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Alberto Baños PhD.
Microbiology

Arancha Aguinaga PhD.
Animal Nutrition

Our Approach

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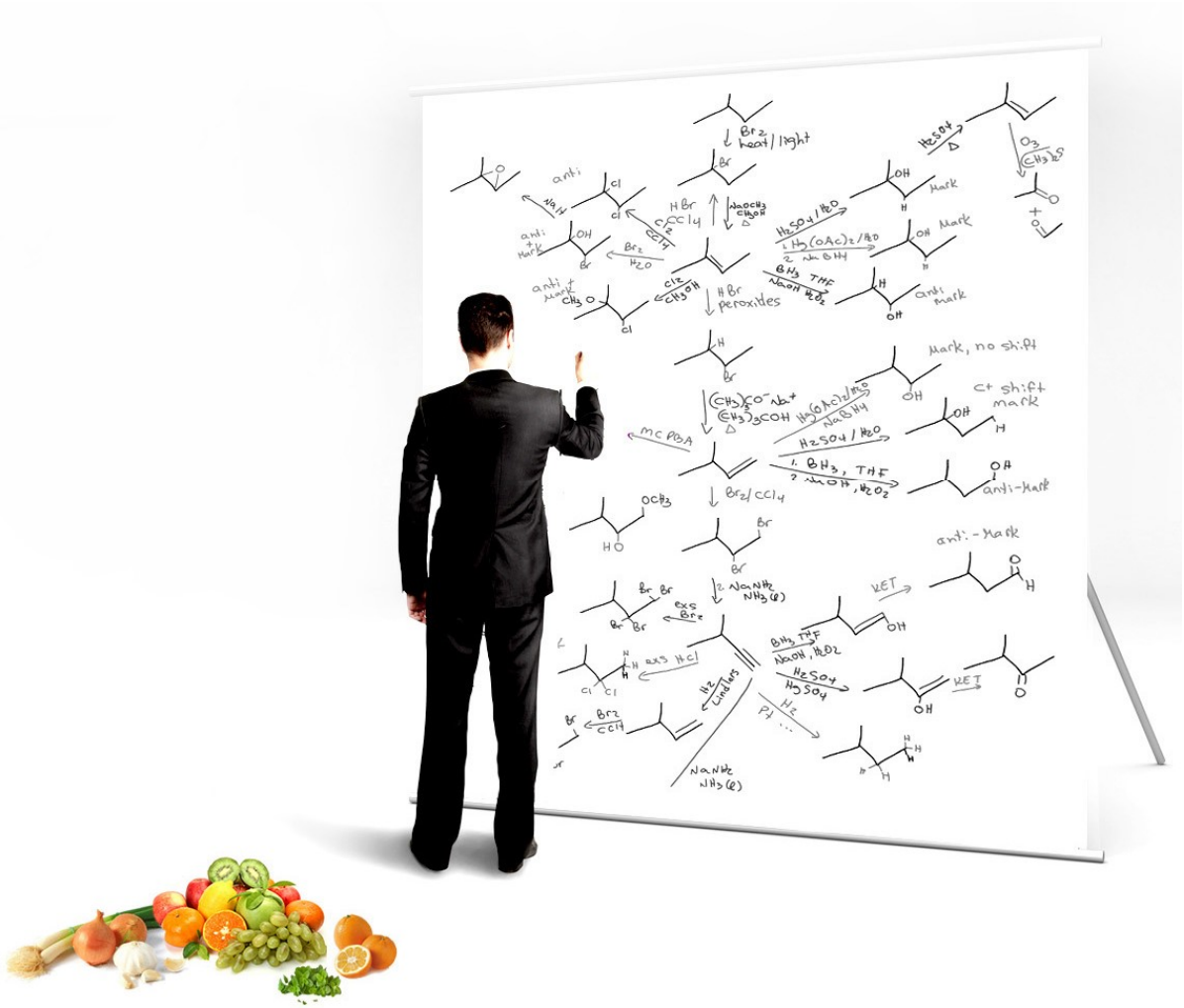

Our Approach

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"Nature as a model"

Alliaceous: the starting point

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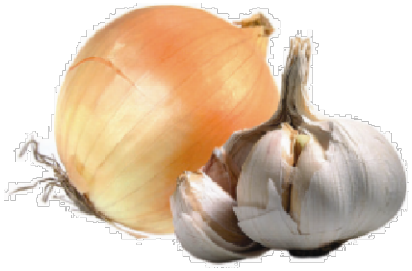
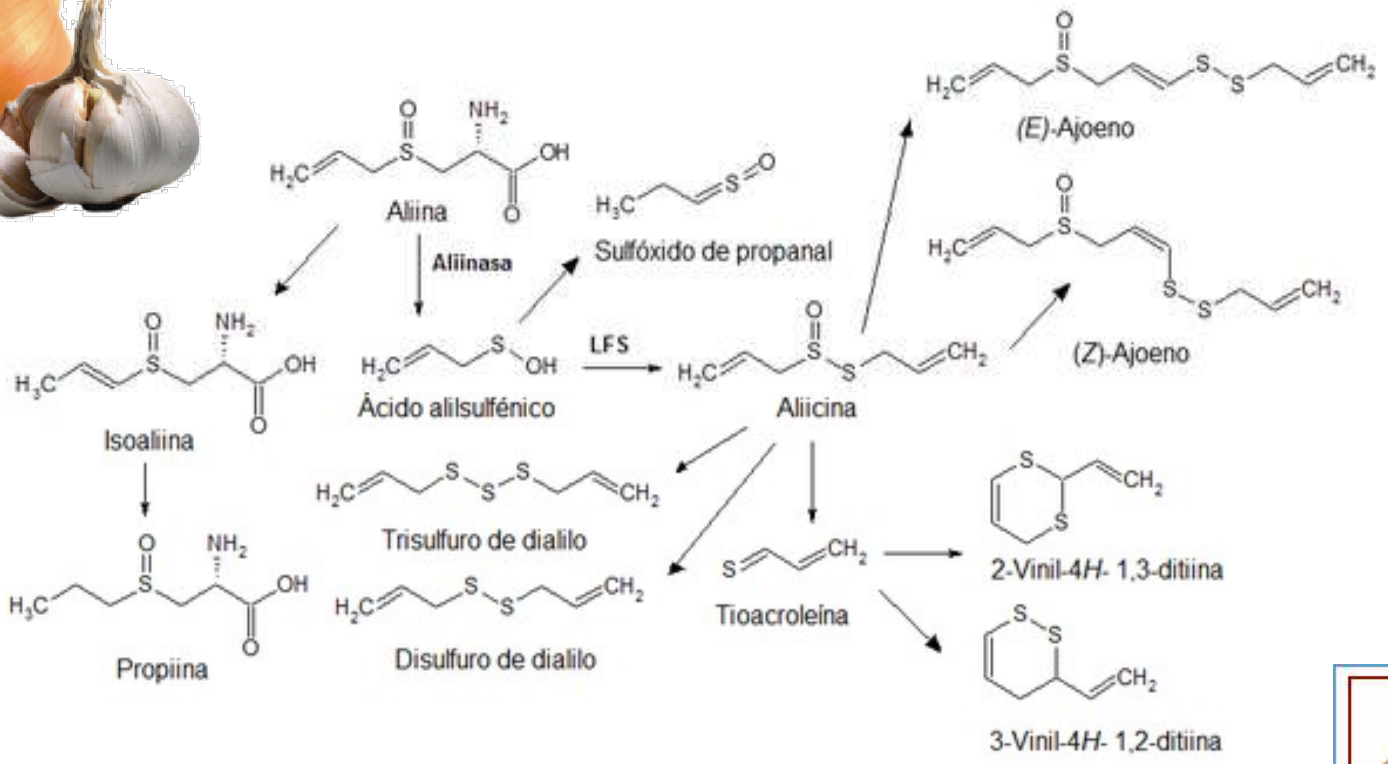
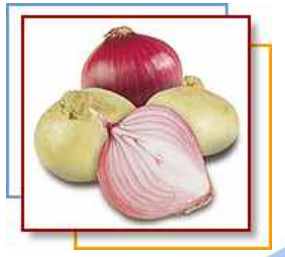
The complex chemistry of Alliaceae:







Instantaneous degradation to other secondary compounds

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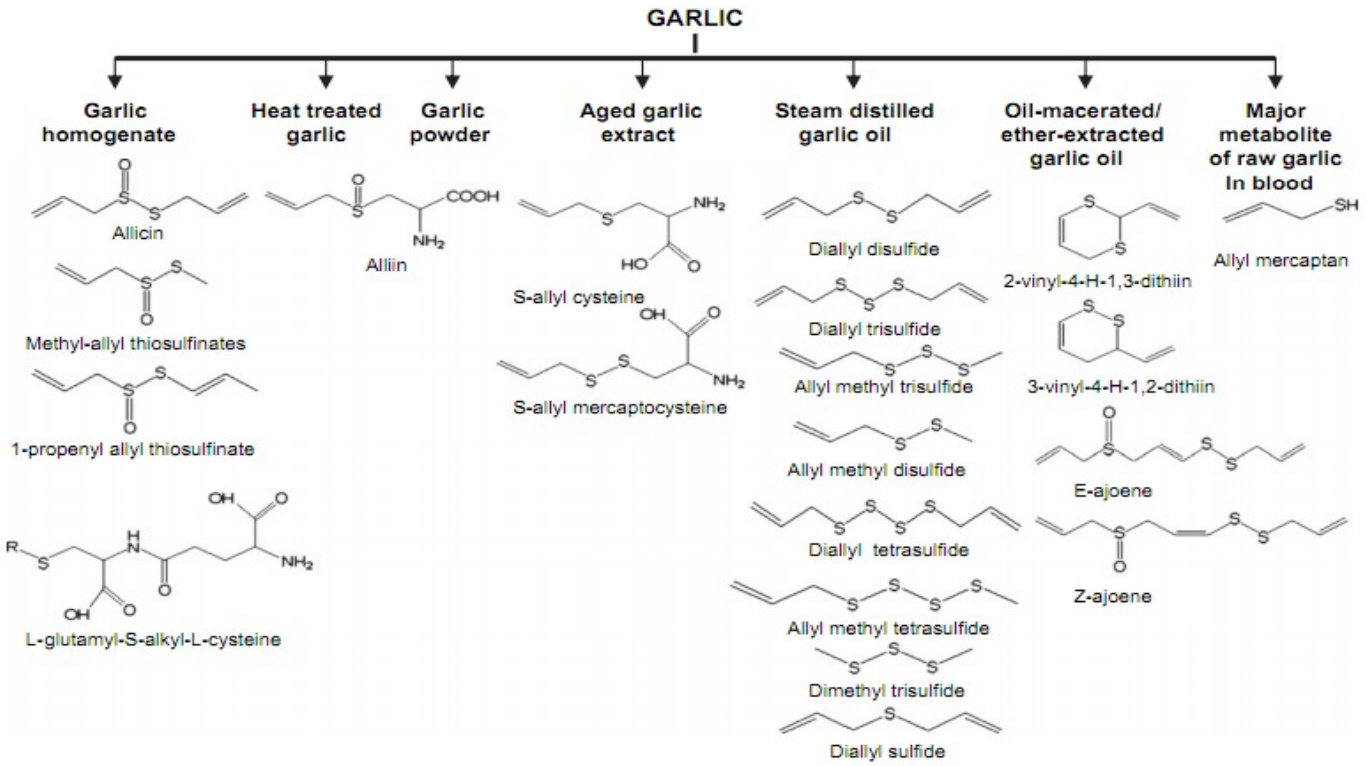
ALLIUM COMPOUNDS:

ALL OF THEM ARE SIMMILAR?

ALL OF THEM HAVE THE SAME PROPERTIES?



WHAT DID WE SEE?

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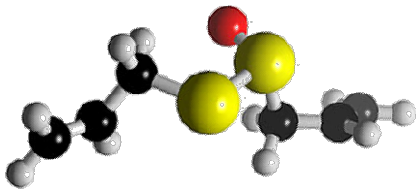
Different extracts having different properties, selecting the most interesting ones.

Our alliaceous extract, in comparison with other garlic extracts is:

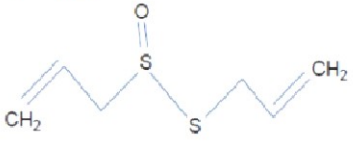
✓ Stable, standardized and traceable

✓Guaranteed quality control and shelf-life.

✓ High thermal resistance.

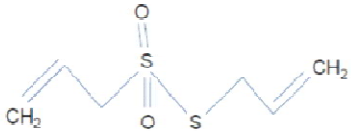


PTS



Propyl propane thiosulfonate

PTSO



Propyl propane thiosulfinate

What makes us different?

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Advantages of PTS / PTSO against other allium compounds:

- ✓ Naturally present in onion
- ✓ Patented
- ✓ Increased thermal stability
- ✓ Standardized
- ✓ Traceable
- ✓ Broad spectrum antimicrobials
- ✓ Anti-parasitic properties
- ✓ Immunomodulatory properties

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- 1. Natural, safe and healthy product.
- 2. Very effective to reduce the presence of parasites, pathogenic bacteria and moulds.
- 3. Increases the defensive capacity of animal against breathing and digestives pathological process.
- 4. Cost-effective solution to improve performance parameters.
- 5. Available in liquid and powder form with different concentrations.
- 6. Compatible with organic acids and most of common vegetable extracts, showing synergism in some cases.
- 7. Patented.
- 9. Scientifically tested in a lot of different trials worldwide.
- 10. QC control methods available for traceability and stability tests. Heat-resistant.

PTS/PTSO Apps

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US 20100035984A1

(19) **United States**

(12) **Patent Application Publication**

Garcia Pareja et al.

(10) **Pub. No.: US 2010/0035984 A1**

(43) **Pub. Date: Feb. 11, 2010**

(54) **USE OF AN ANTIBACTERIAL COMPOUND WHICH IS DERIVED FROM ALLIACEAE, AS A NATURAL ADDITIVE IN ANIMAL FEED**

(75) Inventors: **Pilar Garcia Pareja**, Alhendin (Granada) (ES); **Armando Lara Cambil**, Alhendin (Granada) (ES); **Luis A. Rubio Sanmillan**, Alhendin (ES); **Eduarda Molina` Alcaide**, Alhendin (ES)

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(73) Assignee: **DMC Research Center, S.L.**, Ahendin (Granada) (ES)

(21) Appl. No.: **12/310,987**

(22) PCT Filed: **Sep. 24, 2007**

(86) PCT No.: **PCT/ES2007/000541**

§ 371 (c)(1),
(2), (4) Date: **Mar. 13, 2009**

(30) **Foreign Application Priority Data**

Sep. 27, 2006 (ES) P200602446

Publication Classification

(51) **Int. Cl.**
A61K 31/255 (2006.01)
A61P 1/00 (2006.01)

(52) **U.S. Cl.** **514/517**

(57) **ABSTRACT**

The invention relates to the use of an antibacterial compound, which is derived from alliaceae, as a natural additive in animal feed, intended as an antimicrobial agent in animal feed, and as an alternative, due to its antibacterial nature, to the use of antibiotics as growth promoters, proposing the use of the compound separately (purity greater than 95%), and encapsulated or supported on different inert materials or food coatings, and in that said compound preferably consists of propyl propylthiosulfinate and, in an alternative variant, of propyl propylthiosulfonate.

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Quality control

- Unlike other products of alliaceous available in the market, GARLICON offers a strict quality control that guarantees the richness of its active principles.
- There are also standardized and published methods of analysis to guarantee traceability both in the product and in the feed.

Food Anal. Methods (2015) 8:916–921
DOI 10.1007/s12161-014-9952-1

High-Performance Liquid Chromatography Method for the Monitoring of the *Allium* Derivative Propyl Propane Thiosulfonate Used as Natural Additive in Animal Feed

Paloma Abad · Francisco J. Lara · Natalia Arroyo-Manzanares · Alberto Baños · Enrique Guillamón · Ana M. García-Campaña

Received: 21 May 2014 / Accepted: 29 July 2014 / Published online:

ugr Universidad de Granada

Analytical Methods

ROYAL SOCIETY OF CHEMISTRY

PAPER View Article Online View Journal

CrossMark

Cite this: DOI: 10.1039/c6ay00219f

A rapid and simple UHPLC-ESI-MS/MS method for the screening of propyl propane thiosulfonate, a new additive for animal feed

Paloma Abad,[a] Natalia Arroyo-Manzanares*[b] and Ana M. García-Campaña[b]

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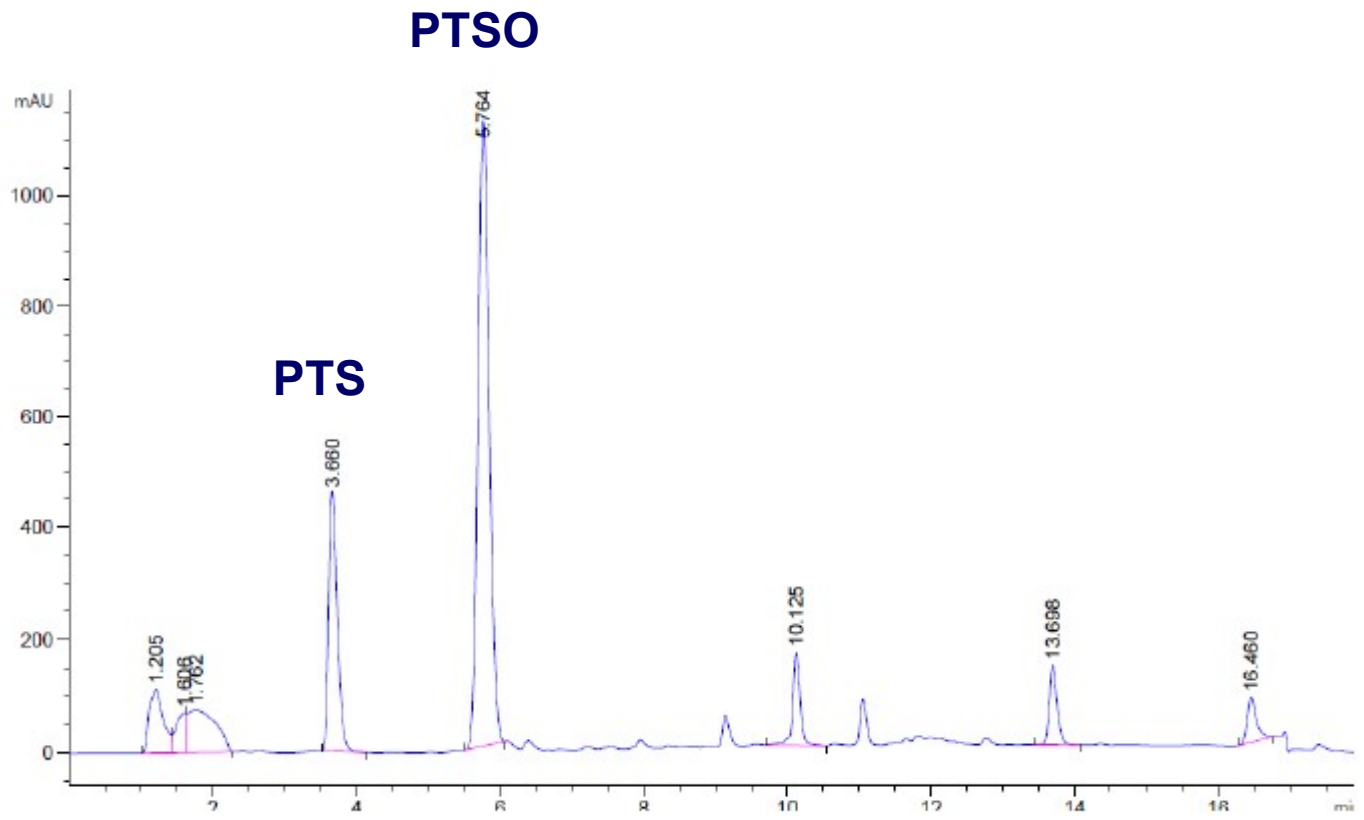

It is observed that 95% of the organosulphur compounds that constitute GARLICON are PTS and PTSO, maintainning this activity at different temperatures and throughout the storage

Comparative with other products

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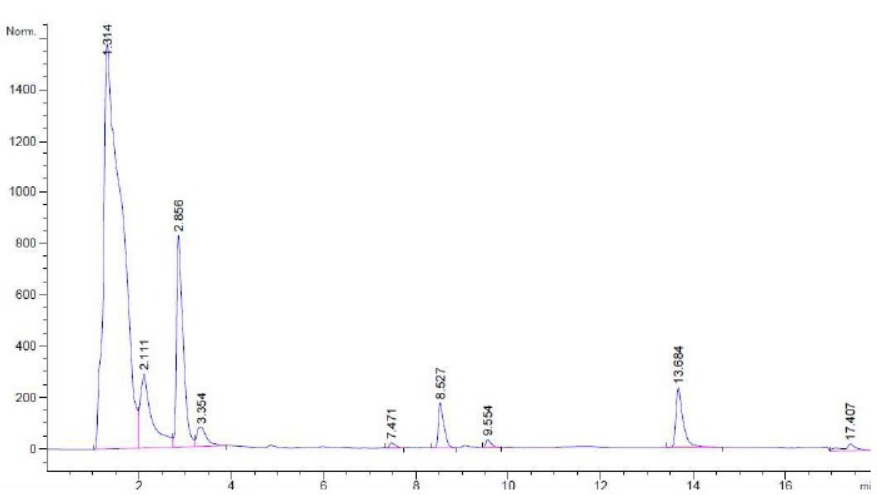
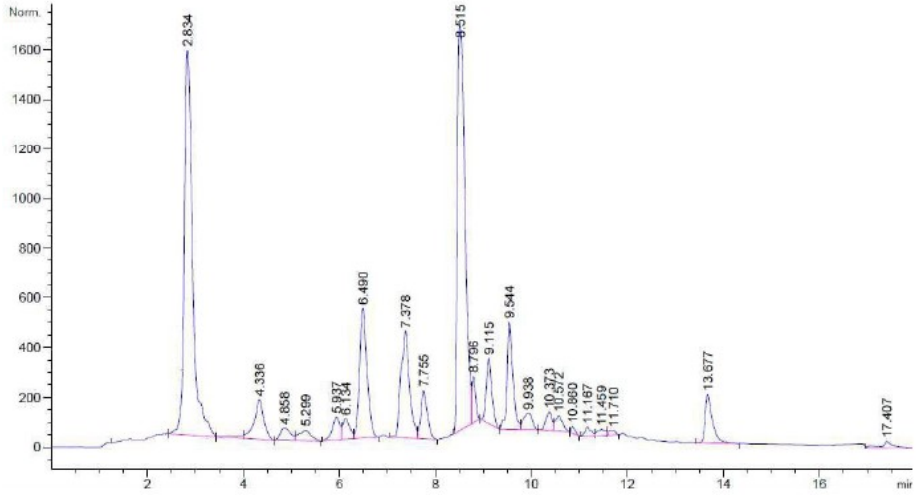
What differentiates us from other alliaceous compounds?

Garlic oil



There is hardly any content in PTS /PTSO

Allicin



It is observed how rapidly allicin is degraded to other compounds such as ajoene

Comparative with other products

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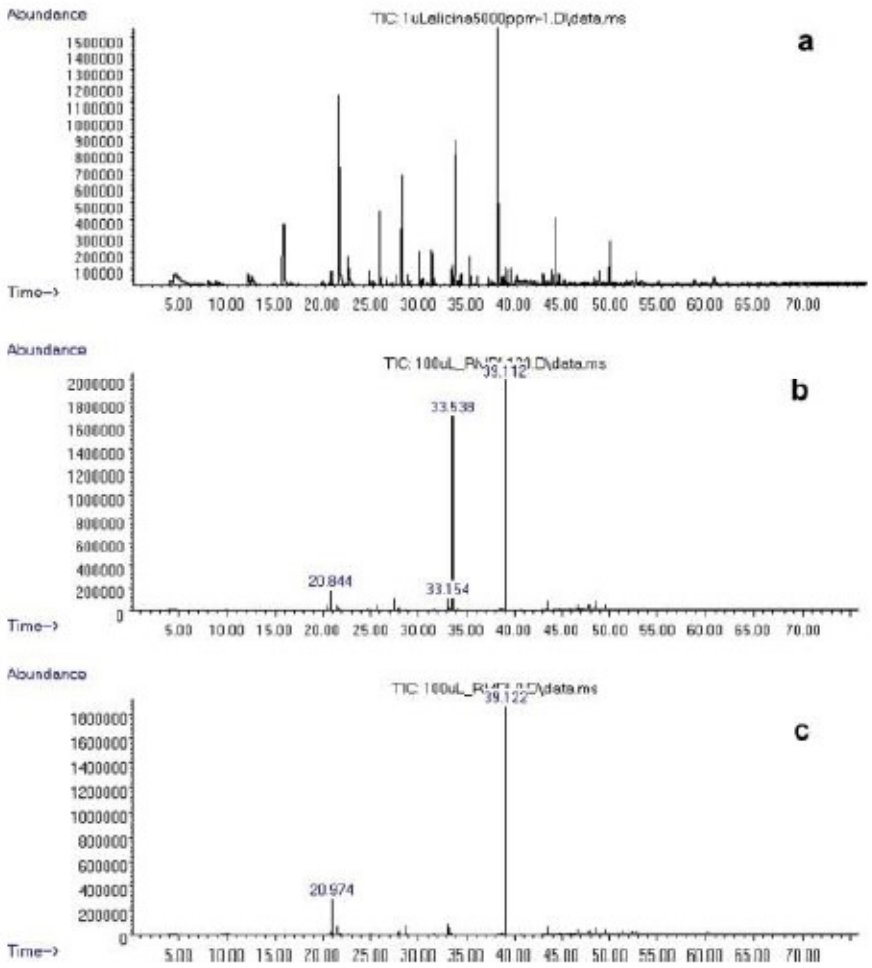
Chinese Extract (Allicin 1%)



Room Temperature (25°C). The product is not pure allicine. High number of peaks should be other thiosulphinates from Allicine degradation.

35°C, 1 min. A lot ot peaks dissapear. High unstability. Degradation compounds in small quantities.

25°C, 1 h. No allicine found. Only small peaks of some thiosulphinates isomers.

TESTED EFFICIENCY

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IMMUNOLOGY, HEALTH, AND DISEASE PS2280

Garlic derivative PTS-O is effective against broiler enteropathogens in vi

CSIC

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British Journal of Nutrition
http://journals.cambridge.org/BJN

Improved resistance to *Eimeria acervulina* infection in chickens due to dietary supplementation with garlic metabolites

Duk Kyung Kim, Hyun S. Lillehoj, Sung Hyen Lee, Erik P. Lillehoj and David Bravo

British Journal of Nutrition / *FirstView* Article / October 2012, pp 1 - 13
DOI: 10.1017/S0007114512000530, Published online: 13 April 2012

Link to this article: http://journals.cambridge.org/abstract_S0007114512000530

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Duk Kyung Kim, Hyun S. Lillehoj, Sung Hyen Lee, Erik P. Lillehoj and David Bravo Improved resistance to *Eimeria acervulina* infection in chickens due to dietary supplementation with garlic metabolites. British Journal of Nutrition, Available on CJO 2012 doi:10.1017/S0007114512000530

Animal (2013), **7:12**, pp 1925–1934 © The Animal Consortium 2013
doi:10.1017/S1751731113001699

animal

***In vitro–in vivo* study on the effects of plant compounds on rumen fermentation, microbial abundances and methane emissions in goats**

G. Martínez-Fernández[1], L. Abecia[1], A. I. Martín-García[1], E. Ramos-Morales[1], G. Hervás[2], E. Molina-Alcaide[1] and D. R. Yáñez-Ruiz[1†]

[1]*Instituto de Nutrición Animal, Estación Experimental del Zaidín (CSIC). C/Camino del Jueves s/n, 18100, Armilla, Granada, Spain;* [2]*Instituto de Ganadería de Montaña (CSIC-ULE), Finca Marzanas s/n, 24346 Grulleros, León, Spain*

Journal of Animal Physiology and Animal Nutrition
DOI: 10.1111/jpn.12256

ORIGINAL ARTICLE

Correlations between changes in intestinal microbiota composition and performance parameters in broiler chickens

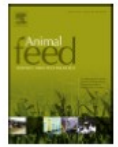
L. A. Rubio[1], M. J. Peinado[1], R. Ruiz[1], E. Suárez-Pereira[2], C. Ortiz Mellet[2] and J. M. García Fernández[3]

1 Depto. de Fisiología y Bioquímica de la Nutrición Animal (INAN, EEZ, CSIC), Granada, Spain
2 Depto. de Química Orgánica, Facultad de Química, Univ. de Sevilla, Sevilla, Spain, and
3 Instituto de Investigaciones Químicas (CSIC), Univ. de Sevilla, Sevilla, Spain

Contents lists available at SciVerse ScienceDirect

Animal Feed Science and Technology

journal homepage: www.elsevier.com/locate/anifeedsci



Garlic derivative PTS-O modulates intestinal microbiota composition and improves digestibility in growing broiler chickens

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M.J. Peinado, R. Ruiz, A. Echávarri, I. Aranda-Olmedo, L.A. Rubio*

Dpto. de Fisiología y Bioquímica de la Nutrición Animal (INAN, EEZ, CSIC), Profesor Albareda, 1, 18008 Granada, Spain

RESEARCH ARTICLE

Response of the rumen archaeal and bacterial populations to anti-methanogenic organosulphur compounds in continuous-culture fermenters

Gonzalo Martínez-Fernández[1,2], Leticia Abecia[1], A. Ignacio Martín-García[1], Eva Ramos-Morales[1], Stuart E. Denman[2], Charles J. Newbold[3], Eduarda Molina-Alcaide[1] and David R. Yáñez-Ruiz[1,*]

[1]Animal Nutrition, Estación Experimental del Zaidín (CSIC). C/Camino del Jueves s/n, Armilla, 18100, Granada, Spain, [2]CSIRO, Agriculture Flagship, Queensland Bioscience Precinct, 306 Carmody Road, St. Lucia, QLD, Australia and [3]IBERS, Aberystwyth University, Aberystwyth, UK

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- **Legal definition:** Seasoning and flavouring premix for organoleptic feed additives.
- Its **active ingredients** are standardized, homogenized: each batch has the same active content. This is only achieved with a high quality control and is the guarantee of the repeatability of the results.
- **Stable product** with high thermal resistance
- Long shelf life
- Manufactured under the **ISO 9001** and **FAMI QS** certification

ISO FAMIQS

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FUNCTIONS OF GARLICON:

1. MODULATION OF THE MICROBIOTA:

1. It is a product that modulates the intestinal / ruminal flora:
    - Reduces number of Enterobacteriaceae (E. coli, Salmonella) ->lower incidence of diseases
    - Respect the number of beneficial bacteria (lactobacilli, bifidobacteria)> better use of nutrient energy
2. The effect on the flora results in the improvement of the animal's immune system capacity to face challenges (infections, stress).
    - The intestinal mucosa is responsible for 70% of the immune response, any alteration of the mucosa has effects on health and productivity
3. As a consequence of the better mucosal state, there is a direct relationship between the use of the product and the improvement of animal growth. That is, animals improve their zootechnical performance.

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FUNCTIONS OF GARLICON:

2. NATURAL ANTIMICROBIAL

- Helps control and prevent intestinal infectious processes of different etiology:
- Gram-positive and Gram-negative bacteria
- Protozoa, coccidiosis and other intestinal infestations
- Mycosis

3. MODULATION OF THE IMMUNE SYSTEM

- Immunomodulatory effect
- Anti-inflammatory (Inhibits the genesis of pro-inflammatory cytokines)
- Immunopotentiator (greater efficiency in vaccine processes)
- Increased resistance to viral infections

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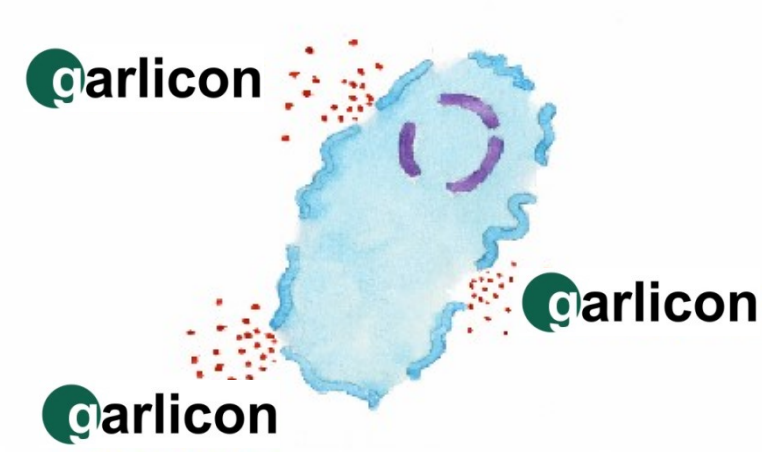
MECHANISMS OF ACTION

- **Mechanism of action: Bactericidal:**

  -Alteration membrane permeability (thiol groups) opening pores and cell lysis

  -Inhibition of metabolic pathways involving cysteine and disulfide groups.

- **Modulation of the intestinal microbiota:** The effect on flora results in improving the ability of the animal's immune system to face challenges (infections, stress).



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- Products Safety has been assessed and confirmed with in vitro and in vivo tests.
- No genotoxicity neither hepatic alterations.

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Toxicological evaluation of an *Allium*-based commercial product in a 90-day feeding study in Sprague–Dawley rats

P. Mellado-García [a], M. Puerto [a], S. Pichardo [a], M. Llana-Ruiz-Cabello [a], R. Moyano [b], A. Blanco [c], A. Jos [a], A.M. Cameán [a, *]

[a] Area of Toxicology, Faculty of Pharmacy, Universidad de Sevilla, Profesor García González nº 2, 41012, Seville, Spain
[b] Department of Pharmacology, Toxicology and Legal and Forensic Medicine, University of Córdoba, Campus de Rabanales Carretera Madrid-Cádiz s/n, Córdoba, 14071, Spain
[c] Department of Anatomy and Comparative Pathology and Anatomy, University of Córdoba, Campus de Rabanales Carretera Madrid-Cádiz s/n, Córdoba, 14071, Spain

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No hematological alterations during 90 days consumption

Haematology data summary

		Male				Female			
		Group 1 (0 mg/kg/day)	Group 2 (25 mg/kg/day)	Group 3 (100 mg/kg/day)	Group 4 (400 mg/kg/day)	Group 1 (0 mg/kg/day)	Group 2 (25 mg/kg/day)	Group 3 (100 mg/kg/day)	Group 4 (400 mg/kg/day)
		N = 10	N = 10	N = 10	N = 10	N = 10	N = 10	N = 10	N = 10
RBC 10^6/µl	Mean	8.93	9.01	9.06	8.88	7.84	8.03	7.79	7.82
	St. Dev.	0.47	0.27	0.24	0.51	0.38	0.53	0.26	0.75
		F(36.3) = 0.4320 p = 0.7313; N.S.				**F(36.3)=0.4390 p = 0.7266; N.S.**			
HGB g/dL	Mean	15.1	15.2	15.1	15.2	13.7	13.8	13.7	13.6
	St. Dev.	0.6	0.6	0.5	0.6	0.6	0.7	0.5	1.2
		KW = 0.2028 p = 0.9771; N.S.				**KW = 0.3069 p = 0.9587; N.S.**			
HCT %	Mean	64.6	71.6	70.8	71.2	65.7	66.4	65.3	65.5
	St. Dev.	20.3	2.8	2.6	2.8	3.0	3.9	2.4	5.5
		KW = 1.299 p = 0.7293; N.S.				**F(36.3) = 0.1553 p = 0.9255; N.S.**			
MCV fL	Mean	79.4	79.5	78.2	80.6	83.8	82.8	83.9	83.9
	St. Dev.	2.4	2.4	3.9	4.3	1.9	2.0	2.2	2.4
		F(36.3)=0.7989 p = 0.5026; N.S.				**F(36.3) = 0.5824 p = 0.6307; N.S.**			
MCH pg	Mean	16.9	16.8	16.7	17.1	17.5	17.2	17.5	17.5
	St. Dev.	0.4	0.6	0.8	0.8	0.5	0.4	0.5	0.4
		F(36.3) = 0.7272 p = 0.5424; N.S.				**F(36.3)=1.101 p = 0.3625; N.S.**			
MCHC g/dL	Mean	21.3	21.2	21.4	21.3	20.8	20.8	20.9	20.8
	St. Dev.	0.2	0.2	0.2	0.3	0.3	0.3	0.2	0.2
		F(36.3) = 1.1190 P = 0.3543; N.S.				**F(36.3)=0.4240 p = 0.7371; N.S.**			
PLT 10^3/µl	Mean	894	886	895	880	813	901	957	939
	St. Dev.	208	184	133	152	260	261	99	128

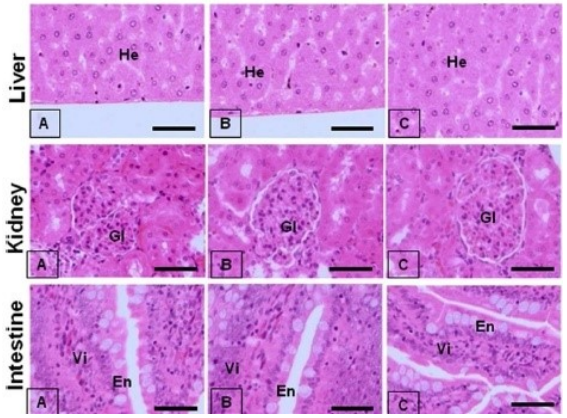
No alterations in blood biochemistry

Clinical biochemistry of Sprague—Dawley male and female rats fed with different doses of Proallium AF® in the diet for 90-day. Values are mean ± SD for 10 rats/sex/group. The differences between control and treated groups for male and female rats were evaluated by Kruskal—Wallis test (K.W.) or by ANOVA test (F values). N.S. (Not significant), and the significance levels observed are *p < 0.05 in comparison to control group values, and# p < 0.05 when 25 mg/kg/d and 400 mg/kg/d were compared.

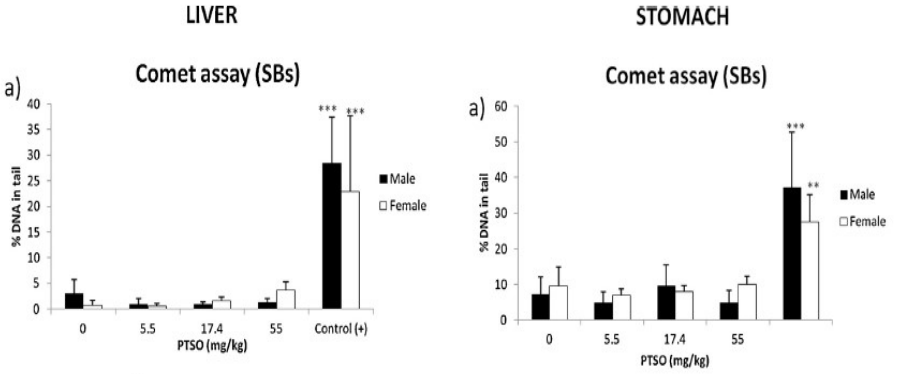
Clinical biochemistry data summary

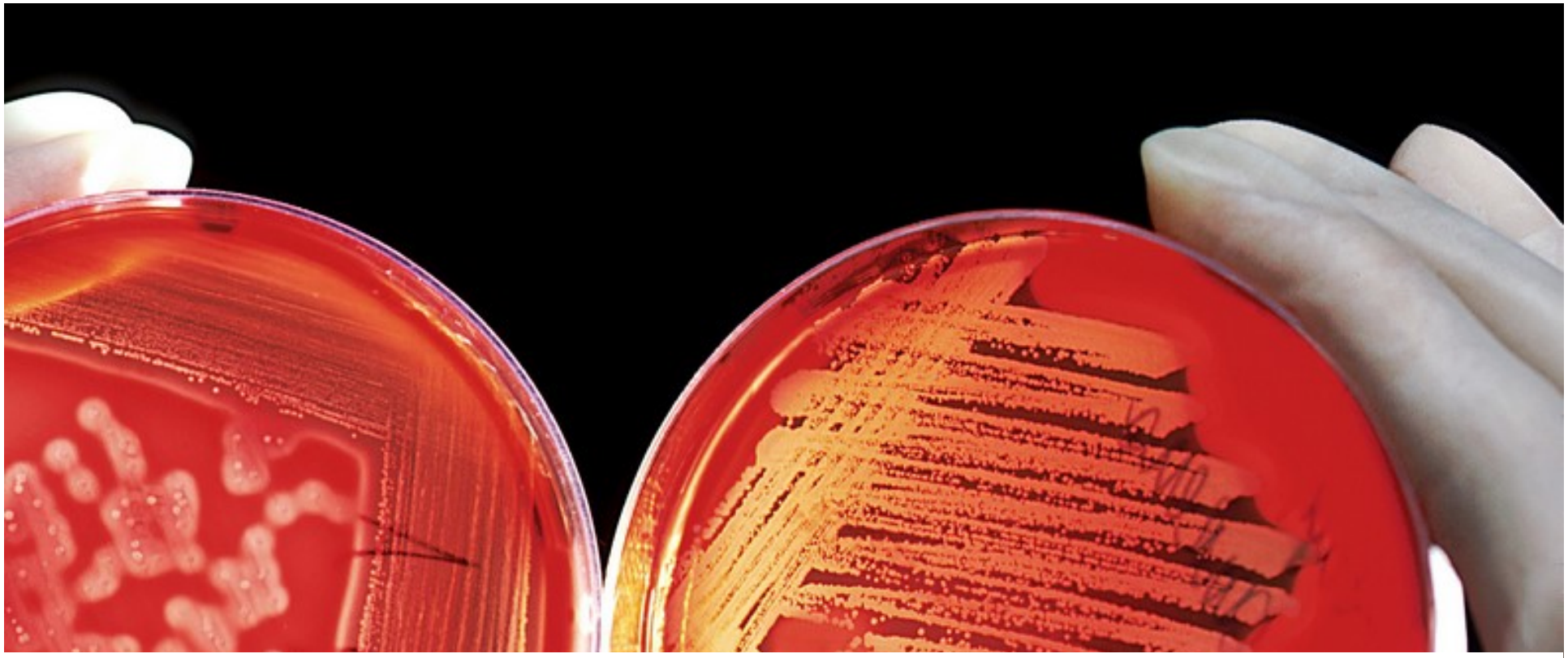
		Male				Female			
		Group 1 (0 mg/kg/day)	Group 2 (25 mg/kg/day)	Group 3 (100 mg/kg/day)	Group 4 (400 mg/kg/day)	Group 1 (0 mg/kg/day)	Group 2 (25 mg/kg/day)	Group 3 (100 mg/kg/day)	Group 4 (400 mg/kg/day)
		N = 10	N = 10	N = 10	N = 10	N = 10	N = 10	N = 10	N = 10
GLUC tmg/dL	Mean	134.2	121.3	110.3*	115.8	135.7	149.3	126.9	129.1
	St. Dev.	28.6	14.0	9.2	10.7	13.1	28.7	15.3	16.7
		F(36.3)=3.450 *p<0.05				**F(36.3)=2.695 p = 0.0604; N.S.**			
BUN mg/dl	Mean	13.95	13.79	15.62	16.01#	13.48	13.94	14.75	13.99
	St. Dev.	1.47	1.88	2.15	1.4	6.56	2.26	4.23	1.41
		F(36.3)=4.214 *p<0.05				**F(36.3)=0.3381 p = 0.7979; N.S.**			
CREAT mg/dL	Mean	0.26	0.25	0.27	0.26	0.30	0.32	0.29	0.32
	St. Dev.	0.07	0.03	0.06	0.04	0.03	0.04	0.03	0.04
		F(36.3)=0.1482 p = 0.9302; N.S.				**F(36.3)=1.946 p = 0.1396; N.S.**			
Bile acids µMol	Mean	31.0	28.9	32.2	29.9	39.0	36.3	29.6	27.8
	St. Dev.	13.1	6.5	12.5	12.1	14.0	25.3	18.5	11.2
		F(36.3)=0.1562 p = 0.9250; N.S.				**F(36.3)=0.8702 p = 0.4655; N.S.**			
CHOL mg/dL	Mean	77.3	78.3	75.3	74.3	91.0	94.3	96.0	85.4
	St. Dev.	13.7	16.2	8.3	11.1	12.8	12.8	17.7	8.8
		F(36.3)=0.2070 p = 0.8909; N.S.				**F(36.3)=1.213 p = 0.3190; N.S.**			
TRIGL mg/dL	Mean	98.0	119.2	109.4.4	109.3	97.8	85.0	78.5	70.3*
	St. Dev.	17.4	28.8	25.2	25.9	30.7	19.3	16.8	7.3
		KW = 4.251 p = 0.2356; N.S.				**F(36.3) = 3.269 *p<0.05**			
AST U/L	Mean	222.3	226.9	190.9	200.9	192.3	190.5	163.7	157.2
	St. Dev.	36.4	36.7	23.9	40.2	41.5	61.3	28.1	29.0

No alterations in the study of pathological anatomy



Absence of liver and stomach DNA damage in rats





ANTIMICROBIAL ACTIVITY

In vitro studies

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Bactericidal activity

Salmonella enterica subsp. *enterica* -	MIC_{90}/MBC_{90} (µl/ml)
Typhimurium (28)	2.5 / 5
Rissen (10)	1.25 / 2.75
Derby (7)	2.5 / 5
O:4[5],12:i:- (8)	1.67 / 2.5
Anatum (3)	2,5 / 5
Enteritidis (5)	2.25 / 4.1
Newport (1)	1.25 / 1.67
London (1)	1.25 / 1.67
Kapemba (1)	1.25 / 1.67
Cholerasuis (1)	1.25 / 1.67
Global results	**2.5 / 5**

* Study conducted by Universitity of León (Spain)

In vitro studies

DOMCA

Table 1. Minimum Bactericidal concentration (MBC)

	Strain	Inhibition halo (mm)	MBC (µg/mL)
1	*Salmonella enterica* subsp. *enterica* (grupo E1) ser. London (CECT 4376).	**38**	**1**
2	*Salmonella enterica* subsp. enterica ser. *Typhimurium* (CECT 4156).	**39**	**4**
3	Salmonella enterica subsp. enterica (9,12:g,m:-) serovar. enteritidis (CECT 7160).	**36**	**2**
4	*Salmonella enterica* subsp. *enterica* (9,12:g,m:-) serovar. enteritidis (CECT 7159)	**36**	**2**
5	*Salmonella enterica* subsp. Arizonae - *Salmonella choleraesuis*. Arizonae (CECT 4395)	**39**	**4**
6	*Salmonella enterica* subsp. *enterica* ser. Derby. (CTC1022)	**42**	**1**
7	*Salmonella enterica* subsp. *enterica Typhimurium*/ DT014 (DSM – 26529).	**39**	**1**

In vitro studies

DOMCA

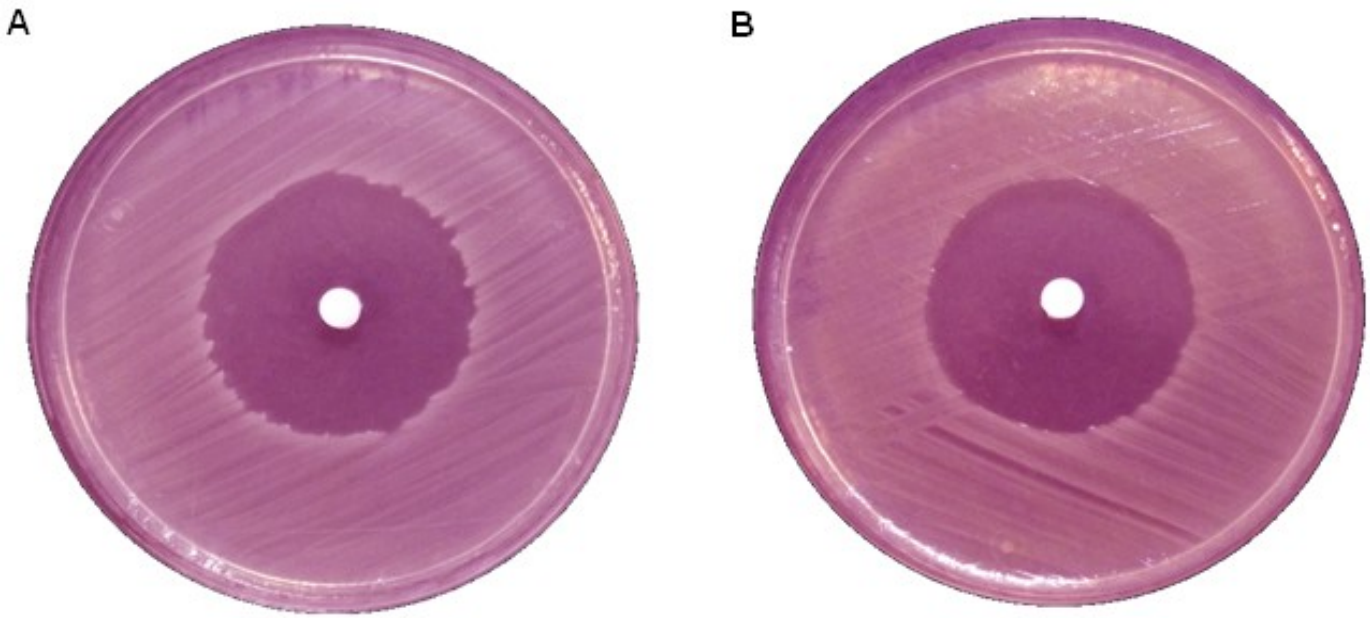


Figure 1. Well Diffusion assay. Inhibition clearance zones prouced by Alliaceae extract (GARLICON 40®) against: **(A)** *Salmonella enterica* CECT 4376 y **(B)** *Salmonella enterica* CECT 4156.

In vitro studies

DOMCA

Bactericidal activity

Campylobacter spp. (50 isolated)	MIC_{90}/MBC_{90} (µl/ml)
Campylobacter coli (18)	50
Campylobacter jejuni (25)	50
Campylobacter upsaliensis (8)	50
Global results	**50**

Otras cepas	MIC_{90}/MBC_{90} (µl/ml)
S. Intermedius (dog isolated) (9)	0.262/0.348
S. aureus (human isolated) (77)	1.67/2
Staphylococcus spp. (global results) (86)	1.67/2
MSSA (human isolated) (35)	1.67/2
MRSA (human isolated) (42)	1.67/2
P. aeruginosa (human isolated) (28)	1.919/2.5
Pasteurella multocida (swine isolated) (16)	0.125/0.4
Brachyspira hyodysenteriae (47 field isolates)	12,5/100

In vitro studies

DOMCA

Antifungal activity

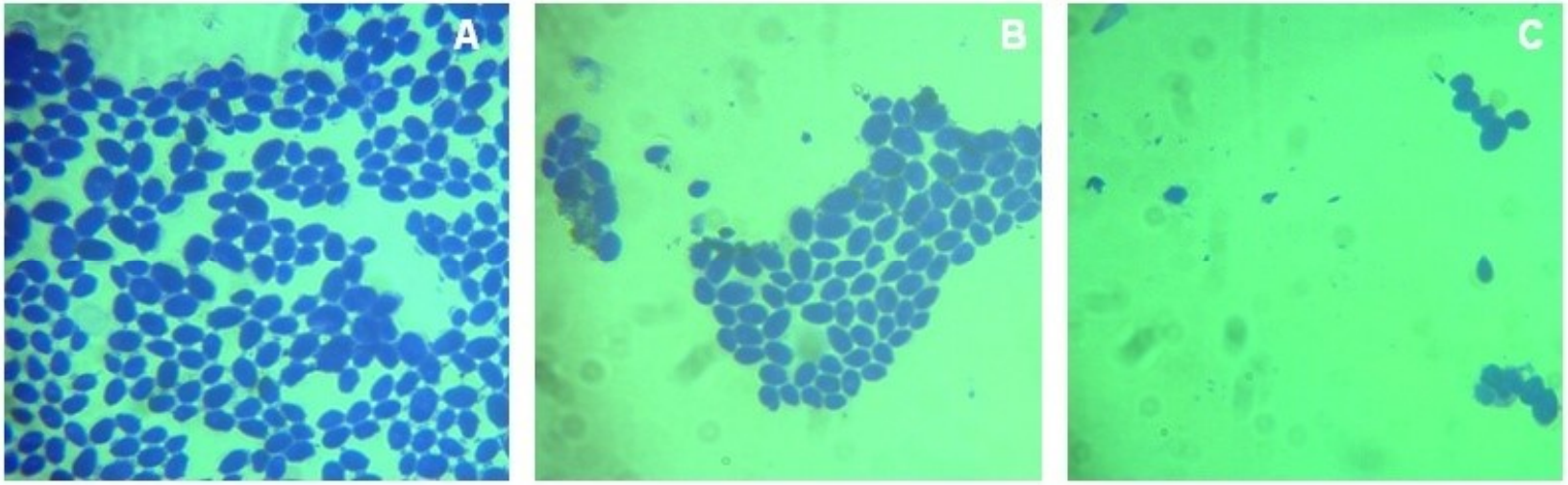
Agar diffusion Method	Growth inhibition halo (mm)	
Strain	**100 ppm**	**50 ppm**
Candida magnoliae	38	30
Candida krusei	29	18
Candida parapsilosis	17	15
Cryptococcus *spp.*	22	20
Aspergillus niger	35	29
Aspergillus flavus (aflatoxina B1producer)	20	16
Aspergillus flavus (aflatoxina B2 producer)	22	18

* Study conducted by DMC Research Center. CSIC (Spain)

In vitro studies

DOMCA

Antifungal activity



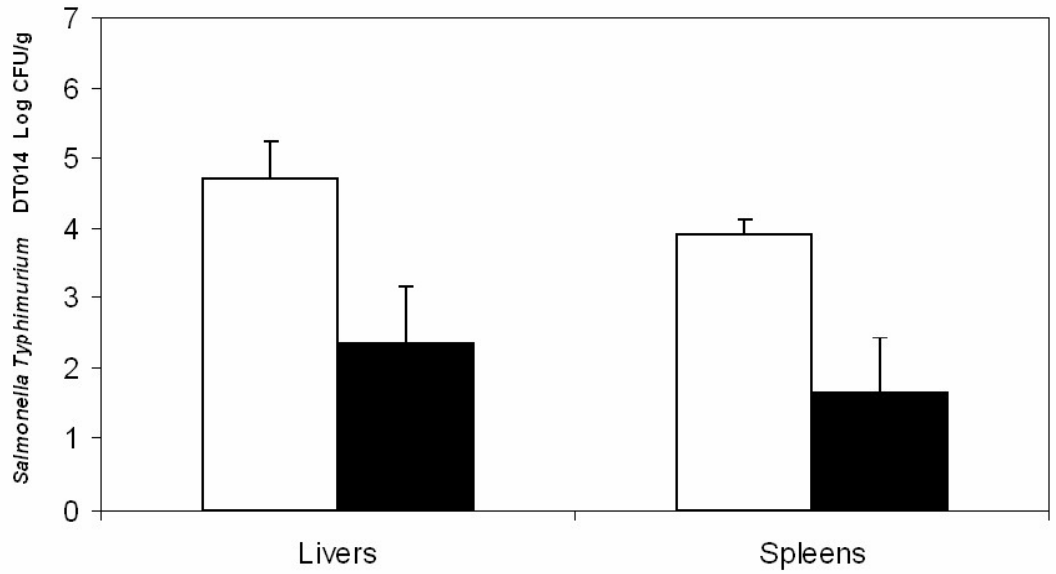
Microscopic image (x1000) of the cellular reduction of *Z.balli* after exposure to different concentrations of Garlicon. A) Control , B) 250 ppm for one hour C) 1000 ppm for one hour.

* Study conducted by DMC Research Center.

Resistance to *in vivo* infections

DOMCA

Resistance to *Salmonella* infections



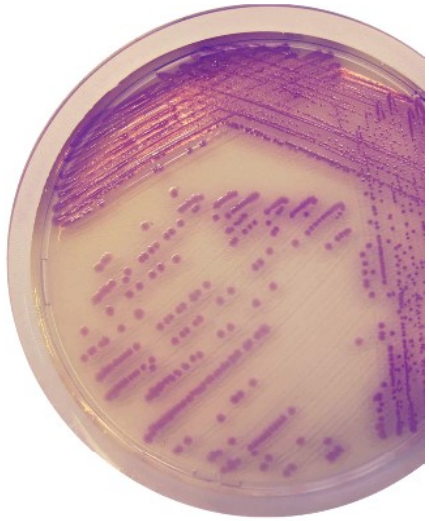
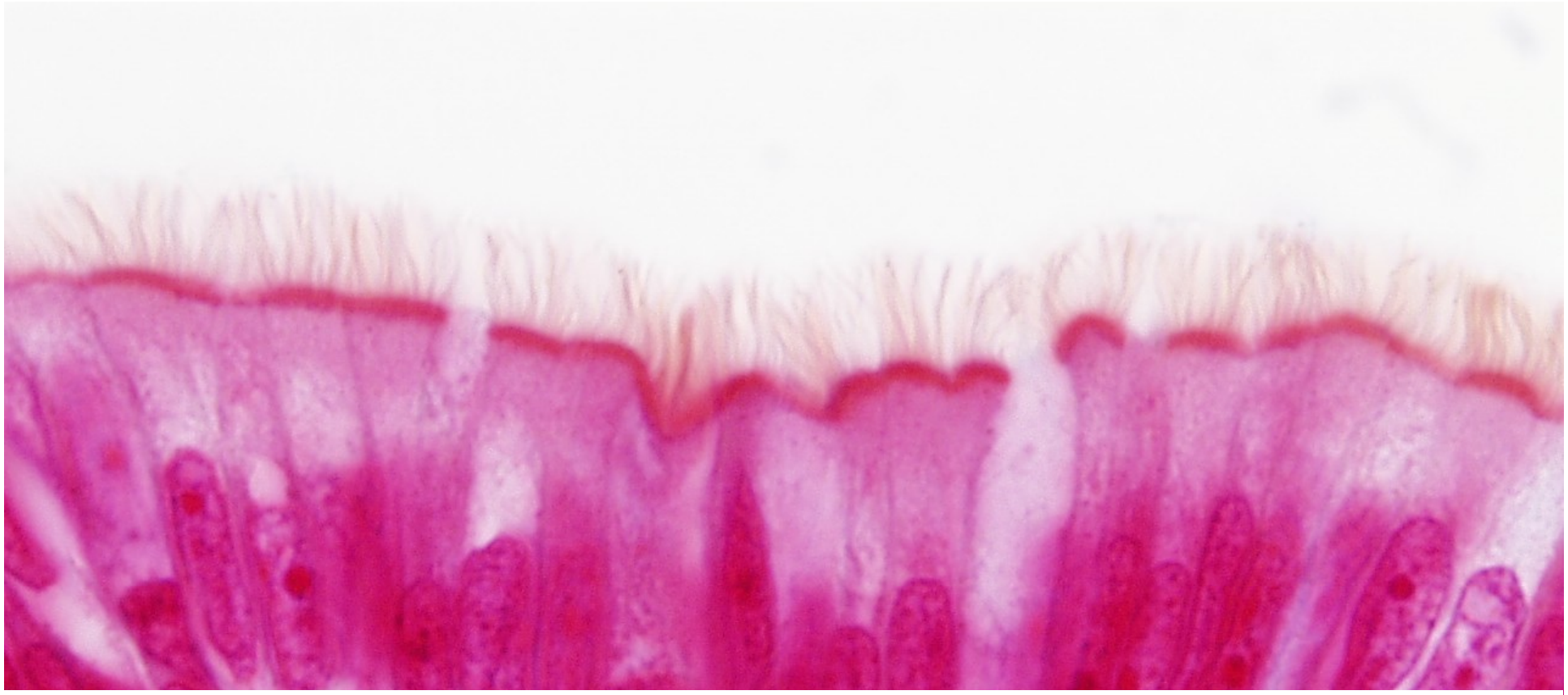
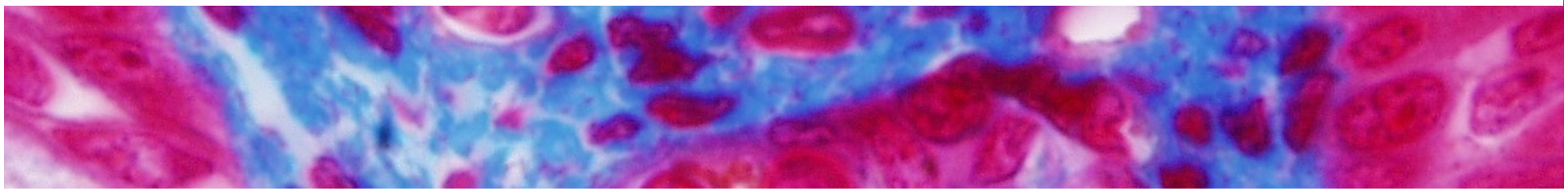


Figure. *In vivo* protection against *Salmonella enterica subsp. enterica* serovar *Typhimurium* infection in spleens and livers after oral dosing of mice with natural compound from Alliaceae **(**150 ppm of Garlicon 40) for 3 days before Salmonella infection. (*) P 0.05, indicating the statistically significant difference between the numbers of bacteria infecting organs compared with placebo-fed control mice.



INTESTINAL MICROBIOMA MODULATION

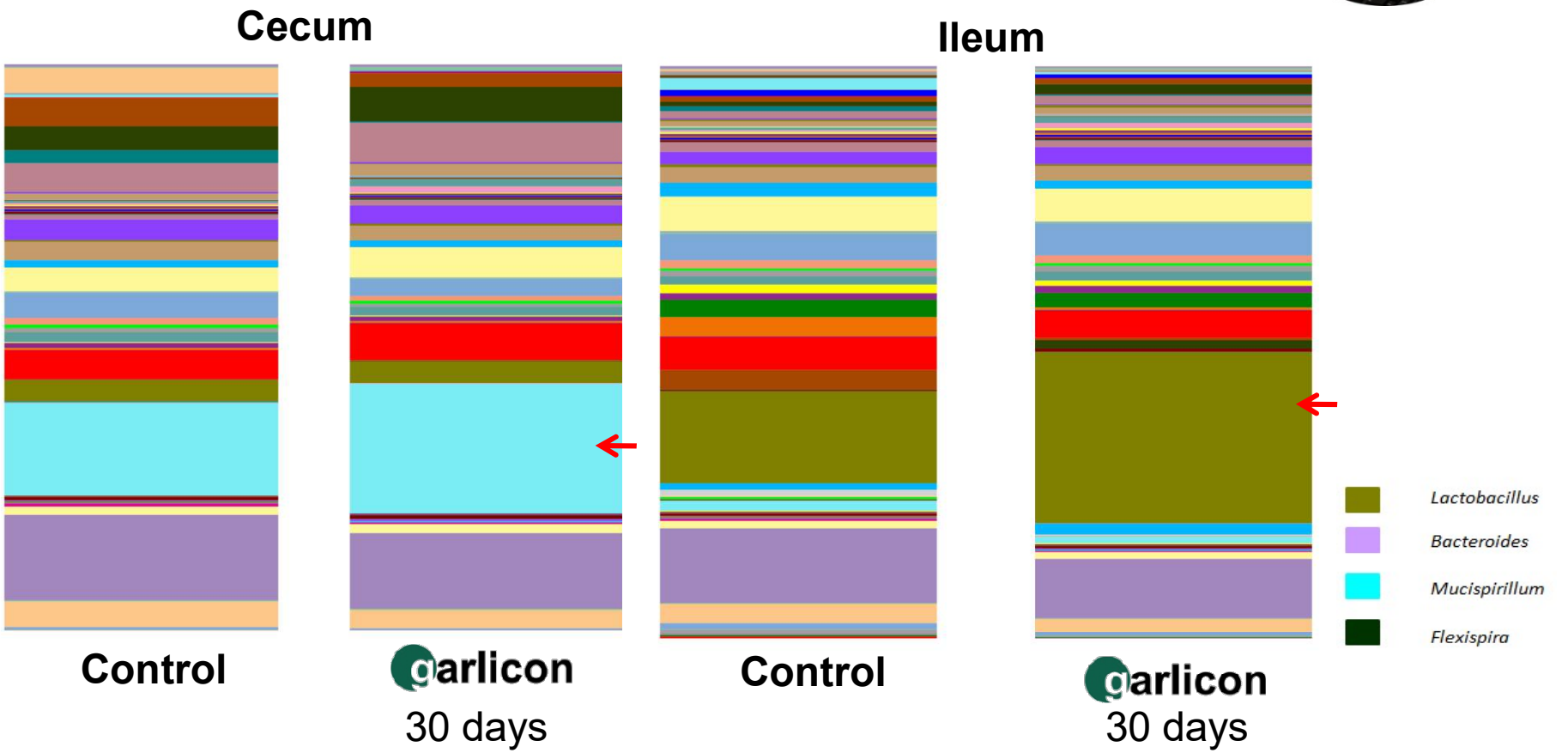


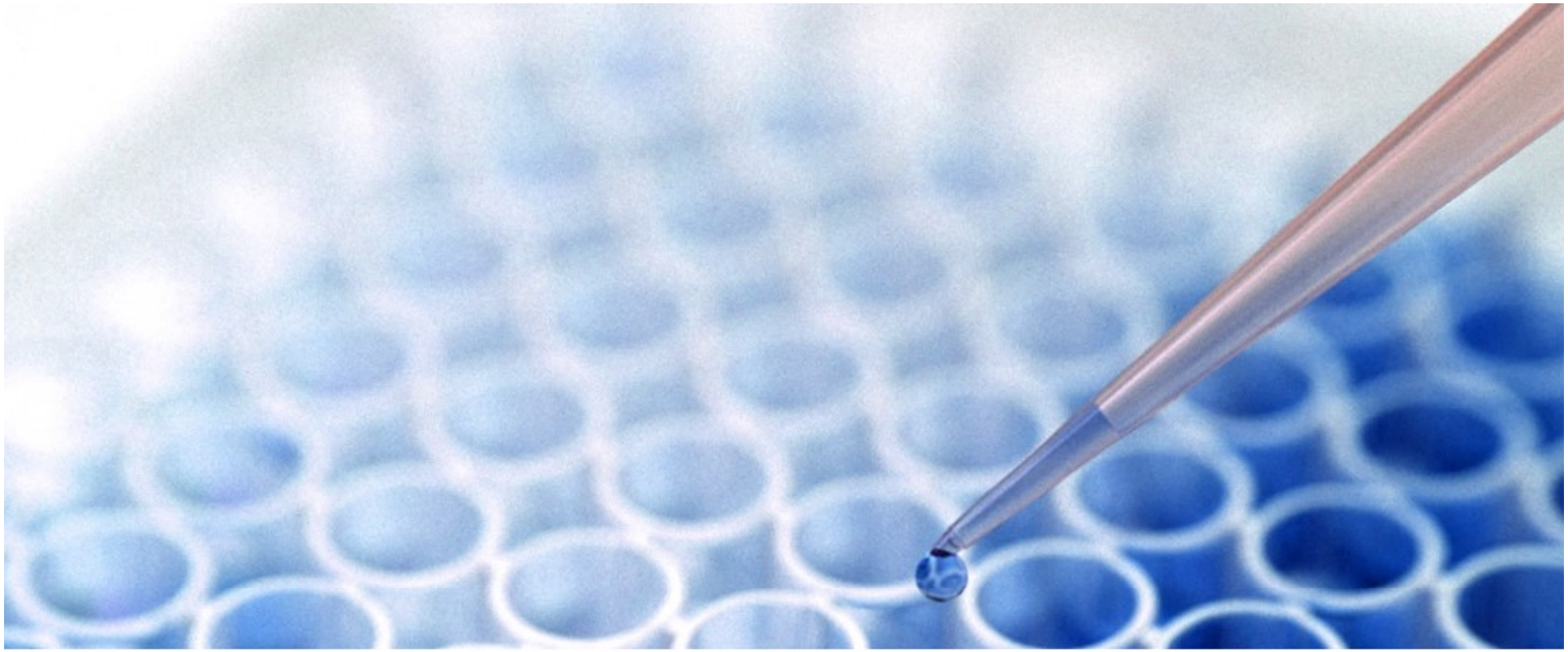
Intestinal microbiota modulación

DOMCA

Modulation of microbial communities of ileum and cecum of laying hens by massive sequencing techniques:

- Modulation of immune response
- Increased of digestibility and nutrient absorption efficiency

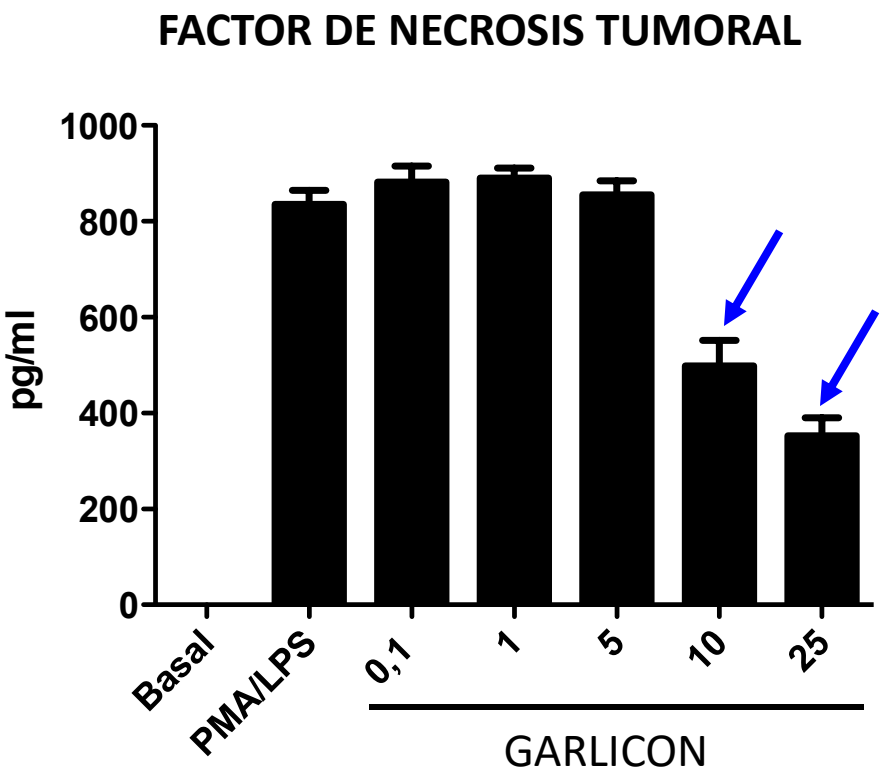




IMMUNITY

Capacidad inmunomoduladora *in vitro*

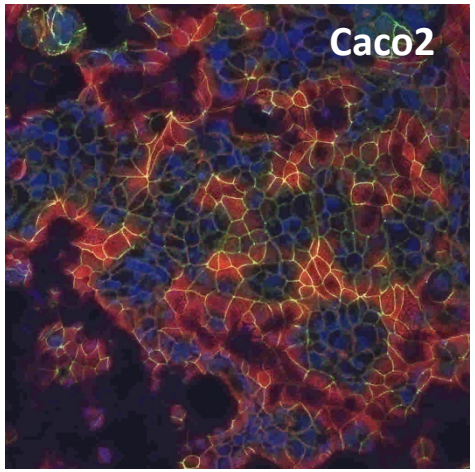
DOMCA

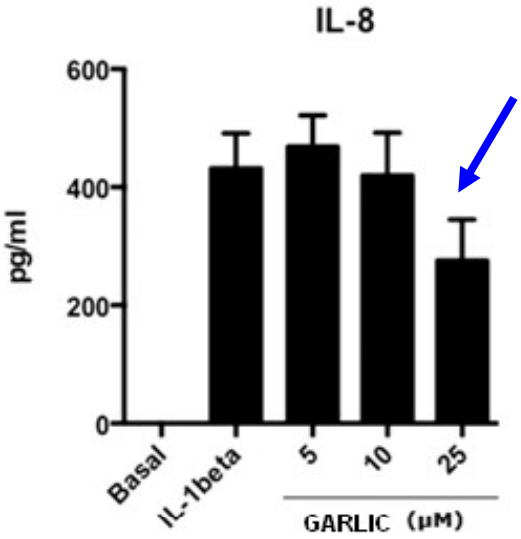


Evaluation of immunosuppressive activity of GARLIC on human acute monocytic leukemia cell line (THP-1).

In vitro immunomodulatory capacity

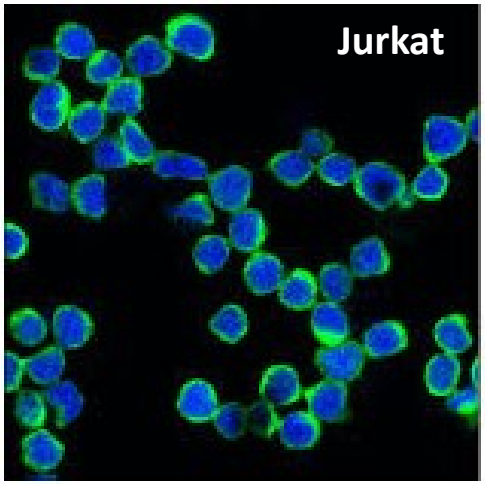
DOMCA

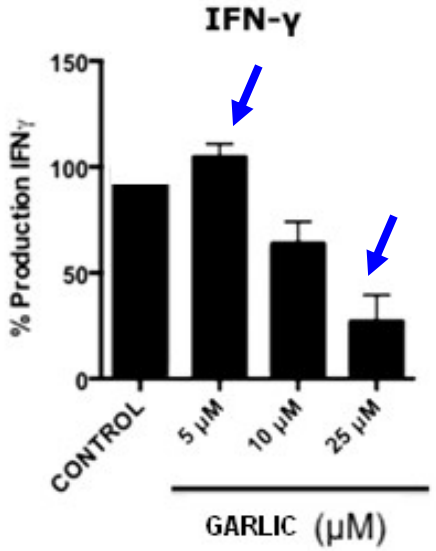




ANTI-INFLAMMATORY ACTIVITY

Immunomodulatory activity in the production of IL-8 in epithelial cells of human colorectal adenocarcinoma (Caco-2).





IMMUNOMODULATOR ACTIVITY

Modulation of the immune function in the production of IFN-γ in human leukemic T lymphocytes.

In vivo studies in intestinal inflammatory model

DOMCA

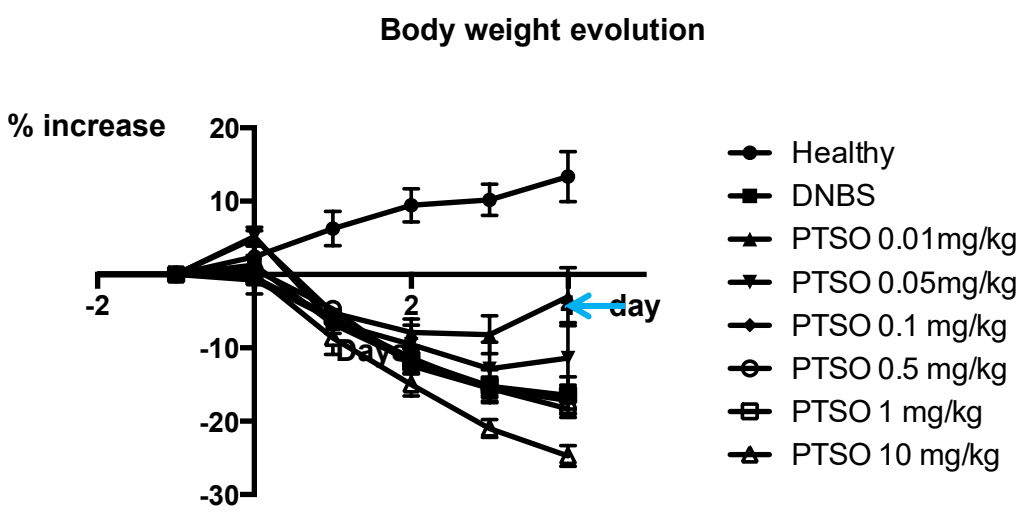


Figure . Evolution of the body weight of mice with colitis DNBS treated with different doses of PTSO..

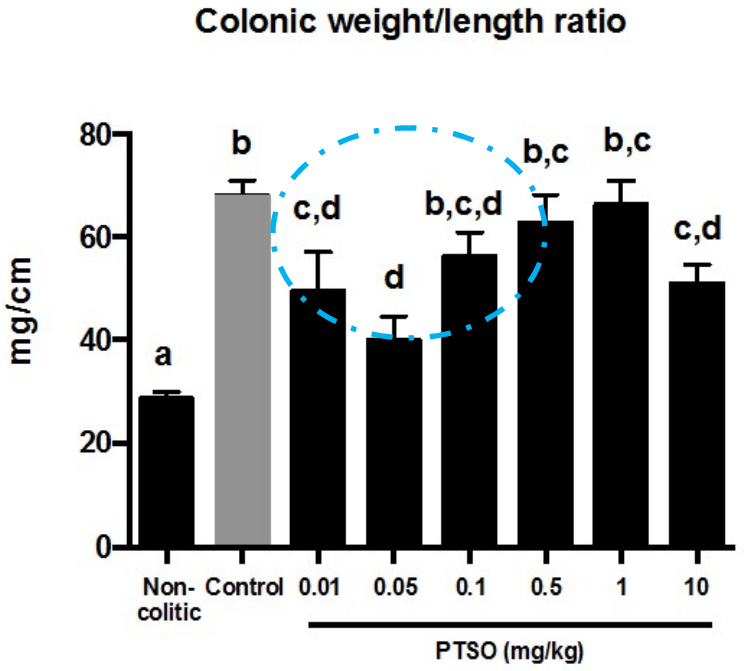


Figure: Effects of PTSO treatment on the weight / length ratio of the colon in mouse DNBS colitis. Groups with different letters indicate significant differences.

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Facultad de Medicina

In vivo studies in intestinal inflammatory model

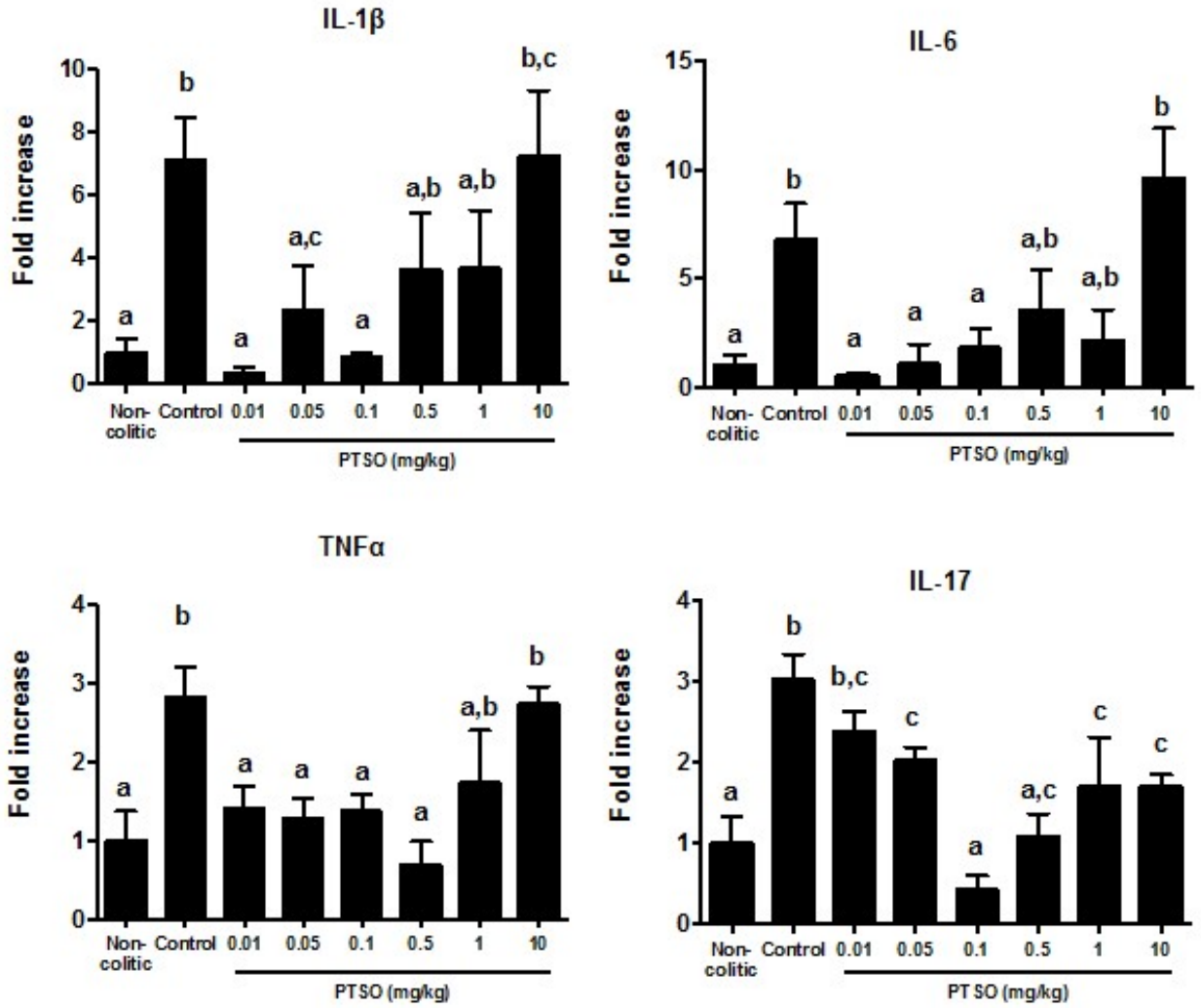


Figure. (Left): Influence of PTSO on the expression of IL-1β or TNF in mouse colitis. The groups with different letter differ statistically ($p < 0.05$). **Figure (Right**): Influence of PTSO on the expression of IL-6 or IL-17 in mouse colitis. Groups with different letters indicate significant differences.

POULTRY

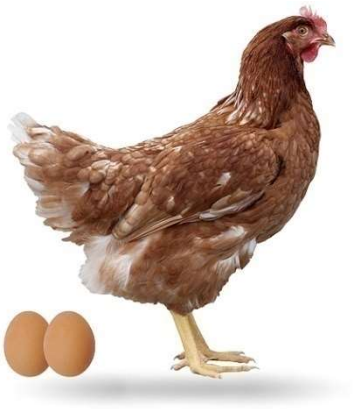
NATURAL SOLUTIONS

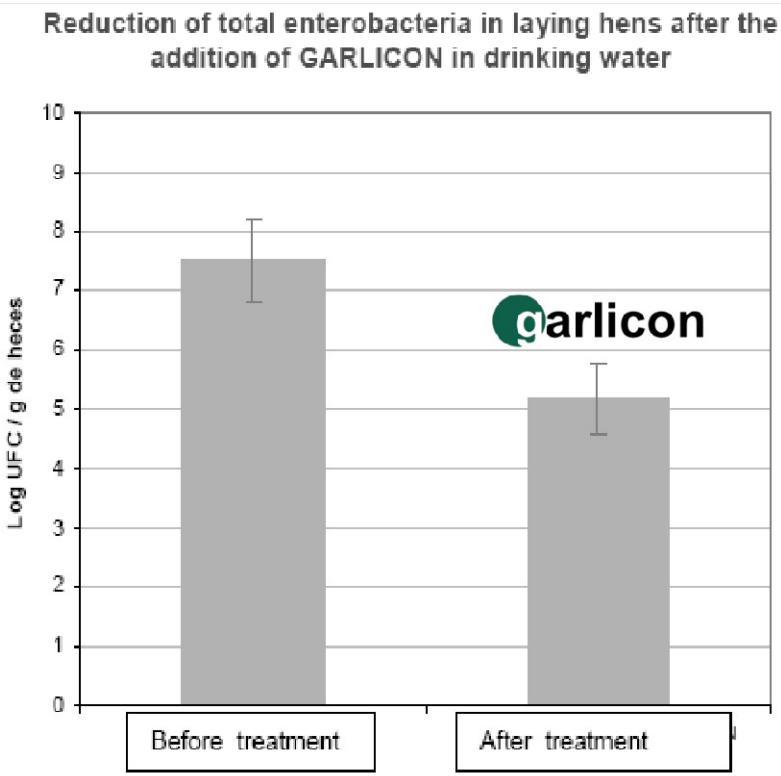
Salmonella: reduction in laying hens

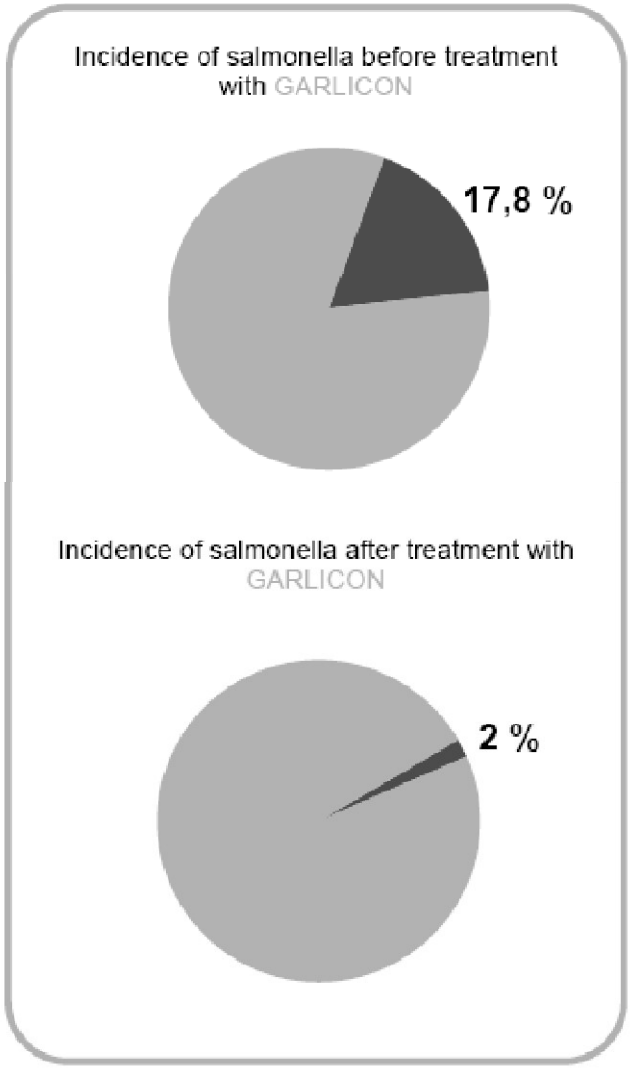
DOMCA

Total enterobacteria	Log UFC / g dregs
Before treatment	7,51
After treatments with GARLICON	5,18

33.000 hens, 150 ppm in drinking water, 7 days







Increase in productivity in laying hens

DOMCA

n = 33

Control

Dieta normal
ad libitum

n = 33

Antibiotic

Oxitetraciclina
(300mg/L)
Drinking water

n = 33

garlicon

Garlicon 40
(300mg/L)
Drinking water

20 days treatment

STOOLS

ILEUM & CECUM

EGGS

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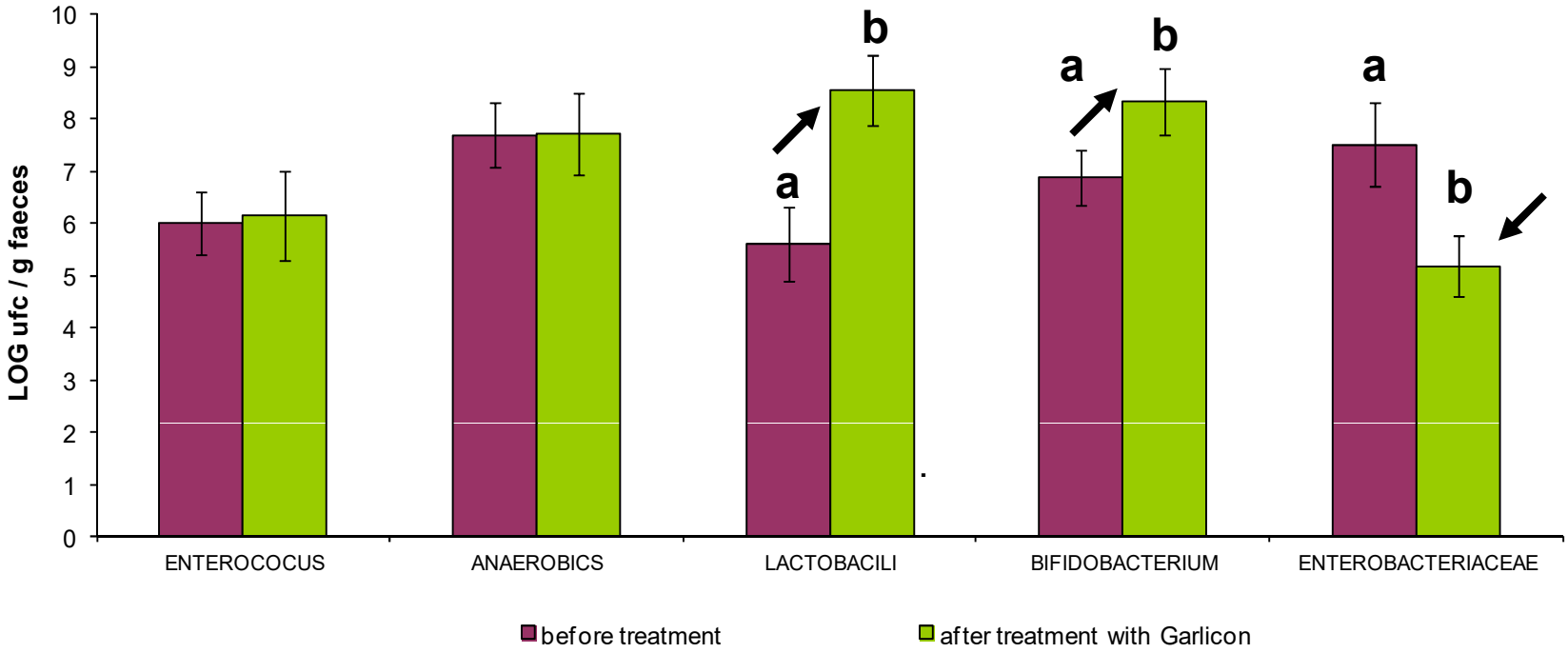
Variations in the intestinal microbiota of layers after administration of GARLICON in drinking water

DOMCA



- Beneficial groups are increased while decreasing pathogenic groups.

- GARLICON® seems to be effective increasing population of lactic acid groups by a direct elimination of competitive microorganisms that could be pathogens in certain situations.

* Study conducted by DMC Research Center/Prebia Feed Extracts (Spain)

Microbiota modulation

DOMCA

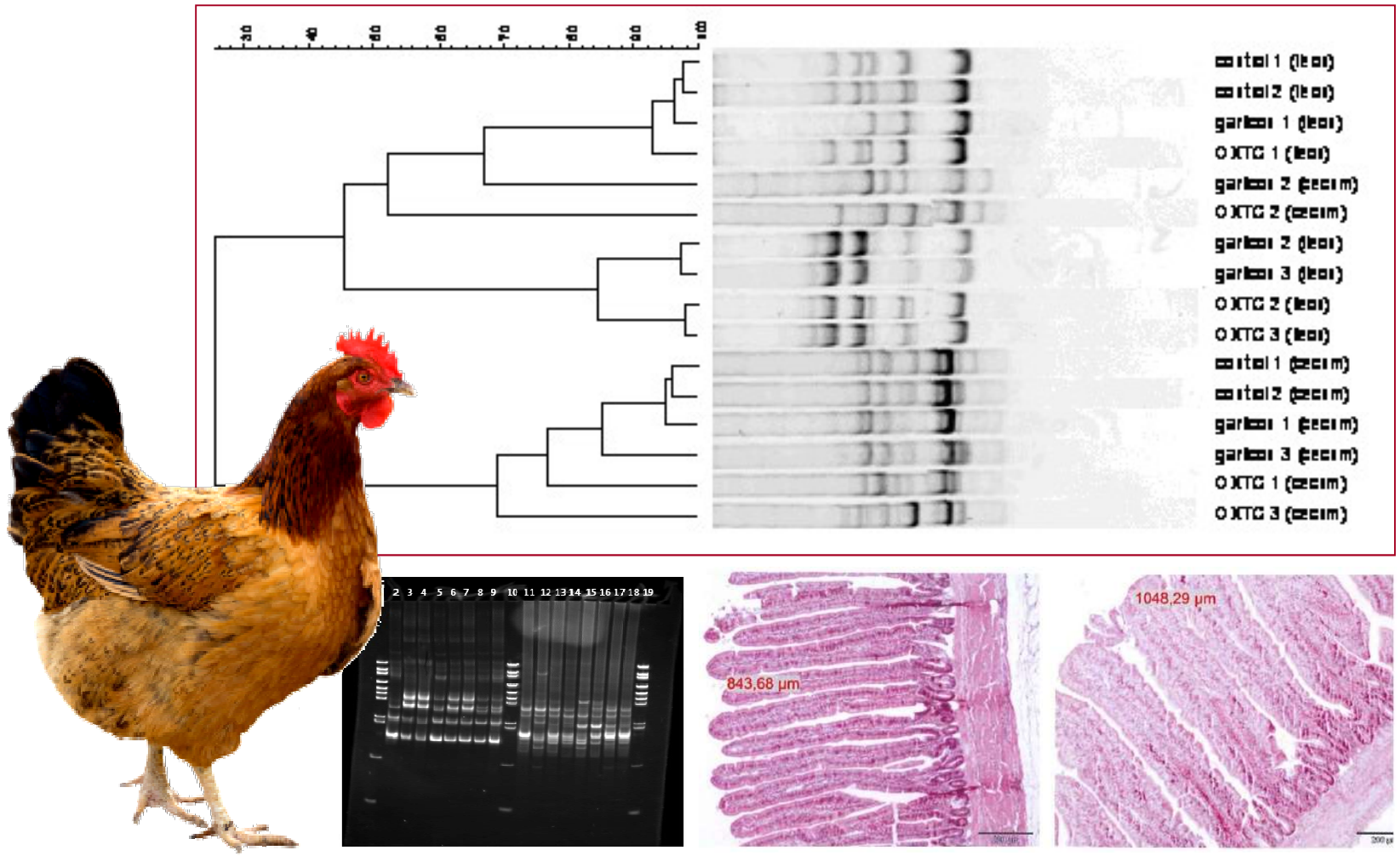


Figure 5. Influence of the extract of alliaceae on microbiota of the intestinal tract. • Analysis by molecular biology techniques (ARISA).

DOMCA

Research of antimicrobial residues in eggs: Analysis of oxytetracycline and organosulfur compounds (thiosulfonates)

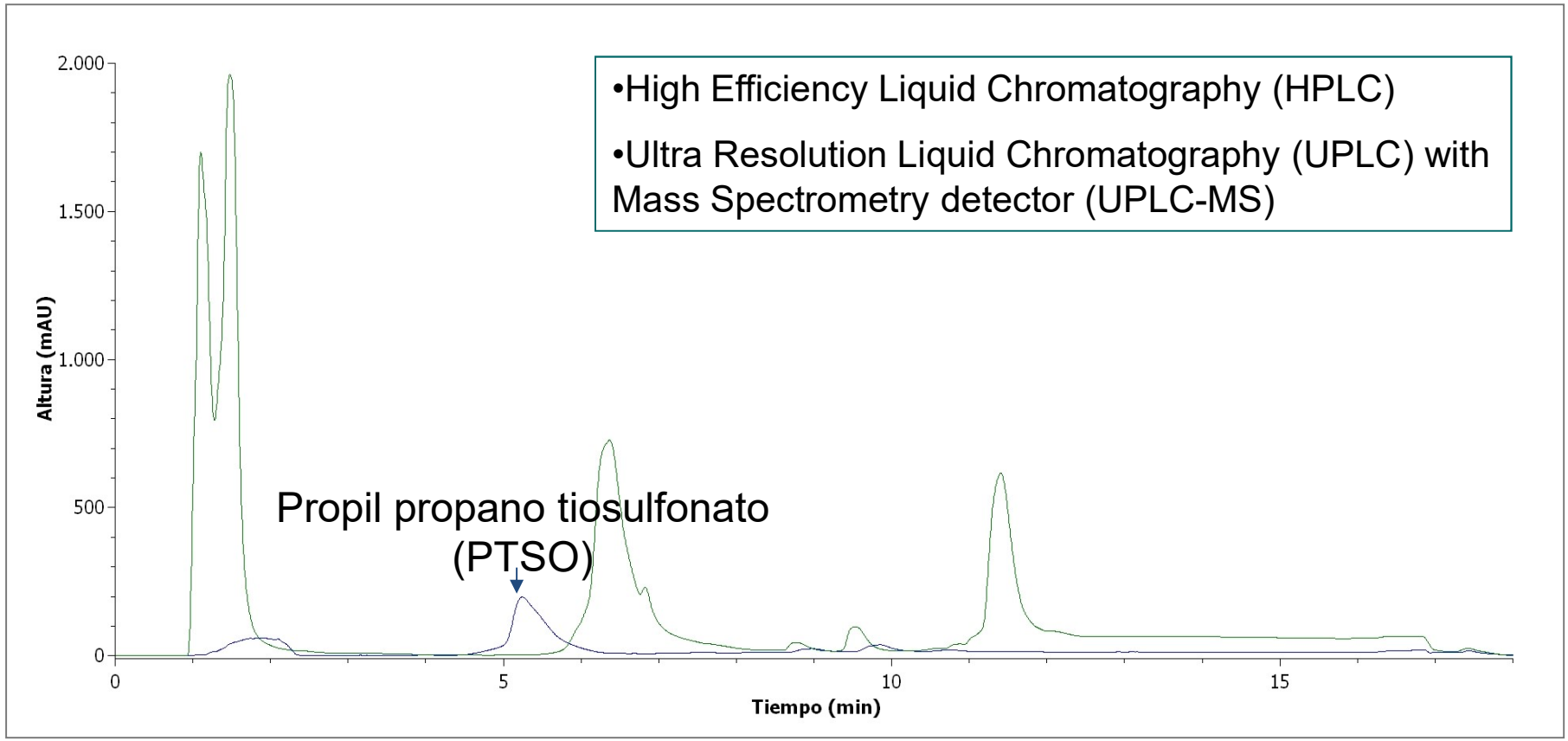
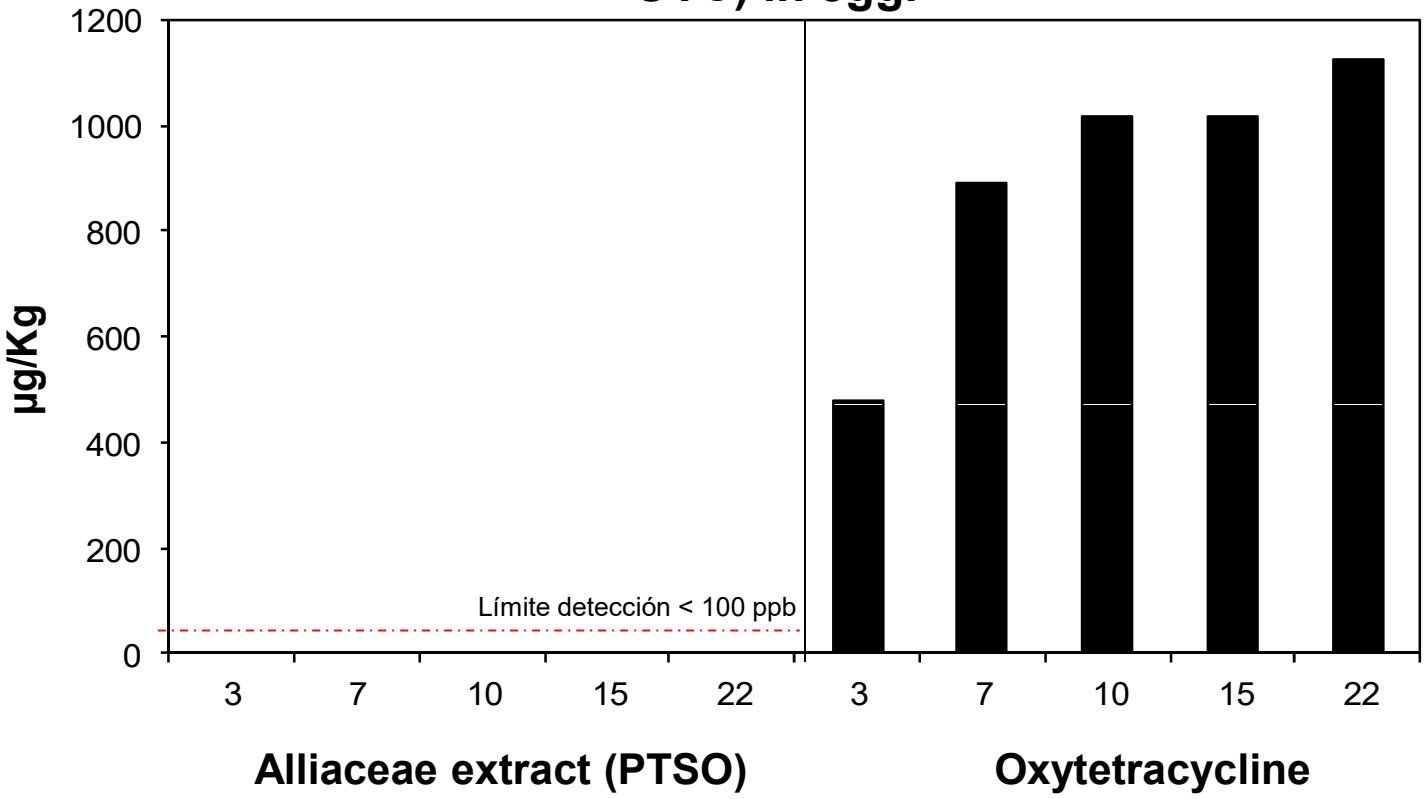


Figure 6. Chromatograms PTSO (blue) and egg (green)

Increase in egg production

DOMCA

Figure 7. Determination of antimicrobial residues (PTSO and OTC) in egg.



Garlicon leaves no residue in the egg or meat, so it does not require safety time and can be delivered to the animals until the last day before slaughter or while laying the eggs.

Increase in egg production

DOMCA

Figure 8. Cumulative average weight of the egg.

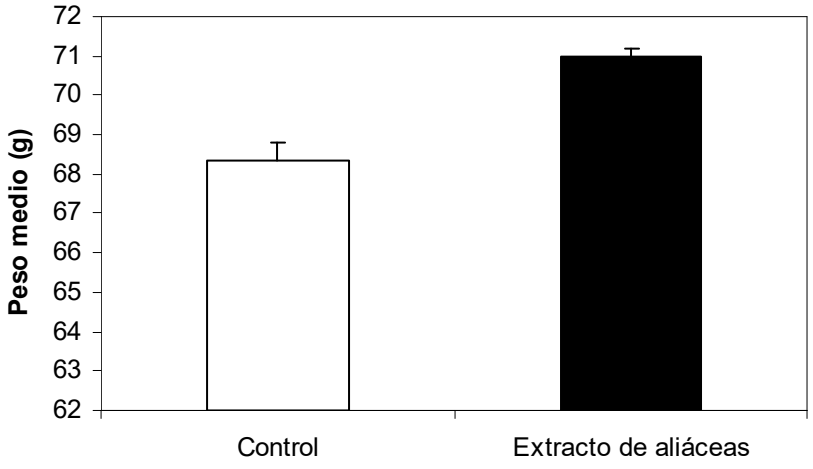
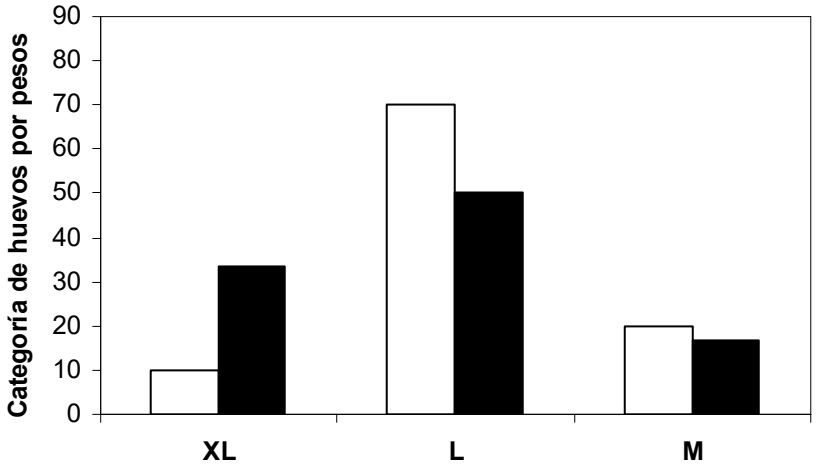


Figure 9. Distribution of egg categories according to size



•**Blank bars**: control group eggs.

• **Black bars**: eggs from hens that received the extract of alliaceae (300 mg / L GARLICON 40®).

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Nutritional improvements in egg

DOMCA

Figure 11. ω-3 Fatty acids

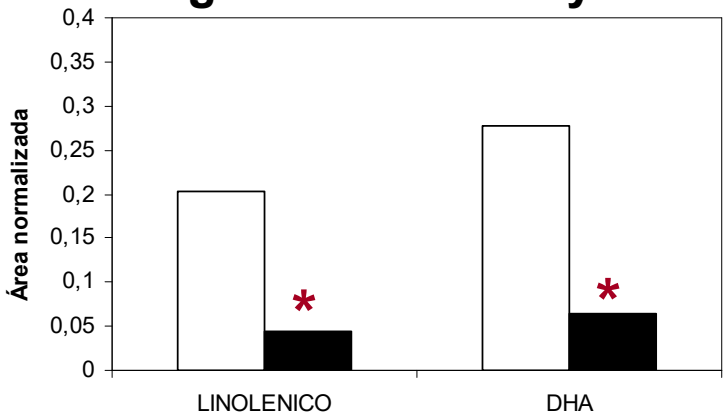
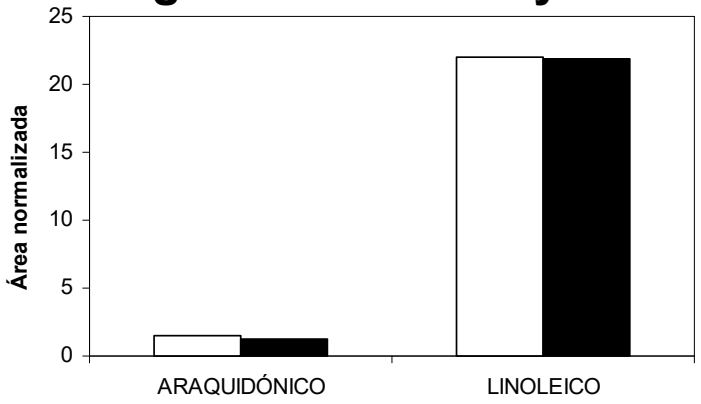


Figure 12. ω-6 Fatty acids

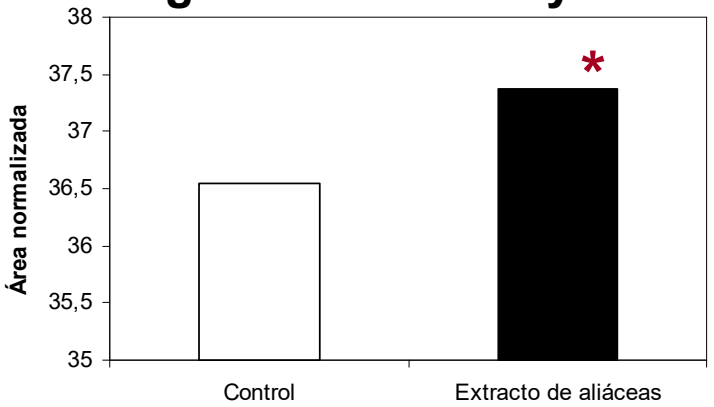


•Blank bars: control group eggs.

•Bars in black: eggs from chickens that received the extract of alliaceae.

P <0.05 indicates significant statistical differences between the treated and control groups.

Figure 13. ω-9 Fatty acids



Nutritional improvements in egg

DOMCA

Figure 13. Saturated fatty acids

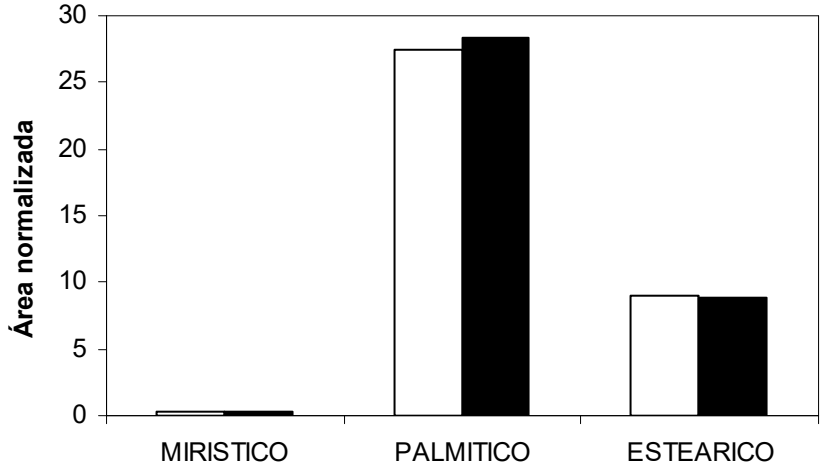
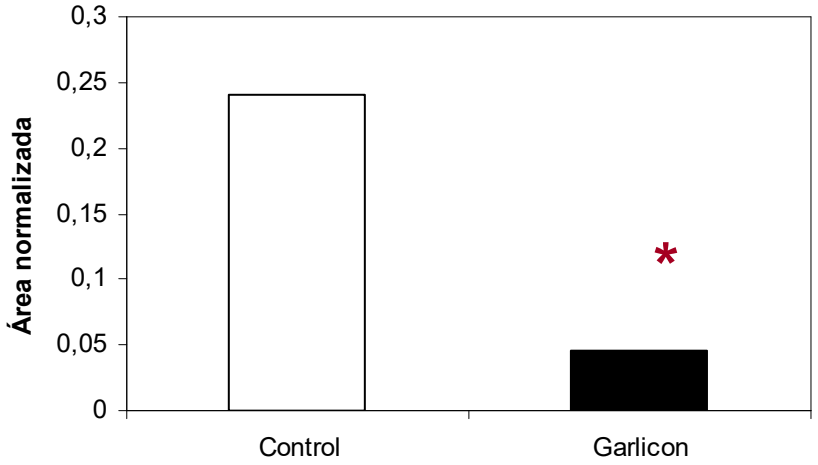
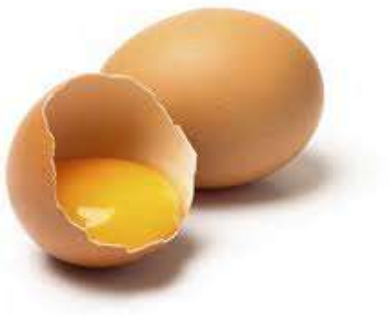


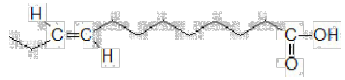
Figure 14. Trans fatty acids (elaidic)



- **Blank bars:** control group eggs.
- **Black bars:** eggs from chickens that received the extract of alliaceae (300 mg / L GARLICON 40®).

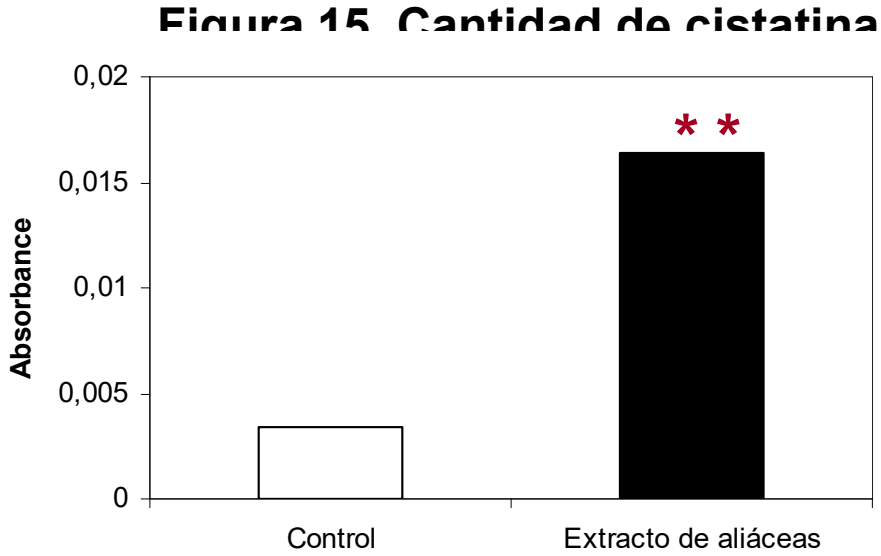
P <0.001 indicates significant statistical differences between the treated and control group.

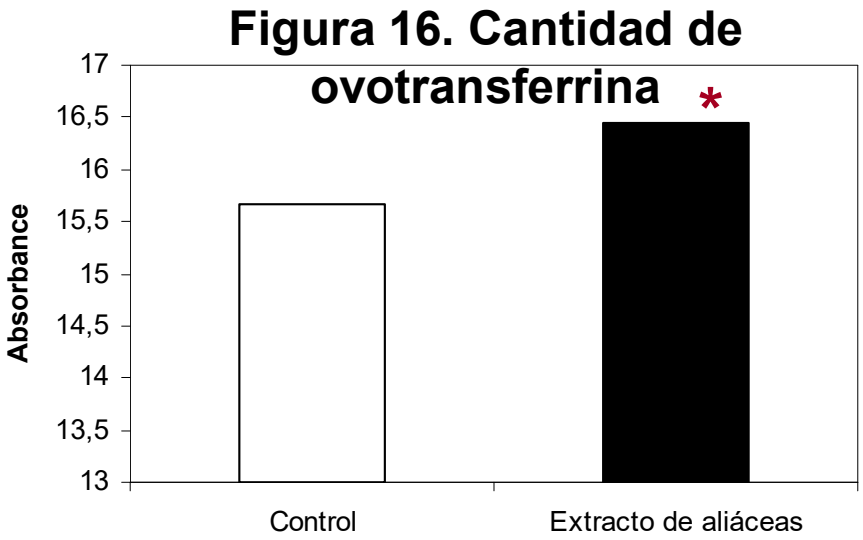




Nutritional improvements in egg

DOMCA





Barras en blanco: huevos del grupo control. **Barras en negro:** huevos procedentes de gallinas que recibieron el extracto de aliáceas (300 mg/L de GARLICON 40®)**. * P <0,01** indica diferencias significativamente estadísticas entre el grupo tratado y control. **** P <0,001** indica diferencias significativamente estadísticas entre el grupo tratado y control.

Improvement of productivity in layer hens

DOMCA

Evaluación de compuestos organosulfurados de Aliáceas en la mejora sanitaria y productiva de gallinas ponedoras

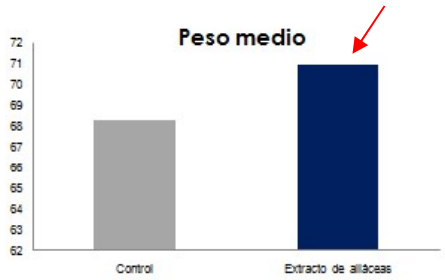
JJ. ARIZA[1], N. LORENZO-VIDAÑA[2], J.D. GARCÍA-LÓPEZ[1], C. NÚÑEZ[1], P. ABAD[2], E. GUILLAMÓN[2] Y A. BAÑOS*[1]

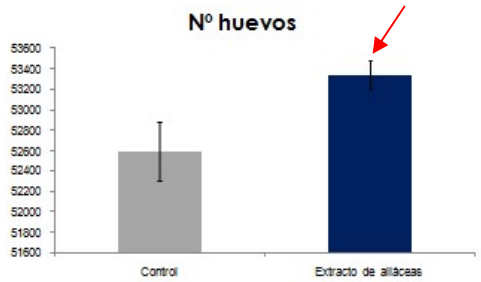
1. Departamento de Microbiología y Biotecnología. DMC Research.
2. Departamento Técnico. DOMCA SAU. Camino de Jayena 82. 18620 - Alhendín, Granada, España.

DMC RESEARCH

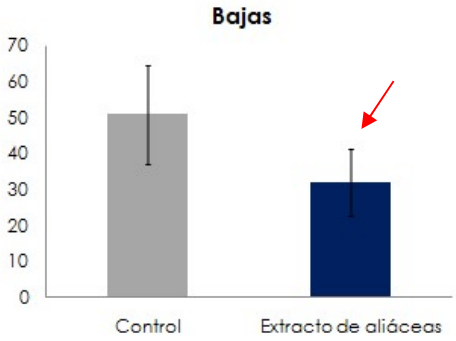
3.000 animales
Aplicación en agua de bebida. 150 ppm
Duración = 15 días

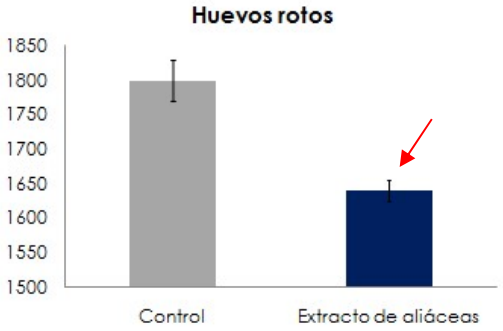
- Mejora de la productividad: número y peso medio del huevo





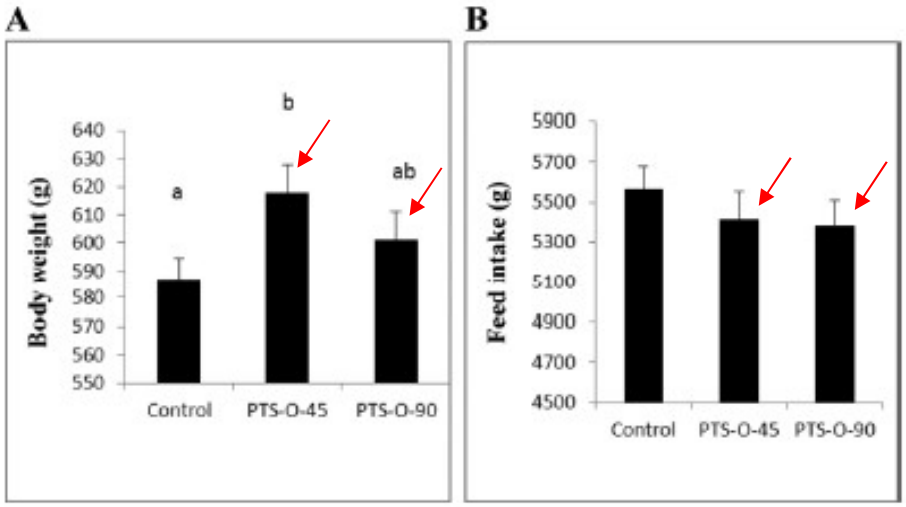
- Reducción en número de bajas y huevos rotos





Increase in productive parameters in broilers

DOMCA



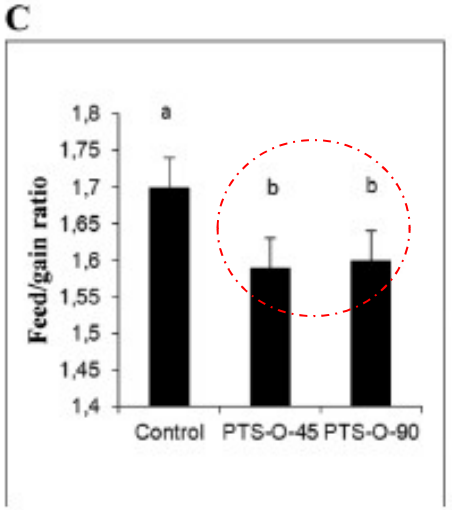


Figure. Effects of dietary inclusion of propyl propane thiosulfonate (PTS-O) on final BW (A), feed intake (B), and feed:gain ratio (C) of chickens in experiment 1. a,b Bars with different letters were significantly different ($P < 0.01$). Values are means (8 replicates of 6 birds each) with their SD in bars.

IMMUNOLOGY, HEALTH, AND DISEASE PS2280

Garlic derivative PTS-O is effective against broiler enteropathogens in vivo

M. J. Peinado, R. Ruiz, A. Echávarri, and L. A. Rubio[1]

Dpto. de Fisiología y Bioquímica de la Nutrición Animal (IFNA, EEZ, CSIC), Profesor Albareda, 1, 18008 Granada, Spain

CSIC

Increase in weight gain

DOMCA

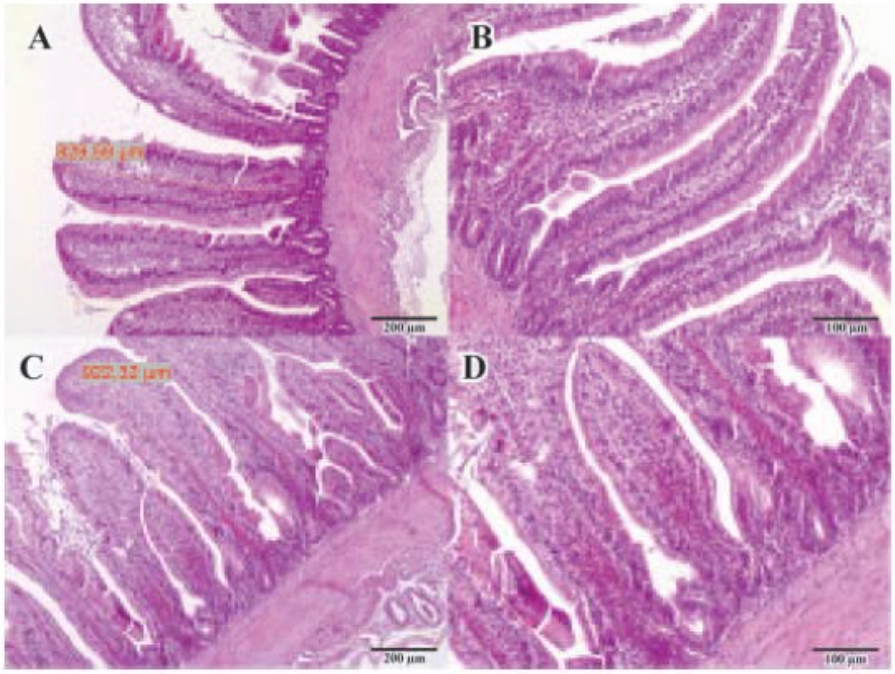


Figure 2. Light microscope photograph showing the histological structure of ileal sections of broiler chickens fed on the control (A and B) or propyl propane thiosulfonate (PTS-O-90; C and D) diets. Villi in PTS-O birds were taller and wider than those of controls. For specific measurements, see Table 6. Bars represent 200 (A and C) or 100 (B and D) μm. Color version available in the online PDF.

Table 6. Morphology[1] of the ileal sections of 21-d-old broiler chickens fed on control or experimental (propyl propane thiosulfonate; PTS-O-90 diets in experiment 1

Item	Control	PTS-O
Villus height, μm	785[a]	937[b]
Crypt depth, μm	96	105
Villus height/crypt depth	8.7	8.9
Villus width, μm	131[a]	276[b]
Villus surface area, μm^2	325,940[a]	807,766[b]
Mucosal thickness, μm	47[a]	66[b]
Muscular layer thickness, μm	172[a]	204[b]

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Reduction of enteropathogens

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Table 3. Effect of propyl propane thiosulfonate (PTS-O) dietary addition on the $\log_{10}$ number of copies per milligram of intestinal content of *Salmonella* spp., *Campylobacter jejuni*, and *Clostridium perfringens* in the ilea of birds in experiment 1[1,2]

Item	Control	PTS-O-45	PTS-O-90	Pooled SD
Salmonella spp.	3.48[a]	3.48[a]	2.77[b]	0.49
C. jejuni	4.12	4.42	3.83	0.59

Table 4. Effect of propyl propane thiosulfonate (PTS-O) dietary addition on the $\log_{10}$ number of copies per milligram of intestinal contents of enterobacteria and *Escherichia coli* in the crop, ileal, and cecal contents of birds in experiment 1[1,2]

Item	Control	PTS-O-45	PTS-O-90	Pooled SD
Crop				
Enterobacteria	5.54[a]	5.07[b]	4.4[b]	0.70
E. coli	5.48[a]	4.79[b]	4.02[b]	0.81
Ileum				
Enterobacteria	4.41[a]	4.14[a]	3.59[b]	0.60
E. coli	3.16	3.34	3.03	0.76
Ceca				
Enterobacteria	5.58	5.73	5.62	0.53
E. coli	5.90[a]	5.84[a]	5.41[b]	0.63

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Modulation of gut microbiota

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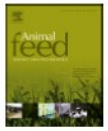

Garlic derivative PTS-O modulates intestinal microbiota composition and improves digestibility in growing broiler chickens

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ORIGINAL ARTICLE

Correlations between changes in intestinal microbiota composition and performance parameters in broiler chickens

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Table 2
AMEn (cal/g), ileal apparent N digestibility and fecal apparent digestibility of energy, N, fat, aNDFom-NDF, ADFom-ADF and total NSP of growing broiler chickens fed on PTS-O supplemented diets.

	Control	PTS-O-45	PTS-O-90
AMEn (cal/g)	3321a	3356b	3448c
Ileal			
N	0.83	0.83	0.82
Fecal			
Energy	0.84^{a}	0.85^{b}	0.88^{c}
N	0.76^{a}	0.77^{a}	0.80^{b}
Fat	0.94^{a}	0.95^{b}	0.96^{b}
aNDFom- NDF	0.66^{a}	0.68^{b}	0.73^{c}
ADFom-ADF	0.39^{a}	0.43^{b}	0.53^{c}
NSP	0.59^{a}	0.62^{a}	0.72^{b}

PTS-O propyl propane thiosulfonate.
Means in each row with different superscript letters (a, b, c) differ ($P<0.05$).

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British Journal of Nutrition
http://journals.cambridge.org/BJN

Improved resistance to *Eimeria acervulina* infection in chickens due to dietary supplementation with garlic metabolites

Duk Kyung Kim, Hyun S. Lillehoj, Sung Hyen Lee, Erik P. Lillehoj and David Bravo

British Journal of Nutrition / *FirstView* Article / October 2012, pp 1 - 13
DOI: 10.1017/S0007114512000530, Published online: 13 April 2012

Link to this article: http://journals.cambridge.org/abstract_S0007114512000530

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USDA

In vitro Anti-coccidial effect (*Eimeria acervulina*)

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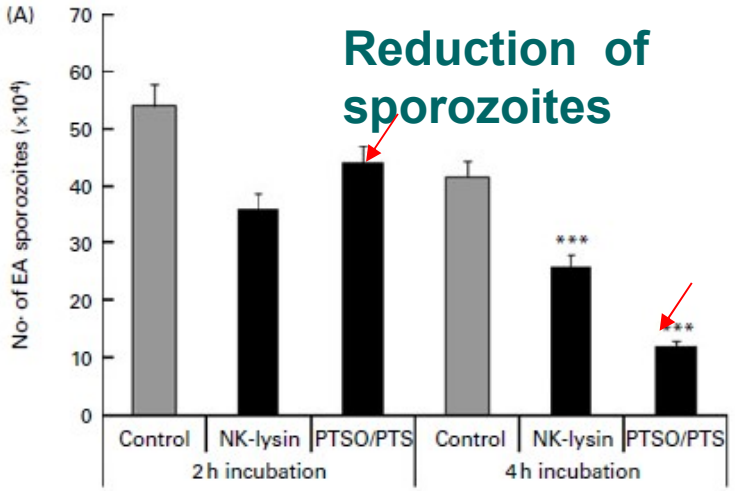
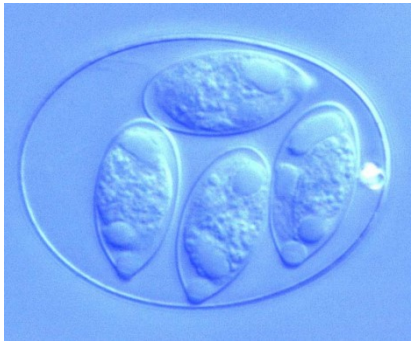
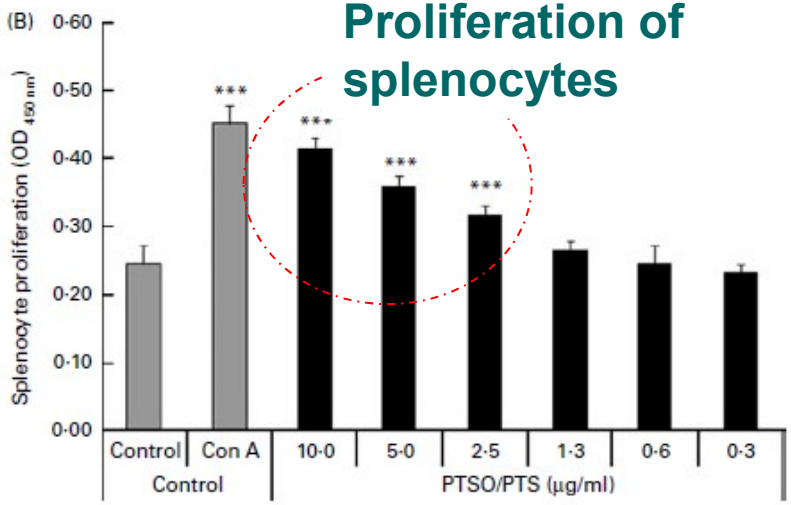


Reduction of sporozoites





Proliferation of splenocytes

Fig. 1. Effect of propyl thiosulphinate oxide/propyl thiosulphinate (PTSO/PTS) *in vitro*. (A) *Eimeria acervulina* (EA) sporozoites ($1{\cdot}0 \times 10^6$/ml) were incubated with PBS (control), 10 μg/ml of PTSO/PTS or 5·0 μg/ml of chicken recombinant natural killer (NK) lysin, for 2 or 4 h at 4°C, and viability was measured by trypan blue exclusion by counting a minimum of 100 sporozoites. (B) Spleen cells were treated with the indicated concentrations of PTSO/PTS, concanavalin A (Con A; 5 μg/ml) or medium (control) for 48 h and viable cell numbers were measured using 2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulphophenyl)-2H-tetrazolium. Values are means, with standard deviations represented by vertical bars (n 3). Mean values were significantly different from those of PTSO/PTS-treated with control groups according to the Student's t test: * $P<0{\cdot}05$; *** $P<0{\cdot}001$. OD, optical density.

* Study conducted by Animal Parasitic Diseases Laboratory. United States Department of Agriculture, animal and Natural Resources Institute. USA

In vivo Anti coccidial effect (*Eimeria acervulina*)

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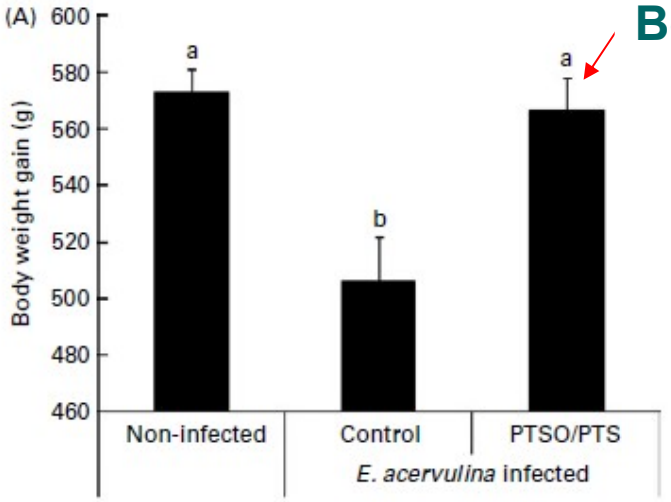
Body weight gain



Reduction of oocysts

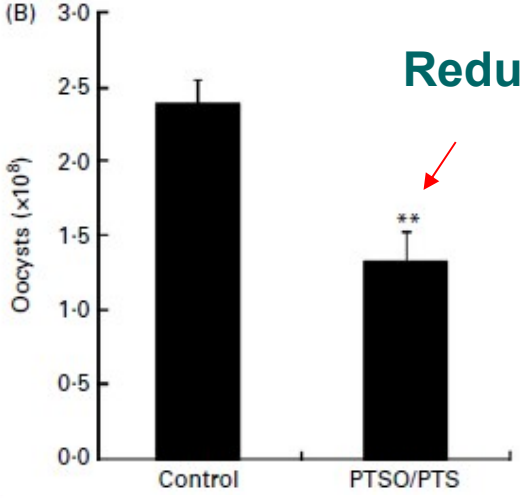


Fig. 2. Effect of dietary propyl thiosulphinate oxide/propyl thiosulphinate (PTSO/PTS) on body weight gain and faecal oocyst excretion following experimental *Eimeria acervulina* infection. Chickens were fed from hatch with non-supplemented or PTSO/PTS-supplemented diets and either uninfected or orally infected with $1{\cdot}0 \times 10^4$ oocysts of *E. acervulina* at 10 d post-hatch. (A) Body weights (twenty birds/group) were measured in non-infected and infected chickens on the non-supplemented diet (control), and in infected chickens on the PTSO/PTS-supplemented diet at 0 and 10 d post-infection. Values are means, with standard deviations represented by vertical bars. ^{a,b} Mean values with unlike letters were significantly different according to Duncan's multiple-range test ($P<0{\cdot}05$). (B) Faecal samples (twenty birds/group) were collected from chickens on the non-supplemented (control) and PTSO/PTS-supplemented diets between 6 and 9 d post-infection and total oocyst numbers were determined using a McMaster chamber. Values are means, with standard deviations represented by vertical bars. ** Mean value was significantly different from that of the control group ($P<0{\cdot}01$; Students *t* test).

* Study conducted by Animal Parasitic Diseases Laboratory. United States Department of Agriculture, animal and Natural Resources Institute. USA

Anti coccidial effect (*Eimeria acervulina*)

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Inmunostimulation post-challenge

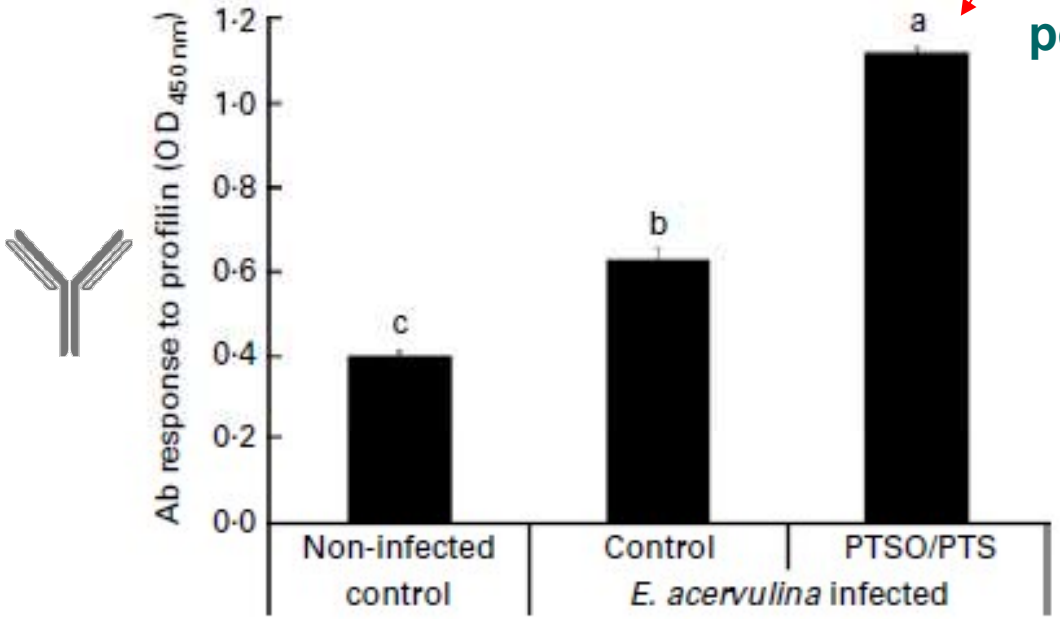

Fig. 3. Effect of dietary propyl thiosulphinate oxide/propyl thiosulphinate (PTSO/PTS) on profilin antibody (Ab) levels. Chickens were fed from hatch with non-supplemented (control) or PTSO/PTS-supplemented diets and orally infected with $1{\cdot}0 \times 10^4$ oocysts of *Eimeria acervulina* at 10 d post-hatch. Peripheral blood (four birds/group) was collected at 10 d post-infection and sera were analysed for anti-profilin Ab levels by ELISA. Values are means, with standard deviations represented by vertical bars (n 4). ^{a,b,c} Mean values with unlike letters are significantly different according to Duncan's multiple-range test ($P < 0{\cdot}05$). OD, optical density.

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RUMINANTS

Rumen microbiota modulation and inmunoestimulant

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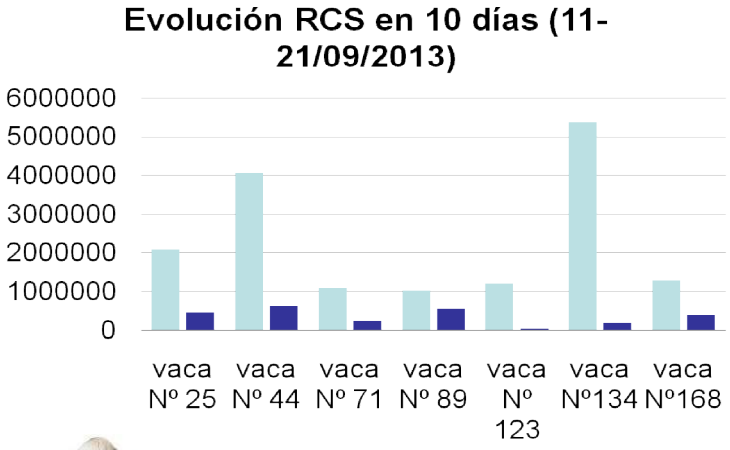
-**Reduction of somatic cells (rumen boluses)**

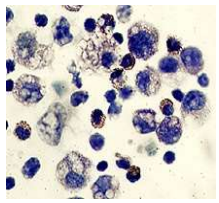
-**Coccidios reduction**

-**Methane reduction**

-**Lactoreplacement: Immunomodulator**

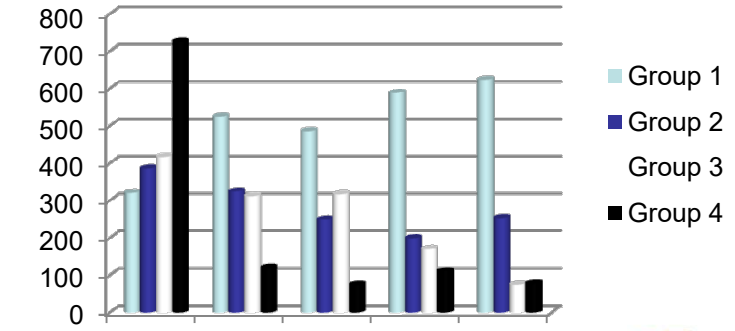
Somatic cells reduction in cows





Garlicon bolus application with several cows with clinical mastitis and evolution of RCS 10 days after the application of the bolus.

Coccidia counts in faeces



group 1, Control
group 2 Garlicon 5 ml/head;
group 3 Garlicon 10 ml;
group 4 Garlicon f 20mL.

PTS incorporated into the diet of cows and goats produce a modulation of the population of rumen bacteria by reducing the emission of CH4 into the atmosphere.

Coccidia reduction in lambs

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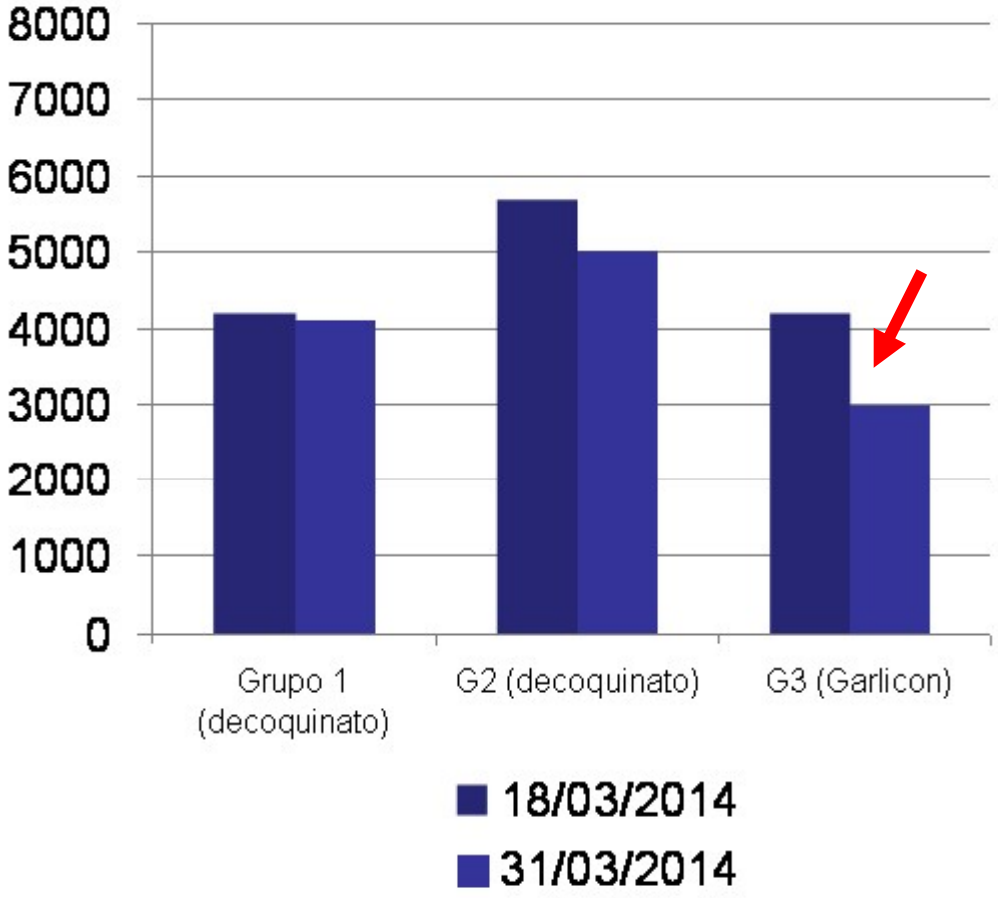

2 weeks, fed with 100 ppm of decoquinate or 100 ppm of Garlicon

Parasites and coccidia reduction

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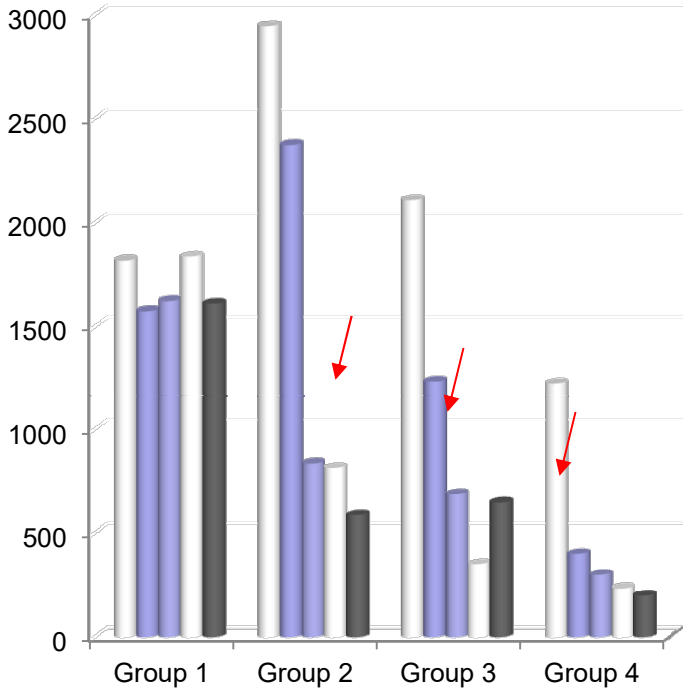
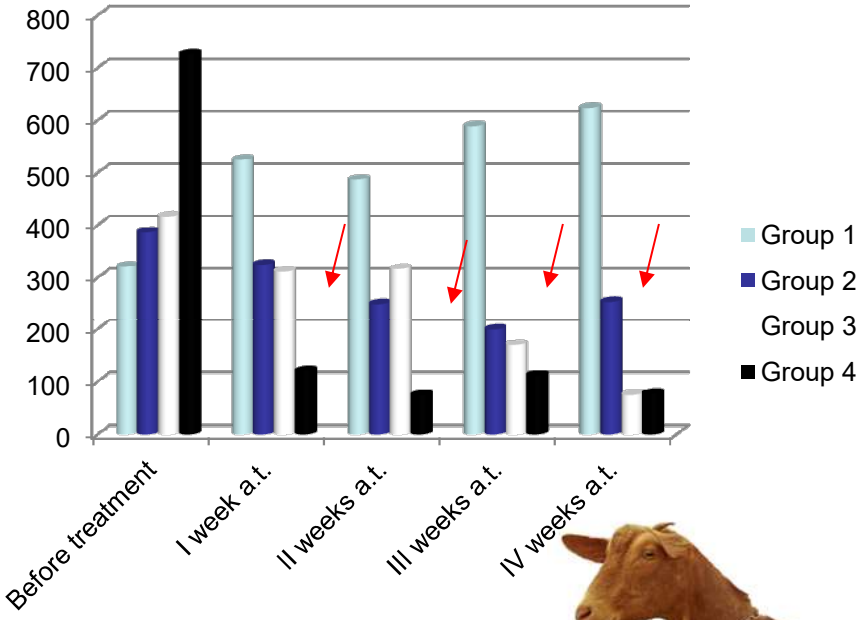
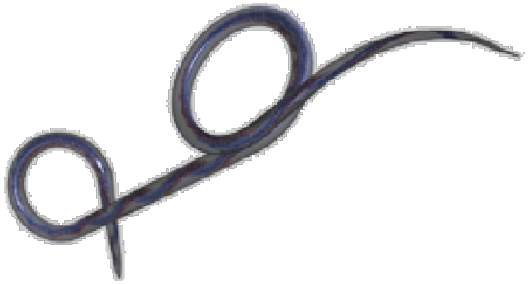
Haemonchus contortu in faeces



Coccidia counts in faeces





group 1, Control

group 2 Garlicon 5 ml/head;

group 3 Garlicon 10 ml;

group 4 Garlicon f 20mL.

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