Determination of the microbiological load in organic, industrial and transfer type eggs in the central-west region of the State of Veracruz

Determinación de la carga microbiológica en huevo tipo orgánico, industrial y de traspatio en la región centro-occidente del Estado de Veracruz

JIMENEZ-HERNANDEZ, Magdalena†*[1], NAVA-VALENTE, Noemi[1], DEL ANGEL-CORONEL, Oscar Andrés[1] and FRIAS-FRIAS, Rocío[2]

[1]Tecnológico Nacional de México-Instituto Tecnológico Superior de Huatusco. Programa de Maestría en Ingeniería[1]. Av. 25 Poniente no. 100 Col. Reserva Territorial, Huatusco, Veracruz. México. C.P. 94100
[2]Tesista de la División de Ingeniería en Industrias Alimentarias. Instituto Tecnológico Superior de Huatusco. Tecnológico Nacional de México. Av. 25 Poniente no. 100 Col. Reserva Territorial Huatusco, Veracruz. México C.P. 94100

ID 1er Autor: *Magdalena, Jimenez-Hernandez* / **ORC ID**: 0000-0003-1988-5753, **CVU CONACYT ID**: 904566

ID 1er Coautor: *Noemi, Nava-Valente* / **ORC ID**: 0000-0002-1598-5821, **CVU CONACYT ID**: 332980

ID 2do Coautor: *Oscar Andrés, Del Angel-Coronel* / **ORC ID**: 0000-0002-0848-907X, **CVU CONACYT ID**: 226585

ID 3er Coautor: *Rocío, Frias-Frias*

DOI: 10.35429/EJRG.2019.8.5.16.26 Received March 10, 2019; Accepted June 30, 2019

Resumen

El objetivo del presente trabajo fue determinar la carga microbiológica en huevo de traspatio, tipo orgánico e industrial de la región centro-occidente del estado de Veracruz. Se evaluó la presencia de microorganismos patógenos en los tres sistemas de producción y se determinó la calidad microbiológica de cada uno de estos sistemas. Lo cual es de suma importancia considerando que en huevo con alta carga microbiológica, pueden estar presentes microorganismos como *Salmonella spp S. enteritidis*, etc., los cuales son considerados agentes causales de infecciones entéricas en la población. Aunado a lo anterior, los sistemas de explotación antes mencionados, representan una fuente de ingreso económico relevante para las familias que habitan esta región. Para el análisis microbiológico, se tomaron diferentes muestras de cascaron y de la parte interna (clara y yema). Se evaluó la presencia de *Salmonella spp* aplicando el procedimiento establecido en la Norma Oficial Mexicana NOM-114-SSA1-1994. Además se realizaron pruebas físicas para evaluar si los defectos físicos en huevo pueden correlacionarse con la presencia de *Salmonella spp* u otras bacterias. **Contribución:** Actualmente es poca la información que existe sobre la calidad microbiológica, en huevo proveniente de los diferentes sistemas de explotación que se consumen en la región centro-occidente del estado de Veracruz, particularmente para el sistema orgánico y de traspatio.

***Salmonella spp.* Huevo de Traspatio, Huevo Tipo Orgánico**

Abstract

The objective of this work was to determine the microbiological load in backyard egg, organic and industrial type of the central-western region of the state of Veracruz. The presence of pathogenic microorganisms in the three production systems was evaluated and the microbiological quality of each of these systems was determined. Which is of the up most importance considering that in eggs with high microbiological load, microorganisms such as *Salmonella spp, S. enteritidis*, etc., may be present, which are considered causal agents of enteric infections in the population. In addition to the above, the aforementioned exploitation systems represent a source of economic income relevant to the families that inhabit this region. For microbiological analysis, different samples of shell and the internal part (white and yolk) were taken. The presence of *Salmonella spp* was evaluated by applying the procedure established in the Official Mexican Standard NOM-114-SSA1-1994. In addition, physical tests were performed to assess whether physical defects in eggs can be correlated with the presence of *Salmonella spp* or other bacteria. **Contribution:** Currently, there is little information on microbiological quality, in eggs from the different exploitation systems consumed in the central-western region of the state of Veracruz, particularly for the organic and backyard system.

***Salmonella spp*, Backyard egg, Organic type egg**

Citation: JIMENEZ-HERNANDEZ, Magdalena, NAVA-VALENTE, Noemi, DEL ANGEL-CORONEL, Oscar Andrés and FRIAS-FRIAS, Rocío. Determination of the microbiological load in organic, industrial and transfer type eggs in the central-west region of the State of Veracruz. ECORFAN Journal-Republic of Guatemala. 2019, 5-8: 16-26

* Correspondence to Author (email: magdalena_jimenez@itshuatusco.edu.mx)
† Researcher contributing first author.

Introduction

In 2017, Mexico ranked the first place of consumption per capita of the Mexican is 22.7 Kg, almost one egg a day. (National Poultry Farmers Union, 2018). Mexico ranks as the fourth largest egg producer worldwide, after China (National Poultry Farmers Union, 2018).
Eggs that are produced industrially or traditionally can be contaminated internally or externally (Minorl et al 1984). Enterobacteria of the genus Salmonella spp. They have been reported as pathogens for humans and in wild and domestic animals, poultry such as chickens. There are more than 2,500 enteric Salmonella serovars of which approximately 250 have been isolated from poultry, and around 40 serovars are the most commonly found (World Health Organization, 2017).

In eggs, Salmonella species can be found in the shell, but they can penetrate inside if adequate conservation conditions are not maintained, (Carbajal, 2006). To reach this food these bacteria use different routes, one of them is when they are present in the chicken where the egg comes from, here the contamination occurs internally, (Suárez M.et al., 2000). The other possibility is that the microorganism is present in the shell and its origins can be: animals, people who handle, distribute or are in contact with eggs, (Méndez, et al., 2011).

In this work the microbiological load of different egg production systems was determined, such as, backyard, organic and industrial type, in order to know the importance of each of them, as well as the health risks that may arise. This will improve their production, applying Good Poultry Practices (BPA), raising the quality of the egg produced in each of the aforementioned systems. The different samples used for the analysis were taken from the internal and external part of the different egg batches. (hull, white and yolk).

The presence of Salmonella spp was evaluated by applying the procedure established in Standard NOM-114-SSA1-1994. On the other hand, physical tests were carried out to assess whether the presence of physical defects in organic, industrial and backyard eggs can be correlated with the presence of Salmonella spp. or other bacteria.

The risk in backyard egg production, without BPA and without proper management in bird feeding, can present a high microbial load including pathogenic bacteria, as is the case in production systems that develop in the target region. study. In addition to the above, this system represents an economic income for the families that are engaged in this activity, so it is important to evaluate its microbiological quality.

Materials and methods

The experimental phase began with the collection of organic, industrial and backyard eggs that are produced or marketed in the central-western region of the state of Veracruz. Egg collection was carried out in different parts of the area, for example: backyard were collected in Atlanca, Tenexcalco, Tehuipango, Acatepec Chalchiltepec, all these locations belong to the Municipality of Huatusco, Coauixtlauac, belonging to the municipality of Zongolica, Potrerillo market , from the municipality of Orizaba. While the organic type egg was found in the Costco super market, the brands that were purchased were Vegetatarian from the state of Jalisco, certified organic egg (Finca Guayacán) certified by bio-agricet, the closest market to this region is located in Xalapa, other organic type eggs were obtained at the Chilcuahutla Farm, the industrial egg was purchased at the Aurrera supermarket of the commercial chain belonging to the municipality of Tomatlán and at the municipal capital of Huatusco.

On each collection point, a batch of 12 eggs was analyzed, from this, 3 samples were obtained per batch, each sample contained 4 eggs. The analysis that was elaborated to the egg included two stages that began with the measurement of external variables (shell) later internal variables were measured (white and yolk). To determine the presence of salmonella spp, the procedure established by Standard NOM-114-SSA1-1994 was used. Method for the determination of Salmonella spp in food.

The analysis that was elaborated to the egg included two stages that began with the measurement of external variables (shell) later internal variables were measured (white and yolk). To determine the presence of salmonella spp, the procedure established by Standard NOM-114-SSA1-1994 was used. Method for the determination of Salmonella spp in food

Physical analysis

As part of the physicochemical analyzes, each egg was weighed with an analytical balance (Electronic Analytical Balance Mod. 20002). Subsequently, the diameters of each egg, larger diameter (polar diameter) and smaller diameter (equatorial diameter) were measured for this purpose a vernier (Truper) was used, these data serve to know the size of the egg. Egg diameter data were used to determine the volume and surface area of each egg according to the methodology and equation proposed by Narushin (2005)

The response variables were: egg weight, larger diameter (DMa), smaller diameter (DMe). DMa and DMe measurements were used to determine the theoretical volume coefficient (Kv) and surface coefficient (Ks), according to the Narushin methodology (2005) with expressions:

$$Kv = 0{,}6057 - 0{,}0018 * (DMe)$$

Ovoscopy: The technique used to observe the state of eggs is ovoscopy, which consists of keeping the egg backlit for study in a dark room or box. With the present technique, the internal state of each egg was observed, obtaining corresponding data:

Air chamber: With the air chamber we can know the freshness of the egg if the air chamber is larger, its freshness is lower and vice versa. This variable was measured by placing a ruler on the upper left, with which the diameter of the air chamber was obtained and its size determined.

Position of the yolk: it was obtained with the observation of its position, by means of the ovoscope that allows visualizing the internal part of the egg without reaching its destruction.
Fractures: Fractures with greater size and thickness were observed with the naked eye, through the ovoscope the smallest ones were visualized by placing the egg against the light and turning at the same time to observe possible fractures.

Determination of the microbiological load for shell, white and egg yolk.

The microbiological analysis of the egg samples was performed according to NOM 114-SSA1-1994, in which 25.0 g sample is considered as an analytical unit, in a 1: 9 proportion of sample / broth. This amount can be varied as long as the same 1: 9 ratio is maintained in the pre-enrichment medium. The work was carried out under a laminar flow hood (Figursa Industry Mod.CFH-90).

Lactose broth was prepared as a pre-enrichment medium for culturing bacteria, to achieve a stable physiological condition in Salmonella spp. present in the samples.

For the pre-enrichment of the shell in lactose broth, sterile bags were used, placing in each bag 4 eggs without breaking them and pouring the amount of 36ml of lactose broth, maintaining the same 1: 9 ratio (4:36) of sample / broth.

The eggs remained 5 minutes in the bag promoting so that the impurities of the shell remain in the lactose broth. It was left to incubate (Memmert Incubator Mod. 30-1060) for 24 hours at a temperature of 37 ° C
After 24 hours of incubation the samples were observed to detect the possible growth of bacteria

Análisis de la clara y yema

For this step a batch of 12 eggs was used from which 4 eggs were taken from each sample in triplicate. They were poured into test tubes, to which numbers 1-4, 5-8 and 9-12 were assigned, according to the number of eggs contained in each sample.

4 eggs were poured into a beaker and perfectly homogenized.

15 ml were taken, to make a proportional analysis, for which 135 ml of lactose broth were taken, this proportion remained 15: 135 sample / broth.

150 ml flasks previously sterilized were used for this phase.

The sample was seeded in the lactose broth and incubated for 24 hours at a temperature of 37 ° C / 24 hours. (Memmert Incubator Mod. 30-1060).

Differential Staining (Gram Staining)

Samples from each of the bags previously incubated by the Gram stain technique were analyzed in triplicate and determined whether they corresponded to Salmonella spp.

Selective enrichment for clear and yolk shell

After 24 hours of incubation of the analysis of the shell, white and yolk in medium lactose pre-enrichment broth, a selective enrichment was prepared, the selective sterile cystine selenite medium was prepared which is specific for the growth of Salmonella spp, It is used to increase populations of Salmonella spp and inhibit other microorganisms.

Subsequently, 9 ml of selective medium (selenite cystine) were taken per sample and added to each test tube.

Subsequently, from each bag, a 1 ml sample was taken with a micropipette.

It was poured into test tubes containing 9 ml of the cystine selenite medium.

The thread of the tubes was perfectly closed, sealing them with tape to prevent the substance from spilling and each tube was labeled. Subsequently, it was stirred to properly homogenize the sample to the selective medium. Finally, the tubes were incubated at 37 ° C for 24 hours.

Figure 1 Comparison of tubes in different phases, Witness, Pre enrichment and Selective enrichment
Source: Self Made

Finishing the microbiological study in the shell, it is destroyed, its thickness is measured and the same procedure is repeated with the samples of white and yolk.

Multiple tubes for shell, white and yolk

To perform this method, a phosphate buffer was prepared, according to NMX-F-286-1992, which was sterilized at 120 ° C. Three test tubes were prepared with 9 ml of phosphate buffer, a 1000 µl aliquot of the sample previously incubated with cystine selenite was added. Subsequently, the same procedure was repeated with the tube already homogenized, taking an aliquot of 1000 µl of the tube and poured into a new test tube with 9 ml phosphate buffer, serial dilutions 0.1, 0.01 and 0.001 were prepared. Subsequently, they were incubated at 37 ° C for 24 hours. The optical density (OD) of each suspension at a wavelength of 620 nm was determined. Calibrating the device with a phosphate buffer blank. The measurements were taken every hour, being a total of 4 readings.

Pouring on plate

The solid medium (bright green agar) was prepared and sterilized at 121 ° C. The plates were subsequently prepared under laminar flow hood (Figurine Industry Mod. CFH-90). Samples with selenite were diluted 0.1 ml in phosphate buffer tubes.

This dilution allowed to obtain a better concentration of the sample and to observe quantifiable colonies. Once the bright green bile agar solidified, a 10 µl aliquot of the previously diluted sample was taken and poured into the Petri dishes. The cultures were incubated at 37 ° C for 24 hours.

If after 24 hours of incubation, no growth is observed, another 24 hours are expected, according to NOM-210-SSA1-2014. After the growth of the plaque bacteria, the colonies that were grown were counted, then the colonies that developed on the plates were counted; The following equation was used to determine the UFC Colony Formation Units / ml:

$$\frac{ufc}{ml} = \frac{number\ of\ plaque\ colonies}{dilution\ factor\ x\ plate\ volume}$$

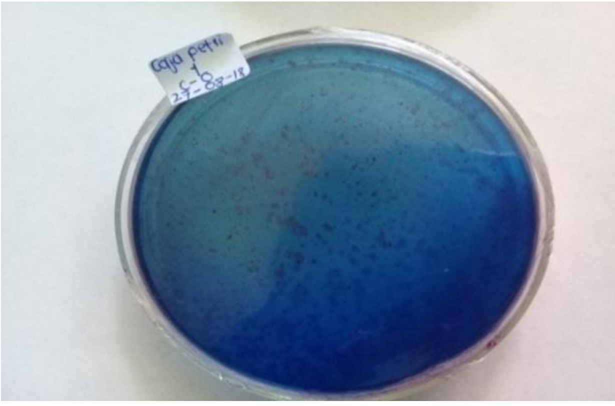

Figure 2 Plate incubated for 24 hours, in which colonies are observed
Source: Self Made

Results and Discussion

Physical analysis of organic, industrial and backyard type eggs

The results of the variance analysis did not show significant statistical differences (P≤0.05) in the volume, surface and weight parameters. The volume of the three types of eggs is statistically similar, but in the backyard egg there is a notable variability in the standard deviation, the same happens in the surface parameter. Regarding the recorded weight of the different types of eggs, it is observed that they are not significantly different, according to the Tukey mean comparison test (P≤0.05).

The thickness of the shell in the three types of egg production systems showed significant statistical differences (P≤0.05). The backyard eggs showed a greater thickness of the shell, while the organic eggs showed a lower thickness, those that presented an intermediate value were the eggs from the industrial production system.

In a study conducted by Hernández-Bautista et al., (2013) the properties of physical parameters of the eggs of 4 lines of hybrid hens were evaluated, of which 40 were housed in individual cages where food was provided and 80 were raised When fully grazing, a similar behavior in the parameters of egg weight and egg diameter was observed in these two systems, which may be due to the age of the birds. This explains the data that were obtained in the analysis of variance and the variability that is observed in the backyard eggs when not knowing exactly the age of the hen.

The aforementioned study also points out that the eggs of grazing chickens showed a better shell thickness, possibly due to the lower laying rate of grazing hens favors the increase in shell thickness.

In the analysis of variance with respect to the thickness of the shell of the organic type egg reflects a smaller thickness of the shell according to Muriuri and Harrison (1991). The high ambient temperature increases the chicken's body temperature by reducing the quality of the shell (shell thickness). According to Roland (1977) in the United States, the total egg produced by shell problems (thin shell and fractured) is not collected mainly in the southern states. The organic type eggs that were manipulated in the experimental phase are from organic farms in Mexico located in the north of the country with a warm climate that reduces the quality of the shell, which produces a smaller thickness.

According to Muriuri and Harrison (1991), the high ambient temperature reduces the quality of the shell. The organic type eggs manipulated for the experimental phase are from ecological farms in northern Mexico with a warm climate that reduces the quality of the shell that produces a smaller thickness of it.

Egg ovoscopy of three production systems

The technique used to observe the state of eggs is ovoscopy.

The results obtained in the analysis of variance in table 1, showed no statistically significant differences (P≤0.05). The air chamber in the three types of production systems is statistically equal, only a notable variability in the standard deviation in the backyard egg is observed.

Type of egg	Air chamber (cm)
Industrial	2.492±0.373 [a]
Organic	2.584±0.719 [a]
Backyard	2.474±2.479 [a]

* Means with the same letter in each row are not significantly different according to the Tukey mean comparison test (P≤0.05)

Table 1 Determination of the air chamber in industrial egg, organic type and backyard

ISSN-On line: 2414-8849

According to the previous data, no statistically significant differences were shown (P≤0.05) but with a wide variability. The variability observed in the backyard egg can be due to or attributed to the fact that they have a lower laying rate and therefore, the way in which the eggs are collected is different compared to the industrial eggs Juárez and Ochoa (1995).

Regarding the fractures in the ovoscopy of the industrial and backyard eggs, no fractures were observed while in the organic type eggs there was a percentage of 9.37% of fractures, this is consistent with the previous results in the physical tests. of the shell thickness where a smaller thickness is observed with respect to the other egg production systems, consequently a greater fragility.

Microbiological analysis in eggshell

To determine the microbiological load in eggshell, first a pre-enrichment was previously described in the methodology. The results of the analysis of variance showed significant statistical differences (P≤0.05) between the microbiological load observed in the different types of eggs evaluated. Table 2.

For the 0.1ml dilution it was observed that the industrial eggs were the ones with the lowest microbiology load (0.117 absorbance units), while in the backyard eggs and organic eggs the highest values of this variable were recorded (0.421 and 0.368 units , respectively).

With respect to the 0.01 dilution, significant differences were also observed (P≤0.05) between the three types of eggs, the industrial type again being the one with the lowest microbiological load value, while the backyard eggs maintained the highest concentration for this treatment; in the case of organic type eggs, these recorded an intermediate microbiological load in relation to the previous types.

Finally, for the dilution to 0.001 the industrial type eggs showed the highest values of microbiological load with respect to the organic and backyard types that were statistically equal according to the Tukey test (P≤0.05).

Dilution (ml)	Type of Egg * (Absorbance Units)		
	Backyard	Organic	Industrial
0.1	0.421±0.148 a	0.368±0.078 a	0.117±0.0155 b
0.01	0.053±0.014 a	0.048±0.010 ab	0.028±0.0051 b
0.001	0.011±0.004 b	0.012±0.006 b	0.038±0.0275 a

* Means with the same letter in each row are not significantly different according to the Tukey mean comparison test (P≤0.05).

Table 2 Determination of the microbiological load by DO620 spectrophotometry for organic, industrial and backyard eggs

According to the previous results, the lower concentration of microorganisms in industrial-type eggs can be attributed to the hygiene conditions with which this type of production system is managed, since it is characterized by a total control of food, health and cage care, as well as the handling of bird times and movements to avoid stress during the laying season (SAGARPA., 2009).

On the other hand, the management and control in the production of organic type egg and backyard is less strict, since in both cases the flocks of hens are freely grazed and there is little control over the laying sites. According to Cuca-García et al., (2011).

Microbiological analysis in egg white and yolk

The determination of the microbiological load of the white and yolk was made starting with a pre-enrichment of the sample, following with an enrichment in a selective medium such as selenite cystine incubated for 24 hours and consequently the realization of multiple tubes to determine the optical density at a wavelength of 620nm.

Table 3 shows the results of the analysis of variance showed significant statistical differences (P≤0.05) between the microbiological load observed in the white and yolk of the different types of eggs evaluated. For dilution 0.1 the analysis of variance showed no statistically significant differences (P≤0.05) between the three types of egg, the microbiological load of the backyard egg and organic type showed great variability.

In the 0.01 dilution, statistically equal results were observed according to the Tukey test (P≤0.05).

ISSN-On line: 2414-8849

Finally, in the 0.001 dilution, statistically significant results were shown (P≤0.05), the highest microbiological load in this dilution was the industrial one (0.0490 ± 0.034 a), the one with the lowest microbiological load was the organic one (0.0122 ± 0.005 b) while the backyard was at an intermediate point (0.0168 ± 0.031 ab)

Type of Egg * Absorbance Units			
Dilution	Backyard	Organic	Industrial
0.1	0.3229±0.283 a	0.2274±0.156 a	0.0626±0.023 a
0.01	0.0416±0.033 a	0.0639±0.090 a	0.0306±0.006 a
0.001	0.0168±0.031 ab	0.0122± 0.005 b	0.0490±0.034a

* Means with the same letter are not significantly different according to the Tukey mean comparison test (P≤0.05)

Table 3 Determination of the microbiological load in egg white and yolk by DO620 spectrophotometry for backyard, industrial and organic type eggs

According to the results discussed above; no statistically significant differences were shown (P≤0.05) but it was observed in the backyard egg and organic type there is great variability in the standard deviation. According to Mancera (2005) in a study conducted in Mexico City to identify Salmonella enteritidis in eggs for consumption. 131 isolates considered in 12 different bacterial genera were obtained:

Acinetobacter spp., Alcaligene spp., Bacillus spp., Branhamella spp., Edwardsiella spp., Hafnia spp., Klebsiella spp., Serratia spp., Shigella spp., Staphylococcus spp., Yersinia spp., obtaining a strain of Salmonella enteritidis, which was classified as not typifiable. This would partially explain the results obtained in Table 3 where similar microbiological loads were observed in the 0.1ml and 0.01ml dilutions of the three egg production systems, which does not mean that the inside of the egg is contaminated with Salmonella spp, it is possible There are also other types of bacteria that are not pathogenic. In this regard, the results of the analysis of variance of the microbiological load of the exterior and interior of organic type eggs did not show significant statistical differences (P≤0.05), in the results of the microbiological load of the shell. In the results of the white and yolk the results of the analysis of variance showed significant statistical differences (P≤0.05), the lowest microbiology load was found in dilution 0.1 (0.2274 ± 0.15 b).

	Dilution		
	0.1	0.01	0.001
Shell	0.3679±0.07 a	0.0476±0.01 a	0.0.117±0.006 a
Clara and Yema	0.2274±0.15 b	0.0639±0.09 a	0.0122±0.005 a

* Means with the same letter are significantly different according to the Tukey mean comparison test (P≤0.05)

Table 4 Microbiological load outside and inside of organic egg by spectrophotometry DO_{620}

This can be attributed to the fact that hens that are raised in this type of production have access to the outside. Access to pasture, despite not being specifically required for organic birds, allows birds to forage in search of plants, insects, and generally results in better health (Baier, 2015). Preventive practices and healthy living conditions, such as keeping feeding systems and drinking fountains clean, are critical for reducing diseases and the presence of pathogenic organisms such as Salmonella spp and E. coli (Baier, 2015).

	Dilution		
	0.1	0.01	0.001
Shell	0.4207±0.14 a	0.0532±0.01 a	0.0115±0.004 a
Clara and Yema	0.3229±0.28 a	0.0416±0.03 a	0.0168±0.031 a

* Means with the same letter are not significantly different according to the Tukey mean comparison test (P≤0.05)

Table 5 Microbiological load outside and inside backyard egg by spectrophotometry DO_{620}

The comparison of the internal and external bacterial growth of the backyard eggs was made from the dilutions shown in Table 5. The optical density was determined by spectrophotometry at a wavelength of 620 nm. The results of the analysis of variance did not show significant statistical differences (P≤0.05) between the microbiological load inside and outside the backyard egg and the corresponding dilutions. The above data can be attributed to the fact that under backyard conditions there is little or no sanitary management, the backyard bird feeding consists of what the birds can collect as leaves, tender herbs, fodder, insects, food leftovers, fruits and tortilla which should be the day to avoid digestive diseases (Cuca-García et al., 2011). According to a study conducted in Ecuador in the city of Loja, the presence of salmonella spp. In backyard chicken eggs marketed in different parts of that city, the presence of Salmonella spp.

In the microbiological analysis of suspicious samples, the confirmation disc was applied, its result being negative, despite this the egg if it contains a microbiological load in this study and genotyping was no longer continued. The results shown in table 5 present a microbiological load, this does not mean that it is salmonella spp. if there are other microorganisms present in the sample, which can cause interference, for example, between gram negative and positive species, multiple forms of the O chain or O antigen can be found, it is very variable in its composition between different families, species and even within the same species of gram-negative bacteria. However, despite the specificity of the antibodies (responsible for recognizing the component of interest) for a particular antigen (component of interest), nonspecific binding with other bacteria can occur.

There are studies that indicate that this phenomenon in gram-negative bacteria may be due to the antigenic similarity of lipid A among gram-negative bacterial species (Mutharia, 1984). The microbiological load of the exterior and interior of the industrial egg was determined by spectrophotometry, whose optical density was measured at a wavelength of 620 nm.

The results of the analysis of variance showed significant statistical differences ($P \leq 0.05$) in the microbiological load of the shell in the 0.1ml dilution (0.1170 ± 0.015 a) the highest microbiological load was shown while in the others they were of lesser load and similar . In the interior of the industrial egg in the white and yolk, no significant differences were found according to the Tukey mean comparison test ($P \leq 0.05$).

	Dilution		
	0.1	**0.01**	**0.001**
Shell	0.1170±0.015 a	0.0285±0.005 b	0.0382±0.027 b
Clara and Yema	0.0626±0.023 a	0.0306±0.006 a	0.0489±0.034 a

* Means with the same letter are not significantly different according to the Tukey mean comparison test ($P \leq 0.05$).

Table 6 Microbiological load outside and inside of industrial egg by spectrophotometry DO_{620}.

Table 6 of the microbiological load of the industrial egg showed statistically significant differences in the shell according to the Tukey mean comparison test ($P \leq 0.05$).

In a study carried out in Mexico City on consumption eggs (Industrial) Of the 400 samples taken from different companies, 131 bacterial isolates were obtained, 88 being yolk (67%), 26 of white (20%) and 17 of shell with membranes (13%).

Comparison of the number of colonies present in plaque by the method of pouring in plaque inside and outside the egg

The present comparison was obtained from the realization of multiple tubes using only the 0.1 ml dilution. This dilution was made to have a better concentration of the culture, obtaining isolated colonies that can be quantified and proportional to the start culture, consequently a medium of solid culture (bright green agar) to manipulate it in the plate pouring process according to NOM-114-SSA1-1994, this bright green agar allows quantifying the colonies present in the culture. The entire procedure was handled in the laminar flow hood to avoid cross contamination. The results presented in table 7 did not show statistically significant differences ($P \leq 0.05$). The three types of eggs have a statistically equal number of colonies, but a standard deviation with a wide variability in the two internal and external variables (shell, white and yolk) in backyard eggs.

	Type of egg		
	Organic type	**Backyard**	**Industrial**
Shell	96.28±85.79 a	112.09±85.09 a	18.33±9.46 a
Yolk and white	105.96±64.06 a	114.00±105.82 a	22.50±23.85 a

* Means with the same letter per row are not significantly different according to the Tukey mean comparison test ($P \leq 0.05$).

Table 7 Determination of the number of colonies present in each production system

The results showed no statistically significant differences ($P \leq 0.05$). All three types of eggs have a statistically equal number of colonies. About industrial eggs, work was found on the type of microorganisms present in the interior and exterior (shell, white and yolk) while in the organic types no work was found on microorganisms present in them, only their physical and sensory properties. In a study by Neira Solís (2016) About the microbiota in eggs and derivatives: identification and development, in this work we studied two production systems the backyard and the industrial.

ISSN-On line: 2414-8849

Commercial eggs showed great variability in the shell microbiota, being higher in backyard eggs than in industrial eggs, in no case was a genus of salmonella spp found, with respect to transport through the shell it was observed that microorganisms tend to adhere to the surface of the egg and can easily cross the natural protective barriers and thus multiply inside the egg, in the yolk the microorganisms find a liquid medium where they multiply easily, for this reason in the white and yolk there is a greater number of colonies than in the shell

Comparison of the number of colonies per plate discharge at different collection points

The present comparison of numbers of colonies in organic eggs from different collection points was obtained from the 0.1 ml dilution. This dilution was made to have a lower and better concentration of the culture, then a solid culture medium was made to analyze the samples on account. viable (plate pouring technique) according to NOM-114-SSA1-1994, the bright green agar (solid medium) allows quantifying the colonies present in the culture. All the analysis was performed under laminar flow hood to avoid cross contamination. This comparison was made to identify the collection point with the highest risk of contamination.

The results of the analysis of variance showed statistically significant differences in the eggshell, the highest value of the number of colonies occurred in the Finca de Chilcuahutla4 (202.50 ± 3.54 a) and the lowest in the Finca de Guayacán (10b). Regarding Clara and yolk, the analysis of variance did not show statistically significant differences, but with a notable variability in the Chilcuahutla Estate (125 ± 141.42 a).

Collection Points				
Variables	**Chilcuautla Farm**	**Jalisco**	**Huatusco**	**Guayacán Estate**
SHELL	202.50±3.54 ^{a}	186±91.5 ^{a}	48.38±27.9 ^{b}	10 ^{b}
WITHE AND YEMA	125±141.42 ^{a}	124.4±50.43 ^{a}	105.0±67.64 ^{a}	78.75±49.8 ^{a}

* Means with the same letter per row are not significantly different according to the Tukey mean comparison test (P≤0.05).

Table 8 Comparison of the number of organic type colonies in the differentcollection points

In the organic type system of the shell variable there is variability between the collection points as it has a high value and a very low value, the Guayacán Estate has an organic certification provided by bio-agricert Annex1.as well as the Jalisco for the certification of cage free hen. The Finca Chilcuahutla is certified by Rainforest and Amsa Starbooks despite this it has a high number of colonies.

In the backyard eggs there was great variability in the different collection points. In the case of the shell, it did not show statistically significant differences (P≤0.05). The data are statistically equal, the collection point of Atlanca presented a greater number of colonies (238.50 ± 54.45 a) but at the same time the various points presented very high standard deviations.

In the case of the white and yolk the results of the analysis of variance showed significant statistical differences (P≤0.05). The one with the highest number of colonies was Tehuipango (242.50 ± 10.61 a), the ones that indicated the lowest number of colonies were Mercado de Potrerillo (10 bc) and Chalchiltepec (20.00 ± 7.07 c). The results obtained in table 9 show collection points with a number of colonies that are too high and others that are too low or zero, this variability was presented in the clear and yolk of the different collection points, this can be attributed to the care that each place gives birds including feeding and laying sites.

Collection points (Number of colonies)							
Variables	**Atlanca**	**Tenexcalco**	**Tehuipango**	**Potrerillo Market**	**Coauixtlahua**	**Chalchiltepec**	**Acatepec**
Shell	238.50 ± 54.45 ^{a}	187.50± 17.68 ^{a}	112.17± 99.41 ^{a}	99.00 ^{a}	83.33± 80.36 ^{a}	56.25±58.34 ^{a}	47.83± 48.18 ^{a}
Clara and yolk			242.50±10.61 ^{a}	10.00 ^{bc}	212.50± 88.39 ^{ab}	20.00± 7.07 ^{c}	60.00±39.69 ^{bc}

* Means with the same letter per row are not significantly different according to the Tukey mean comparison test (P≤0.05).

Table 9 Comparison of the number of backyard egg colonies

Conclusions

The three egg production systems that were compared showed statistically equal physical parameters, such as volume, surface and weight. Only the shell thickness parameter was different, the one with the smallest shell thickness was the organic type egg. In the microbiological analysis of the shell of these egg production systems, a lower microbiological load was observed in the industrial eggs, while in the backyard and organic type eggs the microbiological loads were similar. Backyard eggs showed great variability in the standard deviation.

In the case of the microbiological analysis of the yolk of the three production systems, no statistically significant differences were shown. The three production systems indicated similarities in the microbial load. Organic and backyard type eggs recorded a wide variability in the standard deviation, this happened because they had too many values dispersed with respect to the average, they included quite low and very high values.

In the comparison of the number of colonies present in plaque by the method of pouring in plaque of the interior and exterior (shell, white and yolk) of the egg the results that were observed in the production systems were statistically equal, with a notable variability in egg Backyard and Organic this is attributed to the fact that in some collection points no colonies were found, in others low values were located while some had very high values. The white and yolk in the different production systems presented a higher value of the microbial load than the shell, the yolk is a nutrient rich medium where microorganisms multiply easily.

Organic type eggs have a low microbial load, the birds are cared for so that the eggs have a lower load or there is no microbial load, there are rules to take care of this type of system but not all ecological farms meet these requirements, for For this reason, farms were found where there were eggs with a high number of colonies and with a low or zero value, another factor that affects this type of egg was the fractures that were observed as it could be attributed to the presence of microorganisms.

Something similar happened with the Traspatio eggs, although they are higher than the organic ones, they are statistically equal, since they show variability between the collection points. This helps us to know the state of each production system, with this we can conclude that the Traspatio system can be improved, to reduce the microbiological load since at several collection points there was no microbial load or it was low everything depends on the care and Chicken feed. With the aforementioned we can improve the Backyard system to help the economy of families that depend on this practice. Until now, the amount of microorganisms present in the types of eggs consumed in the state of Veracruz had not been determined, particularly for organic and backyard production systems.

References

Baier, A. (2015). *Hoja de Datos: Producción de Aves Orgánicas para Carne y Huevos.* Estados Unidos: Especialista en Agricultura NCAT.

Carbajal, A. A. (2006). Calidad nutricional de los huevos y relación con la salud. *revista de nutricion practica*, 73-76.

Cuca-García J.M.1, G.-A. D.-P. (2011). Producción y Manejo de Aves Domesticas. *Universidad Autónoma Chapingo*, 237.

Juárez, A. y. (1995). Rasgos de producción de huevo y calidad de cáscara en gallinas criollas de cuello desnudo, en climatropical. . *Archivos de Zootecnia.*, 79-84.

Mancera Martínez Arturo, J. V. (2005). Identificación de Salmonella Enteritidis en huevo para consumo en la ciudad de México. *Técnica Pecuaria en México*, 229-237.

Méndez, I., Badillo, C., Ortíz, G., & Faccini, A. (2011). Caracterización microbiológica de Salmonella en alimentos de venta callejera en un sector universitario de Bogotá. *Médicas UIS*, 24:26-33.

Minorl., L. A. (1984). *Serology of salmonella.* london: bergman.

Muriuri, H. K. (1991). Effect of peripheral foot cooling on metabolic rate and thermoregulation on fed and fasted chicken hens ina hot environmet. *Poultry Sci.*, 74-79.

Mutharia, L. C. (1984). Monoclonal Antibodies Specific for Escherichia coli J5 Lipopolysaccharide: Cross-Reaction with OtherGram-Negative Bacterial Species. . *American Society for Microbiology, Infection and Immunity* , 631-636.

Narushin, V. (2005). Production, Modeling, and Education Egg Geometry Calculation using the Measurements ofLength and Breadth. *Poultry Science*, 482-489.

Neira Solis, C. (2016). *Microbiota en huevos y derivados: identificacion y desarrollo.* oviedo, españa.
Organizacion Mundial de la Salud, O. (septiembre de 2017). Obtenido de www.who.int/mediacentre/factsheets/fs139/es/

Roland, D. (1977). The extent of unconllected eggs due to indadecuate shell. *Poultry Sci.*, 1517-1521.

SAGARPA, S. (1ª Edición, 2009). Manual de Buenas Prácticas Pecuarias Producción de Huevo para Plato. *Subdirección de Promoción y Regulación de Inocuidad Pecuaria*.

Suárez, M. y. (2000). Presencia de Salmonella serovariedad Enteritidis en productos de origen avícola y su repercusión en salud pública . *Iatreia*, 13,238-245.

Union Nacional de Avicultores, U. (2018). *Situacion de la avicultura Mexicana.* http://www.una.org.mx/index.php/panorama/situacion-de-la-avicultura-mexicana.

ISSN-On line: 2414-8849