

VOL II

# Estudos em Ciências Agrárias e Ambientais

Eduardo Spers  
(Organizador)



EDITORA  
ARTEMIS

2024

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## APRESENTAÇÃO

O campo das Ciências Agrárias e Ambientais desempenha um papel fundamental na compreensão e solução dos desafios contemporâneos relacionados à produção de alimentos, à conservação ambiental e ao bem-estar animal. Em um mundo em constante transformação, questões como a sustentabilidade dos agroecossistemas, o manejo eficiente dos recursos naturais e a saúde pública se tornam cada vez mais relevantes. É com este espírito que apresentamos o volume II da coletânea "Estudos em Ciências Agrárias e Ambientais", que reúne pesquisas de autores de diversas partes do mundo, cada um contribuindo com sua perspectiva e expertise únicos.

Os quinze artigos que compõem este volume abordam uma variedade de tópicos, refletindo a riqueza e a diversidade das Ciências Agrárias. Desde práticas conservacionistas que buscam melhorar e manter agroecossistemas, até investigações sobre o uso de fitohormonas e fertilização na produção vegetal, o uso de tecnologias de processamento de madeira e a promoção do bagre armado - cada estudo traz à tona questões cruciais que impactam tanto a produção agrícola quanto a saúde ambiental.

Neste volume, também exploramos a crescente relevância dos produtos agrícolas locais, especialmente em tempos desafiadores como os que vivemos, marcados pela pandemia da COVID-19. A importância de circuitos curtos de proximidade se torna evidente, promovendo não apenas a segurança alimentar, mas também a resiliência das comunidades.

Além disso, as contribuições da veterinária destacam a importância do cuidado animal e da saúde pública, ilustrando a interconexão entre os seres humanos, os animais e o meio ambiente.

Esperamos que esta coletânea não apenas informe, mas também inspire debates e colaborações futuras entre pesquisadores, profissionais e estudantes da área. Juntos, podemos avançar em direção a um futuro mais sustentável e equilibrado, em que conhecimento e pesquisa sejam os pilares para soluções efetivas.

Agradecemos a todos os autores e colaboradores que tornaram este trabalho possível. É nossa esperança que os estudos aqui apresentados contribuam para um entendimento mais profundo das questões agrárias e ambientais, e que possam servir de base para novas investigações e práticas inovadoras.

Eduardo Eugênio Spers

## SUMÁRIO

### SUSTENTABILIDADE E PRÁTICAS CONSERVACIONISTAS

#### **CAPÍTULO 1.....1**

PRÁTICAS CONSERVACIONISTAS PARA MELHORIA E MANUTENÇÃO DOS AGROECOSSISTEMAS

Eliana Batista

Glêvia Kamila Lima

 [https://doi.org/10.37572/EdArt\\_3010242771](https://doi.org/10.37572/EdArt_3010242771)

#### **CAPÍTULO 2.....17**

PROS AND CONS OF USING FORESTRY AS A COMPENSATION MECHANISM FOR GREENHOUSE GASES EMISSIONS ON NEW ZEALAND PASTORAL FARMS

Phil Journeaux

 [https://doi.org/10.37572/EdArt\\_3010242772](https://doi.org/10.37572/EdArt_3010242772)

#### **CAPÍTULO 3.....32**

STRUCTURAL AND CHEMICAL CHARACTERISTICS OF WOOD GREENERY ORIGINATING FROM BOSNIA AND HERZEGOVINA

Srđan Ljubojević

Ladislav Vasilišín

Goran Vučić

 [https://doi.org/10.37572/EdArt\\_3010242773](https://doi.org/10.37572/EdArt_3010242773)

#### **CAPÍTULO 4.....47**

THE CHOICE OF OPTIMAL TECHNOLOGY FOR EXTRACTING WOOD GREENERY FROM FOREST DENDROMASS

Srđan Ljubojević

Ladislav Vasilišín

Goran Vučić

 [https://doi.org/10.37572/EdArt\\_3010242774](https://doi.org/10.37572/EdArt_3010242774)

**CAPÍTULO 5..... 61**

PLAN DE ACCIÓN PARA LA PROMOCIÓN DEL BAGRE ARMADO (HYPOSTOMUS PLECOSTOMUS) EN VILLAHERMOSA TABASCO

María Patricia Torres Magaña

María Rivera Rodríguez

Ana Laura Fernández Mena

Araceli Pérez Reyes

María del Carmen Hernández Martínez

 [https://doi.org/10.37572/EdArt\\_3010242775](https://doi.org/10.37572/EdArt_3010242775)

**PRODUÇÃO VEGETAL E IMPACTOS AMBIENTAIS**

**CAPÍTULO 6.....70**

FITOHORMONAS Y FERTILIZACIÓN QUIMICA EN LA RENTABILIDAD DE LA PRODUCCIÓN DE SEMILLA DE PASTO BUFFEL ZARAGOZA 115 EN DOS ESTACIONES DEL AÑO BAJO RIEGO EN EL NORTE DE COAHUILA, MEXICO

Pedro Hernández Rojas

Mauricio Velázquez Martínez

Carlos Ríos Quiroz

Víctor Hugo González Torres

Dagoberto Flores Marín

Macotulio Soto Hernández

 [https://doi.org/10.37572/EdArt\\_3010242776](https://doi.org/10.37572/EdArt_3010242776)

**CAPÍTULO 7 ..... 86**

A IMPORTÂNCIA CRESCENTE DOS PRODUTOS AGRÍCOLAS E AGROALIMENTARES LOCAIS: OS EFEITOS DA PANDEMIA COVID-19 NOS CIRCUITOS CURTOS DE PROXIMIDADE

Maria Lúcia Pato

 [https://doi.org/10.37572/EdArt\\_3010242777](https://doi.org/10.37572/EdArt_3010242777)

**CAPÍTULO 8..... 96**

PARÂMETROS FITOTÉCNICOS DE CANA-PLANTA E DE PRIMEIRA SOCA EM SOLO ARGILOSO

Lia Mara Moterle

Renato Frederico dos Santos

Hugo Zeni Neto



Luiz Gustavo da Mata Borsuk

Bruna Sisti Michelin de Polli

 [https://doi.org/10.37572/EdArt\\_3010242778](https://doi.org/10.37572/EdArt_3010242778)

**CAPÍTULO 9..... 100**

SEVERITY OF 'WOOD POCKET' PHYSIOPATHY IN SELECTED PERSIAN LIME PLANTS OF DIFFERENT GENERATIONS

Juan Carlos Álvarez Hernández

José Concepción García Preciado

José Joaquín Velázquez Monreal

 [https://doi.org/10.37572/EdArt\\_3010242779](https://doi.org/10.37572/EdArt_3010242779)

**CAPÍTULO 10..... 108**

THE DILEMMA OF THE DEVELOPMENT OF OIL PALM PLANTATIONS AGAINST FOREST CONSERVATION IN CAMEROON

Mesmin Tchindjang

Guy Donald Abasombe

Rose Ngo Makak

Philippe Mbevo Fendoung

 [https://doi.org/10.37572/EdArt\\_30102427710](https://doi.org/10.37572/EdArt_30102427710)

**SAÚDE ANIMAL E MEIO-AMBIENTE**

**CAPÍTULO 11..... 146**

COMPARACIÓN DE PRUEBAS DIAGNÓSTICAS DIRECTAS PARA LA DETECCIÓN DE *Babesia bigemina* EN BOVINOS

Azul Gisela Comas González

Julio Vicente Figueroa Millán

José Juan Lira Amaya

Rebeca Montserrat Santamaría Espinosa

Grecia Martínez García

Carmen Rojas Martínez

Jesús Antonio Álvarez Martínez

 [https://doi.org/10.37572/EdArt\\_30102427711](https://doi.org/10.37572/EdArt_30102427711)

**CAPÍTULO 12 ..... 168**

OZONOTHERAPY AS AN ASSISTANT IN THE TREATMENT OF MASTITIS, IN LACTATING COWS

Gabriel Gerardo Aguirre Espíndola

Mari Carmen Larios Garcia

José Alfredo Galicia Domínguez

Sandra Ortiz González

 [https://doi.org/10.37572/EdArt\\_30102427712](https://doi.org/10.37572/EdArt_30102427712)

**CAPÍTULO 13 ..... 178**

DIAGNOSTICS IN A PUG DOG WITH ALLERGY REACTION ON RABIES VACCINE, CLINICAL PICTURE AND ATOPIC DERMATITIS- CASE REPORT

Danijela Videnovic

 [https://doi.org/10.37572/EdArt\\_30102427713](https://doi.org/10.37572/EdArt_30102427713)

**CAPÍTULO 14 ..... 187**

PARASITIC CONTAMINATION OF PUBLIC PLACES IN BELGRADE AND ITS CONTROL RESULTS OF A THIRTY-YEAR STUDY (1993-2023)

Ivan Pavlovic

Aleksandra Tasic

Vesna Kovačević Jovanović

Dara Jovanovic

Zoran Tambur

 [https://doi.org/10.37572/EdArt\\_30102427714](https://doi.org/10.37572/EdArt_30102427714)

**CAPÍTULO 15 ..... 216**

DISEÑO CONCEPTUAL DE UN SISTEMA DE PRODUCCIÓN DE ALIMENTOS BALANCEADOS PARA GANADO PORCINO

Oralio Hernández Alvarado

Adolfo López Zavala

César Chávez Olivares

Efraín Zúñiga Morales

 [https://doi.org/10.37572/EdArt\\_30102427715](https://doi.org/10.37572/EdArt_30102427715)

**SOBRE O ORGANIZADOR ..... 228**

**ÍNDICE REMISSIVO ..... 229**

# CAPÍTULO 12

## OZONOTHERAPY AS AN ASSISTANT IN THE TREATMENT OF MASTITIS, IN LACTATING COWS<sup>1</sup>

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**ABSTRACT:** The action of Ozone (O<sub>3</sub>) as a germicide, against different pathogens has been studied; and has revealed the biological effects with therapeutic benefits [1-5]. Ozone inhibits growth and causes the death of gram negative and gram-positive bacteria, exposed to the ozone concentration of 0.167/ µg /min/L, at different times, produced ultra-structural changes in the bacteria, showing deformation and sudden damage with surface destruction, collapse and cell lysis [6]. The present work was developed in the “El Salado” zootechnical

<sup>1</sup> This article was published in 2019 by Juniper Publishers. (<https://juniperpublishers.com/jdvs/pdf/JDVS.MS.ID.555876.pdf>). Some authors have been eliminated from the original paper due to political issues.

post, belonging to the Faculty of Veterinary Medicine and Zootecnics of the “Benemérita Universidad Autónoma de Puebla”, in the production module of Milk Bovines, which has cows of the Holstein- Frisean and Jersey breeds, under a system of intensive handling, being milked twice a day (every 12 hours), in a 4-box fishbone milking parlor and with a Westfalia brand milking equipment; where, after identification of subclinical mastitis, by performing the California Mastitis Test on each cow, the readings were made and three experimental series were applied, using different concentrations of the ozone-oxygen mixture, in the affected mammary quarters of nine cows in production. After 4 days of treatment with the ozone / oxygen mixture, a 50% improvement was observed in the affected rooms, reflected in a reduction of somatic cells and leukocytes per milliliter of milk; being the dose that obtained better results, the one of 11µg of ozone in a mixture of ozone- oxygen in 300ml of volume, that were instilled in the affected breast rooms. It is concluded that the effect of intramammary administration of the ozone / oxygen mixture (MOO) is presented as an adjuvant in the innovative treatment of subclinical and clinical bovine mastitis, representing a real alternative, being necessary to establish effective doses and volumes for said end; Therefore, the indiscriminate use of antimicrobials would be avoided, establishing this ozone therapy as an effective, safe, cost-effective method, with zero days of withdrawal and without the risk of having drug residues in milk.

**KEYWORDS:** Ozone therapy. Subclinical mastitis. Milk quality.

## 1 INTRODUCTION

Mastitis is a serious disease suffered by milk-producing animals, causing large economic losses, due to the reduction in milk production, as well as its low nutritional value. Mastitis is characterized by the occurrence of physical, chemical and bacteriological changes in milk including pathological abnormalities of the mammary gland tissue. Mastitis affects the quality of milk in terms of its composition and production. The extent of the various changes in milk composition depends on the inflammatory response [7]. Somatic cells are the main epithelial cells that have detached from the glandular lining and white cells (leukocytes) that penetrate the mammary gland in response to a specific damage or infection. Somatic milk cells include 75% of leukocytes, such as neutrophils, macrophages, lymphocytes and 25% of epithelial cells. During inflammation (mastitis) the greatest increase in SC is due to the influx of neutrophils into the milk to fight the infection and has an estimated 90% [8,9]. The most precise relationship between intramammary infection and somatic cell count (SCC) can be assessed at the quarter level when the SCC exceeds 200,000 cells / ml [10]. In addition, the CCS in healthy rooms is consistently low and usually below 200,000 cells / ml [11]. In subclinical mastitis, the pathogens do not cause sufficient destruction of the alveolar tissue, so that it is reflected in the characteristics of the milk, but the SCC is increased indicating the infection of the milk [12].

In clinical mastitis, clinical signs can be recorded as weak, moderate and severe [13]. *Staphylococcus aureus* is considered one of the most virulent bacterial species that cause bovine mastitis (MSC) and clinical mastitis (MC) [14]. The administration of intramammary antibiotics is the most common method used for the treatment of bovine mastitis. However, at present this treatment is having few results regarding the recovery of the infection, mainly due to the bad management and the development of resistance by pathogens. Such is the case of the identification and characterization of methicillin-negative coagulase *Staphylococcus*-resistant in bovine mastitis [15,16]. The treatment of acute bovine mastitis during the period of breastfeeding represents one of the greatest economic losses of livestock production. This is related to the cost of treatments (veterinary service and drugs), the decrease in production and the sale price of milk, as well as the increase in somatic cell count in the milk sample volume, the increase in the number of cows discarded and the increase in losses due to the presence of antibiotic residues in milk and meat [17-19].

A range of production systems coexist in Mexico, ranging from the most traditional, in the hands of farmers in isolated regions of the country, to large modern companies, vertically and horizontally integrated. The technology required by the various systems is very different and MVZs must be very aware of the scope and limitations of each method or input they apply or recommend. The monitoring of the quality of products has a wide social demand and also plays a strategic role in the protection of our internal market, access to markets abroad and in making our country more attractive for these important economic activities. Therefore, this problem must be addressed by trained professionals, who can provide effective alternatives, easy to apply and with affordable costs, which guarantee the quality of food of animal origin. The presence of mastitis is a very serious problem for dairy farming and is one of the main causes of economic losses for the farmer and / or producer. In the United States, annual losses are estimated at around \$ 2,000,000. ([20], University of Oviedo, Gijón, Asturias). World losses, annual due to mastitis, have been estimated at US \$ 35 billion [21,22]. The greatest losses result from the reduction in milk production due to subclinical mastitis. In other studies, conducted in herds located in the highlands of Mexico, the prevalence of subclinical mastitis has been estimated at 20.80% in Tizayuca, Hidalgo, and 81.10% in stables around the Federal District [23-31].

Hygiene in cowsheds and milking place are very important and everyone knows that the lower the level of hygiene, the greater the risk of infection. Dr. Carlos Concha Bascuñan, M.V.Z. specialist of the University of Chile, recommends the application of drying knobs or treatments only in the case of cows with positive results to bacteriological

cultures carried out with the secretion existing during the period, has found that more than half of the cows, in The dry period is bacteriologically negative and all they need is a good external nipple sealer. By routinely applying antibiotics to all cows when they are dried, even in completely healthy udders, we soon create resistance, which will make it difficult to treat clinical cases. The Nordic countries, with a very careful policy “prohibition of the prophylactic use of antibiotics” as a treatment for cows on drying without having carried out bacteriological tests, has allowed them to present only 10 to 20% resistance to penicillins, versus 40 60% of the rest of Europe [32-39]. Due to the above, the use of ozone as a treatment in subclinical mastitis, will allow us to have zero days of withdrawal in milk, perfectly sterilize the room of bacteria, viruses and fungi, due to its potent oxidizing action, it will not allow the resistance effect of microorganisms and We will have the possibility to enjoy a milk free of antibiotics, anti-inflammatory, with moderate somatic cells and with a higher protein quality, both milk and derivatives.

## 2 MATERIAL AND METHODS

The realization of this work was carried out in the “El Salado” zootechnical post, belonging to the Faculty of Veterinary Medicine and Zootechnics of the Benemérita Universidad Autónoma de Puebla, which has cows of the Holstein-Frisean and Jersey breeds, under an intensive management system, being milked twice a day (every 12 hours), in a 4-box fishbone milking place.

### 2.1 INCLUSION CRITERIA:

- Cows with clinical and bacteriological diagnosis of clinical mastitis.
- Cows diagnosed with subclinical mastitis.

### 2.2 EXCLUSION CRITERIA:

- Cows that are under antibiotic or other therapeutic treatment.
- Cows that show signs of worsening of the clinical picture during treatment with the MOO.

### 2.3 EXPERIMENTAL SERIES GROUPS:

The animals were divided into experimental series of 3 cows in each group.

Experimental series 1: Dose of 11mg of ozone in 300ml of volume.

Experimental series 2: Dose of 35mg of ozone in 300ml of volume.

Experimental series 3: Dose of 50mg of ozone in 300ml of volume.

## 2.4 JUSTIFICATION FOR THE SELECTION OF MOO CONCENTRATIONS FOR EACH EXPERIMENTAL SERIES:

The treatments of the MOO by intramammary route will be carried out every 24 hour after discharging the milk from the room and after disinfecting the room with an antiseptic solution.

## 2.5 STUDY SUBSTANCES AND / OR REFERENCE:

The MOO, obtained from an Ozone Generator for Medical Use, Model MEDIC-O3 PORTATIL, Manufactured by OZONO CARBAR ´S. Serial No. BRJ17-960. The measurement was carried out by means of an ozone analyzer model MINI-HICON BENCH with serial number 170107 with a capacity of up to 400 g / Nm<sup>3</sup> with temperature and pressure compensator, of American origin.

Equipment Accuracy + - 2 µg / ml.

Maximum Standard Deviation: 0.11%

## 2.6 METHOD OF ADMINISTRATION AND / OR EXPOSURE AND REASON FOR BEING EMPLOYED:

Intra-mammary route: After disinfection of the nipple area of the room, with an iodine-based disinfectant solution, an intra- mammary cannula is placed, which is connected with the MOO- loaded syringe, it is slowly discharged until the volume of 300 ml is completed determined for the application. It will be applied every 24 hours for 4 consecutive days.

## 2.7 MEASUREMENTS TO BE PERFORMED:

- a. Review of the medical history of each cow registered in the medical control cards of the production module.
- b. Identification of race, age, days of milk production.
- c. Registration of physiological constants.
- d. Carrying out the mastitis California test.
- e. Performing the somatic cell count test.
- f. Identification of the rooms affected by clinical mastitis and their antimicrobial treatment.
- g. Observation of the response to ozone treatment or recurrence.

## 2.8 SOMATIC CELL COUNT (SCC)

Milk samples were taken from the affected rooms and SCC counting was performed using the Porta Check SCC method; taking as a reference, the count greater than 200,000 cells / ml and apparently without morphological changes in milk, will be classified as subclinical mastitis (SCM). While a SCC from 5,000,000 cells/ml, accompanied by morphological changes of milk such as lumps and color changes, it will be classified as clinical mastitis (CM). This diagnostic method will be performed before and after the end of the treatment cycle with the substance under test (Table 1).

Table 1: This diagnostic method will be performed before and after the end of the treatment cycle.

		07-11-18								10-11-18					
		California Mastitis Test				Ozone Dose		Type of Mastitis		Type of Mastitis California Mastitis Test				Ozone Dose	
No. Cow	c) Clínica	AI	AD	PI	PD		No. Cow		AI	AD	PI	PD			
	s) ubclínical														
4	s)	-	2	1	-	11µg/300ml	4	s)	-	-	T	-	11µg/300ml		
69	s)	-	T	2	2	11µg/300ml	69	s)	-	-	1	1	11µg/300ml		
49	s)	2	1	2	2	11µg/300ml	49	s)	2	1	T	1	11µg/300ml		
28	s)	-	3	2	-	35µg/300ml	28	s)	2	2	2	T	35µg/300ml		
160	s)	-	3	3	2	35µg/300ml	160	s)	-	-	-	-	35µg/300ml		
38	c)	C	-	-	-	35µg/300ml	38	c)	2	-	-	-	35µg/300ml		
19	s)	-	-	3	-	50µg/300ml	19	s)	-	-	2	3	50µg/300ml		
77	s)	2	-	2	-	50µg/300ml	77	s)	1	-	1	-	50µg/300ml		
99	s)	2	-	-	-	50µg/300ml	99	s)	2	-	-	-	50µg/300ml		

## 3 RESULTS

After 4 days of treatment with the ozone / oxygen mixture, a 50% improvement is shown in the affected fourth.

The cow No. 38 that presented clinical mastitis in the fourth AI, after treatment improved, showing in the California mastitis test a clear positive reading 2, with reduction in the number of leukocytes and somatic cells in said room.

It can be seen that the most effective dose of the ozone / oxygen mixture is 11 to 35µg / ml, in a volume of 300ml (Table 2).

Table 2: Milk sample from thermo tank.

No. of Cow	The Affected Fourth	SCC at the Beginning	SCC the End
4	AD	1,720,000/ml	630,000/ml
38	AI	>3,000,000/ml	950,000/ml



24	PD	873,000/ml	230,000/ml
Milk sample from thermo tank			
SCC at he beginning		SCC the end	
281,000/ml		253,000/ml	

## 4 DISCUSSION

Taking into account the high incidence of mastitis in the dairy farming industry, as well as the economic losses due to said disease, it is appropriate to carry out its diagnosis in the field and treat in the best way and in each case in particular, being a good option, the administration of correct doses of the ozone-oxygen mixture. Several works show that the Ozone / Oxygen Blend has a high germicidal power and in the present work, an effectiveness of the MOO was obtained as a treatment for bovine mastitis. Antibiotic treatments are not entirely effective because of the high incidence of resistance developed by the microorganisms that cause bovine mastitis. The use of ozone in gas has no withdrawal period or leaves metabolites in milk. Therefore, it is rational to study and determine the optimal MOO concentrations for the treatment of clinical and subclinical mastitis in cattle, according to the causative germs.

## 5 CONCLUSION

The effect of intramammary administration of the ozone / oxygen mixture (MOO) as an innovative treatment of subclinical and clinical bovine mastitis is investigated, establishing effective doses for this purpose. Avoid the indiscriminate use of antibiotics, establish an effective, safe, profitable method, with zero days of withdrawal and without risk of having residues of medicines in the milk. The sterilization of treated breast rooms is checked by physical and biological methods. A practical, viable and low-cost application protocol is established to control the presence of clinical mastitis in the dairy herd.

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## SOBRE O ORGANIZADOR

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## ÍNDICE REMISSIVO

### A

Agricultural systems 17  
Animal welfare 187, 189, 190, 200, 203, 205, 208  
Atopic Dermatitis 178, 179, 185, 186  
Atributos 216, 217, 220, 221, 224, 225, 226

### B

Babesia bigemina 146, 147, 148, 149, 150, 152, 153, 165, 166, 167  
Bagre armado 61, 62, 63, 64, 65, 66, 67, 68, 69  
Belgrade 45, 178, 180, 187, 189, 194, 195, 197, 198, 199, 200, 201, 203, 204, 206, 207, 210, 211, 212, 213, 214  
Biodiversidade 1, 2, 4, 6, 109, 110  
Buffel Z115 71, 75, 77, 78, 81, 83

### C

Cana-de-açúcar 96, 97, 99  
Carbon farming 17, 28, 30  
Chemical composition 32, 33, 35, 37, 40, 45  
Circuitos curtos de proximidade 86, 88, 93, 94  
Citrus latifolia 101, 102, 107  
Conifers 32, 33, 34, 37, 40, 41, 43, 44, 46, 50  
Conservation 108, 109, 112, 113, 120, 122, 123, 133, 134, 135, 138, 141, 142, 144  
Contamination control 187  
COVID-19 4, 86, 87, 88, 90, 91, 92, 93, 94, 95

### D

Deciduous trees 32, 33, 37, 40, 41, 43  
Deforestation 108, 109, 111, 112, 119, 120, 121, 122, 123, 126, 127, 131, 132, 133, 138, 139, 142, 143, 145  
Desempenho 96, 97  
Diagnóstico 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 158, 159, 162, 164, 165, 166  
Dilemma 108, 109, 113, 134, 141  
Diseño 71, 72, 74, 216, 217, 218, 219, 221, 223, 224, 225, 226, 227  
Dog 178, 179, 180, 181, 182, 183, 184, 185, 187, 188, 189, 192, 195, 196, 197, 198, 199, 200, 201, 202, 206, 207, 208, 209, 210, 212, 213, 215

## E

Elaeisfarming 109, 110, 114, 115, 123, 125, 127, 129, 130, 131, 133

Epidemiology 187, 209

## F

Fertilización 70, 71, 72, 73, 75, 76, 79,

Fitohormonas 4, 70, 71, 72, 74, 75, 77, 78, 80, 81, 82, 83, 84, 85

Food allergens 178, 179, 180, 181, 184

Forest dendromass 47, 48

Forestry offsets 17

Frotis 146, 147, 148, 150, 151, 152, 154, 155, 158, 159, 160, 161, 162, 163, 164

## G

Genótipos 76, 96, 97, 98, 99

Greenhouse gas mitigation 17

## I

Impacts 29, 52, 109, 111, 116, 117, 118, 121, 122, 125, 126, 127, 128, 130, 131, 132, 144, 145, 207

Inhalant allergens 178, 179, 180, 183

## L

Legislative enforcement 187

## M

Manejo 1, 2, 4, 6, 7, 8, 14, 15, 16, 69, 74, 81, 82, 83, 84, 218

Milk quality 169, 175

Modelo de studio 62

## O

Ozone therapy 169, 174, 175, 176

## P

PCR 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 158, 159, 160, 161, 162, 163, 164, 165, 166

Peletizado 217

Persian lime 100, 101, 102, 103, 104, 105, 106

Plan de acción 61, 62, 68  
Plantas de cobertura 1, 9, 10, 11, 12, 15, 16  
Porcino 216, 217, 219, 221, 226, 227  
Práticas sostenibles de pesca 61, 62  
Preservação 1, 2, 3, 4, 6, 7, 8, 10, 14, 90  
Processing technology 48, 50, 56  
Produção local 86, 90  
Produtividade 1, 3, 4, 6, 9, 10, 13, 14, 16, 96, 97, 98, 99  
Promoción 61, 62, 67, 68, 69  
Public education 187  
Public hygiene 187

## R

Rentabilidad 70, 71, 72, 83, 84, 218

## S

SAT 178, 184  
Sectorial spot 101, 102  
Segurança alimentar 86, 88, 110  
Semilla 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85  
Stray dogs 187, 188, 189, 197, 198, 199, 201, 203, 204, 205, 206, 207, 214  
Structural characteristics 33  
Subclinical mastitis 169, 170, 171, 173, 174  
Sustentabilidade 1, 2, 6, 7, 13, 89, 94, 110

## T

Tahiti lime 101, 107  
Triturado 216, 217, 223

## U

Urban health 187

## W

Wood greenery 32, 33, 35, 38, 39, 40, 41, 42, 43, 44, 46, 47, 48, 50, 53, 54, 55, 56, 60

## Z

Zoonotic parasites 187, 188, 189