

Lysozyme, an powerful protein supplement for prevention of brood diseases and stimulation of colony development: a report from large scale laboratory-to-field trials on the Canary Islands and Spain

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Background

Honeybee diseases, are a constant threat to the fitness of colonies. While the extensive study of Varroa has yielded many treatments, the damage to colony development from subclinical brood diseases has long been underestimated. Long before outbreaks are visible, the presence of various pathogens is inhibiting the development and reducing the performance of honeybee colonies. Yet, aside from largely prohibited antibiotics, there are no effective solutions available for protecting colonies from common brood diseases, such as Chalkbrood, American- and European foulbrood.

Problem Description

Here we present data from laboratory and in large field trials on the use of egg lysozyme, a natural antibacterial and anti-fungal enzyme, which improves the hygiene around the brood nest and stimulates colony development.

Methodology

Lysozyme has been previously reported by Van Haga et al. (J. Economic Entomol. 2012) for its effectiveness in the treatment of chalkbrood in field trials in Canada. We have undertaken extensive artificial larval rearing experiments to study the effects of Lysozyme in larval food for the treatment of common brood diseases (Chalkbrood, American- and European Foulbrood. Field trials for colony development were conducted in Spain, Romania and Italy, using a commercially available liquid formulation "ApiForte". Currently a large multi-year field trial is ongoing in apiaries, where there have been recent foulbrood outbreaks, on the Canary Islands. Honey samples of nearly two hundred colonies located in these apiaries were taken to quantify the presence of foulbrood spores and disease incidence. Thereafter, a one-year treatment regime consisting in three applications of ApiForte was initiated and spore counts, disease incidence and colony development measured for one year.

Results and Conclusions

Lysozyme has emerged as a natural, effective and powerful tool to prevent and treat chalkbrood and foulbrood infections under field conditions. The product stimulates colony development with visible effects after 4 weeks and reduces disease incidence.

Practical Implications for Beekeepers

Systematic field trials in several countries have shown the positive effect of supplementation with this protein ingredient and confirm prior studies from North America.

Lysozyme provides the beekeepers with a real tool to increase the fitness and resilience of their colonies.

**LYSOZYME, A POWERFUL PROTEIN SUPPLEMENT
FOR PREVENTION OF BROOD DISEASES AND
STIMULATION OF HONEYBEE COLONY
DEVELOPMENT: A REPORT FROM LABORATORY
TESTS TO FIELD EXPERIENCES**



Lysozyme

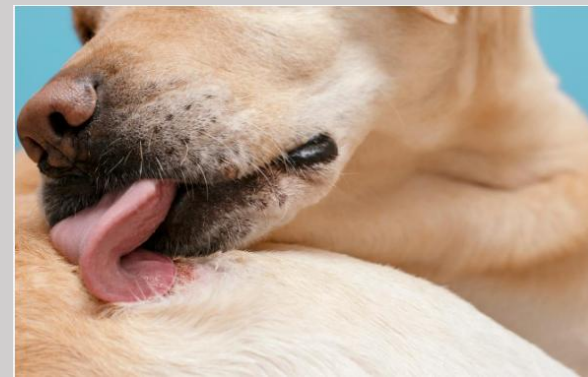
Lysozyme is a protein enzyme that exhibits a **strong antimicrobial activities against bacterial, fungal, and viral pathogens**. It protects against infections acting as a natural antibiotic

In the human body: Lysozyme is a key component of the innate immune system, present in tears, saliva, mucus, sweat and milk

Current applications:

Food preservation: used as a natural preservative to extend the shelf life of products like meat, fish, cheese, wine, vinegar, dairy... (food additive **E1105**)

Pharmaceutical and medical uses: due to its antibacterial and antiviral properties it is used to treat various inflammatory and infectious conditions, including chronic sinusitis, respiratory diseases, sore throats and gum inflammation. Also used as a component of certain drugs (e.g. combination with other medications for HIV to reduce virus levels)



Research with lysozyme

First investigations were carried out at the University of Graz (Austria) between 2012-2014

- Lysozyme was initially tested against American Foulbrood and European Foulbrood in liquid and solid cell cultures.
- It showed a strong antibacterial activity against *Paenibacillus larvae* (AFB) and *Melissococcus plutonius* (EFB). *MIC* was determined
- We moved to artificial larval rearing tests where honeybee larvae were exposed to an infection of foulbrood in the laboratory

The team in Graz



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Freiburg (Brsg.), PhD in Pharmacognosy (ETH Zurich),
Postdoc in USA, drug design based on natural products,
Analytics of active ingredients and pesticide residues in honeybees

 Dr. Ulrike Riessberger-Gallé, zoologist, PhD on American
Foulbrood (KFU Graz), Establishment of artificial larval rearing in
Graz, beekeeper

 Dr. Javier Hernández López,
molecular biologist
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PhD in honeybee immunology and bee
diseases (Graz)



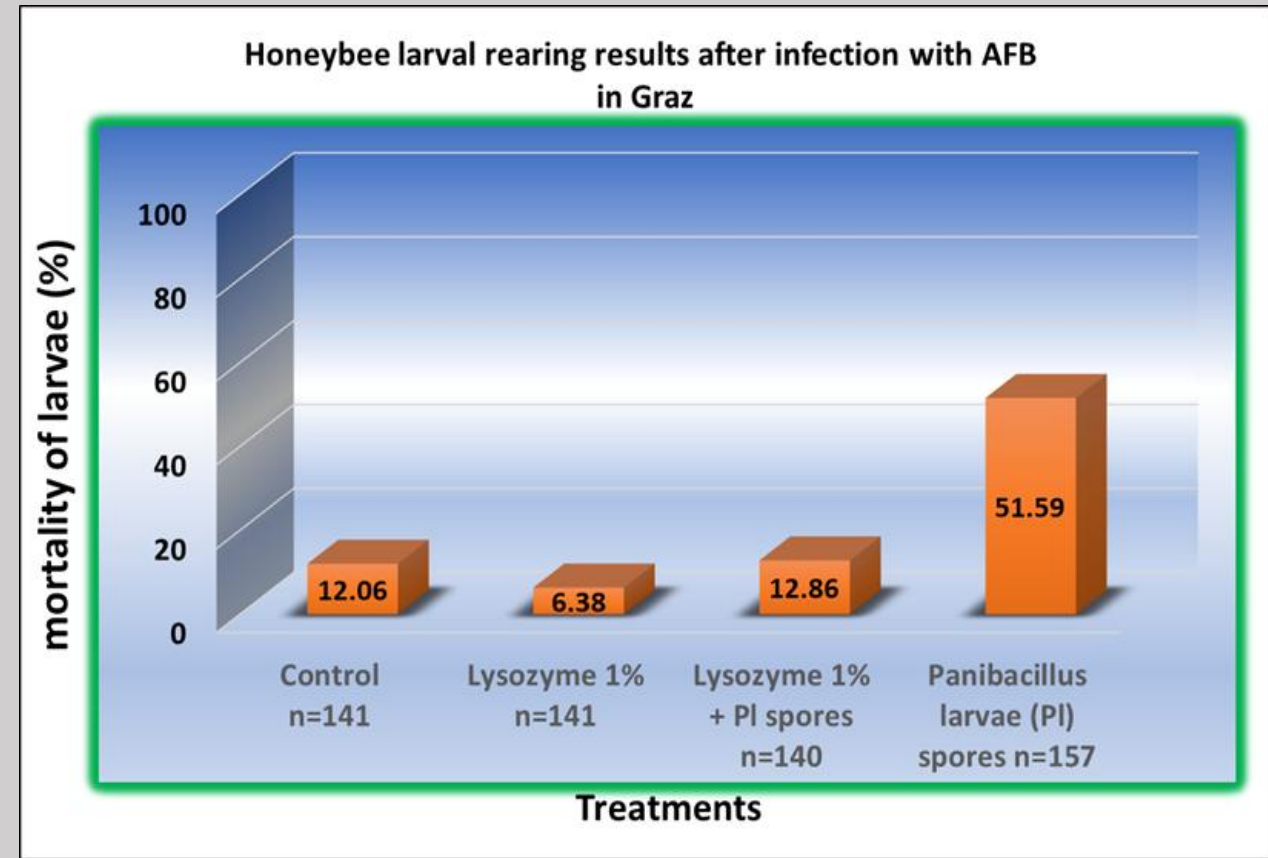
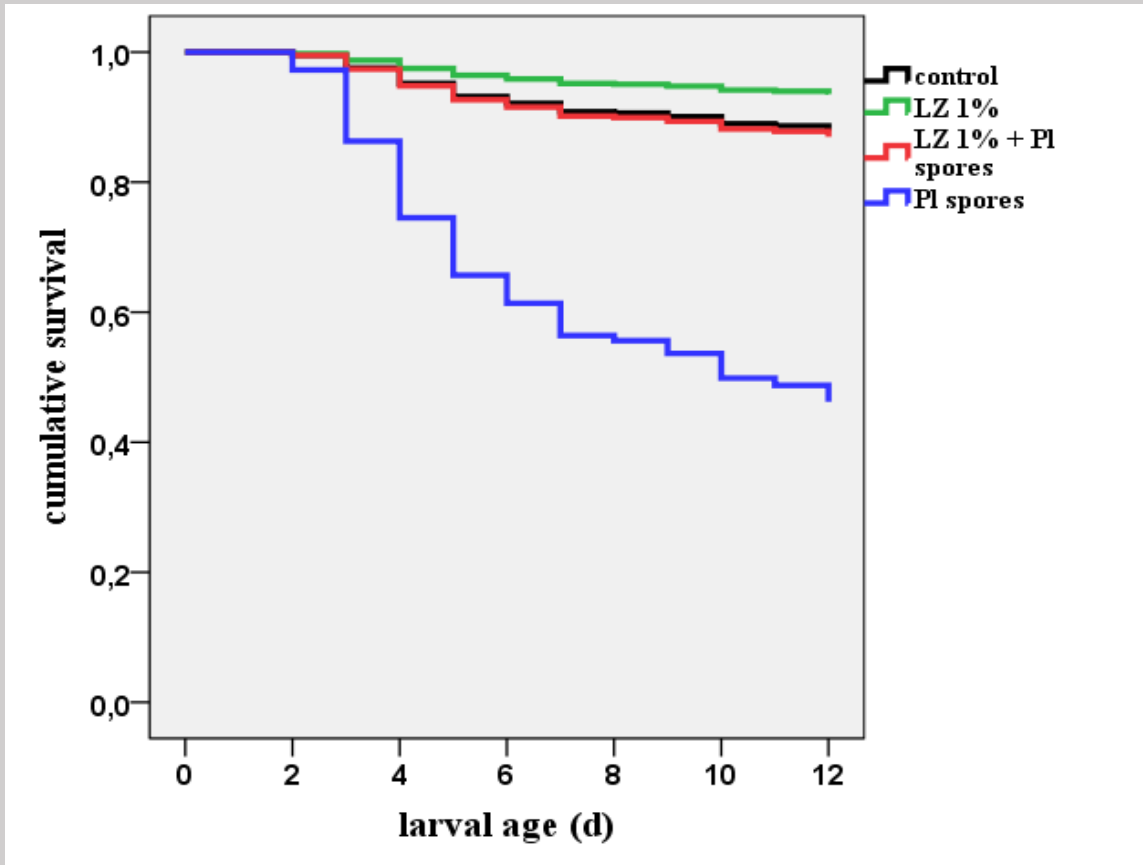
Research with lysozyme

The artificial larval rearing method for honeybees



Research with lysozyme

The artificial larval rearing method for honeybees: results with AFB infections



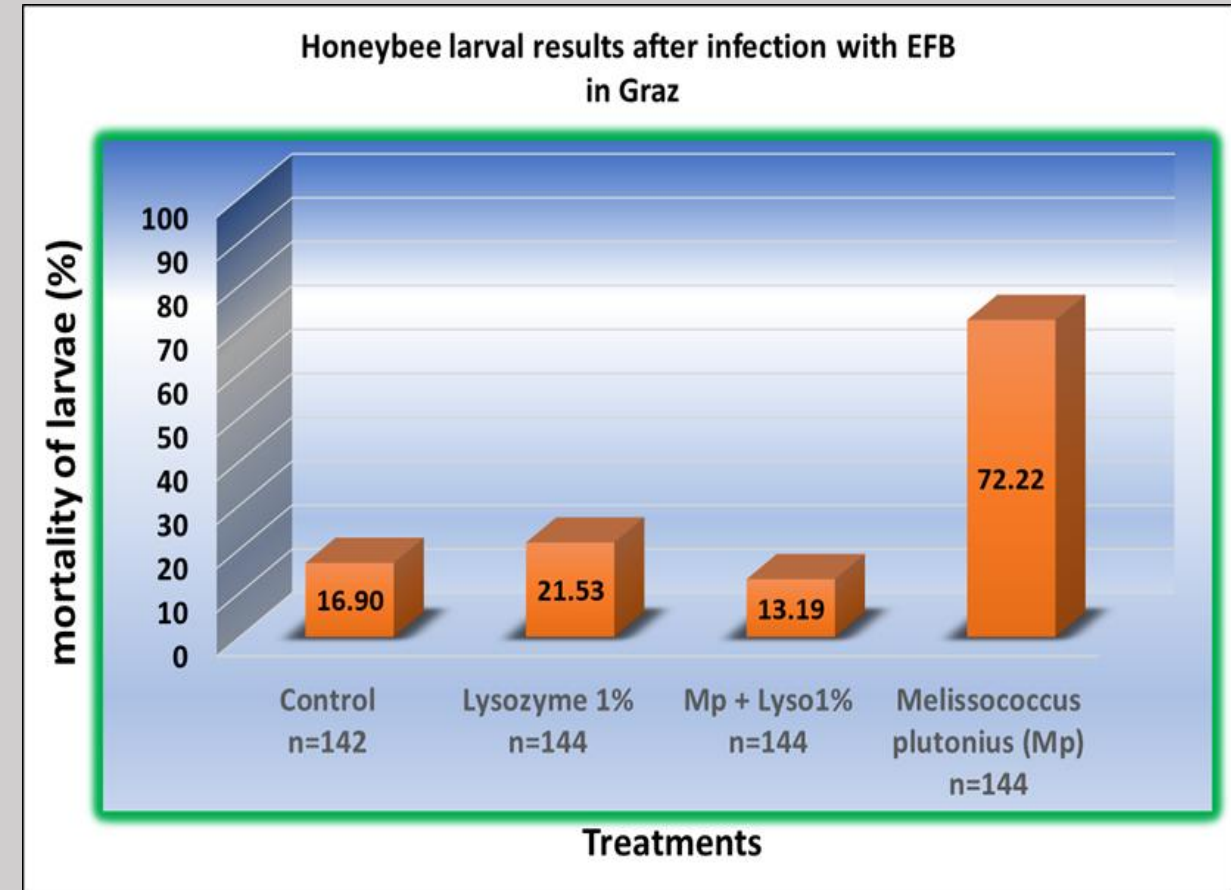
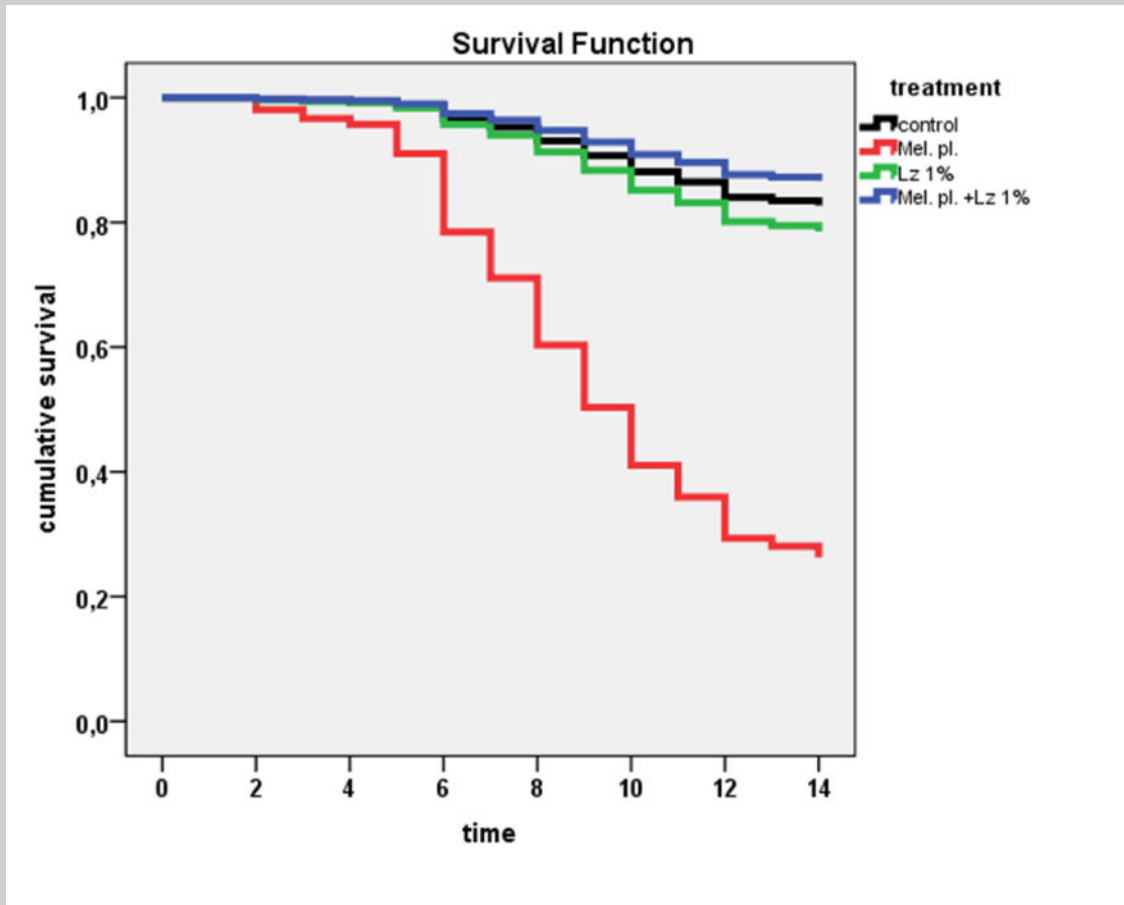
Research with lysozyme

The artificial larval rearing method for honeybees: results with lyso alone or in combination with AFB spores

Treatment	Control	Lysozyme 1,5%	Lyso 1%	Lyso 0,75%	Lyso 1,5% + PI EII	Lyso 1% + PI EII	Lyso 0,75%+ PI EII	PI EII 233/00 spores
Total larvae	141	141	141	139	139	140	140	157
dead larvae day1	0	0	0	0	0	0	0	0
dead larvae day2	1	1	0	0	3	0	0	5
dead larvae day3	0	0	0	0	0	0	4	24
dead larvae day4	2	0	0	0	0	2	6	22
dead larvae day5	1	0	0	0	2	4	2	15
dead larvae day6	2	0	0	0	1	3	8	5
dead larvae day7	2	0	2	0	3	5	2	3
dead larvae day8	1	0	0	0	1	1	0	0
dead larvae day9	2	2	1	1	2	2	0	0
dead larvae day10	4	3	1	0	1	1	1	4
dead larvae day11	0	1	2	3	0	0	2	1
dead larvae day12	2	2	3	2	2	0	2	2
dead larvae day13	0	0	0	0	0	0	0	0
Total mortality	17	9	9	6	15	18	27	81
% mortality	12.06	6.38	6.38	4.32	10.79	12.86	19.29	51.59

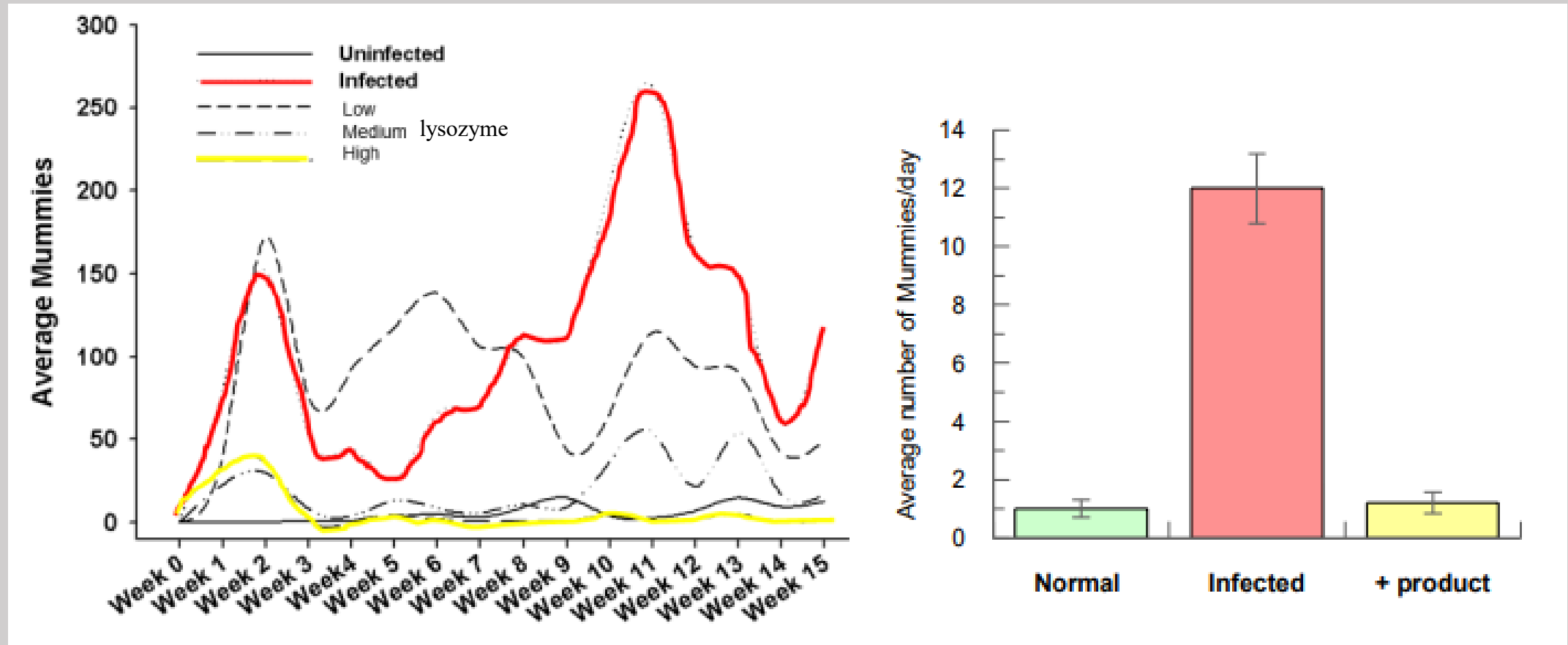
Research with lysozyme

The artificial larval rearing method for honeybees: results with EFB infections



Research with lysozyme

Field experiment where honeybee colonies were exposed to an **infection of chalkbrood** (*Ascosphaera apis*) in Canada (Ash Apiaries) in 2016



Research with lysozyme

Investigations in Gran Canaria

- We had 16 reported cases of American Foulbrood outbreaks in different apiaries with over 230 colonies between 2023 and 2024
- We designed a plan to prophylactically feed all colonies with lysozyme (Apiforte, commercial product) for three times for the following 12-15 months
- Before feeding Apiforte we took honey samples of 200 colonies, each sample consisted in a pool of honey from different combs



Research with lysozyme

- During the visit to an apiary that had suffered an AFB outbreak the previous season, we detected 6 colonies with chalkbrood and one new outbreak of AFB
- We took samples of AFB diseased larvae and nearby honey
- After inspecting the apiary we treated all colonies with Apiforte



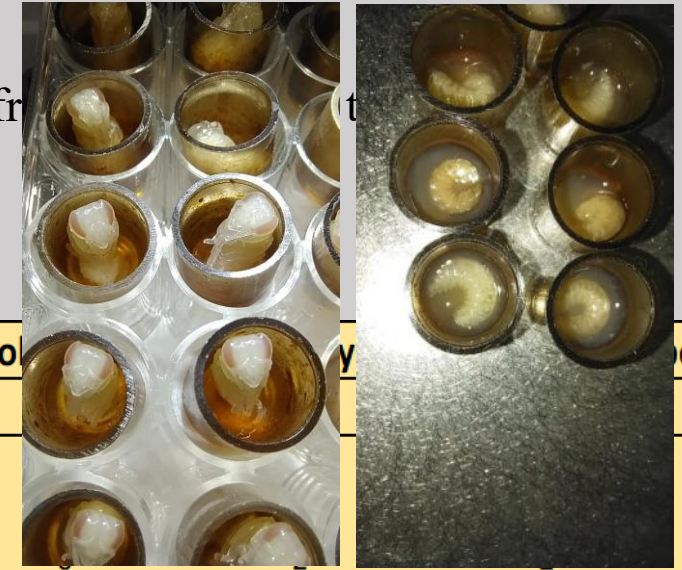
Research with lysozyme

- A rapid field test confirmed an outbreak of AFB against EFB in the laboratory of the IUSA Institute (Vet University) in Gran Canaria
- We later cultured and isolated *Paenibacillus larvae* (**Pl**) bacteria from the field samples in MYPGP-agar
- Spores of Pl were cultured in slunt agar
- We plated all honey samples to determine the presence/absence of Pl spores.

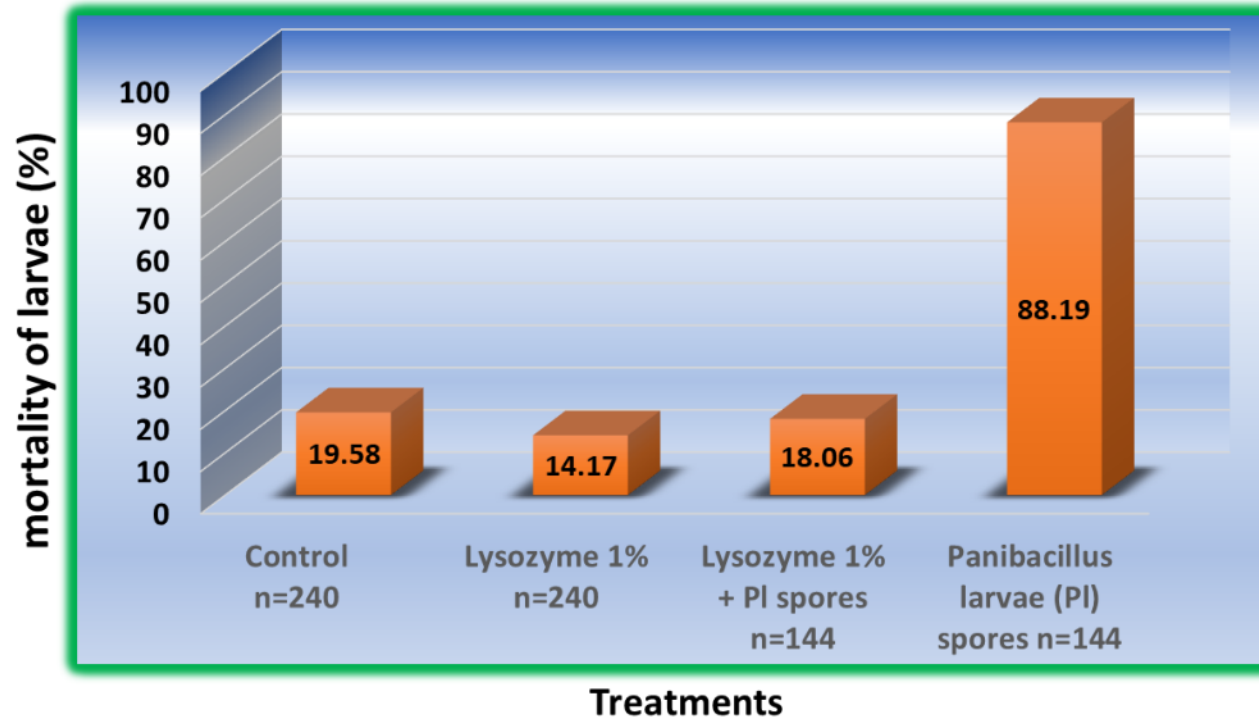


Research with lysozyme

- We carried out the artificial honeybee larval rearing method
- We infected honeybee larvae with a high dose of spores of the *Pl* strain isolated from near 90% mortality.
- We co-exposed a group of larvae to the same dose of AFB and lysozyme



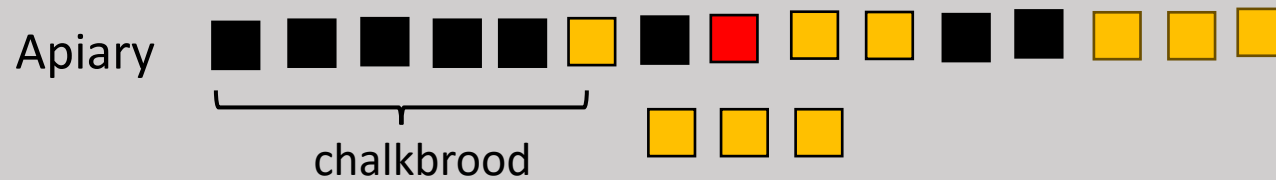
Honeybee larval rearing results after infection with AFB
in Gran Canaria



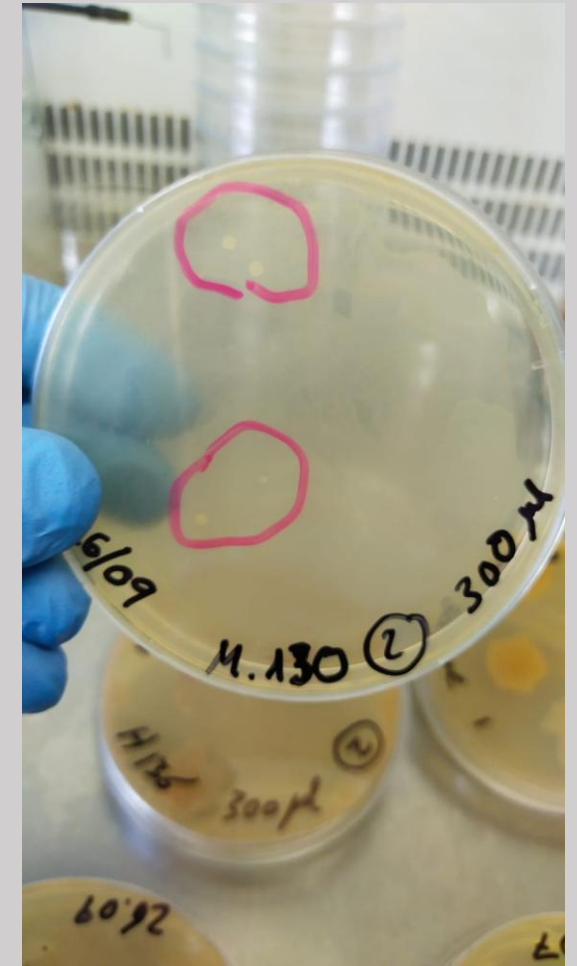
Treatment	Control	Lysozyme 1%	Lysozyme 1% + PI spores	Panibacillus larvae (PI) spores
Total larvae	240	240	144	144
dead larvae day1	0	0	0	0
dead larvae day2	5	5	5	5
dead larvae day3	1	1	1	1
dead larvae day4	1	1	1	1
dead larvae day5	1	1	1	1
dead larvae day6	1	1	1	1
dead larvae day7	1	1	1	1
dead larvae day8	1	1	1	1
dead larvae day9	1	1	1	1
dead larvae day10	1	1	1	1
dead larvae day11	1	1	1	1
dead larvae day12	1	1	1	1
dead larvae day13	1	1	1	1
dead larvae day14	1	1	1	1
dead larvae day15	1	1	1	1
dead larvae day16	1	1	1	1
dead larvae day17	1	1	1	1
dead larvae day18	1	1	1	1
dead larvae day19	1	1	1	1
dead larvae day20	1	1	1	1
dead larvae day21	1	1	1	1
dead larvae day22	1	1	1	1
dead larvae day23	1	1	1	1
dead larvae day24	1	1	1	1
dead larvae day25	1	1	1	1
dead larvae day26	1	1	1	1
dead larvae day27	1	1	1	1
dead larvae day28	1	1	1	1
dead larvae day29	1	1	1	1
dead larvae day30	1	1	1	1
Total	240	240	144	144
% m	4.17	4.17	6.94	6.94

Research with lysozyme

- Out of the 200 honey samples analyzed 28 tested positive for AFB spores
- In one particular apiary with an outbreak detected (red square), we had 6 out of 33 colonies near the infected colony testing positive for AFB spores



- 10 days after application of lysozyme no clinical symptoms of infections were observed
- We are currently analysing new honey samples taken after completing three applications of Apiforte in 200 monitored colonies
- We have not detected new outbreaks of AFB nor chalkbrood in any of the monitored colonies



The company, the product and the distributors








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ApiForte es un pienso complementario para abejas que favorece el desarrollo de un cría sana y fuerte..

ApiForte es basa en un extracto natural de proteínas con una composición de aminoácidos similar a la jalea real ideal para la nutrición de las abejas. Nuestras investigaciones han demostrado que algunas proteínas naturales no sólo tienen un alto valor nutritivo, sino que también realizan una función higienizante dentro de las celdillas de la cría y de las celdas reales.

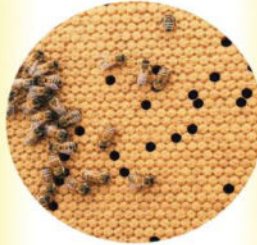
Cuando nutrir con ApiForte:
-Al principio de la primavera, con el pienso de estimulación, añada **ApiForte** para un desarrollo óptimo de la colonia.
-Al final del verano/otoño, la adición de **ApiForte** al jarabe de azúcar utilizado para la nutrición invernal asegura el nacimiento de un mayor número de abejas y más sanas garantizando colmenas libres de enfermedad y fuertes para la próxima primavera.

Instrucciones de uso: por cada colmena diluir 25-50 ml de ApiForte en al menos 250 ml de jarabe de azúcar. Mezclar bien y administrar a la colmena. Si es necesario, repetir una segunda vez al cabo de 2 semanas. La mezcla no puede superar un máximo de 40° C.

Almacenamiento: temperatura ambiente. Conservar en un lugar fresco y seco, no congelar.



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Pienso complementario para sus colmenas

Composición:
 Agua, fructosa, proteínas extraídas de clara de huevo, sorbato potásico

Lot Nr.: *vea la pegatina*

Utilizar preferentemente antes de: *vea la pegatina*

Componentes analíticos:
 Proteína bruta 25%,
 fructosa 18%, agua 52%

N° de producto:
 ITa000033RO

Distribuido por:

 PASIÓN POR LA APICULTURA

Pol. La Concepción,
 Ctra. NII, km 437,
 22520, Huesca,
 Spain



In other EU countries

EUROHONIG®

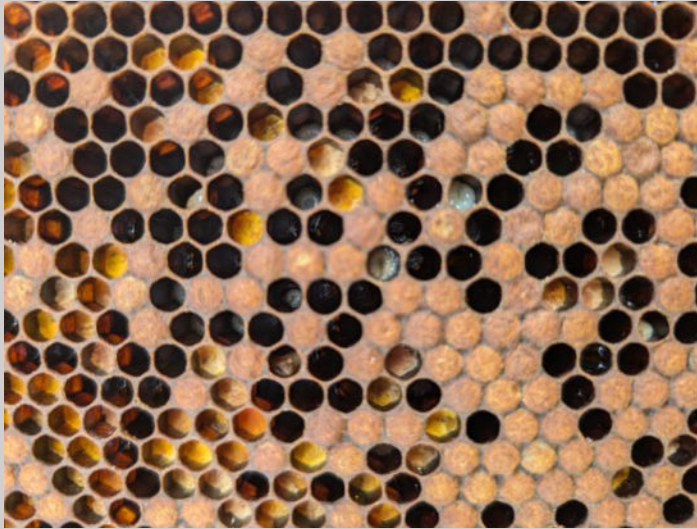
In Spain and Portugal

BIOPYC
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Field experiences of Spanish beekeepers

A case of EFB



3 weeks
after
feeding
Apiforte



Field experiences of Spanish beekeepers

A case of chalkbrood in Burgos



A case of chalkbrood in Guadalajara under supervision of the local veterinarian



To conclude

- The protein lysozyme has a well-known **antibacterial** and **antifungal** activity
- It showed a **strong inhibition** of **AFB** and **EFB bacterial growth** in several laboratory tests (from liquid cultures to larval rearing tests)
- It showed a **very positive effect** when used **to treat apiaries with chalkbrood infections**
- As a result of these investigations a commercial food additive for honeybee colonies was developed (**Apiforte**)
- It has a potent hygienic effect in brood areas, it helps to prevent and treat honeybee brood diseases
- It can be used for queen breeders to boost production of queen cells (results not shown)

Thank you!

EuroHonig Booth B8

