Volume 8, Issue 14 – January – June – 2021

E C O R F A N

Journal - Ecuador

ISSN-On line: 1390-9959

ECORFAN®

ECORFAN Journal - Ecuador, Volume 8, Number 14, June - 2021, is a biannual Journal edited by ECORFAN-Ecuador. Av.18 Marcial Romero Salinas l - Santa Elena, Ecuador. CP. 241550, http://www.ecorfan.org/ecuador/journal.php, journal@ecorfan.org. Responsible editor: Juan Carlos Olives Maldonado. ISSN: 1390-9959. Responsible for the last update of this issue ECORFAN Computer Unit. Imelda Escamilla Bouchán, PhD. Vladimir Luna Soto, PhD. Av.18 Marcial Romero Salinas l - Santa Elena, Ecuador, CP. 241550. Date of last update June 30, 2021.

ECORFAN Journal-Ecuador

Definition of Journal

Scientific Objectives

Support the international scientific community in its written production Science, Technology and Innovation in the Field of Biology and Chemistry, in Subdisciplines of experimental design, commerce, options, rural-flora and fauna, agronomy, natural.

ECORFAN-Mexico SC is a Scientific and Technological Company in contribution to the Human Resource training focused on the continuity in the critical analysis of International Research and is attached to CONACYT-RENIECYT number 1702902, its commitment is to disseminate research and contributions of the International Scientific Community, academic institutions, agencies and entities of the public and private sectors and contribute to the linking of researchers who carry out scientific activities, technological developments and training of specialized human resources with governments, companies and social organizations.

Encourage the interlocution of the International Scientific Community with other Study Centers in Mexico and abroad and promote a wide incorporation of academics, specialists and researchers to the publication in Science Structures of Autonomous Universities - State Public Universities - Federal IES - Polytechnic Universities - Technological Universities - Federal Technological Institutes - Normal Schools - Decentralized Technological Institutes - Intercultural Universities - S & T Councils - CONACYT Research Centers.

Scope, Coverage and Audience

ECORFAN Journal-Ecuador is a Journal edited by ECORFAN-Mexico S.C in its Holding with repository in Ecuador, is a scientific publication arbitrated and indexed with semester periods. It supports a wide range of contents that are evaluated by academic peers by the Double-Blind method, around subjects related to the theory and practice of experimental design, commerce, options, rural-flora and fauna, agronomy, natural with diverse approaches and perspectives , That contribute to the diffusion of the development of Science Technology and Innovation that allow the arguments related to the decision making and influence in the formulation of international policies in the Field of Biology and Chemistry. The editorial horizon of ECORFAN-Mexico® extends beyond the academy and integrates other segments of research and analysis outside the scope, as long as they meet the requirements of rigorous argumentative and scientific, as well as addressing issues of general and current interest of the International Scientific Society.

Editorial Board

Arbitration Committee

Assignment of Rights

The sending of an Article to ECORFAN Journal-Ecuador emanates the commitment of the author not to submit it simultaneously to the consideration of other series publications for it must complement the Originality Format for its Article.

The authors sign the Authorization Format for their Article to be disseminated by means that ECORFAN-Mexico, S.C. In its Holding Ecuador considers pertinent for disclosure and diffusion of its Article its Rights of Work.

Declaration of Authorship

Indicate the Name of Author and Coauthors at most in the participation of the Article and indicate in extensive the Institutional Affiliation indicating the Department.

Identify the Name of Author and Coauthors at most with the CVU Scholarship Number-PNPC or SNI-CONACYT- Indicating the Researcher Level and their Google Scholar Profile to verify their Citation Level and H index.

Identify the Name of Author and Coauthors at most in the Science and Technology Profiles widely accepted by the International Scientific Community ORC ID - Researcher ID Thomson - arXiv Author ID - PubMed Author ID - Open ID respectively.

Indicate the contact for correspondence to the Author (Mail and Telephone) and indicate the Researcher who contributes as the first Author of the Article.

Plagiarism Detection

All Articles will be tested by plagiarism software PLAGSCAN if a plagiarism level is detected Positive will not be sent to arbitration and will be rescinded of the reception of the Article notifying the Authors responsible, claiming that academic plagiarism is criminalized in the Penal Code.

Arbitration Process

All Articles will be evaluated by academic peers by the Double Blind method, the Arbitration Approval is a requirement for the Editorial Board to make a final decision that will be final in all cases. MARVID® is a derivative brand of ECORFAN® specialized in providing the expert evaluators all of them with Doctorate degree and distinction of International Researchers in the respective Councils of Science and Technology the counterpart of CONACYT for the chapters of America-Europe-Asia- Africa and Oceania. The identification of the authorship should only appear on a first removable page, in order to ensure that the Arbitration process is anonymous and covers the following stages: Identification of the Journal with its author occupation rate - Identification of Authors and Coauthors - Detection of plagiarism PLAGSCAN - Review of Formats of Authorization and Originality-Allocation to the Editorial Board-Allocation of the pair of Expert Arbitrators-Notification of Arbitration -Declaration of observations to the Author-Verification of Article Modified for Editing-Publication.

Instructions for Scientific, Technological and Innovation Publication

Knowledge Area

The works must be unpublished and refer to topics of Experimental design, commerce, options, rural-flora and fauna, agronomy, natural and other topics related to Biology and Chemistry.

Presentation of the Content

As a first chapter we present, *Maize: General characteristics, importance and challenges for its production in the state of Hidalgo Mexico*, by GONZÁLEZ-LEÓN, Yared, TÉLLEZ-JURADO, Alejandro, RAMÍREZ-VARGAS, María del Rocío, ANDUCHO-REYES, Miguel Angel and MERCADO-FLORES, Yuridia, with adscription in Universidad Politécnica de Pachuca, as a second article we present, *Physiological effects of hyperprotein diets with the addition of Eisenia foetida in broilers. Proposal for a model for heart disease*, by REYNOSO-OROZCO, Ramón, TORRES-GONZÁLEZ, Carlos, CONTRERAS-RODRÍGUEZ, Sergio Honorio and SÁNCHEZ-CHIPRES David Román, with affiliation in the Universidad de Guadalajara, as the third chapter we present, *Production and effects of green tea kombucha with blueberry and orange blossom honey without caffeine as probiotic inhibitor of pathogenic bacteria*, by LAGUNA-MORALES, Leslie Asenat, SANTIESTEBAN-LÓPEZ, Norma Angelica and MALDONADO-RESÉNDIZ, Jorge Ángel, with affiliation at Benemérita Universidad Autónoma de Puebla, as fourth article we present, *Innovative process of a food supplement made from oregano bagasse. "NUTRIOREG"*, by DELGADO-MARTÍNEZ, Martha Lilia, AGUIRRE-OROZCO, Mario Abelardo, MÁRQUEZ-MONÁRREZ, Olivia and CONTRERAS-MARTÍNEZ, Jesús José, with ascription in the Tecnológico Nacional de México, Campus Delicias.

Content

Article **ECORFAN Journal-Ecuador**
June 2021, Vol.8 No.14 1-7

Maize: General characteristics, importance and challenges for its production in the state of Hidalgo Mexico

El maíz: Características generales, importancia y los retos para su producción en el estado de Hidalgo México

GONZÁLEZ-LEÓN, Yared†, TÉLLEZ-JURADO, Alejandro, RAMÍREZ-VARGAS, María del Rocío, ANDUCHO-REYES, Miguel Angel and MERCADO-FLORES, Yuridia*

Universidad Politécnica de Pachuca, Mexico.

ID 1st Author: *Yared, González-León* / **ORC ID:** 0000-0002-7053-4214, **CVU CONACYT ID:** 605765

ID 1st Co-author: *Alejandro, Téllez-Jurado* / **ORC ID:** 0000-0002-5491-3679, **CVU CONACYT ID:** 30605

ID 2nd Co-author: *María del Rocío, Ramírez-Vargas* / **ORC ID:** 0000-0003-3811-4137, **CVU CONACYT ID:** 266405

ID 3rd Co-author: *Miguel Angel, Anducho-Reyes* / **ORC ID:** 0000-0003-2401-6608, **CVU CONACYT ID:** 89678

ID 4th Co-author: *Yuridia, Mercado-Flores* / **ORC ID:** 0000-0003-3278-2783, **CVU CONACYT ID:** 122168

DOI: 10.35429/EJE.2021.14.8.1.7 Received January 10, 2021; Accepted June 30, 2021

Abstract

The corn production is one of the most important agricultural activities in the world, because it is one of the cereals destined for human and animal consumption, its value has influence at economic, social and cultural level. Its life cycle is divided into vegetative and reproductive, with approximately 232 days of duration. In Mexico during spring-summer and autumn-winter periods is produced, under the temporal and irrigation modalities. In the state of Hidalgo, the Mezquital Valley is the zone with higher production, in where the blackwater and high productivity hybrids are used, however, it is important to mention that the temporal production include a large extension of agricultural soils, in where its main objective is to supply this product locally. As happens at worldwide level, the corn intensive production and other crops, is made by the utilization of chemical supplies, practice used since of the green revolution, which has caused the physicochemical a biological agricultural soil degradation. The aim of this review is described the corn general characteristics and its importance in the Hidalgo Mexico state, as well as analyze the challenges to its production of the sustainable way.

Food, Sustainable agriculture, *Zea mays*

Resumen

La producción de maíz es una de las actividades agrícolas más importantes del mundo, por ser uno de los cereales destinados al consumo humano y animal, su valor tiene influencia a nivel económico, social y cultural. Su ciclo de vida se divide en vegetativo y reproductivo, con aproximadamente 232 días de duración. En México se produce durante los períodos primavera-verano y otoño-invierno, bajo las modalidades temporal y de riego. En el estado de Hidalgo, el Valle del Mezquital es la zona con mayor producción, en donde se utilizan las aguas negras y los híbridos de alta productividad, sin embargo, es importante mencionar que la producción temporal incluye una gran extensión de suelos agrícolas, en donde su principal objetivo es abastecer este producto a nivel local. Como ocurre a nivel mundial, la producción intensiva de maíz y otros cultivos, se realiza mediante la utilización de insumos químicos, práctica utilizada desde la revolución verde, que ha provocado la degradación fisicoquímica y biológica de los suelos agrícolas. El objetivo de esta revisión es describir las características generales del maíz y su importancia en el estado de Hidalgo México, así como, analizar los desafíos para su producción de manera sustentable.

Food, Agricultura sostenible, *Zea mays*

Citation: GONZÁLEZ-LEÓN, Yared, TÉLLEZ-JURADO, Alejandro, RAMÍREZ-VARGAS, María del Rocío, ANDUCHO-REYES, Miguel Angel and MERCADO-FLORES, Yuridia. Maize: General characteristics, importance and challenges for its production in the state of Hidalgo Mexico. ECORFAN Journal-Ecuador. 2021. 8-14:1-7.

*Correspondence to Autor (E-mail: yuridiamercado@upp.edu.mx)
† Researcher contributing as first author.

Introduction

Food has been the main concern of mankind; therefore, throughout history, methods have been sought to obtain enough food to meet the needs of the population. Among the most important crops, corn (Zea mays) is one of the cereals with the highest demand worldwide for human and animal consumption, in addition to being used as raw material in a large number of industrial processes (Sámano, 2013; González-León *et al*., 2020).

In Mexico, this cereal is one of the most widely used ingredients in gastronomy, with an estimated daily consumption of 343 g per capita. Its importance also encompasses social and cultural aspects, being a fundamental part of the traditions of a great variety of indigenous communities. The latest available data indicate that by 2020, 7,481,136.87 hectares were allocated, which generated 21,885,170.16 tons, so more than 35% of the total arable area of the country was used, of this percentage just over 80% is rainfed, and the rest irrigated (Herrera et al., 2005; Vela et al., 2011; López et al., 2012; SIAP, 2019; SIAP, 2020).

Although Mexico is among the first ten countries that generate the highest yields worldwide, it is also an importer (Figures 1 and 2), which is necessary to cover the national demand mainly for white corn used for human consumption, and to a lesser extent for yellow corn used in different industries or in the preparation of animal feed (CIMMYT, 2010; SIAP, 2019). Therefore, it is necessary to look for strategies to face the current challenges of agriculture in a sustainable manner, and corn is one of the crops that could be considered as a sustainable crop. one of the crops that could be considered a priority for food sovereignty.

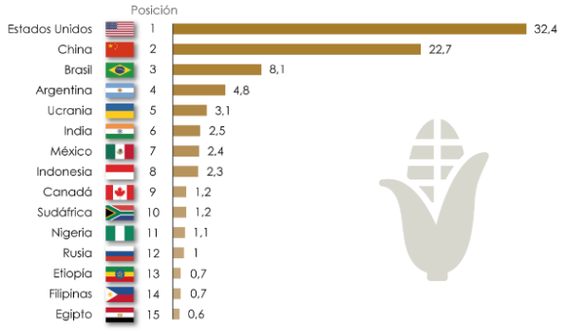


Figure 1 Contribution of different countries to world corn production in millions of tons
Source: (SIAP, 2019)

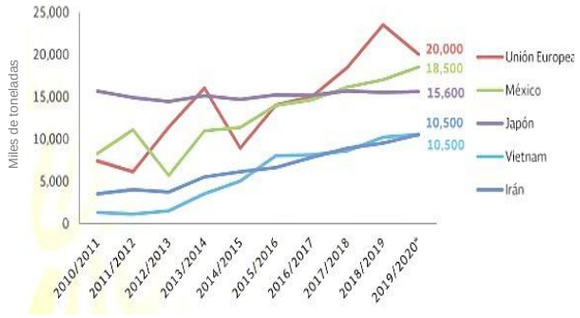


Figure 2 Major corn importing countries during the period 2010-2020
Source: (SIAP, 2019)

General characteristics of corn

Maize originated and diversified in Mexico, where it was domesticated from teosinte (Zea mays spp. parviglumis), a process that involved the selection of morphological variants, mainly in the architecture of the inflorescences, with the capacity to develop in a wide variety of agroclimatic conditions. Their classification has been carried out according to their morphology, under a hierarchical order, as shown in Table 1 (Doebley, 2004; Johnston-Monje and Raizada, 2011; Sánchez-González *et al*., 2018).

Kingdom	Plant
Division	Spermatophytes or phanerogams
Subdivision	Angiosperm
Class	Monocotyledoneae
Subclass	Glumiflorae
Order	Poales
Family	Poaceae or Gramineae
Tribe	Maydeae
Genus	*Zea*
Species	*Zea mays* L
Subspecies	*Zea mays* subsp *Huehuetenangensis* *Zea mays* subsp *Mexicana* *Zea mays* subsp *Parviglumis* *Zea mays* subsp *Mays*

Table 1 Taxonomic classification of maize
Source: (Revelo, 2006)

This plant consists of root, stem, leaves, flowers and fruits (Figure 3). It is robust and can reach up to 4 m in height. It has separate male and female inflorescences within the same plant; the former has a panicle with 20-25 million pollen grains. The female inflorescence has vegetative structures called spadix, which are arranged laterally. Its vegetative cycle is divided into five stages, which are described below (Vela *et al*., 2011; CYMMYT, 2017; CONABIO, 2017).

ISSN-On line: 1390-9959

Nascence: First stage comprising the period from sowing to the appearance of the coleoptile. Its duration can be 6-8 days.

Growth: After germination, a new leaf appears every three days, until generating 5 to 6, so that in the fourth and fifth week there will be a complete foliage.

Flowering: Period that begins between the fourth and sixth week in which the panicle is emitting pollen; with a duration of 5 to 8 days.

Fructification: After fertilization, fruiting begins, where the stigmas take on a brown color. The third week after pollination, the cob takes the definitive size and the formation of grains occurs.

Ripening and drying: In the eighth week after pollination, the grain reaches its maximum physiological maturity starting at 35% moisture, which decreases over time.

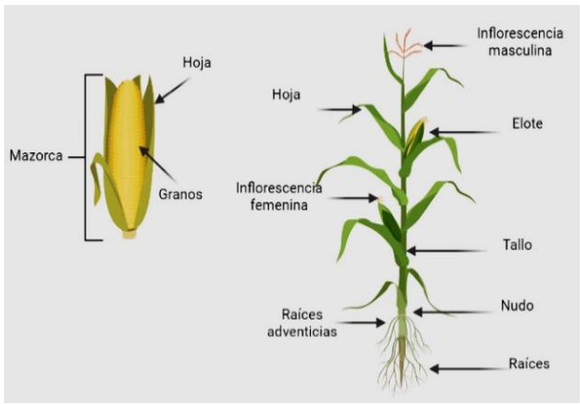


Figure 3 Schematic representation of the structure of the maize plant
Source: (Vela, 2011)

The stages of the maize cycle are the vegetative and reproductive stages, represented by the letters V and R respectively (Figure 4). The first has subdivisions and are designated numerically as V1, V2, up to Vn, where "n" represents the last leaf before the ear. Plant development has the following vegetative stages: emergence (EV), first leaf (V1), second leaf (V2), nth leaf (Vn) and spiking (VT). The reproductive stage comprises: Visible stigmas (R1), blistered grain (R2), milky grain (R3), doughy grain (R4), indentation (R5) and physiological maturity (R6) (Ranere et al., 2009; FAO, 2017).

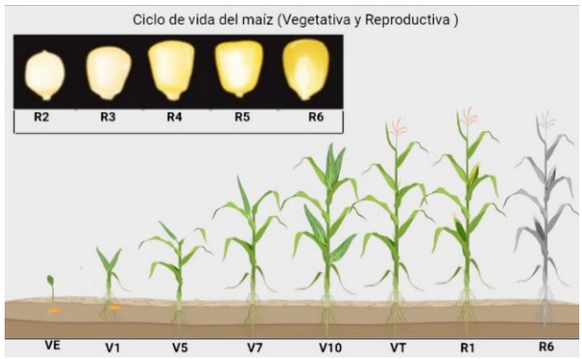


Figure 4 Schematic representation of the structure of the maize plant
Source: (FAO, 2017)

Importance of maize in the state of Hidalgo

In 2020, 392,669.45 ha of the surface area of the state of Hidalgo was used for agriculture, of which 58.8 % was used for corn grain production. Of this percentage, 75.3% was rainfed with a yield of 1.4 t/ha. For the remaining 24.7 %, yields of up to 8.2 t/ha were reported under irrigation (SIAP, 2019; SIAP, 2020).

Based on the above, the cultivation of this cereal is one of the main agricultural activities in the entity, with rainfed production supplying a large number of communities classified as high and very high marginalization, where, although yields are low, mainly creole maize is planted for self-consumption (Mercado-Flores et al., 2016; SIAP, 2020).

On the other hand, although the area allocated to irrigated production is smaller compared to rainfed production, yields are higher, such is the case of the Valle del Mezquital, an area made up of 27 municipalities where the cultivation of this grass is intensively practiced, making use of sewage from the metropolitan area of Mexico City and technological packages that include mechanization programs, improved seeds of high productivity hybrids, in addition to chemical inputs for fertilization and elimination of pests and diseases (Mercado-Flores *et al.*, 2016).

As happens worldwide, the crop is affected by weeds, insects and phytopathogens, with negative impact on productivity. The state of Hidalgo is under phytosanitary control of wireworm, cogollero, elotero, soldado and alfilerillo pests, as well as leafhopper and blind hen. It also includes the diseases coal ear and asphalt spot complex.

ISSN-On line: 1390-9959

The support is provided by the Government of Mexico in the Production for Wellbeing program. In this case, advice and inputs are given that are mostly of chemical origin; however, other health problems that are present in the different regions of the state are not contemplated (Galarza *et al*., 2008; Mercado-Flores *et al*., 2016; OSIAP, 2019).

Aspects to consider that influence maize production

Agriculture is considered the engine of the economy and will always be an indispensable sector because it generates a large number of jobs. However, there are different problems that must be addressed to ensure sufficient supply of this cereal, and thus contribute to food sovereignty (Figure 5) (González-León *et al*., 2020; Giller *et al*., 2021).

Agroecosystems are complex structures where climate is an important factor, since it determines the environmental elements and natural supplies for plant development, such as temperature, water resources, fertilization, as well as the presence of beneficial and harmful organisms (Godfray *et al*., 2010; Skendžić *et al*., 2021).

The increase in temperature in recent years has been associated with climate change. Its influence is reflected in plant development, which depends on the physiological characteristics of species that have been selected to develop in conditions different from the current ones. Another consequence is the arrival of more extensive heat waves at a global level and intense rainfall, only in some areas of the planet. On the other hand, the increase in a few degrees Celsius leads to water stress in plants, due to a deficit in soil moisture, as well as an increase in pests, weeds and diseases. Faced with this problem, agricultural producers, who generally practice traditional tillage, apply a large amount of chemical inputs to counteract the effects of different phytosanitary problems, low soil fertility and low water availability (Streck, 2005; Godfray *et al*., 2010; Rehman *et al*., 2015; Zhao *et al*., 2017; Skendžić *et al*., 2021).

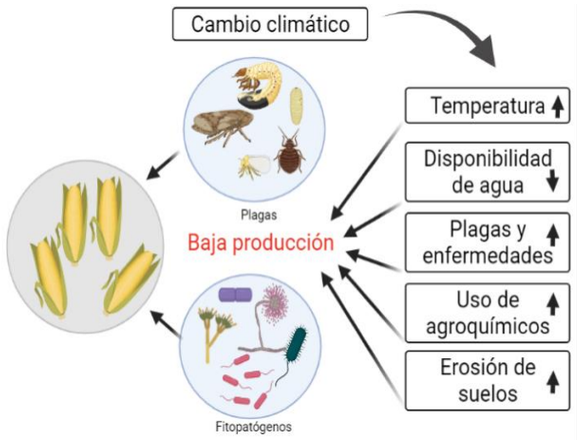


Figure 5 Schematic representation of the effects of climate change on maize production
Source: Image developed at BioRender.com

Numerous studies show that the most viable solution is to increase crop production before allocating more land area. Regenerative agriculture could be the most viable option, because it uses soil conservation as a starting point where the care of ecosystems is fundamental. Its objective is the sustainable production of food without causing negative impacts on the environment, providing social and economic benefits. Among the actions to achieve this is conservation agriculture, where zero or minimum tillage is carried out, which helps to maintain moisture by preserving the residues of the previous harvest, thus reducing dependence on this liquid. On the other hand, soil health is also reestablished by incorporating nutrients and maintaining populations of beneficial organisms. This can be improved with the use of compost and its leachates, green or animal manures, organic and biological fertilizers. In addition, chemical pesticides should be avoided and can be replaced by biological control agents, the use of insect pheromones and crop rotation. Another important aspect is to promote water percolation to avoid surface and subway contamination (González-León et al., 2020; Giller et al., 2021).

Challenges for increasing maize production in the state of Hidalgo

Taking into account that the state of Hidalgo has a mainly agricultural vocation, where maize is a priority crop (Mercado-Flores et al., 2016; SIAP, 2020), an analysis is made of the main challenges to be faced in order to increase the production of this cereal. The discussion is carried out according to the experiences of each of the authors.

ISSN-On line: 1390-9959

As a starting point, it should be considered that the environmental, as well as the economic and social environment, are not the same in irrigated areas compared to rainfed areas. In the first case, the cultivation of this grass is intensive with high productivity hybrids and mainly for economic purposes, while in the second case, the production of creole corn is for local consumption, and in many cases, traditions have a great influence.

On the other hand, the effect of climate change has been important, causing an increase in pests and diseases and directly affecting water availability, since rainfall for rainfed crops is scarce in some regions and very intense in others, while for irrigation, although there is availability of sewage water, levels have decreased considerably.

In this last point, the high organic load of this liquid has caused producers to disagree with the treatment of discharges, even taking into account that there are other pollutants that have important effects on the health of people and the agroecosystem.

In addition, almost all agricultural producers in the state use traditional tillage, with the increasingly frequent addition of chemical fertilizers and pesticides, which has caused soil erosion and environmental contamination that affects the health of the population living in agricultural areas.

Based on the above, the use of regenerative agriculture is proposed (Figure 6), where zero or minimum tillage would help restore the health of eroded soils and reduce dependence on water consumption by also promoting percolation. In the case of irrigation, the treatment of the discharges used is very necessary, so other forms of fertilization will have to be provided, which can also help in rainfed crops.

This could be enhanced with the help of microorganisms to control pests and diseases, as well as inoculants to enhance plant development through biological fertilization, making use of environmentally friendly products that should be designed in such a way that they can counteract the effects of climate change.

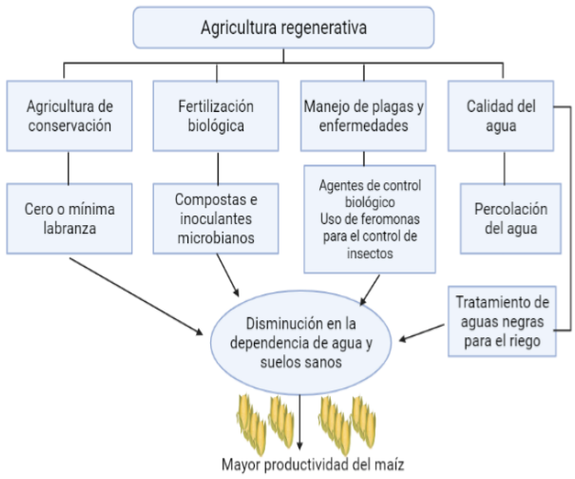


Figure 6 Schematic representation of actions that are part of regenerative agriculture as a strategy to face the challenges of corn production in the state of Hidalgo
Source: Image developed in BioRender.com

Acknowledgments

The authors would like to thank CONACyT for the Master's scholarship awarded to González-León, Y., a student of the Postgraduate Program in Biotechnology at the Polytechnic University of Pachuca.

Conclusion

Maize cultivation in the state of Hidalgo is a priority. Regenerative agriculture could provide solutions to increase the productivity of this cereal; therefore, it is essential that the government, society, producers, and academia work together to develop ecological policies and strategies to confront the ravages caused by climate change and guarantee food sovereignty.

References

CIMMYT. (2010). Centro Internacional de Mejoramiento de Maíz y Trigo. Enfermedades del maíz: Una guía para su identificación en el campo. Accessed August 2021: http://repository.cimmyt.org/xmlui/bitstream/handle/10883/812/94349.pdf?sequence=1&isAllowed=y

CIMMYT. (2017). Centro International de Mejoramiento de Maíz y Trigo. Partes de la planta de maíz. Accessed August 2021: http://maizedoctor.org/es/partes-de-la-planta-de-maiz

CONABIO. (2017). Comisión Nacional para el Conocimiento y Uso de la Biodiversidad Maíces. Accessed August 2021: http://www.biodiversidad.gob.mx/usos/maices/maiz.html

Doebley J. (2004). The genetics of maize evolution. Ann Rev Genet. 38:37–59. DOI: 10.1146/annurev.genet.38.072902.092425

FAO. (2017). Organización de las Naciones Unidas para la Alimentación y la Agricultura. Morfología del maíz tropical. Accessed August 2021: http://www.fao.org/docrep/003/x7650s/x7650s04.htm

Galarza MJM; Miramontes PCU; Cruz DMS; Gómez VMM; Ortíz PME; Entzana TAM; Suárez HCY; Santillán MV. (2008). Situación actual y perspectivas del maíz en México 1995–2012. http://www.financierarural.gob.mx/informacionsectorrural/Documents/Documentos%20de%20SIAP/PerspectivasMa%C3%ADz1996a2012.pdf. Consultado en agosto 2021.

Giller KE; Hijbeek R; Andersson JA; Sumberg J. (2021). Regenerative agriculture: an agronomic perspective. Outlook Agri. 50:13–25. DOI: 10.1177/0030727021998063

Godfray HC; Beddington JR; Crute IR; Haddad L; Lawrence D; Muir J.F; Pretty J; Robinson S; Thomas SM; Toulmin C. (2010). Food security: the challenge of feeding 9 billion people. Science. 327:812–818. DOI: 10.1126/science.1185383

González-León Y; Anducho-Reyes MA; Cartagena-Luna A; Mercado-Flores Y. (2020). Capítulo 10. Agricultura sostenible: herramienta para la soberanía alimentaria. En: "Mujeres en la Ciencia", Handbooks T-VIII. (Marroquín JA; Olivares-Ramírez JM; Cruz-Carpio LE; Bautista-Jiménez A. eds). ECORFAN-Mexico, S.C. México. pp. 142-153. DOI:10.35429/H.2020.8.142.153

Herrera JP; Cadena P; San-Clemente A. (2005). Diversidad de la artropofauna en monocultivo y policultivo de maíz (*Zea mays*) y habichuela (*Phaseolus vulgaris*). Boletín del Museo de entomología de la Universidad del Valle. 6:23–31.

Johnston-Monje D; Raizada MN. (2011). Conservation and diversity of seed associated endophytes in *Zea* across boundaries of evolution, ethnography and ecology. PloS One 6:e20396. DOI: 10.1371/journal.pone.0020396

López MSL; García NG; Ibarra GBN. (2012). El maíz (*Zea mays* L.) y la cultura Maya. Biotecnia. 14:3–8.

Mercado-Flores Y; Cartagena-Luna A; Sanchez-Maya HE; Pérez-Camarillo JP; Ortega-Bernal J; Álvarez-Cervantes J; Anducho-Reyes MA. (2016). El Carbón de la espiga del maíz en el Valle del Mezquital. En: Biotecnología y Alimentos en Hidalgo: Transitando a la Bioeconomía. (Batalla-Díaz L; Gómez-Aldapa CA; Castro-Rosas J; Téllez-Jurado A. eds). Amalgama Arte S.A. de C.V. pp. 105–116.

OSIAP (2019). Organismos de Sanidad e Inocuidad Agroalimentaria para Productores. CAMPAÑAS FITOSANITARIAS. http://osiap.org.mx/senasica/sector-estado/hidalgo/Agricola. Consultado en agosto 2021.

Ranere AJ; Piperno DR; Holst I; Dickau R; Iriarte J. (2009). The cultural and chronological context of early Holocene maize and squash domestication in the Central Balsas River Valley, Mexico. PANS. 106: 5014–5018. DOI: 10.1073/pnas.0812590106

Rehman MU; Rather GH; Gull Y; Mir MR; Mir MM; Waida UI; Hakeem KR. (2015). Effect of climate change on horticultural crops. En: Crop Production and Global Environmental Issues (Hakeem KR. Ed). Springer International Publishing. Cham, Switzerland. pp. 211–239.

Revelo M. (2006). Proyecto de prefactibilidad para la comercialización de maíz. http://repositorio. ute.edu.ec/bitstream/123456789/6982/1/27776_1.pdfConsultado en agosto 2021.

Sámano RMA. (2013). La agroecología como una alternativa de seguridad alimentaria para las comunidades indígenas. Revista mexicana de ciencias agrícolas. 4:1251–1266.

ISSN-On line: 1390-9959

Sánchez-González JJ; Ruiz-Corral JA; García GM; Ojeda GR; Larios LC; Holland JB; Medrano RM; García-Romero GE. (2018). Ecogeography of teosinte. PloS One. 13: e0192676. DOI: 10.1371/journal.pone.0192676

SIAP. (2019). Servicio de Información Agroalimentaria y Pesquera. Reporte del mercado de maíz. https://www.cima.aserca.gob.mx/work/models/cima/pdf/cadena/2019/Reporte_mercado_maiz_070819.pdf Consultado en agosto 2021.

SIAP. (2020). Servicio de Información Agroalimentaria y Pesquera. https://nube.siap.gob.mx/avance_agricola/ Consultado en agosto 2021.

Skendžić S; Zovko M; Živković IP; Lešić V; Lemić D. (2021). The Impact of climate change on agricultural Insect Pests. Insects. 12: 440. DOI: 10.3390/insects12050440

Streck NA. (2005). Climate change and agroecosystems: The effect of elevated atmospheric CO2 and temperature on crop growth, development, and yield. Ciênc. Rural. 35:730–740.

Vela E; Viterí S; Granados M; González A. (2011). El Maíz, de la época prehispánica a la actualidad, alimento y cultura. Arqueología mexicana. 16:97–113.

Zhao C; Liu B; Piao S; Wang X; Lobell DB; Huang Y; Huang M; Yao Y; Bassu S; Ciais P; Durand JL; Elliott J; Ewert F; Janssens IA; Li T; Lin E; Liu Q; Martre P; Müller C; Peng S; Peñuelas J; Ruane AC; Wallach D; Wang T; Wu D; Liu Z; Zhu Y; Zhu Z; Asseng S. (2017). Temperature increase reduces global yields of major crops in four independent estimates. PNAS. 114: 9326–9331. DOI: 10.1073/pnas.1701762114.

ISSN-On line: 1390-9959

Article **ECORFAN Journal-Ecuador**
June 2021, Vol.8 No.14 8-22

Physiological effects of hyperprotein diets with the addition of *Eisenia foetida* in broilers. Proposal for a model for heart disease

Efectos fisiológicos de las dietas hiperproteicas con la adición de *Eisenia foetida* en pollos de engorde. Propuesta de modelo de cardiopatía

REYNOSO-OROZCO, Ramón†*´, TORRES-GONZÁLEZ, Carlos´´, CONTRERAS-RODRÍGUEZ, Sergio Honorio´´´ and SÁNCHEZ-CHIPRES David Román´´´

´Dept. of Cellular and Molecular Biology-CUCBA,´´Undergraduate student of Biology, ´´´Department of Environmental Sciences. ´´´´Dept. Animal Production. University of Guadalajara. Km 15.5 highway to Nogales, Las Agujas, Nextipac, Zapopan, Jalisco, Mexico.

ID 1st Author: *Ramón Reynoso-Orozco* / **ORC ID:** 0000-0002-2072-8192, **CVU CONACYT ID:** 35383

ID 1st Co-author: *Carlos, Torres-González* / **ORC ID:** 0000-0002-9423-0565

ID 2nd Co-author: *Sergio Honorio, Contreras-Rodríguez* / **ORC ID:** 0000-0002-8905-2725

ID 3rd Co-author: *David Román, Sánchez-Chipres* / **ORC ID:** 0000-0002-5273-0393, **CVU CONACYT ID:** 6943

DOI: 10.35429/EJE.2021.14.8.8.22 Received January 15, 2021; Accepted June 30, 2021

Abstract

Proposing animal models that allow predicting results in humans becomes critical when the analogies in physiology between both entities are reviewed. About heart disease, the heart rate in humans is more similar to that of chickens than that of the mouse, rat or other mammalian models generally used to study this disease. In the present work, the ethology on the attraction of chickens to earthworms as a food source was reviewed, in addition hematological, organ and urological parameters were measured in chickens fed with double and triple the protein percentage supplied with *Eisenia foetida* live added to the feed. commercial for the Cobb500 line. The results show a marked attraction depending on the nutritional status of the birds for *Eisenia foetida* and differences in hematological parameters, but not for urological parameters. The morphological characteristics of the heart showed a clear association between three times the protein load in the food and cardiac damage in 2 of 7 animals fed during 7 weeks of study. The present work represents the first contribution with the animal model approach in chickens to study cardiac damage and its possible prediction for humans.

High protein diet, *Eisenia foetida*, Heart disease

Resumen

Proponer modelos animales que permitan predecir resultados en humanos se vuelve crítico cuando se revisan las analogías en fisiología entre ambas entidades. Con respecto a la enfermedad cardíaca, la frecuencia cardíaca en humanos es más similar a la de los pollos que a la del ratón, rata u otros modelos de mamíferos generalmente utilizados para estudiar esta enfermedad. En el presente trabajo se revisó la etología sobre la atracción de los pollos por las lombrices de tierra como fuente de alimento, además se midieron parámetros hematológicos, orgánicos y urológicos en pollos alimentados con el doble y el triple del porcentaje de proteína aportado con *Eisenia foetida* viva adicionada al pienso. comercial para la línea Cobb500. Los resultados muestran una marcada atracción en función del estado nutricional de las aves para *Eisenia foetida* y diferencias en los parámetros hematológicos, pero no en los urológicos. Las características morfológicas del corazón mostraron una clara asociación entre tres veces la carga de proteínas en los alimentos y el daño cardíaco en 2 de 7 animales alimentados durante 7 semanas de estudio. El presente trabajo representa la primera contribución con el enfoque del modelo animal en pollos para estudiar el daño cardíaco y su posible predicción para humanos.

Dieta hiperproteica, *Eisenia foetida*, Cardiopatía

Citation: REYNOSO-OROZCO, Ramón, TORRES-GONZÁLEZ, Carlos, CONTRERAS-RODRÍGUEZ, Sergio Honorio and SÁNCHEZ-CHIPRES David Román. Physiological effects of hyperprotein diets with the addition of *Eisenia foetida* in broilers. Proposal for a model for heart disease. ECORFAN Journal-Ecuador. 2021. 8-14:8-22.

* Correspondence to Autor (E-mail: ramón.reynoso@academicos.udg.mx)
† Researcher contributing as first author.

Introduction

The proposal to use new animal models to predict what will happen in humans (Shanks et al., 2009) makes sense when the analogies between the entities involved are taken into account. Currently high protein and low carbohydrate diets are considered to have positive effects on functional capacity in patients with heart damage, although studies are believed to be necessary and since they are not carried out in animal models, their realization is not very feasible. Excessive consumption of these diets and for prolonged periods it can have some adverse effects on the body, these are aggravated when associated with a sedentary lifestyle (Dos Reis et al., 2018). These effects occur mainly in the liver, kidney, skin and on the intestinal microbiota (Vasconcelos, 2021). However, in the short term, diets high in protein, low in carbohydrates, and intermittent fasting promote greater weight loss and could be adopted as a starter for treatments. However, due to undesirable effects, caution is required. There are controversies in the application of diets, for example, to treat being overweight and obese have become popular and are generally widely adopted. However, they are based primarily on personal impressions and reports published in "gray literature" books and magazines, rather than scientific evidence. Human clinical trials and animal models to study changes in body composition and metabolism to determine efficacy meet many limitations and must be carefully analyzed according to Freire (2020). Regarding dietary patterns, it is important to mention that even with technological advances when meta-analysis studies are intended to be carried out, the limitations are recognized given the heterogeneity of the data (Sanches Machado et al., 2018), and it cannot be conclusive in different perspectives of investigation.

For Gout and Heart Failure, developing study models is very important since it is practically impossible to address them from diagnosis to treatments and their consequences. Many mouse models have been established to investigate the causal mechanisms of hyperuricemia, a condition directly associated with Gout, but no mouse model of spontaneous gout exists, despite the availability of animals with stable and increased serum urate concentrations (Lu et al., 2019).

An alternative can be a Gout model in chickens since it represents a health problem that affects poultry and appears frequently enough (Ejaz et al., 2005), this coupled with the fact that hyperprotein diets develop a picture similar to that of humans (Singh et al., 2013). Combining evolutionary and clinical biological studies, Hong's (2020) group concludes that chicken is a suitable animal model for hyperuricemia.

On the other hand, the development of Gout is associated, among other risk factors, with this type of diet (Ejaz et al., 2005). In addition, that diets directed towards Arterial Hypertension try to minimize the consumption of red meat, among other recommendations, since it is well known that high blood pressure is associated with functional changes in the heart and blood vessels, including altered function of the left ventricle (Sanches Machado et al., 2018). Both are diseases that can be addressed with animal models that can reproduce these health problems from diets rich in protein.

One metabolic explanation for the disease and the association between the quail and chicken models is that as in humans, the gene encoding uricase is also inactive in bird species (Remy et al., 1951). This physiological similarity with humans makes it possible to propose models with birds for the study of Gout. In addition, and given that uric acid is the final degradation product of dietary or endogenous purines and that its metabolism varies from one species to another, but is similar in man, primates and birds (Ejaz et al., 2005). For the problem of Heart Failure, the failure of numerous subsequent clinical studies is recurrent, which demonstrated efficacy in animal models, but not in patients. Although it is not considered among the candidate models for Heart Failure, one analogies that the chicken presents with respect to the human is the closeness in the heart rate values, between 95 and 105 beats per minute and 60 to 80, in man (Riehle and Bauersachs, 2019). Values much closer than those of mouse or rat.

Eisenia foetida is the most widely used earthworm in captivity conditions in vermicomposting due to its wide distribution, tolerance to temperature fluctuation, resistance to handling, ability to live in organic waste, with different degrees of humidity, in addition to its high reproductive capacity and rapid growth, among other advantages (Schubert et al., 2019).

ISSN-On line: 1390-9959

The acceptance among farmers in the application of vermite technology based on the use of earthworms allows to see its greater impact with the increase in sustainable development and strengthening the macroeconomy, as well as at the social and the underlying ecological level (Singh et al., 2020). Another area of knowledge of *Eisenia foetida* that has gained importance is in the diet given its nutritional characteristics when worm flours are made to market as protein ingredients that allow farmers not only to reduce their production costs, but also to improve the efficiency of production (Bahadori et al., 2017; Bollido, 2021), and for poultry farmers an excellent alternative to soy and fish meals, for Khan's group (2016) there is very little information on the use of worm meal in the diet of poultry. Mimicking wildlife conditions and providing a preferred feed as part of environmental enrichment whereis included *Eisenia foetida* can enhance the natural behavior of broilers without causing frustration. Although poultry are considered predatory of worms and risky for vermicomposting (https://lombritec.com/lombriz-roja-californiana-depredadores), no ethological studies of attraction for this type of food have been carried out in chickens fattening. The purpose of the present work is to study the effects of based hyperprotein diets *Eisenia foetida* live in broilers on physiological parameters that may influence the development of Heart Failure and/or Gout.

Metodology

Experimental test

The experimental test was developed in the Behavioral Test Booth of the facilities of the University Center for Biological and Agricultural Sciences, of the University of Guadalajara, which is located at the coordinates of 20º25'30 " to 20º57'00 " north latitude. and 103º19'30 " to 103º39'20 " west longitude, at a height of 1,548 meters above sea level. The average annual temperature is 22 ° C, with a maximum of 36.1 ° C and a minimum of 11 ° C. It has an average rainfall of 906.1 millimeters (García, 2004).

Test attraction by Eisenia foetida

In the experiment attraction Eisenia foetida from broilers line cobb500. 100 chickens 24 hours old were used in pens 2 m^2 where they were provided 20 g in trays feeder type live earthworm. Three tests were carried out with worms for three consecutive days twice/day. It is important to mention that in the first contact the group that responded was that of the chickens that presented nutritional deficiency with an average of 10.5% g less in weight. Chickens that showed interest and took worms and later ate them were marked with a black marker on the nape of the neck. In the 6 attraction experiments, the same 17 chickens plus 4 that showed interest until the third day of contact, allowed to establish a 21% attraction towards *Eisenia foetida* as a preliminary test to start the hyperprotein test with a different flock.

Groups with diets

From a different flock, 21 33-day-old birds of the Cobb500 genetic line were used, with an average weight of 1.26 Kg, randomly distributed in 3 batches with 7 birds each, the control group received the recommended diet for chickens fattening according to the cobb500 manual, 2018. The second group also received 12.5 g of earthworm, *Eisenia foetida*, alive and the same weight of feed was withdrawn. The third group received in addition to the fattening feed 25 g of worm and the same 25 g of commercial feed was withdrawn. It is important to mention that of the groups fed with *Eisenia foetida*, an excessive attraction was noted for two of the birds in the 25 g group and another 8 with moderate attraction between both groups; the other 4 birds showed almost no such behavior. The birds were housed on a floor with a chip bed, which when they reached the age of 33 days they were transferred to quail cages and 7 were housed per cage. At 45 days of age, they were placed in cages for laying hens and one per cage was placed to administer the corresponding diets. At day 60, given that the birds were unable to stay in the "laying" cages, and their weight gain, they were moved one per pen on a floor with chip bed until slaughter. The experiment lasted 7 weeks and the birds were euthanized at the end of the test.

ISSN-On line: 1390-9959

Ingredient (%)	Start	Finish
Oil	6.00	6.00
Sorghum	48.18	59.28
Soybean paste	35.92	26.34
Ca Carbonate	1.21	1.10
Dicalcium Phosphate	0.68	0.28
Premix	8.00	7.00

Table 1 Dietary characteristics broilers inclusion percentages for ingredients of the diets provided

Nutrient	Initiation	Completion
Protein (%)	21.46	18.01
Metabolizable energy (Mcal / Kg of weight)	2.92	3.07
Calcium (%)	0.96	0.78
Phosphorus	0.48	0.39

Table 2 Nutritional content in the diets provided

Preparation of Fresh Vermi (Eisenia foetida)

The vermi was grown in animal manure, such as livestock waste, poultry excrement. The cultivation was carried out in cement beds 1 m long x 40 cm high x 1 m wide, with integrated drainage through a 3-inch diameter pvc pipe, with a cement bed and covered with shade mesh and in the open. The food was prepared by precomposting to eliminate pathogens with a temperature of 20 to 40 °C and a pH of 6 to 9, added every 15 days and watered with tap water. With a volume of approximately 50 Kg/bed.

To feed the chickens, the corresponding grams of *Eisenia foetida* of any stage of development were taken indiscriminately.

Bromatological Analysis of Eisenia foetida

From 200 g of Live earthworms were desiccated at 40 ºC in a drying oven for 48 hrs and the corresponding tests were carried out for: Moisture and volatile matter using the method of the Official Methods of Analysis of the Association of Official Analytical Chemists (AOAC) 934.01; Protein (% NX6.26) AOAC 655.04; Ethereal extract AOAC 920.39; Ashes AOAC 942.05; Crude fiber AOAC 962.09; ELN by difference and Dry matter by difference

Feeding System

During the development of the birds the feed was provided in the morning in a controlled way, according to a feeding program where they were increased according to their requirement and an appropriate consumption was guaranteed of nutrients according to the NRC (1992) tables.

The food was served in tray-type feeders for the first 33 days, and later it was offered in quail cage feeders. The water was initially supplied in vitrolero drinkers and later bell drinkers were used. After 33 days the weight gain was recorded weekly.

The live earthworm *Eisenia foetida* was included in the diet in such a way that it was ingested by the birds prior to consumption of the forage to guarantee total and controlled consumption, and according to the technical reference of the avian lineage. A vaccination schedule was carried out according to the study area and they received a dose of vaccine with live Newcastle virus, intraocularly.

Body Weight Gain

The birds of the three treatments were weighed every seven days with an OHAUS® model T21P digital scale, to obtain weight gain up to the week of slaughter.

Sampling

At the beginning of the experimental test, 21 days later and at the time of sacrifice, blood was obtained to evaluate the hemograms acquired using the MISTIQ18 equipment (Orphée, Switzerland). At the beginning and 21 days later during the test, the blood was extracted by puncture of the vein (clavicular) and in the sacrifice it. By means of a ventral incision, the organs were exposed and the interior of the body was photographed to record any evidence of evident damage related to the treatments. The dimensions of the joint were measured, it was cut in its proximal portions. The heart, liver, spleen were removed. The organs were previously weighed and in the case of the heart an apical cut was made to observe the internal chambers and they were photographed for their registration.

Joint Dimension

The dimensions of the femuro-tibial were measured with a vernier, first antero-posterior and then lateral to calculate the difference in the total dimension with an approximation that allows measurement in mm^2.

ISSN-On line: 1390-9959

Obtaining the serum

After obtaining the serum by intravenous puncture or by gravity in the carotid vein, it was cooled at 4 ° C for 24 hrs and subsequent centrifugation at 2,500 rpm for 10 min. Serum was obtained and the clot was discarded.

Hematology

The heparinized blood sample was analyzed with the MISTIQ18 (Orphée, Switzerland) chemical veterinary accountant of hemograms. The equipment works by automatically sucking through an exposed needle in the apparatus approximately 70 μL of blood sample, it is an automated multi-parameter hematology analyzer designed for diagnostic use in vitro, which analyzes blood cells from different species, which they provide the necessary reference to the clinical diagnosis. The reading methods of the MISTIQ18 (Orphée, Switzerland) are through electrical impedance and colorimetry to run the parameters of leukocytes, erythrocytes, platelets and hemoglobin appropriate for the qualitative and quantitative analysis of the visible components in the blood of the individual.

Procedure for Faeces

For the collection of excreta, a plastic was spread in the pens where the birds were placed and the faeces deposited on the plastic were absorbed with a rubber pipette. They were transferred into 15 mL conical tubes and frozen until their use for urinalysis.

Urinalysis

The stool samples were placed in a water bath for thawing at 35 °C for ~ 3hrs. Each sample was diluted ~ 1:2 w/v, homogenized and allowed to settle, then the test strip was immersed for at least 10 seconds, allowed to dry and was introduced into the slot of the automatic equipment "KONTROLab 11Vet", which was thrown the results after one min. The parameters that are measured are: color, leukocytes, ketone, nitrites, urobilinogen, bilirubin, proteins, glucose, specific gravity, blood cells, pH, and ascorbic acid.

Statistic analysis

The effect of the treatments was analyzed with a repeated measures anova, where the experimental unit was 7 animals, and for each treatment 7 repetitions were used, when there were statistically significant differences, the means were compared with the Holm-Sidak procedure with an $\alpha = 0.05$.

Results

Ethology by Eisenia foetida

It seems common to know that all chickens accept worms 100% without restrictions. However, when we added to the diet of the 33-day-old chickens, it became apparent that not all the animals accepted said food. The result was that of one n = 14 exposed animals, only 6 showed interest and of these 2 were voracious, they even seemed to have addiction to *Eisenia foetida*. Therefore, an additional test was performed with 100 10-day-old chicks and no response was found on the first exposure to worms. Since 44 chicks were found to have an average weight of 10.5% (257 vs 230 g) less, the worms were exposed separately to a ration of 20 g of earthworm. The experiment was carried out in six-fold 3x at day 10 and 3x at day 11 of age. In the first contact, and without such a forceful attraction, an n = 17 animals attracted by the food was obtained and in the third contact with the worms another 4 chicks joined the group adding 21 in total, that is, 47.7%, It should be noted that in the 3 subsequent contacts the same behavior was confirmed by the total number of chickens.

Bromatological Analysis

Table 2 shows the results of the Bromatological study for *Eisenia foetida* carried out in our laboratory where it can be seen that the moisture and volatile matter content expressed as a percentage represents more than 84%, followed by the dry matter obtained by weight difference. It is interesting to note that although the percentage of Wet Base for Protein occupies 9.41%, it is equivalent to 60.25% in Dry Base, the highest content in the sample.

ISSN-On line: 1390-9959

Determination	Method	Result BH (%)	Result BS (%)
Moisture and volatile matter	AOAC 934.01	84.38	0.00
Protein (% NX6.26)	AOAC 655.04	9.41	60.25
Ethereal extract	AOAC 920.39	1.21	7.54
Ashes	AOAC 942.05	1.54	9.90
Fiber crude	AOAC 962.09	0.10	0.62
ELN (Nitrogen Free Extract)	By difference	3.36	21.69
Dry matter	By difference	15.62	100

Table 3 Results of the Bromatological Analysis for *Eisenia foetida*

Weight gain

In Fig. 1, regarding total body weight, there were no statistically significant differences between the treated groups. Feed conversion was measured weekly but It did not present statistically significant differences (Data not shown).

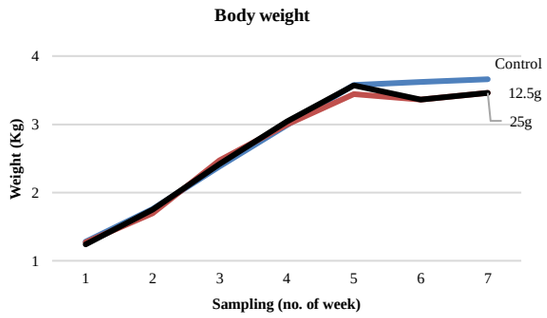


Figure 1 Body weight in Kg of the treatment groups during the 7 weeks of the study. The groups did not show representative statistical differences

Weight of Organs at Sacrifice

Parameter	Treatments					
	Control		12.5 g		25 g	
	Average	Dev. Std.	Average	Dev. Std.	Average	Dev. Std.
Body weight (Kg)	3.66	0.21	3.46	0.21	3.46	0.25
Heart (g)	16.3	2.4	15.78	2.0	17.17	2.7
Liver (g)	46.45	6.0	44.82	3.2	42.05	3.2
Spleen (g)	4.1	1.2	3.44	0.5	3.24	0.7

Table 4 Body and organ weight at slaughter

In table 3 it can be observed that, although there are no statistically significant differences in the total body weight of the chickens in the three experimental groups, the differences in weight of the organs show a tendency to increase the size of the heart in the group of 25 g of *Eisenia foetida*. added to the diet.

The difference is 5.33% higher compared to the control group and 8.52% compared to the group added with 12.5 g of worm, which showed the lowest value and was 3.19% lower than the control group. It is very important to show the photographs that show the lateral deterioration of the myocardium Fig. 2a and 2b, corresponding to the chickens that showed the greatest voracity for worms. As well as Fig. 2c of a bird from the control group.

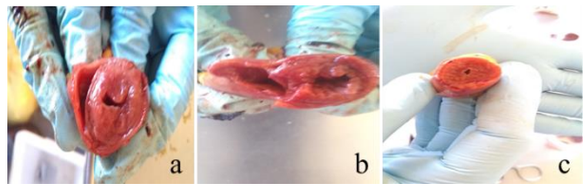


Figure 2 Photographs showing the left lateral myocardial damage of chickens with the addition of 25 g of Eisenia foetida (a and b) and of a chicken fed the commercial diet

In the same table 3 it is observed how the liver shows a slightly more considerable decrease in weight (9.47%) for the group added with 25 g of worm compared to the control (46.45 g) and a little less decrease, more than double, for the group with 12.5 g (3.5%) of the roundworm added to the diet.

The spleen shows a similar trend with 20.97% less weight for the group added with 25 g of earthworm and almost 16.1% in the group added with 12.5 g of *Eisenia foetida*, With a weight of 4.1 g for this organ of the control group (Table 3). The relative weight of the spleen in broilers of the control group was significantly higher compared to the worm treatments.

Treatment											
		Control				12.5 g of E. f.				25 g of E. f.	
Chicken no.	Body weight (Kg)	thickness (mm²)	weight (g)/thickness (mm²)	Chicken no.	Body weight (Kg)	thickness (mm²)	weight (g)/thickness (mm²)	Chicken no.	Body weight (Kg)	thickness (mm²)	weight (g)/thickness (mm²)
Chicken no 66	3.48	590	169.5	Chicken no 93	3.64	540.33	148.4	Chicken no 88	3.26	556.26	170.6
Chicken no 68	4.1	857.49	209.1	Chicken no 92	3.48	595.11	171	Chicken no 86	3.3	570.21	172.7
Chicken no 76	3.54	646.6	182.6	Chicken no 96	3.2	629.01	196.5	Chicken no 84	3.72	558	150
Chicken no 77	3.64	570.18	156.6	Chicken no 94	3.58	686.66	191.8	Chicken no 85	3.24	553.04	170.6
Chicken no 98	3.46	599.76	173.3	Chicken no 97	3.2	588.53	183.9	Chicken no 87	3.28	564.75	172.2
Averages		652.806	178.2			607.928	178.3			560.452	167.2
Desv. Std.		117.8	19.62			54.2	19.31			6.93	9.69

Table 5 Ratio of total body weight to the thickness of the femoro-tibial joints of chickens treated with *Eisenia foetida*

Joints

Table 4 Ratio of total body weight to the thickness of the femoro-tibial joints of chickens treated with *Eisenia foetida*.

ISSN-On line: 1390-9959

Joints thickness

Table 4 shows the results of the relationship that exists between the total body weight of the chickens with respect to the thickness of the femoro-tibial joints, comparing the weight in g with respect to the dimensions in mm^2. Since there is no uniform weight-to-joint ratio, this ratio is averaged and the group fed 25 g of *Eisenia foetida* shows a decrease in joint thickness of almost half (9.69 mm^2) compared to the other two treatment groups (control 19.62 mm^2 and 19.31 mm^2 for the group fed 12.5 g).

Hematology

Table 5 shows the results obtained from the hematological test where we could divide the parameters to be determined into three blocks, all of which show an average plus-minus the standard deviation.

Parameters							
	Erythrocytes	Hemoglobin	Hematocrit	MCV	MCHC	(STD)RDW_SD	RDW_CV
	Average/DS	Average/DS	Average/DS	Average/DS	Average/DS	Average/DS	Average/DS
Control	2.66/.33	16.35/2.5	25.7/3.9	95.6/10	66.53/10	46.22/2.9	14.5/1.8
12.5 g of *E. f.*	2.94/.24	15.35/6.0	25.43/9.2	98.75/5.9	59.37/4.4	47.31/6.7	13.95/2.4
25 g of *E. f.*	2.92/.41	16.8/2.2	30.64/5.2	104.3/6.8	55.12/3.4	54.78/15	14.7/2
	% Leukocytes	% Lymphocytes	% Monocytes	% Granulocytes	# Lymphocytes	# Monocytes	# Granulocytes
	Average/DS	Average/DS	Average/DS	Average/DS	Average/DS	Average/DS	Average/DS
Control	14.45/7.5	94.67/1.7	4.45/1.4	0.375/.28	13.7/6.0	0.5/.14	0.25/.36
12.5 g of *E. f.*	84.01/31	94.5/2.1	4.72/1.6	0.72/.6	20.03/25	1.29/2.2	0.36/.47
25 g of *E. f.*	11.06/3.8	94.3/2.6	4.35/1.2	1.35/1.5	10.57/4.2	0.45/.25	0.22/.13
	Platelets x10^3/µL	MPV (*fL*)	PDW (*fL*)	Thrombocytes (%)	P_LCR (%)	P_LCCx10^3/µL	
	Average/DS	Average/DS	Average/DS	Average/DS	Average/DS	Average/DS	
Control	108.25/16	13.25/1.1	17.05/5.3	0.137/.02	46.12/7.8	49.5/11	
12.5 g of *E. f.*	108.25/23	13.39/.57	16.54/3.8	0.144/.03	44.4/4.2	49.4/14	
25 g of *E. f.*	115.2/15	13.76/.27	20.12/3.3	0.152/.02	48.96/2.9	56/9.4	

Table 6 Hematological parameters of Cobb500 chickens fed with Eisenia foetida at the time of slaughter

Results of the hematological test in chickens that received diets supplemented with 25 g/day or 12.5 g/day of *Eisenia foetida*, at the time of slaughter. Represented as an average and its Standard Deviation of an n = 7 and in some cases lower, since the sample was not processed properly. (Red blood cell distribution width) RDW_CV; (Red Cell Distribution Repeated Precision) (STD) RDW_SD; (Mean corpuscular hemoglobin concentration) MCHC; (Mean Corpuscular Volume) MCV; (Mean platelet volume) MPV; (Platelet distribution width) PDW; (Higher platelet range) P_LCR; (Major platelets) P_LCC.

Group of 25 g of *Eisenia foetida* seems to present anemia at the beginning of the experiment and the results show a clear recovery when the number of erythrocytes per µL of blood is measured, in addition, in the final part of the experiment approximately 300,000 more erythrocytes are observed than the control for each µL of fluid and only 20,000 cells more than group fed 12.5 g of worm.

The hemoglobin (HB) test measures, in this case, the amount of the molecule in the blood. Here, almost 0.5 g/dL more HB was obtained in group 3 compared to the control and 1.5 g/dL compared to group 2. The hematocrit measures the amount of blood made up of red blood cells. At the end of the experiment, approximately 5% more erythrocytes were observed in group of 25 g of added worm compared to the other two groups. The MCV blood test measures the average cell size of the erythrocytes and in our results it was found that the size of the erythrocytes is more than 10 femptoL greater than the control and almost 5 femptoL than group of 12.5 g of *Eisenia foetida*. In the amount of intracellular Hemoglobin (Humans = 28 to 32 pg/cell). It is possible to see 4.3 pg of HB more in the erythrocytes of the control group compared to group 2 and 5.1 compared to group of 25 g of added worm. According to these results, it seems that HB is found in the extracellular space in chickens treated with 25 g of *Eisenia foetida*.

Regarding immune cells, one of the individuals showed disproportionately high values for the 12.5 g group and the DS extends too much except for the percentage of lymphocytes. An immunostimulatory effect is observed in the dose of 12.5 g of added worm and an immunosuppressive effect in the diet with 25 g of *Eisenia foetida*. If the number of immune cells obtained with the cytometer is observed, there are 3,000 more lymphocytes/µL in the control group compared to group 3 and 10,000 cells/µL, that is, twice in group of 12.5 g of *Eisenia foetida* compared to group of 25 g of *Eisenia foetida*. In Monocytes, 500 were obtained cells/µL less in group 3 compared to the control and almost three times in group of 12.5 g of *Eisenia foetida* compared to group of 25 g of *Eisenia foetida*. For Granulocytes an average of 300 cells/µL less was found in group 3 or fed 25 g of worms with respect to the control and 140 cells/µL more in group 2 or fed with 12.5 g of worm compared to group of 25 g of *Eisenia foetida*.

In terms of platelet and thrombocyte values, an increase in said parameters was found directly related to the amount of *Eisenia foetida*. included in the diet. Although slightly higher the values are constant except for larger platelets (P_LCC), when the control group is compared with the one fed with 12.5 g more of worm, see Table V.

ISSN-On line: 1390-9959

For Platelets in group 3 of 25 g of *Eisenia foetida* it is observed almost $7x10^3$ cells more than the other two groups.

Stool	Treatment		
Parameter (average)	Control (N = number of individuals)	12.5 g (N = number of individuals)	25g (N = number of individuals)
Leukocytes (cells /µL)	57.8571429	65.7142857	42.1428571
Ketones (mmol / L)	ND-	ND-	ND-
Nitrites	ND	ND	ND
Urobilinogen (µmol / L)	Normal + 33 (1)	Normal + 33 (1)	Normal + 33 (2)
Bilirubin (µmol / L)	NA + 8.6 (1)	NA	NA
Proteins (g / L)	0 + 0.15 (1)	0 + 0.15 (1)	0 + 0.15 (2)
Glucose (mmol / L)	NA + 0.28 (1)	NA	NA
Specific Gravity	1.01785714	1.02071429	1.02071429
Blood cells (cells / µL)	18.57	56.42	38.57
pH	5.85	6	6
color	Transparent + orange (1)	Transparent + yellow (3)	Transparent + orange (2)

Table 7 Results of the urinalysis performed on chicken feces fed with *Eisenia foetida*

In the Uroanalysis carried out on the faeces of the Cobb500 line chickens, a difference of 3 hundredths more was found in the birds fed with worm compared to the controls, triple and double the number of blood cells was observed. in the group supplemented with 12.5 g and 25 g, respectively. The pH remained acidic and only increased by almost 11 tenths to reach 6 in the groups treated with *Eisenia foetida*. No ketones, bilirubin, protein, or glucose were found in the three experimental groups. Urobilinogen showed a value of 33 mg/dL only in a control animal and one more in the 25 g group, none for 12.5 g. For bilirubin, only one individual was found with 8.6 mg/dL belonging to the control group. Regarding the color of the sample, it was found that one individual of the controls presented one orange, 5 transparent and one was not detected; of the group treated with 12.5 g, 3 yellow and 4 transparent were registered, finally for the 25 g group, 2 orange and 5 transparent were obtained.

Discussion

Since our group did not find any literature that demonstrates the attraction of chickens to *Eisenia foetida*, we could propose this study as a pioneer in reporting such contributions.

It is important to mention that after 6 exposures to *Eisenia foetida* the same 17 chicks plus four, for a total of 21 birds, showed interest and consumed the worms provided.

According to the theories that propose how food may have influenced the evolutionary process of species prior to hominids, it is proposed that the provision of an environment with better food resources promoted greater physical and intellectual development (Martínez-Rincón and Cisneros-Redríguez, 2002). It could be said that this theory is in agreement with the results obtained in the pilot test carried out by the attraction of the worm to the chickens, since the birds with 10.5% (257 vs 230 g) less body weight showed their avidity in 5 tests by the consumption of *Eisenia foetida* and that it did not appear in any case in the chickens with greater weight. Similarly, when chickens with 33 days of age were exposed, only 5 showed moderate attraction to *Eisenia foetida* and 2 plus an excessive or voracious attraction.
The results of the bromatological study are shown in table II and are in accordance with what was published by Köse and Ozturk (2017) regarding protein content and where their group proposes worms as a protein source to feed farm birds. On the other hand, the group of Zhenjun et al. (1997) mention that this roundworm contains 20 of the 24 main amino acids that are very important in poultry production. Depending on the 18% protein content, according to the manual for the Cobb500 line, it was decided in the present work to increase the protein content to approximately double, adding 12.5 g of live earthworm, and approximately to triple with 25 g of said worm, given the result of the Bromatological study (62% protein) and according to that reported by Köse and Öztürk (2017). Although we do not adjust to the hyperprotein proportions reported for humans (Buendia et al., 2015; Stamler et al., 2002), we do adjust to the usual in chickens.

In Fig. 1 it can be seen that, in terms of total body weight, there were no statistically significant differences between the treated groups. The results obtained in the present work are consistent with those obtained by Bollido (2021), although an important difference is the amount of worm used, from 2 to 5% compared to our birds that received a supplement of approximately 36 and 54%, in addition that were not found confined to a single development site.

ISSN-On line: 1390-9959

On the other hand, the group of Vargas López (2004) treating non-confined laying hens in family production units in Puebla, Mexico, also found no differences between the commercial diet and that supplemented with *Eisenia foetida* and it does not appear that they controlled the quantity of worm supplied.

The study of risk factors associated with left ventricular mass carried out by Heckbert et al. (2006) find the Body Mass Index and hypertension as the most related in humans and discuss the need for additional studies of risk factors and their treatment in relation to the new development or progression of left ventricular dysfunction, and the association of subclinical changes in cardiac size and function with outcomes such as myocardial infarction and sudden cardiac death. In this regard, it was found in the present study that the two individuals who showed greater voracity towards worms developed greater weight gain (3.86 and 3.6 Kg) at the time of slaughter, as well as frank deterioration on one side of the heart (images a and b) compared to the rest of the experimental chickens (image c).

For the group of Díaz-Rúa (2017), long-term hyperprotein diets in rats show signs of health risk, when the liver transcriptome is studied, with increased inflammation and alterations in acid-base balance and oxidative stress, given the persistence of the altered metabolic state. Although our results only measure total weight and a decrease is observed in this proportional to the consumption of worm protein, it is likely that this weight loss is related to liver deterioration. Although in rats this decrease is not observed and the excess of protein is with casein (Díaz-Rúa et al., 2017). The lack of studies in chickens that compare liver weight with respect to body weight forces us to confront our results with those obtained in models such as mice, where a decrease in liver weight and its relationship with body weight in "nock out" mice is observed. Of proteins involved with the metabolism of fatty acids and in individuals fed with phytol (Landrock et al. 2017), although in our case it is required to study analogous parameters with said metabolic pathway to compare the effect of the diet based on *Eisenia foetida*.

On the other hand, when protein derived from vegetables such as soy or canola flours is used, no significant changes are observed for the heart, spleen and other organs, but an increase in the size of the liver and, as the group of Payvastegan et al. (2017), the group of Manyeula et al. (2020) agree that said increase corresponds to chemical compounds in the food that require detoxification.

The spleen shows a similar trend with 20.97% less weight for the group added with 25 g of earthworm and almost 16.1% in the group added with 12.5 g of *Eisenia foetida*, with a weight of 4.1 g for this organ of the control group (Table III). Our results agree with those obtained by the group of Chen et al., (2019) and where they find a relationship with an immunosuppression phenomenon.

Joint inflammation in humans has tried to relate the parameters of total body mass with blood levels of uric acid and with damage to the cartilage of the knee or its general condition (Crowson et al., 2011), on the other hand, If the diagnosis of Joint Inflammatory Syndrome (AIS) is reviewed, it is found that deepening the evaluations of joint damage, and understanding that AIS has a generally similar behavior according to the disease that gives it origin, facilitates, once correctly interpreted , the way to achieve a nosological diagnosis and certain treatments (Martínez-Larrarte et al., 2007).

In support of this uncertainty, perhaps the model with chickens will allow the carrying out of studies at both histological, cellular and molecular levels that allow associating the consequences of hyperprotein diets with AIS and with uric acid levels in any of the sources to analyze.

If we take into account that HB values are almost 0.5 g/dL more than HB in group of 25 g of *Eisenia foetida* compared to the control and 1.5 g/dL compared to group of 12.5 g of *Eisenia foetida*, and having hematocrit levels that are too high or low may indicate a problem with the blood, dehydration, or other medical conditions and since observations from clinical use of recombinant, pure, artificial hemoglobin solutions support a causal relationship between excess cell-free hemoglobin in the bloodstream with cardiovascular symptoms and events (Rother et al., 2005).

ISSN-On line: 1390-9959

It is not unreasonable to think of one of the causes that generated the deterioration of the heart. If we take into account that HB has a harmful role in stressful or pathological situations and that they can fall into the following categories: (a) Autooxidation of hemoglobin within the rbc, (b) Release of hemoglobin outside its confines in rbc to act as a source of Reactive Oxygen Species (ROS) in the vasculature and tissues, (c) Nitric Oxide (NO) uptake by hemoglobin and (d) Release of HB heme to increase the generation of free radicals. Perhaps we are talking about a kind of generalized low intensity inflammatory reaction (Minihane et al., 2015).

First, the released hemoglobin is closer to the vascular endothelium and can also leak into the tissues as dimers so that the ROS generated in autoxidation easily access the macromolecules and cause damage.

Perhaps similar to the pathogenesis of false cell disease that in addition to inflammation involves heterocellular adhesion between erythrocytes, platelets, endothelium, and leukocytes (de Azevedo-Quintanilha et al., 2020). In the same sense, Kato et al. (2017) hypothesize that intravascular hemolysis products damage the vascular system, reducing nitric oxide (NO) given the release of HB and that they react through dioxygenation to form inert nitrate. Quaye's group (2015) also mentions that HB disaggregates and becomes a dangerous molecule for the survival of cells.

The controlled diet in the omnivorous wild bird Pycnonotus barbatus has demonstrated its effect on physiological parameters (decreased haptoglobin or α-globin concentration, earlier molting and immunostimulation) when fruits or invertebrates, the latter considered hyperprotein, are fed as food. The decrease in haptoglobin is associated with the initial evaluation parameters of intravascular hemolysis and oxidation (Nwaogu et al., 2020).

These conditions can explain a picture of hemolytic anemia caused by malignant hypertension, perhaps prompted by the increase in uric acid levels due to the hyperprotein diet and that exceeds the protective mechanisms of erythrocytes (Phillips and Hendersone, 2018); with the consequent release of hemoglobin that increases oxidative stress in the vessels.

In humans, kidney damage is associated as a cause of Malignant Arterial Hypertension, but also the increase in intravascular hemoglobin levels is associated with hyperproteinuria, which seems to explain a systemic and feedback problem (Morales et al., 2011) that probably generates a long-lasting state of low intensity inflammation (Castro et al., 2017), but no one associates these complications with lifestyle or high protein diets in humans. Probably the reason is that institutions such as the European Food Safety Authority (EFSA) and the European Union Register of Health Claims (http://ec.europa.eu/nuhclaims) do not provide for health claims that specifically address the area of health benefits of suppressing or controlling low-intensity inflammation (Minihane et al., 2015). For Kato group (2017) understanding how these components' complexity of sickle erythrocytes is compounded by genetic and environmental modulation provides insight into the well-known clinical heterogeneity of sickle cell disease (SCD). Perhaps the best way to address the consequences of this inflammation is through animal models such as broilers in which it can be studied at any point in development and under controlled conditions.

The Nwaogu group (2020) proposes the existence of a causal effect of the composition of the diet on the innate immune function, since in thebirds *Pycnonotus barbatus* that were found in better conditions when they were fed with fruits than when they were fed with invertebrates, confirming that innate immunity is specific to nutrients and, furthermore, it is well established that nutrients modulate intestinal immunity (Farré et al., 2020). In our results the possibility is observed that the amount of nutrients with 12.5 g of *Eisenia foetida*. it induces immunostimulation, but not to a greater quantity of protein from this source (Table V).

Thrombocythemia or increase in the number of platelets, induced by supplementation with 25 g of *Eisenia foetida*, is a factor that can contribute to the formation of clots and with it to high blood pressure and a high risk of arterial thrombosis (Tefferi et al., 2018). Our results are in agreement with epidemiological studies that present leukocytosis and high levels of total HB as risk factors and increase in episodes of acute pain in acute chest syndrome (de Azevedo-Quintanilha et al., 2020).

ISSN-On line: 1390-9959

Added to all the hematological parameters collected in this study, they allow a more complete view of the physiological alterations suffered by hyperprotein diets and in an environmental enrichment model (Ipema et al., 2020).

Trying to establish study models, as in this case, requires making determinations in a longer period of time, that is, 7 weeks. Since the general approach in the experimental work with broilers is given in function of productive parameters, they are estimated in much shorter treatment time, for example the group of Bollido (2021) during 8 weeks; Vargas López (2004) for 90 days in free-range hens measuring these productive parameters. However, the toxicological evaluation for 4 generations of the worm meal in the feed, with an experimental diet containing 200 g of earthworm protein per kg. It did not reveal any deleterious effect on the health of the rats (Ibáñez et al., 1993). Therefore, if the experimentation time is extended further, the effect of the addition of live earthworms in broilers could be reported.

The use of animals by the biomedical sciences as models to help understand and predict responses in humans, in toxicology and pharmacology in particular, remains the primary tool for biomedical advancements and a source of significant controversy. In general, animals have performed exceptionally well as predictive models for humans when used correctly (Shanks et al., 2009).

In the case of urinalysis, and although the detection equipment is designed for pure urine, the present study attempted to search for biomarkers within the 10 parameters determined by the device. Perhaps observation by phase contrast microscopy should be attempted according to the Recommendations for Urinary Sediment of Chile (Lagos and Pinto, 2013) and compared with our results in the different parameters of the urinalysis. The primary objective should be to separate elements of urine that can be disturbed by contact with solid waste material (Baños-Laredo et al., 2010). The pH of our results is suggestive of the formation of urates in their form of sodium, potassium, magnesium and ammonium salts of uric acid, since their generation occurs between 5.5 and 6 of this parameter (Jiménez and Ruiz, 2010).

On the other hand, in humans the reductions in urinary pH ($p = 0.07$) in studies with meta-analysis for hyperprotein diets (HP) are considered an independent risk factor for developing nephrolithiasis (Schwingshackl and Hoffmann, 2014), we consider that This contradiction requires further study in the broiler model.

A limitation of the earthworm in the chicken feed ration is its dry matter content, since large volumes would be needed to satisfy the requirements or include it in commercial concentrates in the form of flour (Rodríguez et al., 1995). Its carbon footprint implications and environmental impact should probably also be analyzed (Vauterin et al., 2021).

In this way the use of *Eisenia foetida* live on diets represents a better option with multiple extra benefits as a live food. In addition, the worm is the only animal in the world that does not transmit or suffer from diseases (Pineda, 2006). Although exposure to fluoroquinolone and organophosphate pesticides is known to cause oxidation of their proteins through carbinylation, thus decreasing nutritional quality (Márquez-Lázaro et al., 2021). On the other hand, the humus produced in its cultivation could be recommended as other humic substances that have proven to provide quality to broiler meat (Hudák et al., 2021).

The importance of this organism has been recognized since the time of Hansen and Darwin; Currently it is exploited by means of a technique called vermiculture that consists of the breeding and management of earthworms in captivity conditions with the basic purpose of obtaining with it two products of great importance for man: humus as a fertilizer-amendment for agricultural use and protein, either as fresh meat or flour for a food supplement (Nalunga et al., 2021; http://ri.ues.edu.sv/id/eprint/1624/3/13101281TT.pdf

Acknowledgments

Our working group is grateful for the essential help they provided to carry out this work to: Muñoz-Urias A, Pedroza-Uribe NV, Guerrero-García FC, Loya-Romero MG, Ordoñez Sánchez CA, Jiménez Plasencia C, Raya Rodríguez LA, Gómez Ramírez BA, Noa -Pérez M.

ISSN-On line: 1390-9959

Conclusions

There is a differential behavior regarding the attraction to *Eisenia foetida* of broilers of the Cobb500 line. Those who showed voracity and were fed 25 g/day of worm have the probability of developing marked heart damage, perhaps from the geometry of the left ventricular mass due to diet. Diets with 12.5 and 25 g/day of *Eisenia foetida* induce low-intensity inflammation symptoms when measuring joints, organ size, hematological and probably urological parameters. Determining the management and the conditions to develop a model of cardiac damage in the Cobb500 chicken line and according to the results obtained in the present work seems plausible and requires more studies to compare the consequences of hyperprotein diets based on *Eisenia foetida* live compared to diets of the same type in humans.

References

Bahadori, Z.; Esmaielzadeh, L.; Karimi-Torshizi, M.A.; Seidavi, A.; Olivares, J.; Rojas, S.; Salem, A.Z.M.; Khusro, A.; López, S. (2017). The effect of earthworm (*Eisenia foetida*) meal with vermi-humus on growth performance, hematology, immunity, intestinal microbiota, carcass characteristics, and meat quality of broiler chickens. *Livest. Sci*., 202:74-81. https://doi.org/10.1016/j.livsci.2017.05.010**.**

Baños-Laredo, M.E., Núñez-Álvarez, C.A. and Cabiedes, J. (2010). Urinary sediment analysis. *Reumatología Clínica*. 6(5): 268-272. DOI: 10.1016/S2173-5743(10)70062-9

Bollido, M.E. (2021). Growth Performance of Broiler Chicken Fed with Fresh Vermi Supplementation under Total Confinement Management System. *Journal of the Austrian Society of Agricultural Economics* 17(04):463-471.

Buendia, J.R.M., Bradlee, L. Singer, M.R. and. Moore, L.L. (2015). Diets Higher in Protein Predict Lower High Blood Pressure Risk in Framingham Offspring Study Adults. *American Journal of Hypertension*. 28(3):372-379. DOI: 10.1093/ajh/hpu157

Castro, A.M., L.E. Macedo-de la Concha, and C.A. Pantoja-Meléndez. 2017. Low-Grade Inflammation and Its Relation to Obesity and Chronic Degenerative Diseases. *Revista Médica del Hospital General de México* 80(2): 101–5.

De Azevedo-Quintanilha, Isaclaudia G. et al. 2020. "Haem Oxygenase Protects against Thrombocytopaenia and Malaria-Associated Lung Injury." Malaria Journal 19(1): 1–16. https://doi.org/10.1186/s12936-020-03305-6.

Díaz-Rúa R, Keijer J, Palou A, van Schothorst EM, Oliver P. Long-term intake of a high-protein diet increases liver triacylglycerol deposition pathways and hepatic signs of injury in rats. *J Nutr Biochem*. 2017;46:39–48.

Dos Reis Padilha, G., Karina Sanches Machado, K. d'A., Ronchi Spillere, S. and Corrêa Souza, G. (2018). Dietary Patterns in Secondary Prevention of Heart Failure: A Systematic Review. *Nutrients* 10(7): 1–19.

Chen Y, Han S, Wang Y, Li D, Zhao X, Zhu Q,Yin H. (2019). Oxidative Stress and Apoptotic Changes in Broiler Chicken Splenocytes Exposed to T-2 Toxin. *BioMed Research International*. Article ID 5493870, 9 pages. https://doi.org/10.1155/2019/5493870

Crowson, C.S., Myasoedova, E., Davis, J.M., Matteson, E.L., Roger, V.L., Therneau, T.M., Fitz-Gibbon, P., Rodeheffer, R.J. and Gabriel, S.E. (2011). Increased Prevalence of Metabolic Syndrome Associated with Rheumatoid Arthritis in Patients without Clinical Cardiovascular Disease. Journal of Rheumatology. 38(1):29-35.

de Azevedo-Quintanilha, I.G., Medeiros-de-Moraes, I.M., Ferreira, A.C. Reis, P.A., Vieira-de-Abreu, A., Campbell, R.A., Weyrich, A.S., Bozza, P.T. Zimmerman, G.A.and Castro-Faria-Neto, H.C. (2020). Haem oxygenase protects against thrombocytopaenia and malaria-associated lung injury. *Malar J*. 19:234. https://doi.org/10.1186/s12936-020-03305-6

Ejaz, S, Kim, B.S, and Lim, C.W. (2005). Gout Induced by Intoxication of Sodium Bicarbonate in Korean Native Broilers. *Drug and Chemical Toxicology*. 28(2): 245-261.

ISSN-On line: 1390-9959

Farré R, Fiorani M, Rahiman SA, Matteoli G. Intestinal permeability, inflammation and the role of nutrients. Nutrients. 2020;12(4):1–18.

Freire, R. (2019). Scientific evidence of diets for weight loss: Different macronutrient composition, intermittent fasting, and popular diets. *Nutrition*. 69:110549. doi: 10.1016/j.nut.2019.07.001.

García, E. 2004. Modificaciones al sistema de clasificación climática de Kopen. 5ª Edición. México: *Instituto de geografía, UNAM*. http://www.publicaciones.igg.unam.mx/index.php/ig/catalog/book/83

Heckbert, S.R., Wendy Post, W., Pearson, G.D.N., Arnett, D.K., Gomes, A.S., Jerosch-Herold, M., Hundley, G., Joao A. Lima, J.A. and Bluemke, D.A. (2006). Traditional cardiovascular risk factors in relation to left ventricular mass, volume, and systolic function by cardiac magnetic resonance imaging: The Multi-Ethnic Study of Atherosclerosis. *J Am Coll Cardiol*. 48(11): 2285–2292. doi: 10.1016/j.jacc.2006.03.072

Hong, F., Zheng, A., Xu, P., Wang, J., Xue, T., Dai, S., Pan, S., Guo, Y., Xie, X., Li, L., Qiao, X., Liu, G. and Zhai, Y. (2020). High-Protein Diet Induces Hyperuricemia in a New Animal Model for Studying Human Gout. *Int. J. Mol. Sci*. 21:2147. doi:10.3390/ijms21062147

Hudák, M., Semjon , B., Marcincáková, D., Bujnák, L., Nad, P., Koréneková, B., Nagy, J., Bartkovský, M., and Marcincák, S. (2021). Effect of Broilers Chicken Diet Supplementation with Natural and Acidified Humic Substances on Quality of Produced Breast Meat. *Animals*. 2021, 11, 1087. https://doi.org/10.3390/ani11041087

Ibáñez, I.A., Herrera, C.A., Velásquez, L.A., and Hebel, P. (1993). Nutritional and Toxicological Evaluation on Rats of Earthworm (Eisenia fetida). Meal as Protein Source for Animal Feed. *Animal Feed Science and Technology*. 42(1-2): 165-172. https://doi.org/10.1016/0377-8401(93)90031-E.

Ipema, A.F., Bokkers, E.A.M., Gerrits, W.J.J., Kemp, B., Bolhuis, J.E. (2020). Long-term access to live black soldier fly larvae (*Hermetia illucens*) stimulates activity and reduces fearfulness of broilers, without affecting health. *Sci Rep.* 10(1):1–13. https://doi.org/10.1038/s41598-020-74514-x

Jiménez García, J., and Ruiz Martín, G. (2010). Estudio de los elementos formes de la orina. Estandarización del sedimento urinario. *Labcam*. 2010. 1–96 p. https://www.abm.org.ar/docs/campanas/erc/Estandarizacion_del_sedimento_urinario.pdf

Kato, G.K., Steinberg, M.S. and Gladwin, M.T. (2017). Intravascular hemolysis and the pathophysiology of sickle cell disease. *J Clin Invest*. 1;127(3):750-760. doi: 10.1172/JCI89741.

Khan, S.R., Khan, U.W. and Sultan, A.A. (2018). Evaluating the nutritive profile of three insect meals and their effects to replace soya bean in broiler diet. *Journal of Animal Physiology and Animal Nutrition.* 102(2): e662-e668. https://doi.org/10.1111/jpn.12809

Köse, B. and Öztürk, E. (2017). Evaluation of Worms as a Source of Protein in Poultry. *Selcuk J Agr Food Sci*. 31(2): 107-111. DOI: 10.15316/SJAFS.2017.27

Lagos, R.G. and Pinto, P.P. (2013). Recomendaciones para el análisis del Sedimento Urinario Instituto de Salud Pública. ;(1):1–14. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0213005X10004209%5Cnhttp://dspace.ucuenca.edu.ec/bitstream/123456789/5564/1/TESIS.pdf%5Cnhttp://www.ispch.cl/sites/default/files/documento/2013/04/RECOMENDACIONES PARA EL ANÁLISIS DEL SEDIMENTO URINARIO.PDF

Landrock, D., Milligan, S., Martin, G., McIntosh, A., Landrock, K.K., Friedhelm, Schroeder, F. and Kier, A.B. (2017). Effect of Fabp1/Scp-2/Scp-x Ablation on Whole Body and Hepatic Phenotype of Phytol-Fed Male Mice. *Lipids*. 52(5):385–397. doi:10.1007/s11745-017-4249-y.

ISSN-On line: 1390-9959

Lu, J., Dalbeth, N., Yin, H., Li, C., Merriman, T.R. and Wei, W.H. (2019). Mouse models for human hyperuricaemia: a critical review. *Nature Reviews, Rheumatology Reviews*. 15.413.

Manyeula, F., Mlambo, V., Marume, U. and Sebola, N.A. (2020). Partial replacement of soybean products with canola meal in indigenous chicken diets: size of internal organs, carcass characteristics and breast meat quality. *Poult Sci*. 99(1):256–62. http://dx.doi.org/10.3382/ps/pez470

Martínez-Larrarte, J.P., Reyes Pineda, Y. and Prada Hernández, D.M. (2007). Aspectos teórico-prácticos de la inflamación en las enfermedades reumáticas. *Rev Cubana Med Gen Integr v*. 23(2). http://scielo.sld.cu/scielo.php?script=sci_arttext&pid=S0864-21252007000200014

Martínez-Rincón, C. y Rodríguez-Cisneros A. (2002). Influencia de la alimentación en el comportamiento humano a través de la historia. *Nutrición*. 21(7): 80-86. https://www.elsevier.es/es-revista-offarm-4-articulo-influencia-alimentacion-el-comportamiento-humano-13034832

Márquez-Lázaro, J., Díaz-Pineda, K., Méndez-Cuadro, D. and Rodríguez-Cavallo, E. (2021). Fluoroquinolone antibiotics and organophosphate pesticides induce carbonylation on *Eisenia fetida* muscle proteins. *Science of The Total Environment*. 758: 143954. https://doi.org/10.1016/j.scitotenv.2020.143954

Martinez Rincon, C., and Cisneros Rodriguez Á. (2002). No Title Influencia de la alimentación en el comportamiento humanoa través de la historia. Https://WwwElsevierEs/Es-Revista-Offarm-4-Pdf-13034832. 2002;21(0):80–6.

Minihane, A.M., Vinoy, S., Russell, W.R., Baka, A., Roche, H.M, Tuohy, K.M., et al. (2015). Low-grade inflammation, diet composition and health: Current research evidence and its translation. *Br J Nutr*. 114(7):999–1012. doi: 10.1017/S0007114515002093.

Morales, E., González, R., Gutiérrez, E., Segura, J. and Praga, M. Hipertensión arterial maligna . Una visión actualizada. 2017;4(1):34–43.

Nalunga, A., Komakech A.J., Jjagwe, J., Magala, H. and Lederer, J. (2021). Growth characteristics and meat quality of broiler chickens fed earthworm meal from *Eudrilus eugeniae* as a protein source. *Livestock Science*. 245. 104394. https://doi.org/10.1016/j.livsci.2021. 104394

NRC. 1992. National Research Council.

Nwaogu, C.J., Galema, A., Cresswell, W., Dietz, M.W. and Tieleman, B.I. (2020). A fruit diet rather than invertebrate diet maintains a robust innate immunity in an omnivorous tropical songbird. *J Anim Ecol*. 89(3):867–83.

Öztürk, E. and Köse, B. (2017). Evaluation of Worms as a Source of Protein in Poultry. *Selcuk J Agric Food Sci*. 31(2):107–11.

Payvastegan, S., Farhoomand, P., Daneshyar, M. and Ghaffari, M. (2017). Evaluation of Different Levels of Canola Meal on Performance, Organ Weights, Hepatic Deiodinase Gene Expressionand Thyroid Morphology in Broiler Chickens. *J.Poult.Sci*.,54:282-291.

Phillips, J., and Henderson, A.C. (2018). Hemolytic Anemia: Evaluation and Differential Diagnosis. American Family Physician. 98(6): 355-361.

Pineda, J.A. (2006). Lombricultura. Instituto Hondureño del Café. 1a. ed.- (Tegucigalpa): (Litografía López). http://www.asocam.org/sites/default/files/publicaciones/files/aa3947fa7e7b1775e4a2dc2261ead741.pdf

Quaye, I.K. (2015). Extracellular hemoglobin: the case of a friend turned foe. *Front. Physiol*. 6:96. doi: 10.3389/fphys.2015.00096

Remy, C., Richert, D.A. and Westerfeld, W.W. (1951). The determination of xanthine dehydrogenase in chicken tissues. *J. Biol. Chem*. 192, 649–657.

Riehle, C. and Bauersachs, J. (2019). Small animal models of heart failure. *Cardiovasc Res*. 115(13):1838–49.

ISSN-On line: 1390-9959

Rodríguez, L., Salazar, P. and Arango, M.F. (1995). Lombriz roja californiana y azolla-anabaena como sustituto de la proteína convencional en dietas para pollos de engorde. *Livestock Research for Rural Development*. 7(3). https://www.fao.org/ag/Aga/agap/frg/FEEDback/lrrd/lrrd7/3/5.htm

Rother, R.P., Bell, L., Hillmen, P. and Gladwin, M.T. (2005). The Clinical Sequelae of Intravascular Hemolysis and Extracellular Plasma Hemoglobin. A Novel Mechanism of Human Disease. *JAMA*. 293(13):1653-62. doi: 10.1001/jama.293.13.1653.

Sanches Machado, K.D'A., Ronchi, S.S., Zuchinali, P. and Corrêa Souza, C. (2018). Mediterranean Diet and Other Dietary Patterns in Primary Prevention of Heart Failure and Changes in Cardiac Function Markers: A Systematic Review. *Nutrients*. 10(1).

Schubert, R.N., Morselli, T.B.G.A., Tonietto, S.M., Henriquez, J.M.O., Trecha, R.D., Eid, R.P., Rodriguez, D.P., Piesanti, S.R., Maciel, M.R.S., and Lima, A.P.F. (2019). Edaphic Macrofauna in Degradation of Animal and Vegetable Residues. *Braz J Biol*. 79(4):589-593. doi: 10.1590/1519-6984.184765.

Schwingshackl, L., and Hoffmann, G. (2014). Comparison of high vs. normal/low protein diets on renal function in subjects without chronic kidney disease: A systematic review and meta-analysis. PLoS ONE, 9(5). https://doi.org/10.1371/journal.pone.0097656

Shanks, N., Greek, R., and Greek, J. (2009). Are Animal Models Predictive for Humans? *Philosophy, Ethics, and Humanities in Medicine* 4(1): 1–20.

Singh, N., Ghosh, R.C., Singh, A. (2013). Prevalence and haematobiochemical studies on naturally occurring gout in Chhattisgarh. Adv. Anim. Vet. Sci.; 1(38):9-11.

Singh, A., Karmegam, N. and Shankar. G. (2020). Earthworms and Vermicompost : An Eco-Friendly Approach for Repaying Nature ' s Debt. *Environmental Geochemistry and Health* 42(6): 1617–42. https://doi.org/10.1007/s10653-019-00510-4.

Stamler, J., Liu, K., Ruth, K.J., Pryer, J. and Greenland, P. (2002). Eight-Year Blood Pressure Change in Middle-Aged Men Relationship to Multiple Nutrients. *Hypertension*. 39:1000-1006. DOI: 10.1161/01.HYP.0000016178.80811.D9

Tefferi, A., Vannucchi, A.M. and Barbui, T. (2018). Essential thrombocythemia treatment algorithm. *Blood Cancer J*. 8(1):4–9. http://dx.doi.org/10.1038/s41408-017-0041-8

Vasconcelos, Q.D.J.S., Bachur, T.P.R. and Aragão, G.F. (2021). Whey protein supplementation and its potentially adverse effects on health: a systematic review. *Appl Physiol Nutr Metab*. 46(1):27-33. doi: 10.1139/apnm-2020-0370. Epub 2020 Jul 23.

Vargas López, S., García Martínez, A.R. and Palma Guarneros H.R., and Librado Pérez, M. (2004). Integración de la lombricultura en la producción de aves de traspatio en Puebla, México. https://fci.uib.es/Servicios/libros/conferencias/seae/Integracion-de-la-lombricultura-en-la-produccion.cid221661

Vauterin A, Steiner B, Sillman J, and Kahiluoto, H. (2021). The potential of insect protein to reduce food-based carbon footprints in Europe: The case of broiler meat production. *Journal of Cleaner Production*. 320 (2021) 128799

Zhenjun, S., Xianchun, L., Sun Lihui, S. and Chunyang, S. (1997). Earthworm as a potential protein resource. *Ecol. Food Nutr*. 36:221–236.

ISSN-On line: 1390-9959

Article **ECORFAN Journal-Ecuador**
June 2021, Vol.8 No.14 23-38

Production and effects of green tea Kombucha with blueberry and orange blossom honey without caffeine as probiotic inhibitor of pathogenic bacteria

Producción y efectos de Kombucha de té verde con arándano azul y miel de flor de azahar sin cafeína como probiótico inhibidor de bacterias patógenas

LAGUNA-MORALES, Leslie Asenat†, SANTIESTEBAN-LÓPEZ, Norma Angelica* and MALDONADO-RESÉNDIZ, Jorge Ángel

Benemérita Universidad Autónoma de Puebla. Facultad de Ciencias Biológicas, Puebla, Mexico.

ID 1st Author: *Leslie Asenat, Laguna-Morales* / **ORC ID:** 0000-0001-6103-1749

ID 1st Co-author: *Norma Angelica, Santiesteban-López* / **ORC ID:** 0000-0001-7700-4139, **CVU CONACYT ID:** 240825

ID 2nd Co-author: *Jorge Ángel, Maldonado-Reséndiz* / **ORC ID:** 0000-0003-1787-3660, **CVU CONACYT ID:** 1087863

DOI: 10.35429/EJE.2021.14.8.23.38 Received January 20, 2021; Accepted June 30, 2021

Abstract

Kombucha is a probiotic drink of Asian origin, whose symbiotic relationship between acetic bacteria and yeasts provides a wide range of compounds with antioxidant and antimicrobial power (against Gram-negative and Gram-positive bacteria); thus, improving digestion and preventing chronic diseases. Blueberry (*Vaccinium corymbosum*) of the *Ericaceae* family is one of the most studied and used fruits due to its high antioxidant benefits. In turn, orange blossom honey is one of several products rich in flavonoids obtained from the *Citrus sinensis* plant. Due to the high amount of phenolic compounds in these products, a homemade kombucha based on blueberry and orange blossom honey was developed to increase the beneficial properties of this probiotic, generating a value-added product. First, the tea fungus (SCOBY) was obtained in a caffeine-free medium of green tea and white sugar, through a process called "First fermentation". Then we performed a "Second fermentation" by adding the fruit and honey, giving it flavor and increasing its antioxidant properties. Finally, we tested it pleasant taste by means of a sensory evaluation of 30 people, which was statistically analyzed.

Kombucha, Caffeine-free, Green tea, Antioxidant

Resumen

La Kombucha, es una bebida probiótica de origen asiático, cuya relación simbiótica entre bacterias acéticas y levaduras aporta una amplia gama de compuestos con poder antioxidante y antimicrobiano (contra bacterias Gram negativas y Gram positivas); mejorando así la digestión y previniendo enfermedades crónicas. El arándano azul (*Vaccinium corymbosum*) de la familia *Ericaceae* es uno de los frutos más estudiados y utilizados debido a los beneficios de su alto poder antioxidante. A su vez, la miel de flor de Azahar es uno de los varios productos ricos en flavonoides obtenidos de la planta *Citrus sinensis*. Debido a la gran cantidad de compuestos fenólicos de estos productos, se desarrolló una kombucha casera a base de arándano azul y miel de flor de Azahar para aumentar las propiedades benéficas de este probiótico, generando un producto de valor agregado. Primero se obtuvo al hongo del té (SCOBY) en un medio sin cafeína de té verde y azúcar blanca, mediante el proceso denominado “Primera fermentación”. Después realizamos una “Segunda fermentación” agregado la fruta y miel, confiriéndole el sabor y aumentando sus propiedades antioxidantes. Finalmente, se probó la obtención de un sabor agradable, mediante una evaluación sensorial a 30 personas que se analizó estadísticamente.

Kombucha, Sin cafeína, Té verde, Antioxidante

Citation: LAGUNA-MORALES, Leslie Asenat, SANTIESTEBAN-LÓPEZ, Norma Angelica and MALDONADO-RESÉNDIZ, Jorge Ángel. Production and effects of green tea Kombucha with blueberry and orange blossom honey without caffeine as probiotic inhibitor of pathogenic bacteria. ECORFAN Journal-Ecuador. 2021. 8-14:23-38.

* Correspondence to Autor (E-mail: norma.santiesteban@correo.buap.mx)
† Researcher contributing as first author.

Introduction

One of the most important issues of our time is the strengthening of the immune system to resist or prevent diseases. Due to the current Covid-19 pandemic, several measures were taken to take care of the population and prevent the spread of the virus. According to the survey conducted by Sami et al. in 2021, out of 312 people surveyed in 2020, a total of 137 supported the consumption of functional foods to prevent an infection (Sami *et al.,* 2021).

Probiotics are a type of functional food based on microorganisms, whose consumption in adequate amounts, produces positive effects on human health. Their importance for the reinforcement of the immune system is thanks to the effects they exert through the brain and intestine, via the vagus nerve; these neuroendocrine pathways allow us to regulate not only the composition of the intestinal microbiota, which has immunomodulatory and anti-inflammatory functions, but also to demonstrate reductions in stress and depressive symptoms (Interián-Gómez *et al.,* 2021).

These foods also help in the regulation of homeostasis and the formation of regulatory T cells, which help in the production of metabolites that inhibit TNF-α (tumor necrosis factor alpha) and the cancer-related NF-kB protein complex (Darmawan *et al.,* 2020).

Due to these and other functions, the consumption of probiotics has become increasingly popular. Among these foods, we can highlight Kombucha, a probiotic beverage associated with protection against various pathologies produced by radical oxygen species (ROS), antimicrobial effects, detoxifying properties by accumulation of organic acids (such as acetic, gluconic, lactic and glucuronic acid), generation of bioactives such as vitamins, help against damage caused by antibiotics and contribution of tea polyphenols. It is worth mentioning that the natural flavors of this beverage are obtained from herbs and fruits, so the right choice of these elements could enhance its beneficial properties. (Uțoiu *et al.,* 2018).

On the other hand, caffeine is a popular compound consumed around the world, mainly in coffee, black tea and green tea. Currently, it is found in various products and foods; the compound is associated with many benefits and adverse effects around the amount of consumption, age, gender, lifestyle, and nutrition. Several studies, such as Doepker in 2018 have shown that caffeine in adequate doses avoids the negative effects mentioned above, however, in tea infusions it usually exceeds it, being counterproductive to the beneficial effects of this type of beverage (Doepker *et al.,* 2018).

Blueberry is one of the most popular fruits due to its high composition with polyphenols that confer antioxidant properties. It also contains flavonoids that represent up to 60% of the weight of the fruit, in addition to demonstrating that its anthocyanins reduce biomarkers and the risk to suffer diseases such as cardiovascular risks, diabetes Mellitus type 2, coronary heart disease, hypertension and neurological impairment. (Kalt *et al.,* 2020).

Orange blossom honey also contains flavonoids such as aringenin, hesperetin, kaempferol, chrysin, galangin and quercetin, as well as phenolic acids such as p-coumaric and caffeic acid at 3.64 mg/ 100 g (Escriche et al., 2011). Flavonoids possess anti-inflammatory, anti-atherogenic and anti-thrombotic properties (Parmenter *et al.,* 2021).

The compounds are present in different proportions in green tea, blueberries and orange blossom honey; for this reason, this article seeks to describe probiotics and their influence, highlighting the Kombucha, its origins, composition, antimicrobial properties and benefits, in addition to the description of the fruits and elements that, when added during fermentation, could enhance its benefits.

Showing thus, the production process of homemade Kombucha based on green tea without caffeine, which makes it different from other processes, avoiding the possible adverse effects of the consumption of this compound in the long run.

ISSN-On line: 1390-9959

Firstly, the bacterial consortium (SCOBY) was obtained, to later manufacture a homemade probiotic, in its two versions: in its natural form and a second flavored version that shares the properties of the three elements used, achieving the enhancemgent of its antioxidant properties, thus improving its antibacterial activity, taste, smell and pleasant texture, monitored through a sensory study and its statistical analysis.

Functional foods

The term currently known as "functional food" has its origin in Japan. Country where started its development in the 1980s, when the Japanese government launched and funded large-scale, systematic research programs on the development, analysis, regulation and molecular design of food functions. That project resulted in the introduction of a new concept that would describe those foods that were specifically developed to improve health and prevent disease, thus creating FOSHU (Valenzuela *et al.*, 2014).

FOSHU (Food with Specific Health Uses), is a category that since 1991 grouped foods such as prebiotics, probiotics, phytoestrogens, phytosterols, nutraceuticals, antioxidants, omega fatty acids, supplementary foods, among others. Foods that are intended to improve health and from which specific effects can be observed (Saarela, 2011).

This is how the concept of "functional foods" is described by several authors as those beverages and foods capable of providing health benefits through specific substances that can be consumed within a basic daily diet, regulating processes and preventing diseases (Saarela. 2011).

Probiotics and prebiotics

Probiotics are one of the best-known functional foods consumed by humans. The term "Probiotic" derived from Greek roots, means "for life". It was first used in 1965 by Lilly and Stillwell to describe "Substances secreted by one microorganism that stimulate the growth of another" (Lilly & Stillwell, 1965), mainly related to antibiotics.

Over the years, the definition gained a more general meaning, obtaining different hypotheses on the various beneficial effects it provides to health (Schrezenmeir & de Vrese, 2001).

The various definitions and studies on them allowed the consolidation of two clear ideas, the first is that probiotics are living organisms; and the second is that the positive effects they provide will depend on the adequate consumption of a dose of these microorganisms.By 2006, FAO/WHO gives a definition based on these two ideas, describing probiotics as those live microorganisms that administered in adequate amounts (> 6-7 log CFU/g) are capable of conferring health benefits to the host. Their functionality of course depends on the strain used; most of this type of commercialized products are made based on Gram-positive bacteria, as well as lactic acid bacteria, but Gram-negative bacteria such as Acetobacter and bifidobacteria have also been used (Nelson, 2017).

Among the benefits they have provided, we have reduction of Helicobacter pylori infections, reduction of allergy symptoms and constipation, inhibition of some harmful bacteria in the microbiome, effects on metabolism, cancer prevention and cholesterol reduction (Schrezenmeir & Vrese, 2001). In 2019, Plaza-Diaz et al. showed its role in the prevention of various problems such as digestive disorders, diarrhea caused by drugs (such as antibiotics), irritable bowel syndromes, diseases associated with Clostridium difficile, inflammatory diseases, gastric ulcers, atopic dermatitis and allergic rhinitis (Plaza-Diaz *et al.*, 2019). They also prevent and reduce the symptoms of respiratory diseases and their possible complications, a role mainly described in the Lactobacillus and Bifidobacterium genera (Carbonell, 2021).

The second most popular functional food that we will define, are prebiotics, which have been described as "a non-digestible food ingredient that beneficially affects the host by electively stimulating the growth and/or activity of one or a limited number of bacteria in the colon", this term was introduced by Gibson and Roberfroid in 1994, where they exchanged "pro" (from the definition of probiotic) meaning "before" for "pre" meaning "for" (Gibson & Roberfroid, 2004).

ISSN-On line: 1390-9959

The main difference between these two health beneficial foods is their main component, the probiotic has living organisms while the prebiotic has non-digestible substrates. This is the reason why many prebiotics are made from vegetables and fruits, since cellulose (the main component of these) is not digestible by humans, because we dispense with the enzyme cellulase. It is important to mention them since many probiotics are cultivated on prebiotic bases to stimulate their development and enhance their activity.

The origins of Kombucha tea

Kombucha is a fresh drink of Asian origin with a sweet and slightly sour taste obtained from the fermentation of an infusion of black or green tea leaves, through the cultivation of *Manchurian fungus*, a symbiotic association of bacteria (mainly *Acetobacter spp* and *Gluconobacter spp*) and heterofermentative yeasts commonly called "Tea Fungus", also named SCOBY (Heredia *et al.,* 2021).

This probiotic beverage originated in northwest China in Manchuria during the Tsin dynasty around 220 B.C., used for its benefits as a detoxifier and energizer. Thanks to the expansion of trade routes, Kombucha made its way to Eastern Europe and Russia appearing in different countries. In the early 20th century, researchers in Switzerland noted that Kombucha was equally beneficial as Yogurt consumption (Jayabalan *et al.,* 2014).

Kombucha preparation process

Its preparation is easy and not very elaborate, so it is possible to make it at home. Black tea is usually used, but green tea and oolong (also called blue tea) are also useful. According to Martinez and collaborators in 2018, it is usually used 5 g of tea leaves per liter of water.

Since this beverage is made from living microorganisms it requires a carbon source as substrate, the source in this case is sugar. The best sugar for this fermentation is refined white sugar, since it is of high purity (> 99.9%), obtained mainly from sugar beet or sugar cane, depending on the refining process we can say that it has a high quality and contains less minerals or inorganic elements that affect its purity and flavor.

The main compound of this type is sucrose, one of the carbohydrates most used by our acetobacter.

For Kombucha, 5 to 20% sugar of the total volume to be made is required (Pohl, & Stecka, 2011). Some authors such as Martinez et al. state that minimum 50 g of sugar per liter is sufficient to start the fermentation process (Martinez *et al.*, 2018). First, the water is heated to boiling point, then an infusion is prepared with the tea leaves, it is left to steep to obtain the concentrate, and finally sucrose is added until dissolved. The mixture is cooled and then the SCOBY is added, it is important that the preparation is at least 20° C before adding the "Tea Fungus" to avoid damaging or killing it. If there is no SCOBY, it can be generated from a mother ferment of another Kombucha, i.e., a minimum of 10% of the total volume that we intend to ferment is added with the volume of another previous Kombucha (Heredia *et al.,* 2021).

The process requires clean and sterile areas to avoid unwanted contamination. After adding to the SCOBY it should be covered with a cloth (Abel, & Andreson, 2020). The average fermentation time is from 7 to 14 days depending on the type of tea and conditions such as temperature, darkness, pH, type of carbon source. Authors such as Uțoiu have described for cases of obtaining a new SCOBY, that at room temperature it could take from 7 to 30 days (Uțoiu *et al.,* 2018).

If it is desired to obtain a Kombucha with added flavor and properties it is important to perform a second fermentation, repeating the previous steps by adding the fruits, herbs or seeds for the process This can be seen in more detail in the methodology section of the present research. The temperature can be from 21 °C to 25° C. The optimum pH described in literature is 3.2 in green tea and 3.5 in black tea, reaching a maximum of 4.2 without the overproduction of acetic acid changing the flavor and properties (Martínez *et al.,* 2018).

ISSN-On line: 1390-9959

The SCOBY

It is called SCOBY to the acronym of "Symbiotic Colony Of Bacteria and Yeast", this consortium known as tea fungus is a mixed culture of acid-acetic bacteria of which we find the genera *Bacterium, Gluconoacetobacter, Gluconobacter, Halomonas, Herbaspirillum, Komagataeibacter,* the presence of these can vary from one Kombucha to another, but thanks to its film we always find species such as *Acetobacter xylinum, Acet. Xylinoides or Bacterium gluconicum*, and yeasts such as *Schizosaccharomyces pombe, Saccharomyces ludwigii, Zygosaccharomyces rouxii, Candida spp.* or *Pichia membranaefaciens*

The base of SCOBY to carry out the fermentation process is a floating cellulosic film that is usually seen as a thin film on the medium with tea, ethanol and organic acids (mainly acetic acid and gluconic acid) (Chen & Liu, 2000). The longer the fermentation time, the thicker the film will be (González, 2021). The acids in this culture is what gives the beverage antibacterial activity and prevents its contamination by other pathogenic bacteria. This mixture may also contain traces of carbon dioxide (Martinez *et al.*, 2018).

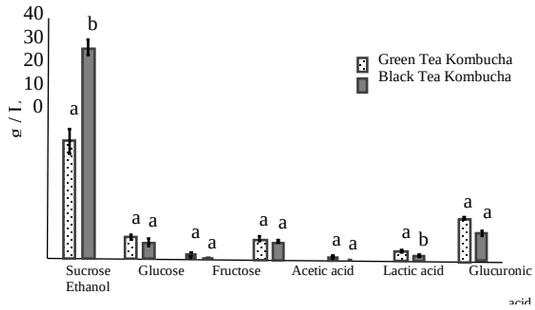
Composition and microbiome of Kombucha

According to Abel, & Andreson in 2020, the probiotic Kombucha beverage possesses compounds such as alcohols, aldehydes, ketones, esters, amino acids, tannins, terpenoids, saponins, flavonoids, phenols, alkaloids, CO2, enzymes, catechins, caffeine, among others (Based on Adzadogo, 2015). Biochemical analysis of Kombucha by Jayabalan and collaborators in 2014 indicated the presence of 14 amino acids, in addition to biogenic amines, purines, proteins, few hydrolytic enzymes, pigments, lipids, ethanol, antibiotically active matter, minerals, anions, DSL, yeast products and bacterial metabolites (Abel & Andreson, 2020).

Its metabolism is capable of producing glucose, fructose, vitamins C, B1 B2, B3, B6, B12, folic acid, organic acids such as acetic, gluconic, lactic, some enzymes, antibiotic active compounds, carbon dioxide, tea catechins and caffeine.

Of their therapeutic effects, they are known to come mainly from polyphenols and secondary metabolites obtained from fermentation, adding properties such as improving digestion, preventing cancer, eliminating toxic substances, relieving arthritis and preventing microbial infections (Kumar & Joshi, 2016).

As mentioned, the compounds vary according to the type of tea, so in Graph 1 (own elaboration of Figure 1 by Cardoso and collaborators in 2020), we can see that green tea has a greater amount of these compounds



Graphic 1 Own elaboration of "Figure 1. Chemical composition of the kombuchas. Results were expressed as mean of three repetitions. Error bars indicate ± standard deviation. Means followed by the same letter, for the same análisis, are not significantly different ($p < 0.05$)."
Source: (Cardoso et al., 2020)

According to Bardales and Pinto in 2021, the chemical composition of this probiotic beverage also varies around the time it takes to ferment, among these compounds, we have acids and alcohol usually increase proportional to the number of days. Thus, after 30 days, the amount of sucrose, glucose and fructose usually decreases, while the amount of gluconic acid, ethanol and acetic acid increases (Bardales & Pinto, 2021).

With respect to yeasts, these are responsible for the hydrolysis of sucrose, thus obtaining glucose and fructose. However, it is thanks to invertase that ethanol can be produced in the glycolysis process. The acetobacteria use this glucose to obtain gluconic acid and ethanol to obtain acetic acid. It is the set of organic acids produced during fermentation that provides a low pH value that confers a natural antimicrobial protection (Gonzalez, 2021).

ISSN-On line: 1390-9959

Some studies show that green tea and lemon balm tea have more stimulating effects than black tea, achieving fermentations in a shorter amount of time (Jayabalan *et al.,* 2014).

Regarding the amount of caffeine found in a beverage, Doepker, et al. in 2018 indicated that the daily intake of 400 mg per day of caffeine does not generate adverse health effects (equivalent to 10 g) in adults, similarly 300 mg in pregnant women and 2.5 mg for adolescents and children. However, when exceeding this dose we can find effects on bones, blood pressure, heart rate, cholesterol, mood, sleep, headache and withdrawal (Doepker *et al.,* 2018). In black tea-based kombucha, caffeine amounts of 16.64 mg/ g have been found, it has also been described that this proportion does not differ much from an infusion to the proportion in a fermentation, this is because, it does not influence the acidity of the beverage (Moya in 2020). In the literature, no standard has been reported for the amount of caffeine allowed or generated in green tea-based kombucha, but we did find in infusions, obtaining levels generally in the range of 141-338 mg/L depending on the brand (Ramiréz-Aristizabal *et al.,* 2016).

According to the FDA, although 400 mg is generally not related as a risk, there is a wide degree of effects on people due to their lifestyles. As we note the range of caffeine in green tea does not exceed the minimum level for consumption, noting that the adverse effects for a kombucha are minimal if the lifestyle is adequate, but in the long run the daily consumption and accumulation could generate effects just like the excessive consumption of a normal tea (FDA, 2018).

Antimicrobial capacity of Kombucha

This beverage, widely consumed in various parts of the world, has demonstrated a wide range of antimicrobial activity. The SCOBY symbiosis present in Kombucha is capable of inhibiting the growth of other highly contaminating bacteria. Activity against *Helicobacter pylori, E. coli, Staphylococcus aureus* and *Agrobacterium tumefaciens* has been reported due to the acetic acid produced during fermentation, in fact, it is considered the main antimicrobial compound.

Other authors such as Grenwalt et al. in 1998 cited by Battikhy collaborators in 2013, demonstrated activity in Kombucha made from green tea against bacteria *E. coli* serotype H10 (non-pathogenic), also for *E. coli* serotype H48 (pathogenic), the *S. typhimurium, Bacillus cereus* and *A. tumefaciens* (Battikh *et al.,* 2013). Table 1 based on Table 1 of Battikh et al. shows the comparison of the antimicrobial activity present in black tea and green tea based kombucha.

TABLE. ANTIBACTERIAL ACTIVITY OF KOMBUCHA[a]

Camellia sinensis type	Tested extracts	pH	Inhibition zone diameter (mm)[b] of target bacteria					
			Staphylococcus epidermidis CIP 106510	*Staphylococcus aureus* ATCC 25923	*Micrococcus luteus* NCIMB 8166	*Salmonella typhimurium* LT2	*Escherichia coli* ATCC 35218	*Listeria monocytogenes* ATCC 19115	*Pseudomonas aeruginosa* ATCC 27853
Black Tea	Fermented infusion (K_{25})[b]	2.59	18.5 ± 2.1	14.5 ± 2.1	16.5 ± 0.7	14.0 ± 1.4	10.5 ± 0.4	18.5 ± 2.1	19.0 ± 1.4
	Neutralized kombucha[c]	7.00	N.A.	9.5 ± 0.7	10.0 ± 0.0	N.A.	N.A.	N.A.	N.A.
	Unfermented infusion[d]	5.14	N. A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
	Acidified infusion[e]	2.59	N.A.	N.A.	N.A.	N.A.	N.A.	27.0 ± 1.4	15.5 ± 0.70
	Heat-denatured Kombucha[f]	2.59	16.5 ± 0.7	13.5 ± 2.1	13.5 ± 2.1	12.0 ± 0.0	13.0 ± 0.0	N.A.	11 ± 0.0
Green Tea	Fermented tea (Kombucha)[b]	2.54	22.0 ± 1.4	12.0 ± 0.0	22.0 ± 2.8	14.0 ± 1.4	14.5 ± 0.7	21.5 ± 2.1	18.0 ± 0.4
	Neutralized kombucha[c]	7.00	12.5 ± 0.7	N.A.	14.5 ± 0.7	N.A.	N.A.	N.A.	N.A.
	Unfermented tea[d]	5.08	10.0 ± 0.0	N.A.	16.0 ± 0.8	N.A.	N.A.	10.5 ± 0.7	N.A.
	Acidified tea[e]	2.54	27.0 ± 0.0	26.5 ± 0.7	20.5 ± 0.7	18.5 ± 0.7	13.0 ± 0.0	23.5 ± 2.1	13.0 ± 0.0
	Heat-denatured Kombucha[f]	2.54	19.0 ± 0.0	16.0 ± 1.4	19.5 ± 0.7	11.0 ± 1.4	12.0 ± 0.0	21.5 ± 2.1	9.0 ± 0.0

a Inhibition zone diameter (mean and standart deviation including wells diameter of 6 mm).
b Fermented infusion (Kombucha) at natural pH value without any adjustment.
c Neutralized kombucha: pH 7 fermented infusion adjusted with 1 M NaOH.
d Unfermented infusion prepared in the same way as that for making Kombucha, and 1 M HCL or 1 M NaOH was used to adjust their pH.
e Acidified infusion with acetic acid according to the acidity of Kombucha samples.
f Heat-denatured fermented infusions were treated at 120 C for 20 min.
N.A., no activity revealed.

Table 1 Own elaboration of "TABLE. ANTIBACTERIAL ACTIVITY OF KOMBUCHA."
Source: (Battikh *et al.,* 2013).

According to Cardoso et al. in 2020, Kombucha green tea inhibits the growth of pathogenic bacteria such as S. aureus, E. coli, Sallmonella and L. monocytogenes with a minimum concentration of 250 µL/mL while black tea only had activity against S. aureus and L. monocytogenes. This is due to the acidity of green tea being higher than that of other types of tea, with a pH of 3.2 and to the phenolic compound verbascoside which is unique to green tea.

Green tea

We denominate tea, to the use of any part of the *Camellia Sinesis* plant, to prepare infusions, by means of a preparation with boiling water. The leaves of this popular plant, native to Southeast Asia (South China, North India, Cambodia and Myanmar) and member of the *Theaceae* family, allow us to produce the famous green tea, whose catechins vary in quantity depending on the type of treatment used to dry them. Other variants of green tea known as black tea, Oolong tea, white tea and red tea are obtained from this same plant (Watson *et al.,* 2018).

ISSN-On line: 1390-9959

It possesses sugars, organic acids, amino acids, non-phenolic metabolites and bioactive phenolic compounds (Das *et al.,* 2019). Amino acids belonging to this tea variety comprise a total of 19, among these the most important are the non-proteinogenic ones such as L-Theanine, or 5-N-ethylglutamine which represents up to 50% of all free amino acids; it also possesses citrulline, ornithine and carnosine (Horanni and Engelhardt, 2013)

Its high antimicrobial and antioxidant activity is due to its xanthine compounds and polyphenols such as flavonoids, catechols, catechin tannins and acids. It has even been found to have a higher susceptibility against large positive than negative bacteria such as *Listeria monocytogenes*. In addition to conferring a pleasant odor and flavor to the taste (Mora *et al.,* 2013).

Kombucha in green tea has presented a total acidity of 0.36% (w / v acetic acid) higher than that of black tea (0.32 %). With respect to total phenolic properties, theaflavins, thearubigins and its antioxidant capacity, it has been found that black tea is 55.7% higher than green tea. Black tea is the beverage with the highest number of antioxidants (Cardoso *et al.,* 2020).

Blueberry or Blackberry

According to SAGARPA in 2017, one of the fruits with the highest antioxidant capacity are the berrys. *Vaccinium spp*. commonly called Blueberry is a berry native to the northeastern United States and eastern Canada currently cultivated in Mexico, it measures 1-2 cm in diameter and has a soft blue/purple coloration.
The literature describes that both the leaves and fruits of this plant possess high antioxidant activity, chlorogenic acid and quercetin glycosides are found in them (Chu *et al.,* 2017).

It possesses glucose, fructose and sucrose, in addition to phenolic compounds such as anthocyanins, quercetin, chlorogenic acids and proanthocyanidins.

Phenolic compounds are the main ones related to their antioxidant activity that allows them to neutralize substances such as free radicals that are related to chronic diseases, damage to the human body and cancer.

They have also demonstrated anti-inflammatory action, neuroprotection, cancer prevention and protection of organs such as the liver. The amount of these compounds in fruits varies according to the ripening stage and species, with anthocyanin being the compound that increases the most during ripening (Toyama *et al.,* 2021).

Orange Blossom Honey

The species *Citrus sinensis* known as Orange Blossom is a tree of Asian origin, from southwest China and the Malay Peninsula, this plant has a high commercial value due to the multiple products obtained from it, from orange to its honey. Currently, it is one of the species cultivated in Mexico, from which different products are exported in large quantities (SAGARPA, 2017).

The honey obtained from the citrus tree or "Orange blossom honey", is a light-colored honey and mild flavor with fresh and floral aroma distinctive of citrus (Kadar *et al.,* 2011). This honey possesses flavonoids such as chrysin, naringenin, kaempferol, luteolin, hesperetin, some galangin, pinocembrin and quercetin; the presence of phenolic acids was also found, specifically caffeic acid and p-coumaric acid (Escriche *et al.,* 2011). Orange tree flavonoids are important substances due to their ability to inhibit the oxidation of low-density lipoproteins in the blood, increase the amount of high-density lipoproteins and inhibit the activity of enzymes directly or indirectly involved in lipid peroxidation pathways such as cyclogenase, NO -synthase and NADH-oxidase (Atanelov *et al.,* 2018).

Studies by Escriche et al. in 2011 demonstrated the abundant presence of some lilac aldehydes and benzenacetaldehyde. Substances that, as seen, are part of the phenolic compounds that confer antioxidant power. Therefore, this honey is usually recommended for its antibacterial and healing properties, prevention of cancer and free radical damage, as well as reducing the risk of suffering chronic diseases (Escriche *et al.,* 2011).

ISSN-On line: 1390-9959

Methodology

Materials and reactives

Extra Special Orange Blossom Honey (370 g). Prometo PRODUCE brand Blueberry (156 g). Premium Green Tea brand STASH (40 g) herbal tea with 20 sachets without caffeine. EPURA brand jug water. Alquimia brand Kombucha lemon flavor black tea base (475 ml). Great Value brand refined white cane sugar (1 kg). Transparent glass jar of 42. 8 cm high with capacity of 4 liters. Electronic scale, Tefal brand, capacity up to 10 K. 100% cotton cloth of 47.5 cm by 34.6 cm. Elastic cloth band. Unireal nitrile gloves. A pewter spoon. Plastic spoon. One soup spoon. Two glass cups. One wooden stick. 1 measuring cup of 250 ml. 2-liter pewter pot. Hermetic refrigerator ACROS brand. 12 Estilo brand glass bottles with a capacity of 5600 ml each. Plastic funnel. AVEDISTANTE pH meter.

First fermentation

Boil 2 liters of Epura brand water for 5 minutes. Remove from heat and add 6 bags of Green Tea. Let stand covered for 10 minutes. Remove the tea bags and add 250 g of Great Value brand refined white sugar. Stir with a pewter spoon and let it stand for 10 minutes.

Subsequently, place the concentrate in the glass container. Add one liter of water at room temperature. Let stand 25 minutes then add 300 ml of commercial Kombucha Alquimia brand. Stir again with a plastic spoon and cover the mixture with the cloth, securing it with a rubber band. It is left to ferment for 7 days at room temperature in a dark and clean place (an airtight refrigerator without energy was used).

On the seventh day add a mixture of 300 g of refined sugar and 150 ml of commercial Kombucha. Stir with a plastic spoon. Let stand 14 days at a temperature of approximately 24 °C. Once the SCOBY has a suitable size, the Kombucha is tasted (it should have a sweet taste with a little sour). If the taste is as desired, the SCOBY is removed from the Kombucha with nitrile gloves to prepare the second fermentation. It is also consumable, as well as natural Kombucha.

NOTE: 2 sachets of Tea are placed per liter. Plastic spoons are used when mixing the concentrate with commercial Kombucha because it is an acidic beverage, if you use metal, the heat can oxidize.

Second fermentation

Once the SCOBY is carefully removed from the Kombucha. Place in a clean ceramic dish (with gloves). With the help of a funnel, pour the Kombucha into bottles with an airtight cap brand Estilo. Place 375 ml of the Kombucha in each bottle (3/4 of kombucha). Then add 30 g of orange blossom honey dissolved in 50 ml of Kombucha. Finally add 25 g of chopped blueberries (equivalent to two tablespoons).

Let it ferment for 4 to 5 days under the same conditions as the first fermentation. If it has the desired flavor, put it in the refrigerator at 4°C to stop fermentation.

To continue producing Kombucha, repeat the steps of the "First fermentation" procedure, but this time add 500 ml of the Kombucha concentrate obtained from the previous procedure. Add the SCOBY we generated and let it stand for 7 days or until the desired flavor is obtained.

Sensory Evaluation

Thirty people over 18 years of age were selected. They were given to taste ml of the two types of kombucha previously refrigerated. A sensory analysis test with a 9-point hedonic scale was applied. We plotted the results for the attributes of color, odor, taste and texture of the two types of Kombucha.

Results and Discussion

Obtaining SCOBY in first fermentation

Recall that the popular beverage known as Kombucha, is generated through the symbiotic relationship of SCOBY (bacteria and yeasts), sustained within a cellulose film that can take 7 to 30 days to grow at room temperature (Uțoiu *et al.*, 2018). This film is synthesized mainly by *Acetobacter xylinum*, a bacterium from the most predominant group of microorganisms in fermentation, of the genus *Acetobacter* (Watawana *et al.*, 2015).

ISSN-On line: 1390-9959

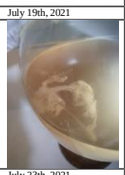
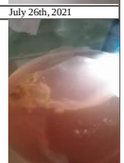
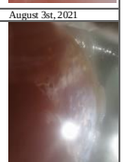
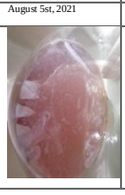
Date	July 7th, 2021	July 8th, 2021	July 9th, 2021	July 10th, 2021
Monitoring photo				
Date	July 11th, 2021	July 12th, 2021	July 13th, 2021	July 14th, 2021
Monitoring photo				
Date	July 15th, 2021	July 16th, 2021	July 17th, 2021	July 18th, 2021
Monitoring photo				
Date	July 19th, 2021	July 20th, 2021	July 21th, 2021	July 22th, 2021
Monitoring photo				
Date	July 23th, 2021	July 24th, 2021	July 25th, 2021	July 26th, 2021
Monitoring photo				
Date	July 27th, 2021	July 28th, 2021	July 29th, 2021	July 30th, 2021
Monitoring photo				
Date	July 31th, 2021	August 1st, 2021	August 2st, 2021	August 3st, 2021
Monitoring photo				
Date	August 4st, 2021	August 5st, 2021	August 6st, 2021	SCOBY- August 6st, 2021
Monitoring photo				

Table 2 Monitoring of SCOBY production from commercial Kombucha.
Source: (Own elaboration)

In Table 2 of the fermentation monitoring for obtaining a SCOBY, we noticed that, throughout the 30 days, the tea fungus took time to form a cellulose film, which on the 30th day reached the size of the surface of the liquid in the jar. It was very thin and with some lumps with a noticeable white-beige coloration.

The tea used for this process was caffeine-free, one of the compounds described as the main stimulator for cellulose synthesis (by bacteria) in green tea (Dufresne & Farnworth, 2000), whose composition is 3-6 % of the dry weight of the tea. Added to this, we have that the starter kombucha was caffeinated black tea. Accordingly, we can state the following, first, the bacteria and yeasts of our starter kombucha were accustomed to a medium with caffeine and xanthines, while the conditions of their new medium were different, since the consortium only possessed other xanthines in smaller amounts for their growth.

Secondly, we must remember that black tea has a higher pH and our starter kombucha was probably grown at an ideal temperature; Therefore, the change to a type of tea with a lower pH, the absence of caffeine and an initial ambient temperature of 21°C in the first 7 days, instead of the ideal 25°C (which was achieved in the remaining 23 days), caused an adaptation process, which is why the cellulose film obtained in Table 2 is thinner than the film obtained with a previous SCOBY used to the medium, as shown in Table 4.

Second fermentation kombucha

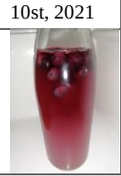
Date	Before fermentation	August 7st, 2021	August 8st, 2021	August 9st, 2021
Monitoring photo				
Date	August 10st, 2021	August 11st, 2021	August 12st, 2021	August 13st, 2021
Monitoring photo				

Table 3 Monitoring of the "Second Fermentation" process from the kombucha obtained in "First Fermentation" to add flavor and enhance properties with blueberry and orange blossom honey.
Source: (Own elaboration)

According to the literature, the optimum pH for fermentation in green tea is 3.2 with a maximum of 4.2. (Martinez *et al.*, 2018). The pH obtained in our Table 2 of natural Kombucha at 23 days was 2.42, having a final pH of 2.12 at 30 days.

ISSN-On line: 1390-9959

Its acidity confirms us the overproduction of acetic acid that can change the flavor and final properties, however, the final pH obtained from the second fermentation in Table 3, was 2.53. The increase in its final pH is due to the addition of blueberry and orange blossom honey, whose pH is 2.48 for the fruit (Stückrah *et al.*, 2007) and 4.14 for the honey (Periago *et al.*, 2019), thus resulting in a higher pH for the second fermentation but obtaining values outside the optimal range of growth for our consortium.

Since the base tea was more acidic due to the use of green tea, we can infer that it possesses antimicrobial activities against pathogenic bacteria such *as S. aureus, E. coli, Sallmonella and L. monocytogenes* (Cardosos *et al.*, 2020), part of the antimicrobial properties described in the literature giving an added economic value to our beverage as a probiotic.

First fermentation with SCOBY previously obtained

Date	August 8st, 2021	August 9st, 2021	August 10st, 2021	August 11st, 2021
Monitoring photo				
Date	August 12st, 2021	August 12st, 2021	August 12st, 2021	August 13st, 2021
Monitoring photo				
Date	SCOBY - August 13st, 2021			
Monitoring photo				

Table 4 Monitoring of the "first fermentation" of green tea based Kombucha with SCOBY previously obtained
Source: (Own elaboration)

For the first fermentation in table 4, with a previously obtained SCOBY, we measure an initial pH of 3.64 and a final pH after 7 days of 2.75 were achieved. The final pH of the natural Kombucha is higher than the pH of the cranberry-honey Kombucha because of the difference in time, ingredients used, and SCOBY obtained. Both are below the optimal growth pH range, however, if it was possible to obtain the desired tea fungus and pleasant flavor.

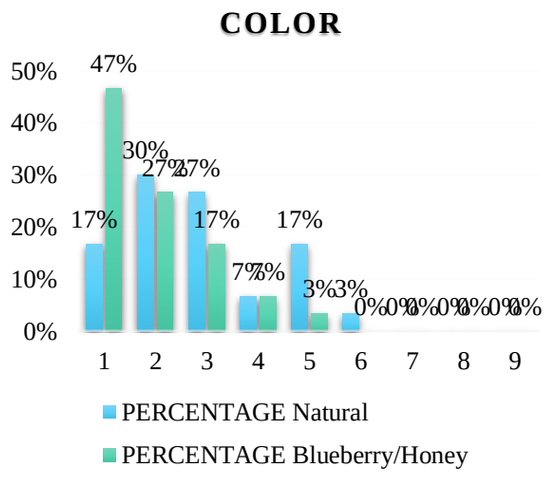
Respect to the SCOBY structure, we note that on the seventh day our cellulose film measures 0.4 millimeters, almost half a centimeter. This consortium is much thicker and stronger than the first cellulose film in Table 2, its coloration is white-beige similar to the first SCOBY, but unlike the previous one, the texture of this film is smooth.

The difference in growth time between the films is remarkable because the first SCOBY took 30 days to regulate itself to its environment in order to grow while the second film already had a tea fungus adapted to this environment, with a good control of the ideal temperature. We were able to obtain a strong SCOBY based on green tea and without caffeine. Also, we can confirm based on the literature, the presence of *Acetobacter xylinum* in both consortia, since in both cases a cellulose film was created.

To slow down fermentation, we must lower the temperature of our Kombucha. The ideal temperature is 4°C to avoid contamination by foodborne pathogens (Murphy *et al.*, 2018). The temperature change allows us to perform the sensory study of the beverage.

Sensory Evaluation

The application of a sensory evaluation using a nine-point hedonic scale allowed us to obtain 4 graphs, where we can compare the characteristics obtained through the production process of a natural Kombucha and the process of the blueberry Kombucha with honey.

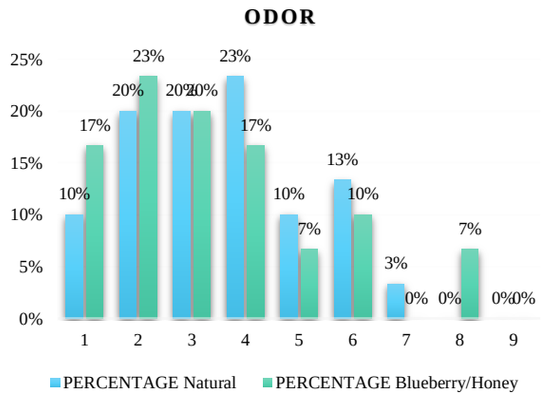


Graphic 2 Percentages obtained from the nine-point hedonic test on the color of natural Kombucha VS. Blueberry Kombucha with orange blossom honey
Source: (Own elaboration)

ISSN-On line: 1390-9959

Graphic 2 corresponds to color acceptance. For flavored kombucha, with a reddish tone, 47% of the population studied liked it very much, 27% liked it very much, 17% liked it moderately, 7% liked it slightly and 3% neither liked nor disliked it. With respect to natural kombucha with a greenish amber shade, we observe that 30% of the population likes it very much, 27% likes it moderately, 17% likes it very much, another 17% neither likes nor dislikes it, 7% likes it slightly and 3% dislikes it slightly.

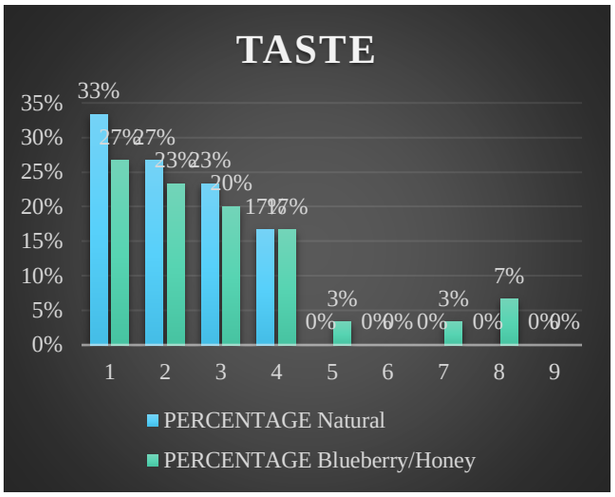
These data indicate that 97% of the population liked the color of the flavored drink, only 3% neither liked nor disliked it. On the other hand, 80% of the population liked the color of the natural drink, 17% neither liked nor disliked it and only 3% disliked it.



Graphic 3 Percentages obtained from the nine-point hedonic test for the odor of natural Kombucha VS. Blueberry Kombucha with orange blossom honey
Source: (Own elaboration)

Graphic 3 corresponds to the comparison of odor. With respect to blueberry kombucha with orange blossom honey, 23% of the population studied liked it very much, 20% liked it moderately, 17% liked it slightly, another 17% liked it very much, 10% indicated that they disliked it slightly, 7% neither liked nor disliked it, and another 7% disliked it very much. For natural kombucha, it was observed that 23% liked it slightly, 20% liked it very much and another 20% liked it moderately, 13% indicated that they disliked it slightly, 10% indicated that they liked it very much and another 10% indicated that they neither liked nor disliked the aroma, finally 3% indicated that they disliked it moderately.

The aroma obtained from the Kombucha resembled the aroma of acetic acid. The study found that 76% liked the aroma of the flavored beverage, 7% neither liked nor disliked it and 17% indicated that they disliked it. For the natural Kombucha, 74% liked it, 10% neither liked nor disliked it and 16% disliked it.



Graphic 4 Percentage of the nine-point hedonic test on the taste obtained from the production of a natural Kombucha VS. Blueberry Kombucha with orange blossom honey
Source: (Own elaboration)

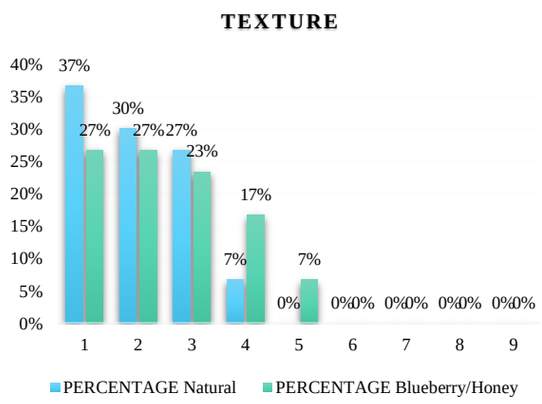
Graphic 4 shows the comparisons of the flavor obtained from the fermentation processes. For the taste of the blueberry kombucha with orange blossom honey fermented for 30 days, 27% of the population indicated that they liked the final taste obtained very much, 23% indicated that they liked it very much, 20% liked it moderately, 17% liked it slightly, 7% indicated that they disliked the taste very much, 3% indicated that they neither disliked it nor liked it, and finally another 3% indicated that they disliked it moderately. With respect to natural kombucha, 33% indicated that they liked it very much, 27% indicated that they liked it very much, 23% liked it moderately and 17% liked it slightly.

In terms of taste, it can be seen, that the cranberry drink with honey obtained 87% of acceptance as a pleasant taste, 3% indicated that it was neither unpleasant nor pleasant and only 10% indicated that it was unpleasant. For the natural drink, 100% indicated that they liked the taste.

ISSN-On line: 1390-9959

During fermentation, a slightly sour taste may predominate, due to the amount of acetic acid being produced. On some occasions, the flavor can be slightly alcoholic, similar to an apple cider (Marsh *et al.*, 2014). According to Ihsani et al., 2021, the ethanol increase in the first 8 days does not exceed 0.32% of its volume, nor does it affect the antioxidant quality, lactic acid levels or pH (Ihsani *et al.*, 2021). This probiotic beverage does not have an alcohol percentage higher than the 0.5% necessary to be considered an alcoholic beverage by the TTB (Alcohol and Tobacco Tax and Trade Bureau) of the United States or by the Mexican Ministry of Health (General Health Law, article 217), so this occasional taste does not affect its main function.

The sweet and fruity flavor it usually acquires depends on the type of sugar, fruit or herb used, as well as the time of the second fermentation. Its aroma is acidic, like vinegars, sometimes becoming fruity (Watawana *et al.*, 2015),



Graphic 5 Percentage of nine-point hedonic test applied for texture of a natural Kombucha VS. Blueberry Kombucha with orange blossom honey
Source: (Own elaboration)

Graphic 5 corresponds to the comparison of the texture of both kombuchas. Both beverages were refrigerated and served cold, obtaining an acceptance for the blueberry kombucha with orange blossom honey, 27% of the population liked it very much, another 27% liked it very much, 23% liked it moderately, 17% liked it slightly and 7% neither liked nor disliked it. The percentages on the texture of natural kombucha indicate that 37% liked it very much, 30% liked it very much, 27% liked it moderately and 7% liked it slightly.

The above data indicate that with respect to the cold texture of our beverage, 93% of the population considered the texture pleasant and only 7% as neither pleasant nor unpleasant, while our natural Kombucha obtained 100% acceptance as a pleasant beverage.

Based on the results obtained from the sensory evaluation, it can be seen that, the beverage with the highest acceptance in color and odor was the flavored Kombucha, but in the categories of flavor and texture, the highest acceptance was obtained by the natural beverage. However, for both probiotic beverages, their rejection rate was low, so both the natural and flavored probiotic have good texture and color. Its odor can be treated when refrigerated since after three days it was noticed that its initial sour aroma decreased drastically as the SCOBY stopped producing more organic acids. Regarding the main category in flavor, its acceptance rate is higher for the natural one, but the flavor was not very low, so the fermentation days could be decreased until the desired acid flavor is obtained. This indicates that its acceptance varies according to consumer tastes.

Acknowledgements

We thank the program of complementary support for the awarding of a scholarship to carry out the Summer of Research 2021, within the framework of the Interinstitutional Program for the Strengthening of Research and Postgraduate Studies of the Pacific "DELFÍN" in virtual mode. As well as to the organizers of the program for the opportunity to do research.

Conclusions

It was possible to develop the production of homemade Kombucha based on caffeine-free green tea with antioxidant properties added by means of a fruit and honey from orange blossom. Thus, obtaining a probiotic beverage with added properties, thanks to the wide variety of microbial species of the consortium and the added fruits.

Despite having different environmental conditions than the initial commercial Kombucha, a SCOBY was obtained and together with it, a Kombucha with enhanced antioxidant properties was produced (shown in Table 2 and 3), a process carried out by means of a second fermentation.

ISSN-On line: 1390-9959

Due to the current situation of COVID-19, it was not possible to perform antimicrobial experiments to test the inhibitory activity of Gram-positive and Gram-negative bacteria of the probiotic beverage, through bacterial growth tests with strains, which could be achieved in other studies; but it did perform a sensory evaluation of natural Kombucha and flavored Kombucha to approximate the added value of the product. Along with this and based on the literature, it was confirmed that the growth of the tea fungus was possible due to the existing broad consortium, which in other studies has demonstrated high effectiveness against pathogens such as Gram-negative and Gram-positive bacteria. Thus, obtaining a probiotic beverage with beneficial properties for the human microbiota, with a very pleasant color and texture, with a characteristic acetic acid odor that is controllable and 87% accepted in taste.

Possible improvements to achieve greater acceptance in flavor would be to reduce the number of days for the first fermentation to obtain SCOBY, since the excess of acetic acid changed the expected organoleptic properties. Also, the temperature control could be improved from the first day, verifying that it is a constant 25°C in the dark, along with this, the field of study for sensory analysis could be expanded, thus yielding even more accurate values.

References

Abel, M., & Andreson, H. (2020). Effect of simultaneous inoculation of commercial yeast starter cultures on Kombucha fermentation. *Agronomy Research, 18((S3))*, 1603–1615. https://doi-org.proxydgb.buap.mx/10.15159/AR.20.140

Atanelov, N., Arutinashvili, N., Burjanadze, G., Kuprava, G., & Koshoridze, N. (2018). Isolation of Flavonoid Extract from Georgian Citrus Sinensis Peel and Its Antioxidative Properties. *Georgian Medical News, 283*, 162–165.

Bardales Espinoza, F. M., & Pinto Perez, C. P. (2021). Estudio de prefactibilidad para la instalación de una planta procesadora de una bebida en base a hojas de té verde y un cultivo probiótico (*Medusomyces gisevi*).

Battikh, H., Chaieb, K., Bakhrouf, A., & Ammar, E. (2013). Antibacterial and Antifungal Activities of Black and Green Kombucha Teas. *Journal of Food Biochemistry, 37(2),* 231–236. https://doi-org.proxydgb.buap.mx/10.1111/j.1745-4514.2011.00629.x

Carbonell Gonzálvez, Á. (2021). Análisis sobre las recomendaciones dietético-nutricionales con relación a la COVID-19.

Chen, C., & Liu, B. Y. (2000). Changes in major components of tea fungus metabolites during prolonged fermentation. *Journal of applied microbiology, 89(5),* 834–839. https://doi.org/10.1046/j.1365-2672.2000.01188.x

Chu, W., Gao, H., Cao, S., Fang, X., Chen, H., & Xiao, S. (2017). Composition and morphology of cuticular wax in blueberry (Vaccinium spp.) fruits. Food chemistry, 219, 436–442. https://doi.org/10.1016/j.foodchem.2016.09.186

Darmawan, G., Tendean, M., Dharmawan, A., William, Tan, H. T., Atmogo, T., & Gunardi, W. D. (2020). The Possible Role of Probiotics in Combating COVID-19. Indonesian *Journal of Gastroenterology, Hepatology & Digestive Endoscopy, 21(3)*, 226–230.

Das, P. R., Kim, Y., Hong, S., & Eun, J.-B. (2019). Profiling of volatile and non-phenolic metabolites—amino acids, organic acids, and sugars of green tea extracts obtained by different extraction techniques. *Food Chemistry*. doi:10.1016/j.foodchem.2019.05.194

Doepker, C., Franke, K., Myers, E., Goldberger, J. J., Lieberman, H. R., O'Brien, C., Peck, J., Tenenbein, M., Weaver, C., & Wikoff, D. (2018). Key Findings and Implications of a Recent Systematic Review of the Potential Adverse Effects of Caffeine Consumption in Healthy Adults, Pregnant Women, Adolescents, and Children. *Nutrients, 10(10),* 1536. https://doi.org/10.3390/nu10101536

Dufresne, C., & Farnworth, E. (2000). Tea, Kombucha, and health: a review. *Food Research International, 33(6),* 409–421.

ISSN-On line: 1390-9959

Escriche, I., Kadar, M., Juan-Borrás, M., & Domenech, E. (2011). Using flavonoids, phenolic compounds and headspace volatile profile for botanical authentication of lemon and orange honeys. *Food Research International, 44(5*), 1504–1513. https://doi-org.proxydgb.buap.mx/10.1016/j.foodres.2011.03.049

Gibson, G. R., Probert, H. M., Loo, J. V., Rastall, R. A., & Roberfroid, M. B. (2004). Dietary modulation of the human colonic microbiota: updating the concept of prebiotics. *Nutrition research reviews, 17(2),* 259–275. https://doi.org/10.1079/NRR200479

González Arranz, S. (2021). Diseño de un nuevo material sostenible a partir de celulosa bacteriana de residuos orgánicos de producción local en un marco de economía circular.

Graph 1: Cardoso, R. R., Neto, R. O., dos Santos D'Almeida, C. T., do Nascimento, T. P., Pressete, C. G., Azevedo, L., Martino, H. S. D., Cameron, L. C., Ferreira, M. S. L., & Barros, F. A. R. de. (2020). Kombuchas from green and black teas have different phenolic profile, which impacts their antioxidant capacities, antibacterial and antiproliferative activities. *Food Research International, 128*. https://doi-org.proxydgb.buap.mx/10.1016/j.foodres.2019.108782

Heredia, A. S. R., Tello, I. P. S., Mancheno, C. I. F., & Andrade, G. I. M. (2021). Utilización de tres sustratos para la determinación de la viabilidad del acetobacter aceti y saccharomyces cerevisiae presentes en el medusomyces gisevi, y su posible aplicación agroindustrial. *ConcienciaDigital, 4(3.1),* 122-141.

Horanni, R., & Engelhardt, U. H. (2013). Determination of amino acids in white, green, black, oolong, pu-erh teas and tea products. *Journal of Food Composition and Analysis, 31(1),* 94–100. doi:10.1016/j.jfca.2013.03.005

Ihsani, N., Hernahadini, N., & Muhammad, F. (2021, febrero). The variation of ethanol concentration and kombucha characterization on several incubation periods. *Journal of Physics: Conference Series, 1764(1)*. DOI:10.1088/1742-6596/1764/1/012008

Interián-Gómez, L., Aguila-Gutiérrez, S. E., Esquivias-López, K. M., Pulido-De la Cruz, V. A., Silva-Arzola, N. J., & Gonzalez-Becerra, K. (2021). Componentes alimenticios, estado de ánimo y su relación con el sistema inmune en COVID-19. RESPYN *Revista Salud Pública y Nutrición, 20(4), 73-86.*

Jayabalan, R., Malbaša, R. V., Lončar, E. S., Vitas, J. S., & Sathishkumar, M. (2014). A Review on Kombucha Tea-Microbiology, Composition, Fermentation, Beneficial Effects, Toxicity, and Tea Fungus. *Comprehensive Reviews in Food Science & Food Safety, 13(4),* 538–550. https://doi-org.proxydgb.buap.mx/10.1111/1541-4337.12073

Kalt, W., Cassidy, A., Howard, L. R., Krikorian, R., Stull, A. J., Tremblay, F., & Zamora-Ros, R. (2020). Recent Research on the Health Benefits of Blueberries and Their Anthocyanins. *Advances in Nutrition (Bethesda, Md.), 11(2),* 224–236. https://doi-org.proxydgb.buap.mx/10.1093/advances/nmz065

Kombucha desde cero | la guía completa. (2020, 25 abril). [Vídeo]. *YouTube*. https://www.youtube.com/watch?v=N3SCuxaIpsQ

Kumar, V. & Joshi, V. 2016. Kombucha: technology, microbiology, production, composition and therapeutic value. *Intl. J. Food. Ferment. Technol. 6,* 13–24.

Lilly, D. M., & Stillwell, R. H. (1965). Probiotics: growth-promoting factors produced by microorganisms. *Science (New York, N.Y.), 147(3659*), 747–748. https://doi.org/10.1126/science.147.3659.747

Maria Saarela. (2011). Functional Foods : Concept to Product: Vol. 2nd ed. *Woodhead Publishing.*

Marsh, A. J., O'Sullivan, O., Hill, C., Ross, R. P., & Cotter, P. D. (2014). Sequence-based analysis of the bacterial and fungal compositions of multiple kombucha (tea fungus) samples. *Food microbiology, 38*, 171–178. https://doi.org/10.1016/j.fm.2013.09.003

ISSN-On line: 1390-9959

Martínez Leal, J., Valenzuela Suárez, L., Jayabalan, R., Huerta Oros, J., & Escalante-Aburto, A. (2018). A review on health benefits of kombucha nutritional compounds and metabolites. CyTA: *journal of food*, *16*, 390-399. doi: 10.1080/19476337.2017.1410499

Mora, Andreína, Parra, Jonathan, Chaverri, José M, & Arias, María Laura. (2013). Determination of the antimicrobial capacity of green tea (Camellia sinensis) against the potentially pathogenic microorganisms Escherichia coli, Salmonella enterica, Staphylococcus aureus, Listeria monocytogenes, Candida albicans and Aspergillus niger. *Archivos Latinoamericanos de Nutrición, 63(3*), 247-253. Recuperado en 16 de julio de 2021, de http://ve.scielo.org/scielo.php?script=sci_arttext&pid=S0004-06222013000300008&lng=es&tlng=en.

Moya Patiño, E. E. (2020). Concentración de cafeína a partir de té negro, hoja de guayusa y café con ganoderma, durante la fermentación del Hongo Kombucha (Medusomyces Gisevi) (Bachelor's thesis, Universidad Técnica de Ambato. *Facultad de Ciencia e Ingeniería en Alimentos y Biotecnología.* Carrera de Ingeniería en Alimentos).

Murphy, T. E., Walia, K., & Farber, J. M. (2018). Safety Aspects and Guidance for Consumers on the Safe Preparation, Handling and Storage of Kombucha -- A Fermented Tea Beverage. *Food Protection Trends, 38(5),* 329–337

Nelson, D. L. (2017). Functional Foods: Sources, Health Effects and Future Perspectives. *Nova Science Publishers, Inc*.

Nutrition Division. (2006). Propiedades saludables y nutricionales y directrices para la evaluación - Informe de la Consulta de Expertos FAO/OMS sobre Evaluación de las Propiedades Saludables y Nutricionales de los Probióticos en los Alimentos (*FAO Food and Nutrition Paper ed., Vols. 1014–2916).* FAO/OMS. http://www.fao.org/3/a0512s/a0512s.pdf

Office of the Commissioner. (2018, 12 diciembre). Spilling the Beans: How Much Caffeine is Too Much? *U.S. Food and Drug Administration*. https://www.fda.gov/consumers/consumer-updates/spilling-beans-how-much-caffeine-too-much

Parmenter, B. H., Dalgaard, F., Murray, K., Cassidy, A., Bondonno, C. P., Lewis, J. R., Croft, K. D., Kyrø, C., Gislason, G., Scalbert, A., Tjønneland, A., Hodgson, J. M., & Bondonno, N. P. (2021). Habitual flavonoid intake and ischemic stroke incidence in the Danish Diet, Cancer, and Health Cohort. *American Journal of Clinical Nutrition, 114(1),* 348–357. https://doi-org.proxydgb.buap.mx/10.1093/ajcn/nqab138

Periago Castón, M. J., Navarro-González, I., Alaminos, A. B., Elvira-Torales, L. I., & García-Alonso, F. J. (2019). Parámetros de calidad en mieles de diferentes orígenes botánicos producidas en La Alpujarra granadina. *Anales de Veterinaria de Murcia, 32,* 59–71. Recuperado a partir de https://revistas.um.es/analesvet/article/view/369371

Plaza-Diaz, J., Ruiz-Ojeda, F. J., Gil-Campos, M., & Gil, A. (2019). Mechanisms of Action of Probiotics. *Advances in nutrition (Bethesda, Md.), 10*(suppl_1), S49–S66. https://doi.org/10.1093/advances/nmy063

Pohl, P., & Stecka, H. (2011). Elemental Composition of White Refined Sugar by Instrumental Methods of Analysis. *Critical Reviews in Analytical Chemistry, 41(2),* 100–113. https://doi-org.proxydgb.buap.mx/10.1080/10408347.2011.538585

Ramiréz-Aristizabal, L. S., Ortiz, A., & Ospina-Ocampo, L. F. (2016). Determinación de compuestos fenólicos (catequinas, cafeína, ácidos orgánicos) en té verde (camellia sinensis) usando cromatografía líquida de alta resolución/ Determination of phenolic compounds (catechins, caffeine, organic acid) of green tea (camellia sinensis) using high performance liquid chromatography resolution. *Vitae, 23,* S245.

ISSN-On line: 1390-9959

SAGARPA, 2017. Frutas del Bosque arándano, frambuesa zarzamora mexicanas. Ciudad de México: *Secretaría de Agricultura y Desarrollo Rural,* pp.1-3.

SAGARPA, 2017. Planeación agrícola nacional 2016-2030. Cítricos, Limón, naranja y toronja mexicanos. Ciudad de México: *Secretaría de Agricultura y Desarrollo Rural,* pp.12-15.

Sami, R., Alshehry, G., Elgarni, E., & Helal, M. (2021). Saudi Community Care Awareness Food Facts, Nutrients, Immune System and Covid-19 Prevention in Taif City among Different Age Categories. *African Journal of Food, Agriculture, Nutrition & Development, 21(1),* 17213–17233. https://doi-org.proxydgb.buap.mx/10.18697/ajfand.96.20440

Schrezenmeir, J., & de Vrese, M. (2001). Probiotics, prebiotics, and synbiotics--approaching a definition. *The American journal of clinical nutrition, 73(*2 Suppl), 361S–364S. https://doi.org/10.1093/ajcn/73.2.361s

Secretaría de Salud. (2021, 1 junio). Reglamentos de la Ley General de Salud. *Ley General de Salud*. https://www.gob.mx/insabi/prensa/reglamentos-de-la-ley-general-de-salud-275018?idiom=es

Stückrath, Rosemarie, & Petzold, Guillermo R. (2007). Formulación de una Pasta Gelificada a Partir del Descarte de Arándanos (Vaccinium corymbosum). *Información tecnológica, 18(2),* 53-60. https://dx.doi.org/10.4067/S0718-07642007000200008

Toyama, Y., Toshima, S., Hirano, T., Yamasaki, M., & Kunitake, H. (2021). Polyphenol contents, antioxidant activities, and anti-cancer cell proliferation properties at each stage of fruit development in intersectional hybrids between highbush blueberry and shashanbo (<italic>Vaccinium bracteatum</italic> Thunb.). *Journal of Berry Research, 1–16.* https://doi-org.proxydgb.buap.mx/10.3233/jbr-210713

Uțoiu, E., Matei, F., Toma, A., Diguță, C. F., Ștefan, L. M., Mănoiu, S., Vrăjmașu, V. V., Moraru, I., Oancea, A., Israel-Roming, F., Cornea, C. P., Constantinescu-Aruxandei, D., Moraru, A., & Oancea, F. (2018). Bee Collected Pollen with Enhanced Health Benefits, Produced by Fermentation with a Kombucha Consortium. *Nutrients, 10(10*). https://doi-org.proxydgb.buap.mx/10.3390/nu10101365

Valenzuela B, Alfonso, Valenzuela, Rodrigo, Sanhueza, Julio, & Morales I, Gladys. (2014). Alimentos funcionales, nutraceúticos y foshu: ¿vamos hacia un nuevo concepto de alimentación?. *Revista chilena de nutrición, 41(2), 198-204.* https://dx.doi.org/10.4067/S0717-75182014000200011

Watawana, M. I., Jayawardena, N., Gunawardhana, C. B., & Waisundara, V. Y. (2015). Health, Wellness, and Safety Aspects of the Consumption of Kombucha. *Journal of Chemistry, 1–11.* https://doi-org.proxydgb.buap.mx/10.1155/2015/591869

Watson, R. R., Preedy, V. R., & Zibadi, S. (2018). Polyphenols: Mechanisms of Action in Human Health and Disease (English Edition) (2.a ed.). *Academic Press. 55*, 715-721, https://doi.org/10.1016/B978-0-12-398456-2.00055-4.

ISSN-On line: 1390-9959

Article **ECORFAN Journal-Ecuador**
June 2021, Vol.8 No.14 39-45

Innovative process of a food supplement made from oregano bagasse. "NUTRIOREG"

Proceso innovador de un suplemento alimenticio elaborado a base del bagazo del orégano. "NUTRIOREG"

DELGADO-MARTÍNEZ, Martha Lilia†*, AGUIRRE-OROZCO, Mario Abelardo, MÁRQUEZ-MONÁRREZ, Olivia and CONTRERAS-MARTÍNEZ, Jesús José

Tecnológico Nacional de México, Campus Delicias, Paseo Tecnológico km. 3.5, C.P.33000. Delicias City, Chihuahua, Mexico.

ID 1st Author: *Martha Lilia, Delgado-Martínez* / **ORC ID:** 0000-0002-5635-6853

ID 1st Co-author: *Mario Abelardo, Aguirre-Orozco* / **ORC ID:** 0000-0002-6899-5230

ID 2nd Co-author: *Olivia, Márquez-Monárrez* / **ORC ID:** 0000-0001-8549-5935

ID 3rd Co-author: *Jesús José Contreras Martínez* / **ORC ID**: 0000-0002-9044-4216

DOI: 10.35429/EJE.2021.14.8.39.45 Received January 25, 2021; Accepted June 30, 2021

Abstract

The South-Central Region of Chihuahua is a highly agricultural and livestock area. It has 41,900 hectares of non-timber forest resource (oregano) (Alarcón, 2005), which is processed and generates waste up to 1,470.17 tons per season cycle, which do not take advantage of its economic and nutritional benefit. The niche of opportunity was born here where it was found that the oregano residue contains 16.44% of protein per 100 g according to studies at the Center for Research in Food and Development of Delicias (CIAD), attached to CONACYT, and in the laboratory of the company Alimentos Concentrados de Delicias. The proportion that is generated from this waste is very rich, it is considered as an area of commercialization opportunity. In this region there are 257 potential clients according to SAGARPA (2017) dedicated to raising cattle. As quoted by Almeida G, (DIGAL 2018) specified that 8363 livestock production units participate directly in the dairy activity with a daily volume of 3,000,000 liters of milk. Of the five dairy basins in the state, Delicias is the most important, therefore, there is the opportunity to establish the NUTRIOREG company as a producer of concentrated feed supplement for livestock.

Innovation, Oregano residues, Nutrioreg

Resumen

La Región Centro Sur de Chihuahua es zona altamente agrícola y ganadera. Cuenta con 41900 Has de recurso forestal no maderable (orégano) (Alarcón, 2005), el cual se procesa y genera desperdicio hasta por 1470.17 toneladas por ciclo de temporada, mismas que no aprovechan su beneficio económico y nutricional. Nace aquí el nicho de oportunidad donde se encontró que el residuo del orégano contiene por cada 100 g un 16.44% de proteína según estudios en el Centro de Investigación en Alimentación y Desarrollo de Delicias (CIAD), adscrito al CONACYT, y en el laboratorio de la empresa Alimentos Concentrados de Delicias. La proporción que se genera de este desecho es muy rica, es considerada como un área de oportunidad de comercialización. En esta región se cuenta con 257 clientes potenciales según SAGARPA (2017) dedicados a la crianza de ganado vacuno. Como lo cita Almeida G, (DIGAL 2018) precisó que en la actividad lechera participan de manera directa 8363 unidades de producción pecuaria con un volumen de 3,000,000 litros diarios de leche. De las cinco cuencas lecheras del estado, Delicias es la más importante, por consiguiente, se tiene la oportunidad de establecer la empresa NUTRIOREG como productora de suplemento alimenticio concentrado para ganado.

Innovación, Residuos del orégano, Nutrioreg

Citation: DELGADO-MARTÍNEZ, Martha Lilia, AGUIRRE-OROZCO, Mario Abelardo, MÁRQUEZ-MONÁRREZ, Olivia and CONTRERAS-MARTÍNEZ, Jesús José. Innovative process of a food supplement made from oregano bagasse. "NUTRIOREG". ECORFAN Journal-Ecuador. 2021. 8-14:39-45.

*Correspondence to Autor (E-mail: mldelgamar@hotmail.com)
† Researcher contributing as first author (ORTEGA-RAMIREZ, Marynor Elena).

Introduction

The State of Chihuahua has an area of 247,460 km^2, divided in 67 municipalities, with 908 agrarian nuclei and 72 rural communities, accounting 10,116,936 hectares of social property in the area according to SEDATU (2014). In the State, there is a surface of 41,900 hectares of non-timber forest resource (oregano) (Alarcón, 2005), which is processed and, like any company, it generates waste up to 1,470.17 tons by time cycle, these wastes are not taken economically and nutritionally advantage of (INEGI, 2015). With the oregano bagasse a total of 36,754 bags of 40 kg of NUTRIOREF will be obtained. Technical scientifical test were performed in the Centro de Investigación en Alimentación y Desarrollo A.C. of Delicias and Hermosillo, Sonora (CIAD), attached to CONACYT. The physicochemical studios obtained in the Alimentos Concretados de Delicias, S.A. de C.V. (ALCODESA, 2019) lab show that the bagasse has a 6.69% of raw fat, 11.19% of fiber and 16.44% of protein; likewise, it has 308.77 kilocalories of caloric intake and a total of 45.7% carbs, in other words, this is established as optimal necessary nutrition to feed the livestock. In the region of Delicias, there is a presence of 257 potential clients according to SAGARPA (2017), which are dedicated to the cattle raising and will be the main clients.

Justification

In the present project are exposed the reasons why it is important to develop this type of research. In the making of the nutritional supplement, it is observed that, after carrying out the oregano oil extraction process, the waste is not used and is thrown away. This is where the fundamental idea of the project is born, the oregano bagasse is the main element of the cattle feed. The physicochemical studies obtained in the Alimentos Concretados de Delicias, S.A. de C.V. (ALCODESA, 2019) lab show that the bagasse has a 6.69% of raw fat, 11.19% of fiber and 16.44% of protein; likewise, it has 308.77 kilocalories of caloric intake and a total of 45.7% carbs. This nutritional supplement will be of great benefit to the livestock of the area.

Attributes: NUTRIOREF has a high nutritional power, for each 100 g it has 16.44% of protein according to the nutritional table of the Centro de Investigación en Alimentos y Desarrollo (CIAD), obtained through the measurement of its properties in technical scientific equipment. The nutritional supplement is a high nutritional quality substitute for the appropriate cattle raising.

Differentiation: The nutritional supplement NUTRIOREG is differentiated in the market from the competing processing companies by offering a lower price of $104.63 pesos for a 40 kg bag, which represents up to 5 times less the price from the companies dedicated to the commercialization of cattle feed.

Problem Statement

There are 257 potential clients in Delicias according to SAGARPA (2017), dedicated to the cattle raising willing to substitute the nutritional supplement they use for NUTRIOREG, due to the high nutritional value provided for the livestock, besides its low cost. NUTRIOREG has a high nutritional power, for each 100 g it has 16.44% of protein according to the nutritional table of the Centro de Investigación en Alimentos y Desarrollo (CIAD), by absorbing the properties it contains. The nutritional supplement NUTRIOREG has as a competitive advantage which allows it to differentiate from the competing companies by offering a lower price of $104.63 pesos for a 40 kg bag, which represents up to 5 times less the price from the companies dedicated to the commercialization of cattle feed, in addition, it provides more energy and nutrients to the livestock, this benefits mainly its feeding.

Theoretical Framework

The State of Chihuahua has an area of 247,460 km^2, divided in 67 municipalities, with 908 agrarian nuclei and 72 rural communities, accounting 10,116,936 hectares of social property in the area according to SEDATU (2014).

ISSN-On line: 1390-9959

In the State, there is a surface of 41,900 hectares of non-timber forest resource (oregano) (Alarcón, 2005), which is processed and, like any company, it generates waste up to 1,470.17 tons by time cycle, these wastes are not taken economically and nutritionally advantage of (INEGI, 2015). With the oregano bagasse a total of 36,754 bags of 40 kg of NUTRIOREF will be obtained. Technical scientifical test were performed in the Centro de Investigación en Alimentación y Desarrollo A.C. of Delicias and Hermosillo, Sonora (CIAD), attached to CONACYT. The physicochemical studios obtained in the Alimentos Concretados de Delicias, S.A. de C.V. (ALCODESA, 2019) lab show that the bagasse has a 6.69% of raw fat, 11.19% of fiber and 16.44% of protein; likewise, it has 308.77 kilocalories of caloric intake and a total of 45.7% carbs, in other words, this is established as optimal necessary nutrition to feed the livestock. In the region of Delicias, there is a presence of 257 potential clients according to SAGARPA (2017), which area dedicated to the cattle raising and will be the main clients.

Innovation description

The amount of waste coming from the extraction of the essence of oregano oil is great and it is used to be thrown away without knowing that these wastes have nutrients that are not manipulated and can be taken advantage of in an effective way. There are 257 potential clients in Delicias according to SAGARPA (2017), dedicated to the cattle raising willing to substitute the nutritional supplement they use for NUTRIOREG, due to the high nutritional value provided for the livestock, besides its low cost. NUTRIOREG has a high nutritional power, for each 100 g it has 16.44% of protein according to the nutritional table of the Centro de Investigación en Alimentos y Desarrollo (CIAD), by absorbing the properties it contains. The nutritional supplement NUTRIOREG has as a competitive advantage which allows it to differentiate from the competing companies by offering a lower price of $104.63 pesos for a 40 kg bag, which represents up to 5 times less the price from the companies dedicated to the commercialization of cattle feed, in addition, it provides more energy and nutrients to the livestock, this benefits mainly its feeding. By processing and marketing it, it will generate an economic apportion to the region, creating more income and employment sources.

ALIMENTOS CONCENTRADOS DE DELICIAS SA DE CV
Quality Control Laboratory
Tels (639) 472-73-93 472-83-66 470-00-12
www.grupoalcodesa.com

ID
90188
DATE
26/04/2019

Customer: 2004 General Public
Sample: 0002 8 Others ingredients — 26/04/2019
Package: PAQ 10 — 26/abr./19 01:10
Report : Full

OBS: **Oregano Saucillo Stove. 11/April/2019 Alejandro Palacios.**

Dry Base Results		
Dry Matter	88.7	%
Ashes	8.7	%
Dcs		%
BMD		%
Energy		
ENL INRA		Mcal/Kg M.S
ENL NRC		Mcal/Kg M.S
In Manto		Mcal/Kg M.S
In Gan		Mcal/Kg M.S
Crude Fat	6.69	%
Starch		%
Starch Dig Rumen		%
% Grains		%
Fast Energy		
CNF		%
Total Sugars		%
Fibers		
ADF		%
NDF		%
NDF Indigestible		% M.S.
NDF digestible		% M.S.
NDF Indigestible		% NDF.
NDF digestible		% NDF.
Crude Fiber	11.19	%
Lignin		%
Proteins		
Crude Fiber	16.44	%
Degradable Protein		%PC
Non-Degrad Protein		%PC
PDIN		%
PDIE		%
PDIA		%

Milk Production	
Milk Production	lt/cow/day
Forage Cons M Dry	kg/cow/day
VRF	kg of M.S.
Degradab Enzymatic	%
Urea Kg/Ton	

Micotoxins		Optimal
Aflatoxin	ppb	Max 20 ppb
Zearalenone	ppb	Max 300 ppb
Vomitoxin	ppm	Max 5 ppm

Minerals	
Calcium	%
Phosphorus	%
Magnesium	%
Potassium	%
Manganese	ppm
Copper	ppm
Zinc	ppm
Arsenic	ppm

Fermentative Quality		Optimal	Quality
PH			
Ammoniacal Nitrogen	%	3.75	Middle
Soluble nitrogen	%	5.00	Good
		60.00	Good

Q.B.P. Mario René Sodi Pérez

Figure 1 Physicochemical analysis
kSource: *ALCODESA, 2019*

Interview with experts

It has been achieved to nail down a commercial alliance with one of the more important companies in the dry oregano processing, to provide with raw material to the NUTRIOREG production, obtaining as a result a major support to the project, the plant is located in the south-central region of the State of Chihuahua.

Manufacturing method

The procedure carried out to explain the process of obtaining the oregano bagasse is, according to W. Niebel Benjamin, (1998), a flow process diagram is a graphic representation of the sequence of all operations, transportations, inspections, delays and storages that happen during all the process.

ISSN-On line: 1390-9959

The flow process diagram includes the information that is considered to be necessary for the analysis, in the figure 2, for example, the required time for the operation and the distance traveled are especially useful to bring out the hidden costs, as well as the distance traveled, temporal storages, delays, etc.

In the flow process diagram attention must be put into:

1. The material handling or transfer.
2. The plant and equipment distribution.
3. The production time and the delays.
4. The storage time.

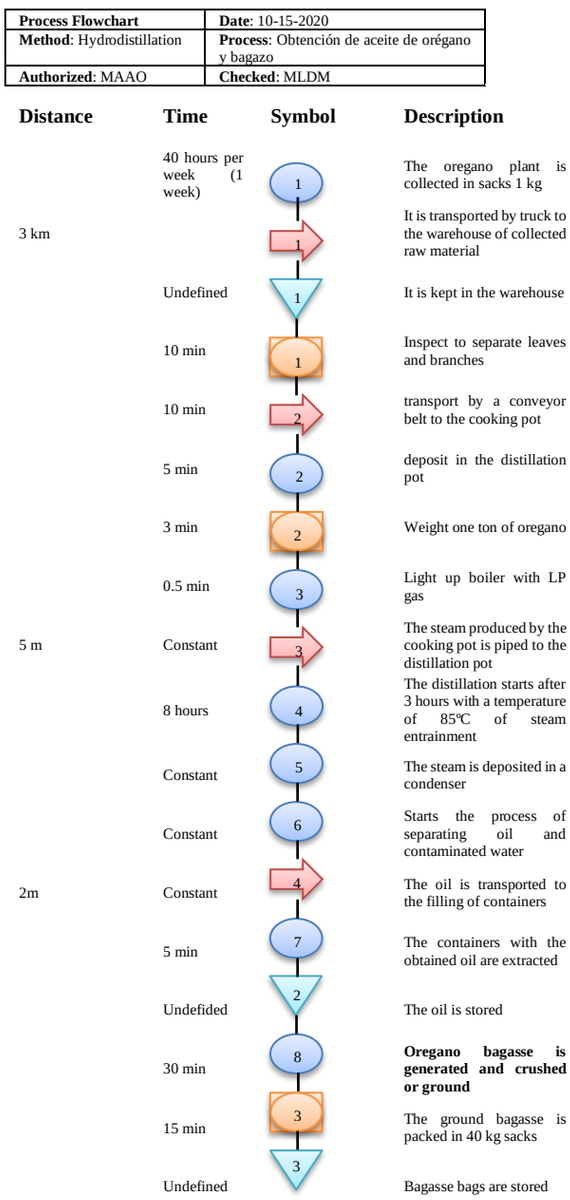


Figure 2 Flow process diagram
Source: Own source

Activity	Symbol	Quantity	Time (min)	Distance (m)
Operation		8	2920.5	
Combined Act.		3	28	
Transport		4	0	3007
Store		3	Undefined	
TOTAL		4	18	2958.5	3007

Table 1 Summary table
Source: Own source

Generalizing, in the table 1 above, it is identified that to produce 40 kg of nutritional supplement were required eight operations, with a time of 2,920.5 minutes, three combined activities with a total time of 28 minutes, four transports traveling a 3,007 meters distance, three storages where the storage time is undefined, in other words, the total time was of 2,958.5 minutes of process and a traveled distance of 3,007 meters, taking into consideration that storages are undefined, this will depend on the sales and advertising made to the NUTRIOREG product.

Potential market

The project of producing and commercializing the nutritional supplement made from oregano bagasse has a potential market in Delicias City, in the State of Chihuahua, where there is a presence of 257 potential clients according to SAGARPA (2017), mainly male people dedicated to the cattle raising and commercialization of its derivates, however, the number of potential clients expands as the project obtains more broadcasting and commercialization taking it to a national and international market; currently, the target market is: small, medium and big livestock producers dedicated to the cattle raising and handling in the south-central region of the State of Chihuahua, and subsequently to any person who owns livestock and is willing to change its current livestock food product for the nutritional supplement NUTRIOREG.

Target market

Mexico is the second world-wide producer of oregano, this brings certain security that the natural resource will not be considered one of the limitations to its production, minimizing the possibility for it to run out.

ISSN-On line: 1390-9959

Annually, near 4 thousand tons of dry oregano are produced in the States of Baja California, Sonora, Chihuahua, Durango, Tamaulipas, San Luis Potosí, Coahuila, Nuevo León y Zacatecas which gives certain security and sustainability to the NUTRIOREG processing plant, allowing it to have sources to obtain the bagasse and keep producing the nutritional supplement.

Method assessment and applicable normativy

Bromatology technical-scientific studies performed in the Centro de Investigación en Alimentación y Desarrollo A.C. (CIAD) and physicochemical studios obtained in the Alimentos Concretados de Delicias, S.A. de C.V. (ALCODESA, 2019) quality control lab, figure 1 and table 2 show that the oregano bagasse has the necessary protein nutrients to the good development in the livestock food. Besides, tests where performed to the bagasse in order to determine the percentage of energy, fiber and dry protein, these calculations were based on The Official Methos of Analysis (AOAC, 2000), through which the amount of calorific energy and carbs was obtained. The statistic evidence resulting from the bagasse analysis stand out the nutritional input for an adequate and balanced nutrition for the development of the livestock, complementing the nutrient intake in the food supplied daily.
Following are shown the norms that must be complied for the NUTRIOREG production:

NormaOficialMexicanaNOM-051-SCFI/SSA1-2010, General specifications for labelled of prepackaged food and non-alcohol drinks. Commercial and health information. Its object is to establish the commercial and health information that the labelled must have for prepackaged food and non-alcohol drinks of national or foreign production, as well as determine the characteristics of that information Norma Oficial Mexicana NOM-182-SSA1-2010, Etiquetado de nutrientes vegetales. In this norm are established the vegetable nutrients that are object of vigilance by the authorities, in order to guarantee to the user its quality and to prevent the potential risk for public, animal and vegetable health, as well as the adverse effects to the environment.

Norma Oficial Mexicana NOM-251-SSA1-2009, Prácticas de higiene para el proceso de alimentos, bebidas o suplementos alimenticios. This norm establishes the minimum requirement of good hygiene practices that must be observed in the production of food, drinks or nutritional supplement and its raw materials, in order to avoid its contamination through all the process.

Norma Oficial Mexicana PROY-NOM-005-SEMARNAT-2012. The norm establishes the requirements to the sustainable exploit of the non-timber forest resources in the forestall ecosystems, cold temperate forests, jungles and arid and semiarid zones – Technical specifications.

Norma Oficial Mexicana NOM-086-SSAL-1994. Goods and services, food and non-alcohol drinks with modifications in its composition, nutritional specifications.

Norma Oficial Mexicana NOM-130-SSA1-1995. Goods and services, food package in hermetically sealed containers and subjected to heat treatment. Sanitary dispositions and specifications.

Different types of oregano used							
Element	Saucillo 1	Saucillo 2	Saucillo stove	Original	Saláices	Without smell	Average
Ni (ppm)	132.785	4.48	5.92	3.785	5.015	5.14	26.1875
Fe (ppm)	158.545	158.575	198.04	117.215	166.95	16.305	135.9383333
Zn (ppm)	20.17	21.12	13.9	13.795	17.855	1.53	14.72833333
Na (%)	0.0047	0.0049	0.00403	0.0025	0.0037	0.0038	0.0040
Cu (ppm)	6.74	7.77	8.2	6.825	7.645	3.51	6.781666667
Mn (ppm)	30.14	30.64	13.47	38.285	15.98	0.36	21.47916667
Ca (%)	1.32785	1.5285	1.3556	1.5073	1.4908	0.1869	1.232825
K (%)	0.9223	0.9999	0.7682	0.7133	0.85365	0.07215	0.721583333
Mg (%)	0.3564	0.3564	0.4232	0.2874	0.25	0.00975	0.280525
N (%)	2.5104	2.3312	2.6304	1.9136	2.1184	2.3008	2.3008

Table 2 Bromatology studies
Source: CIAD Delicias

Financial viability

NUTRIOREG expects to achieve a 16,725 annual tons production of nutritional supplement, it is estimated that to develop the project $ 1,625,178.00 pesos are needed, obtained from partners contribution in $ 731,330.10 pesos and from financed capital with debt in $ 893,847.90 pesos, the short term investment is of $ 349,752.00 pesos for the opening of the company for the first three months, the fixed investment is of $ 1,244,926.00 pesos which includes the employers salary, the local rent, as well as services, freights and a deferred investment of $30,500 pesos that covers the constitutive act and the anticipated paid rent.

ISSN-On line: 1390-9959

The assessment tools show that the business plan is clearly profitable, based on the Net Present Value of $ 1,003,623.17 pesos is possible to generate an Internal Rate of Return of 67.39% in contrast with a Weighted Average Cost of Capital of 20.36% and a Minimum Acceptable Rate of Return of 17.68%, the Internal Rate of Return is bigger than the Weighted Average Cost of Capital, therefore it is possible to cover the cost of the assessment rate and also generate utilities, it was established that the more convenient and reasonable price is of $ 104.63 pesos for a 40 kg bag.

Intelectual property

NUTRIOREG will adopt the patent registry in the IMPI, the legal concept it will adopt will be a patent according to the Diario Oficial de la Federación published on May 18, 2018 in articles 15 to 27. It was requested a patent unity for the State of Chihuahua in the CRODE (Centro Regional de Optimización y Desarrollo de Equipo), the identity patent procedure to back up the technical scientific innovation from the nutritional supplement. Besides, the copyright will be protected through the Sistema Nacional de Propiedad Industrial. The proof of the patent provided to NUTRIORED will allow to exploit the innovation during 20 years.

Results

The present research project develops a solution to the problem that generated the great quantity of oregano bagasse. The nutritional supplement for the livestock is made from the wastes generated in the oil oregano extraction process. Table 3, with nutrimental information, shows the results related to the studies performed in the Centro de Investigación en Alimentación y Desarrollo A.C. and Alimentos Concretados de Delicias, S.A. de C.V. (ALCODESA, 2019) lab, it is proved that the nutritional supplement contains the necessary nutrients to the cattle development. Each bag contains the following nutritional contribution per 100 grams.

Macronutrient element			Micronutrient element		
Na	0.00403%		Ni	5.92	ppm
Ca	1.36%		Fe	198.04	ppm
K	0.77%		Zn	13.9	ppm
Mg	0.42%		Cu	8.2	ppm
Na	2.63%		Mn	13.47	ppm
P	0.18%				
Protein	**16.44%**		**Fiber**	**11.19 %**	

Table 3 Nutritional information of the oregano bagasse
Source: CIAD Delicias

The nutritional supplement gives the livestock a basic nutritional contribution for a balanced diet, complementing the food intake daily, without neglecting the quality of the product. This contributed to the cattle growth and development, table 4 shows the nutrient estimates based on The Official Methos of Analysis (AOAC, 2000), it shows the amount of calorific energy and carbs contained in NUTRIOREG, it satisfices the economic needs of any other supplement existing in the market, offering it by a lower price.

CHO'S:	100%	Moisture	Ashes	Crude Fat	Fiber	Protein
CHO'S=	100	-11.3	-8.68	-6.69	-11.19	=-16.44 - 45.7 %
Calorific Energy (Kcal) = Carbohydrates (4) + Protein (4) + Crude Fat (9)						
KCAL=	45.7 (4)		+ 16.44 (4)	16.69 (9)	=308.77 Kcal	

Table 4 Estimation of calorific nutrients
Source: ALCODESA Delicias, AOAC, 2000

With the obtained results, it is sustained that NUTRIOREG is between the ranges of ideal calorific contribution for a good cattle nutrition.

References

Alarcón, B. M. (2005). El orégano, una tabla de producción para el sureste del estado de Chihuahua. INIFAP- CIFAP- Madera, Chih.

ALCODESA (2019) Alimentos Concentrados de Delicias.

CIAD. (2019). Centro de Investigación de Alimentos y Desarrollo A.C. Cd. Delicias Chihuahua, adscrito a CONACYT.

CIAD. (2019). Centro de Investigación de Alimentos y Desarrollo A.C. Cd. Hermosillo Sonora, adscrito a CONACYT

COFEPRIS (2010). Comisión Federal para la Protección contra Riesgos Sanitarios. http://www.cofepris.gob.mx/MJ/Páginas/Normas-Oficiales- mexicanas.

INEGI. (2015) Instituto Nacional de Estadística y Geografía. Preconteo de Encuesta Intercensal. País México.

Latimer Jr. George W. (2019) The Official Methods of Analysis International. of AOAC INTERNATIONAL

DOF. (2019) Diario Oficial de la Federación. Normas Oficiales Mexicanas.

ISSN-On line: 1390-9959

Rodríguez, R. A. (2011). El Emprendedor de Éxito. México, D. F. McGraw- Hill /Interamericana Editores, S. A. de C. V.

SAGARPA (2018) Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación.

Secretaría de Economía. (IMPI, 31 de mayo de 2019). Blog. Obtenido de La Propiedad Industrial en México:

W. Niebel, Benjamín. (1988) INGENIERIA INDUSTRIAL, Métodos Tiempos y Movimientos, Novena Edición, México, Alfaomega.

SEDATU (2018) Secretaría de Desarrollo Agrario y Territorial Urbano. Gobierno Federal. País México

ISSN-On line: 1390-9959

[Title in Times New Roman and Bold No. 14 in English and Spanish]

Surname (IN UPPERCASE), Name 1st Author†*, Surname (IN UPPERCASE), Name 1st Coauthor, Surname (IN UPPERCASE), Name 2nd Coauthor and Surname (IN UPPERCASE), Name 3rd Coauthor

Institutional Affiliation of Author including Dependency (No.10 Times New Roman and Italic)

International Identification of Science - Technology and Innovation

ID 1st author: (ORC ID - Researcher ID Thomson, arXiv Author ID - PubMed Author ID - Open ID) and CVU 1st author: (Scholar-PNPC or SNI-CONACYT) (No.10 Times New Roman)

ID 1st coauthor: (ORC ID - Researcher ID Thomson, arXiv Author ID - PubMed Author ID - Open ID) and CVU 1st coauthor: (Scholar or SNI) (No.10 Times New Roman)

ID 2nd coauthor: (ORC ID - Researcher ID Thomson, arXiv Author ID - PubMed Author ID - Open ID) and CVU 2nd coauthor: (Scholar or SNI) (No.10 Times New Roman)

ID 3rd coauthor: (ORC ID - Researcher ID Thomson, arXiv Author ID - PubMed Author ID - Open ID) and CVU 3rd coauthor: (Scholar or SNI) (No.10 Times New Roman)

(Report Submission Date: Month, Day, and Year); Accepted (Insert date of Acceptance: Use Only ECORFAN)

Abstract (In English, 150-200 words)

Objectives
Methodology
Contribution

Keywords (In English)

Indicate 3 keywords in Times New Roman and Bold No. 10

Abstract (In Spanish, 150-200 words)

Objectives
Methodology
Contribution

Keywords (In Spanish)

Indicate 3 keywords in Times New Roman and Bold No. 10

Citation: Surname (IN UPPERCASE), Name 1st Author†*, Surname (IN UPPERCASE), Name 1st Coauthor, Surname (IN UPPERCASE), Name 2nd Coauthor and Surname (IN UPPERCASE), Name 3rd Coauthor. Paper Title. ECORFAN Journal-Ecuador. Year 1-1: 1-11 [Times New Roman No.10]

* Correspondence to Author (example@example.org)
† Researcher contributing as first author.

Introduction

Text in Times New Roman No.12, single space.

General explanation of the subject and explain why it is important.

What is your added value with respect to other techniques?

Clearly focus each of its features

Clearly explain the problem to be solved and the central hypothesis.

Explanation of sections Article.

Development of headings and subheadings of the article with subsequent numbers

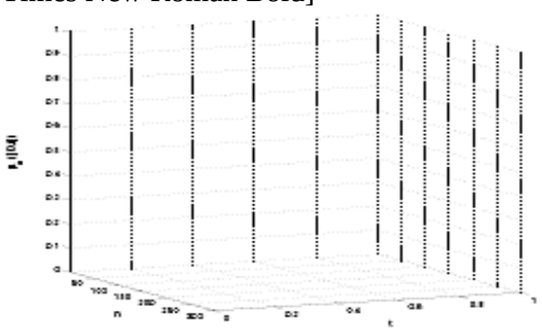
[Title No.12 in Times New Roman, single spaced and bold]

Products in development No.12 Times New Roman, single spaced.

Including graphs, figures and tables-Editable

In the article content any graphic, table and figure should be editable formats that can change size, type and number of letter, for the purposes of edition, these must be high quality, not pixelated and should be noticeable even reducing image scale.

[Indicating the title at the bottom with No.10 and Times New Roman Bold]



Graphic 1 Title and *Source (in italics)*

Should not be images-everything must be editable.

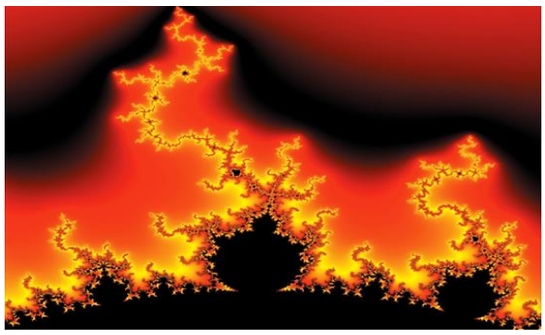


Figure 1 Title and *Source (in italics)*

Should not be images-everything must be editable.

Table 1 Title and *Source (in italics)*

Should not be images-everything must be editable.

Each article shall present separately in **3 folders**: a) Figures, b) Charts and c) Tables in .JPG format, indicating the number and sequential Bold Title.

For the use of equations, noted as follows:

$$Y_{ij} = \alpha + \sum_{h=1}^{r} \beta_h X_{hij} + u_j + e_{ij} \quad (1)$$

Must be editable and number aligned on the right side.

Methodology

Develop give the meaning of the variables in linear writing and important is the comparison of the used criteria.

Results

The results shall be by section of the article.

Annexes

Tables and adequate sources thanks to indicate if were funded by any institution, University or company.

Conclusions

Explain clearly the results and possibilities of improvement.

ISSN: 1390-9959

References

Use APA system. Should not be numbered, nor with bullets, however if necessary, numbering will be because reference or mention is made somewhere in the Article.

Use Roman Alphabet, all references you have used must be in the Roman Alphabet, even if you have quoted an Article, book in any of the official languages of the United Nations (English, French, German, Chinese, Russian, Portuguese, Italian, Spanish, Arabic), you must write the reference in Roman script and not in any of the official languages.

Technical Specifications

Each article must submit your dates into a Word document (.docx):

Journal Name
Article title
Abstract
Keywords
Article sections, for example:

1. Introduction
2. Description of the method
3. Analysis from the regression demand curve
4. Results
5. Thanks
6. Conclusions
7. References

Author Name (s)
Email Correspondence to Author
References

Intellectual Property Requirements for editing:

-Authentic Signature in Color of Originality Format Author and Coauthors

-Authentic Signature in Color of the Acceptance Format of Author and Coauthors

ISSN: 1390-9959

Reservation to Editorial Policy

ECORFAN Journal-Ecuador reserves the right to make editorial changes required to adapt the Articles to the Editorial Policy of the Journal. Once the Article is accepted in its final version, the Journal will send the author the proofs for review. ECORFAN® will only accept the correction of errata and errors or omissions arising from the editing process of the Journal, reserving in full the copyrights and content dissemination. No deletions, substitutions or additions that alter the formation of the Article will be accepted.

Code of Ethics - Good Practices and Declaration of Solution to Editorial Conflicts

Declaration of Originality and unpublished character of the Article, of Authors, on the obtaining of data and interpretation of results, Acknowledgments, Conflict of interests, Assignment of rights and Distribution.

The ECORFAN-Mexico, S.C Management claims to Authors of Articles that its content must be original, unpublished and of Scientific, Technological and Innovation content to be submitted for evaluation.

The Authors signing the Article must be the same that have contributed to its conception, realization and development, as well as obtaining the data, interpreting the results, drafting and reviewing it. The Corresponding Author of the proposed Article will request the form that follows.

Article title:

– The sending of an Article to ECORFAN Journal-Ecuador emanates the commitment of the author not to submit it simultaneously to the consideration of other series publications for it must complement the Format of Originality for its Article, unless it is rejected by the Arbitration Committee, it may be withdrawn.

– None of the data presented in this article has been plagiarized or invented. The original data are clearly distinguished from those already published. And it is known of the test in PLAGSCAN if a level of plagiarism is detected Positive will not proceed to arbitrate.

– References are cited on which the information contained in the Article is based, as well as theories and data from other previously published Articles.

– The authors sign the Format of Authorization for their Article to be disseminated by means that ECORFAN-Mexico, S.C. In its Holding Ecuador considers pertinent for disclosure and diffusion of its Article its Rights of Work.

– Consent has been obtained from those who have contributed unpublished data obtained through verbal or written communication, and such communication and Authorship are adequately identified.

– The Author and Co-Authors who sign this work have participated in its planning, design and execution, as well as in the interpretation of the results. They also critically reviewed the paper, approved its final version and agreed with its publication.

– No signature responsible for the work has been omitted and the criteria of Scientific Authorization are satisfied.

– The results of this Article have been interpreted objectively. Any results contrary to the point of view of those who sign are exposed and discussed in the Article.

Copyright and Access

The publication of this Article supposes the transfer of the copyright to ECORFAN-Mexico, SC in its Holding Ecuador for its ECORFAN Journal-Ecuador, which reserves the right to distribute on the Web the published version of the Article and the making available of the Article in This format supposes for its Authors the fulfilment of what is established in the Law of Science and Technology of the United Mexican States, regarding the obligation to allow access to the results of Scientific Research.

Article Title:

Name and Surnames of the Contact Author and the Coauthors	Signature
1.	
2.	
3.	
4.	

Principles of Ethics and Declaration of Solution to Editorial Conflicts

Editor Responsibilities

The Publisher undertakes to guarantee the confidentiality of the evaluation process, it may not disclose to the Arbitrators the identity of the Authors, nor may it reveal the identity of the Arbitrators at any time.

The Editor assumes the responsibility to properly inform the Author of the stage of the editorial process in which the text is sent, as well as the resolutions of Double-Blind Review.

The Editor should evaluate manuscripts and their intellectual content without distinction of race, gender, sexual orientation, religious beliefs, ethnicity, nationality, or the political philosophy of the Authors.

The Editor and his editing team of ECORFAN® Holdings will not disclose any information about Articles submitted to anyone other than the corresponding Author.

The Editor should make fair and impartial decisions and ensure a fair Double-Blind Review.

Responsibilities of the Editorial Board

The description of the peer review processes is made known by the Editorial Board in order that the Authors know what the evaluation criteria are and will always be willing to justify any controversy in the evaluation process. In case of Plagiarism Detection to the Article the Committee notifies the Authors for Violation to the Right of Scientific, Technological and Innovation Authorization.

Responsibilities of the Arbitration Committee

The Arbitrators undertake to notify about any unethical conduct by the Authors and to indicate all the information that may be reason to reject the publication of the Articles. In addition, they must undertake to keep confidential information related to the Articles they evaluate.

Any manuscript received for your arbitration must be treated as confidential, should not be displayed or discussed with other experts, except with the permission of the Editor.

The Arbitrators must be conducted objectively, any personal criticism of the Author is inappropriate.

The Arbitrators must express their points of view with clarity and with valid arguments that contribute to the Scientific, Technological and Innovation of the Author.

The Arbitrators should not evaluate manuscripts in which they have conflicts of interest and have been notified to the Editor before submitting the Article for Double-Blind Review.

Responsibilities of the Authors

Authors must guarantee that their articles are the product of their original work and that the data has been obtained ethically.

Authors must ensure that they have not been previously published or that they are not considered in another serial publication.

Authors must strictly follow the rules for the publication of Defined Articles by the Editorial Board.

The authors have requested that the text in all its forms be an unethical editorial behavior and is unacceptable, consequently, any manuscript that incurs in plagiarism is eliminated and not considered for publication.

Authors should cite publications that have been influential in the nature of the Article submitted to arbitration.

Information services

Indexation - Bases and Repositories

LATINDEX (Scientific Journals of Latin America, Spain and Portugal)
RESEARCH GATE (Germany)
GOOGLE SCHOLAR (Citation indices-Google)
REDIB (Ibero-American Network of Innovation and Scientific Knowledge- CSIC)
MENDELEY (Bibliographic References Manager)

Publishing Services

Citation and Index Identification H
Management of Originality Format and Authorization
Testing Article with PLAGSCAN
Article Evaluation
Certificate of Double-Blind Review
Article Edition
Web layout
Indexing and Repository
ArticleTranslation
Article Publication
Certificate of Article
Service Billing

Editorial Policy and Management

18 Marcial Romero, CP-241550. Avenue, Salinas l - Santa Elena-Ecuador. Phones: +52 1 55 6159 2296, +52 1 55 1260 0355, +52 1 55 6034 9181; Email: contact@ecorfan.org www.ecorfan.org

ECORFAN Journal-Ecuador

ISSN 1390 - 9959

www.ecorfan.org