.

Analysis	Results
pH	4.05 ± 0.01
Acidity (Citric Acid Exp.)	1.02 ± 0.18
Soluble Solids	84.94 ± 0.03

3.3 Conceptual design of solar dryer

Obtained results allowed to determine the technology and define the geometric shape of the solar dryer and it has the following characteristics:

1. Characteristics of aguaymanto:
   - Manually manageable size.
   - Geometry of similar spheres, in the size range from 2.0 to 2.5 cm.
   - It is a delicate fruit, sensitive to the incidence of direct solar radiation.
   - Initial moisture content is in the narrow range of 79-82%.
2. Drying conditions and requirements:
   - Hot air drying at 60°C.
   - Preferably uninterrupted drying in batches of 100 kg.
   - Final humidity should be of 12%.
3. Selected technology: An indirect solar forced-air-drying is preferred, where a solar collector can be used to reach a drying temperature of 60°C. Ventilation is also regulated with forced air, using photovoltaic energy, according to the schematic displayed in Figure 1.

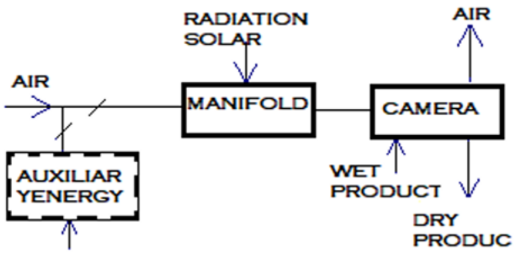

Figure 1. Schematic of an indirect dryer. Taken from Espinoza (1991).

3.3.1 Drying chamber

Given that aguaymanto is fragile but easy to manipulate, the fruit was placed in trays. Trays of 11 kgm^{-2} have been procured. The proposed chamber is of the shape of a parallelepiped (Figure 2), with the following characteristics:

- Flow form: parallel.
- Simple frame tray with millimeter mesh.
- Chamber bottom dimension: 0.60 m.
- Collector width: 1.40 m.
- Chamber height: 1.10 m (which corresponds to 22 trays, with 2 trays per row and 11 trays per holder level. Spaces between tray holders is of 6.50 cm.

Consequently, dimensions of the drying chamber are the ones listed in Table 3.

Table 3. Drying chamber dimensions in $[m]$.

	Inside interior	Outside exterior	Observation
Depth	0.60	0.70	Size difference between inside and outside arise from the insulation
Width	1.40	1.50	
Height	1.10	1.20	

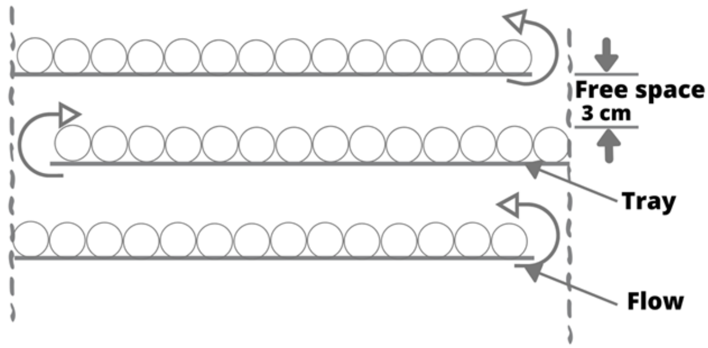

Figure 2. Parallel arrangement of fruit to be dried. Taken from Espinoza (1991)

Flat solar collectors: EThese are devices that allow to transform radiant solar energy into thermal energy in the form of hot air (Espinoza, 1991).

Size: In this part it can be approximated by a drying factor of 1.0 kg water/($m^2 *$day) (Espinoza, 1991).According to the humidity conditions of the aguaymanto, it is necessary to evaporate approximately 70 kg of water.

Estimated area: per day 70 m^2. The result obtained would mean that 70m^2 of flat collector is needed to dry in one day and therefore in three days 23,3m^2 . Hence, the most exact calculations will be done later. For the moment for the conceptual design, the alternative for the collector of 20m^2 and drying in three days is used; besides it is assumed for the width 1.4 m, the length is 16.6 m, which would result in the shape of the solar dryer as shown in Figure 3.

3.3.2 Design calculations for drying 100 kg of aguaymanto

In the design process, it is necessary to carry out some reference or checking calculations (Espinoza, 1991).

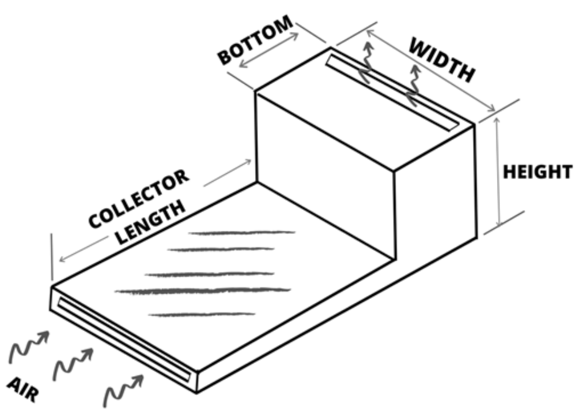

Figure 3. Diagram of the conceptual design of an indirect hybrid solar dryer according to Espinoza (1991).

Amount of energy required: If wanting to dry 100 kg of a product like the aguaymanto from 80% of initial humidity up to 12% of final humidity, there is:

- Initial water quantity: $100 \times 0{,}80 = 80$ kg
- Final water quantity: $100 \times 0/12 = 12$ kg
- Amount of water to evaporate: $80 - 12 = 68$ kg
- Latent heat of water evaporation at solar drying temperature= 2440 kJ/kg water
- Necessary energy : 68 kg water × 2440 kJ/(kg water) = 165920 kJ.

Necessary sunny days: The average annual radiation in Tarma is $5{,}61 kWh/m^2$ of solar radiation (Camayo-Lapa et al., 2017).

- Equivalent energy: 5,61 × 3600 = 20196 kJ/(m^2-day)
- Assuming $20m^2$ of collector: 20196 kJ/(m^2-day)×$20m^2 = 403920$ kJ/day.

Assuming a total efficiency of 40% one has:

- Available energy: 403920 kJ/day ×0,4 = 161568 kJ/day.
- Drying days: 165920 kJ / 161568 kJ/day = 1,03 days.

Therefore, 1.03 days of drying are needed. Hence, by having collectors of 1.40 wide × 2.40 long then 6 collectors are needed, and if placing reflectors like flat mirrors, only 3 solar collectors are needed to dry the product in one day approximately. Checking the solar collector length: Equation 1 is used (Espinoza, 1991); with ΔT the warming of the air through the collector $[°C]$, H solar radiation $[W/m^2]$, L the collector length $[m]$ and v velocity of air through the collector $[m/s]$:

$$\Delta T = (0,131H)\left(1 - e^{\frac{-0,12L}{v}}\right) \tag{1}$$

Given as radiation data of $900W/m^2$, a length of $2{,}40m$, velocity of $1m/s$ the collector can increase by $56{,}2°C$.

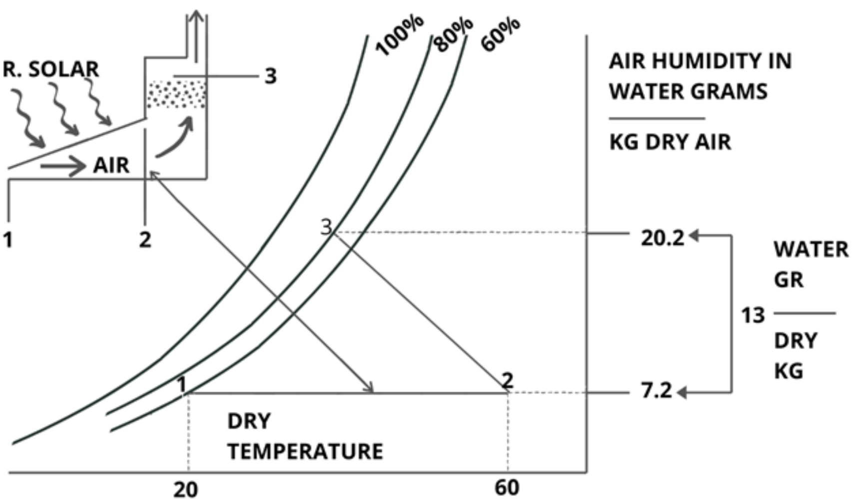

Figure 4. Diagram of the conceptual design of an indirect hybrid solar dryer (according to Espinoza (1991)).

3.3.3 Design of forced air systems

Calculation of the air flow required to dry: For the drying of 100 kg of wet product, from initial humidity of 80% to 12% of final humidity, such as aguaymanto, 24 h. If the average drying speed is $\frac{x_i - x_f}{t} = 7{,}87 \times 10^{-6}$ kg water/((kg dry) s). And if the 100 kg wet product consists of 80 kg water and 12 kg dry matter. The average evaporation rate would be: $7{,}87 \times 10^{-6}$ kg water/(kg dry-s) × 12 kg dry $= 9{,}4 \times 10^{-5}$ water/s.

This amount of water will have to be evaporated in an air stream with a certain flow rate and drying capacity, which is obtained from the psychometric chart for the air pressure and temperature conditions of the place where the drying will take place. According to the diagram Figure 4, 20 kg of forced-air-drying can evaporate 13 g of water, which corresponds to $9{,}4 \times 10^{-5}$ water/s. Thus, the air flow required is $9{,}4 \times 10^{-5}$ kg water/s x 1/(13 g water / kg air $\times$ 1 kg / 1000 g) $= 0{,}00723$ kg air/s $= 7{,}23$ g air/s. An airflow equal to $m = 7{,}23$ g air/s is needed.

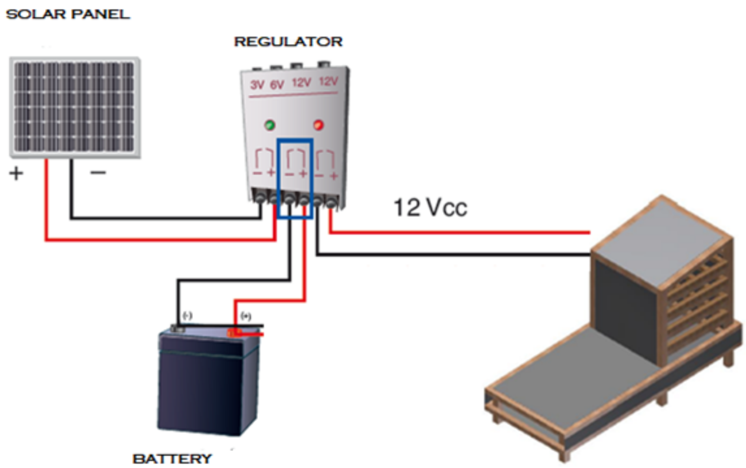

Figure 5. Diagram of the photovoltaic system for use in solar dryers.

Calculation of flow rate (Q) and velocity (v) of the air: The flow rate is calculated from Equation (2)with $\rho = 1{,}2 kg/m^3$ the air density.

$$Q = \frac{m}{\rho} \qquad (2)$$

Therefore, $Q = \frac{0{,}0072\text{kg air/s}}{1{,}2kgm^3} = 0{,}36m^3/s$ is the air flow needed. Recommended air velocity is from 1 to 2 m/s (Espinoza, 1991).

3.3.4 Final dimensions of the solar dryer

Drying chamber: The interior dimensions are 0.60 m, width 1.40 and height 1.10 m and it is expected 0.05 m for the insulation, so the exterior measurements are 0.70 m wide, 1.50 m deep, and 1.20 m high, as it is shown in Table 3.

Solar collector: It needs to be taken into account the dimensions of 1.50 wide, 2.40 long and 0.15 high, which will need 2 reflectors of flat mirrors for each collector, so that in the end it will need 2 units of solar collectors with their respective reflectors of flat mirrors. Likewise, the solar thermal dryer will consist of a photovoltaic system (Figure 5).

Solar panel dimensioning:

- Energy requirement = 404.8 Wh-day.
- Panel Power = Daily Energy Requirement / Daily Solar Incidence Hours.
- Hours of solar incidence in Tarma 5, 6 h-day.
- Panel power = 404.8 Wh-día / 5.6 h-day.
- Panel power = 72.29 W.
- The panel will be 1 module of 80 Wp.

4 Conclusions

Under the drying conditions carried out with an autonomous solar thermal system for indirect dehydrating in aguaymanto, these have nutritional characteristics that show the nutritional quality of the product and that the moisture content obtained guarantees its useful life. Due to the physicochemical characteristics of the aguaymanto, an indirect solar dryer with forced air was selected, where the energy source for the fans and the controls of temperature, humidity and air speed is from a photovoltaic system.The components of the autonomous drying system of 100 kg of aguaymanto per process designed for an average drying time of two days consists of: a collector with the dimensions: bottom of 1.50 m, width 2.40 m and height 0.05 m and 0.05 m is expected for the insulation, which increases its efficiency and reduces the collector area, it will have a reflector of flat mirrors of bottom 1.50 m wide 2.40 m on each side, in a drying chamber whose proposed interior dimensions are bottom of 1.50 m wide 2.40 m; the forced air system and controls is a photovoltaic panel of 80 Wp.

This drying proposal is expected to face the problem of post-harvest deterioration and facilitate the export because it will improve the quality, cost and clean of the aguaymanto. According to the technological development of the metal-mechanic companies of the town, the proposed construction of the solar dryer will allow its local construction. It is also expected that the direct users of this innovative solar drying system will be the small and the medium farmers and agro-industrial of the aguaymanto in our country.

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