

INNODAL
innocuity · naturally

**Preventing the Risk of *Listeria*
with the use of Bacteriocins**

Introduction

Listeria monocytogenes

Listeria monocytogenes is a ubiquitous bacteria that is primarily found in feces, wastewater, soil and contaminated food. It can cause listeriosis, a serious foodborne illness, when consumed on foods containing over 10^3 CFU/g. *Listeria* can survive and grow in a wide range of conditions, including refrigeration temperatures, low pH and high salt concentrations, making it a persistent challenge for food safety.

Listeriosis can cause severe complications such as septicemia or meningoencephalitis and may be fatal. Populations most at risk include pregnant women, individuals over 60 years old and those with weakened immune systems.

According to the CDC, *Listeria* infections result in hospitalizations for about 94% of case and a 20-30% case fatality rate, making it one of the most severe food borne illnesses.



Introduction

The relevance of developing a processing aid against a microbiological target such as *Listeria monocytogenes* is crucial. This microorganism has the highest mortality rate amongst foodborne bacteria in North America. Despite huge repercussions of recalls for the food industry, the number is still high. The cost of a recall for a company is estimated at 10M\$. This estimation does not consider that 55% of consumers will change brands, resulting in significant loss of revenues for the company.

Biological Contamination	Class I	Class II	Class III	Total	%
<i>Listeria monocytogenes</i>	6,906	938		7,844	46%
<i>Salmonella serovas</i>	6,166	428	3	6,597	39%
<i>Escherichia coli</i>	457	174	24	655	4%
<i>Clostridium botulinum</i>	131	217	7	355	2%
Mold		142	84	226	1%
Other	73	1105	223	1139	8%
Total	13,733 80%	3,004 18%	341 2%	17,078	100%

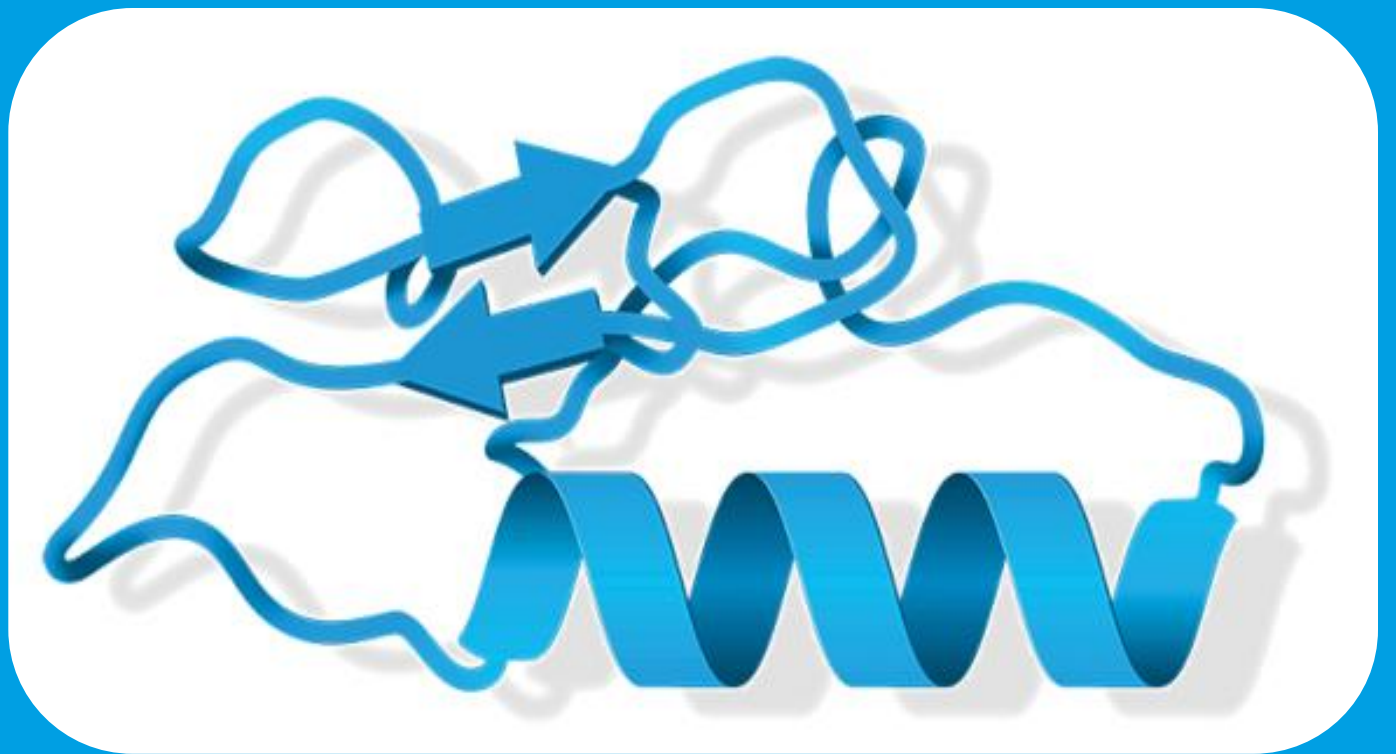
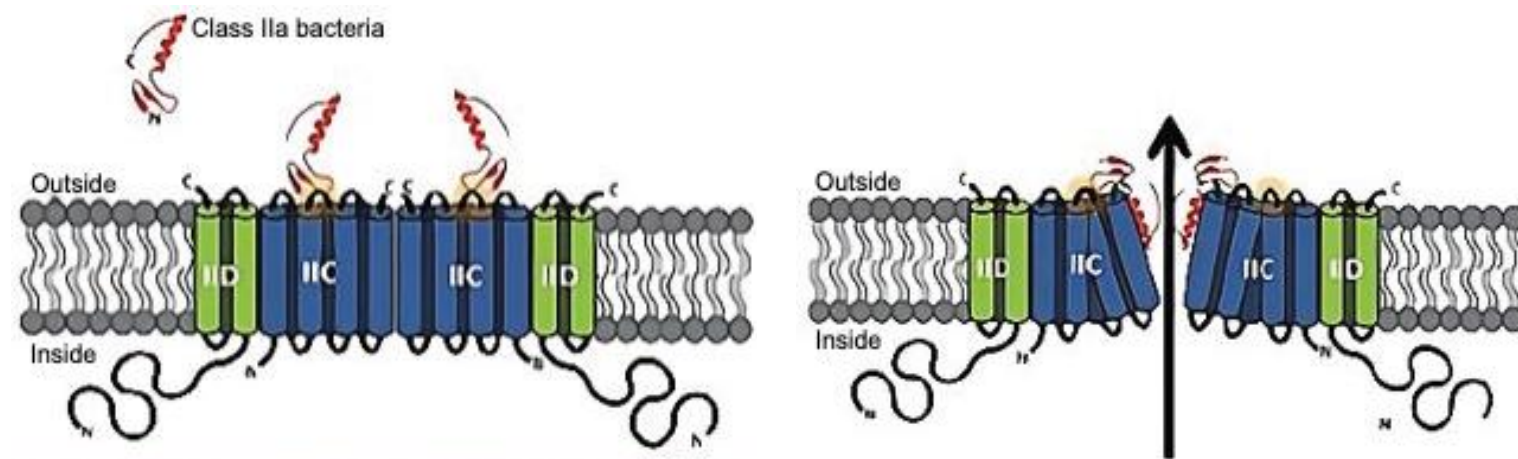
❖ Between 2002-2023, *Listeria* has been the leading cause of food & beverage recalls in the U.S. accounting for 22% of all recalls.

❖ Of these foods recalled, over 60% were processed in some form and almost 70% were chilled or frozen products

INNEO

Characteristics

INNEO (active ingredient pediocin) is a small peptide that inhibits the growth of multiple bacteria. Our laboratory has demonstrated the efficacy of this peptide against ***Listeria monocytogenes***. Pediocins mechanism of action is to destroy bacteria cells by permeabilizing their cell membrane. The molecule has an immediate effect on the pathogenic bacteria. INNEO is a natural solution, given that pediocin is broken down into amino acids by proteases present in food. This characteristic gives INNEO the status of a processing aid.



PEDIOCIN

Pediocin is a protein composed of 44 naturally occurring amino acids. Inneo has the same molecular structure as PA-1 pediocin, except for one amino acid that has been replaced to give the molecule more stability. Pediocin is a bacteriocin produced naturally by lactic bacteria found on foods.

How can INNEO help?

Key Benefits

INNEO offers several key advantages over chemical preservatives. It is a natural solution that is 1,000 times more effective than chemical alternatives, ensuring superior protection. As a clean-label processing aid, it doesn't need to be listed as an ingredient, aligning with consumer preferences. Additionally, its versatile application methods allow for easy integration into existing processes, while its proven effectiveness helps reduce food contamination and related illnesses. By minimizing product recalls, it also helps cut down on food waste and supports broader sustainability goals.

Additional Features

- ❖ Halal / Kosher / Organic Certified
- ❖ Custom made dosage for your operation
- ❖ Functional between 3-8 pH
- ❖ Withstands water hardness up to 400 ppm
- ❖ Shelf life of 2 year at 4C/39F
- ❖ No temperature limitations (can be frozen and supports 212F for 1 hour)



Effective



Natural



Clean Label

Product Application

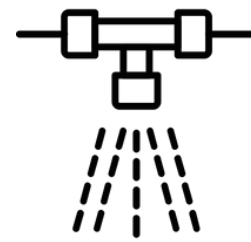
Versatile application methods

INNEO is engineered for versatility and adaptability, making it suitable for a wide range of processing methods and operation sizes. Its application can be customized to target key points within your workflow to ensure maximum coverage and cost-efficiency. During discovery meetings and on-site evaluations, our technical team identifies the most effective application point to address your highest-risk areas. With no minimum contact time required, **INNEO** offers a highly efficient solution for enhancing food safety. It also provides a residual antimicrobial effect, offering continued protection through packaging and into the product's shelf life



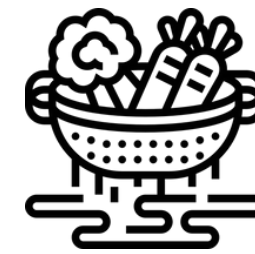
Mixed in Product

Add INNEO directly into your recipe to ensure full coverage. Ideal for products with no defined surface such as sausages, dips, sauces.



Spray

Apply INNEO as a spray application onto products before packaging or freezing. Ideal for meat and seafood with defined surfaces.



Soaking bath

Add INNEO into the water of your products washing bath. Ideal for leafy greens, cut fruits & vegetables and seafood in brine.

Visit our YOUTUBE PAGE
to view the various application
methods that we offer





CHARACTERISTICS

Spectrum of Activity

Pediocin has demonstrated specific spectrum of activity against the bacteria listed below. The minimal inhibitory concentration (MIC) of Inneo against these pathogens is significantly lower than that of antibiotics for the same strains. **Listeria monocytogenes**, for example, is up to 1,000 times more sensitive to Inneo than to a common antibiotic. Additionally, Inneo—and bacteriocins in general—offer a promising alternative for combating multi-drug resistant pathogens. The rising prevalence of antibiotic resistance is a key reason why food processors aim to eliminate all traces of antibiotics throughout the food processing chain.

Strains	Reference	MIC (nM)
<i>Listeria ivanovii</i>	HPB 28	1.7
<i>Leuconostoc mesenteroides</i>	ATCC 23386	1.9
<i>Carnobacterium divergens</i>	ATCC 35677	1.9
<i>Listeria seeligeri</i>	ATCC 35967	4.7
<i>Listeria monocytogenes</i>	ATCC 19111	13.5
<i>Clostridium perfringens</i>	ACC 1-222	37.8
<i>Listeria murrayi</i>	ATCC 25401	151.4
<i>Lactobacillus plantarum</i>	ATCC 8014	605.5

Proof of concept

Challenge test - anti-*Listeria* activity

To prove the efficiency of the Inneo on various types of food, challenge tests have been performed according to Health Canada guidelines ***Listeria monocytogenes*** Challenge Testing of RTE food, 2012. This experiment is a proof-of-concept to demonstrate the antimicrobial activity of Inneo against foodborne pathogen ***Listeria monocytogenes*** in a variety of food matrices.

All matrices were divided into four groups, representing different categories of food products. Each group was inoculated with a cocktail composed of three strains of ***Listeria monocytogenes*** specific for the group.

Group 1: Fish and Seafood

108 (Serotype 4b)
0051 (Serotype 1/2b)
237 (Serotype 1/2a)

Group 2: Poultry and Meat

108 (Serotype 4b)
DSM 19094 (Serotype 1/2a)
0051 (Serotype 1/2b)

Group 3: Fruit and Vegetables

108 (Serotype 4b)
0051 (Serotype 1/2b)
G1-02 (Serotype 1/2a)

Group 4: Cheese and other dairy

108 (Serotype 4b)
0051 (Serotype 1/2b)
ATCC 51772 (Serotype 1/2a)

Proof of concept

INNEO's efficacy on poultry

INNEO has been shown to be effective against *Listeria monocytogenes* in poultry. While Inneo is active for up to 24 hours, the positive effects persist for 4 days.

- **Food matrix:** Diced chicken breast
- **Inoculation level cfu/g :** 10^3
- **Expiration date :** 3 days
- **1.5x Expiration date :** 4 days
- **Storage conditions :** 4°C, aerobically

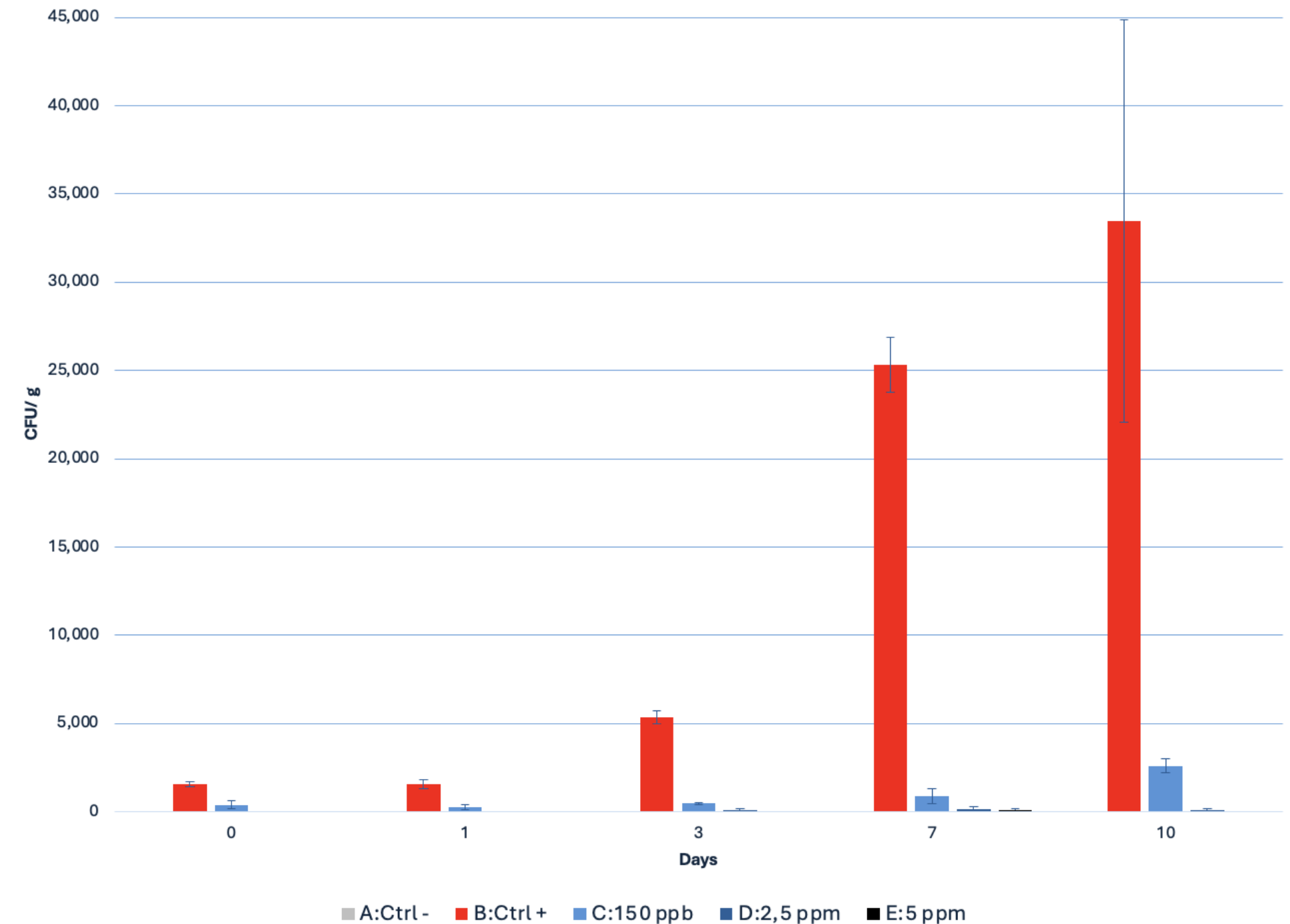


Figure 1 : Enumeration of *Listeria monocytogenes* on diced chicken breasts over 4 days at 4°C. Batch 1: negative control, batch 2: positive control, batch 3: 150 ng/g of Inneo and batch 4: 50 ng/g of Inneo treatment. ****=p-value < 0.0001.

Proof of concept

INNEO's efficacy on pork

INNEO has been shown to be effective against *Listeria monocytogenes* in pork. While Inneo is active for up to 24 hours, the positive effects persist for 7 days.

- **Food matrix:** Diced pork tenderloin
- **Inoculation level cfu/g :** 10^5
- **Expiration date :** 4 days
- **1.5x Expiration date :** 7 days
- **Storage conditions :** 4°C, aerobically

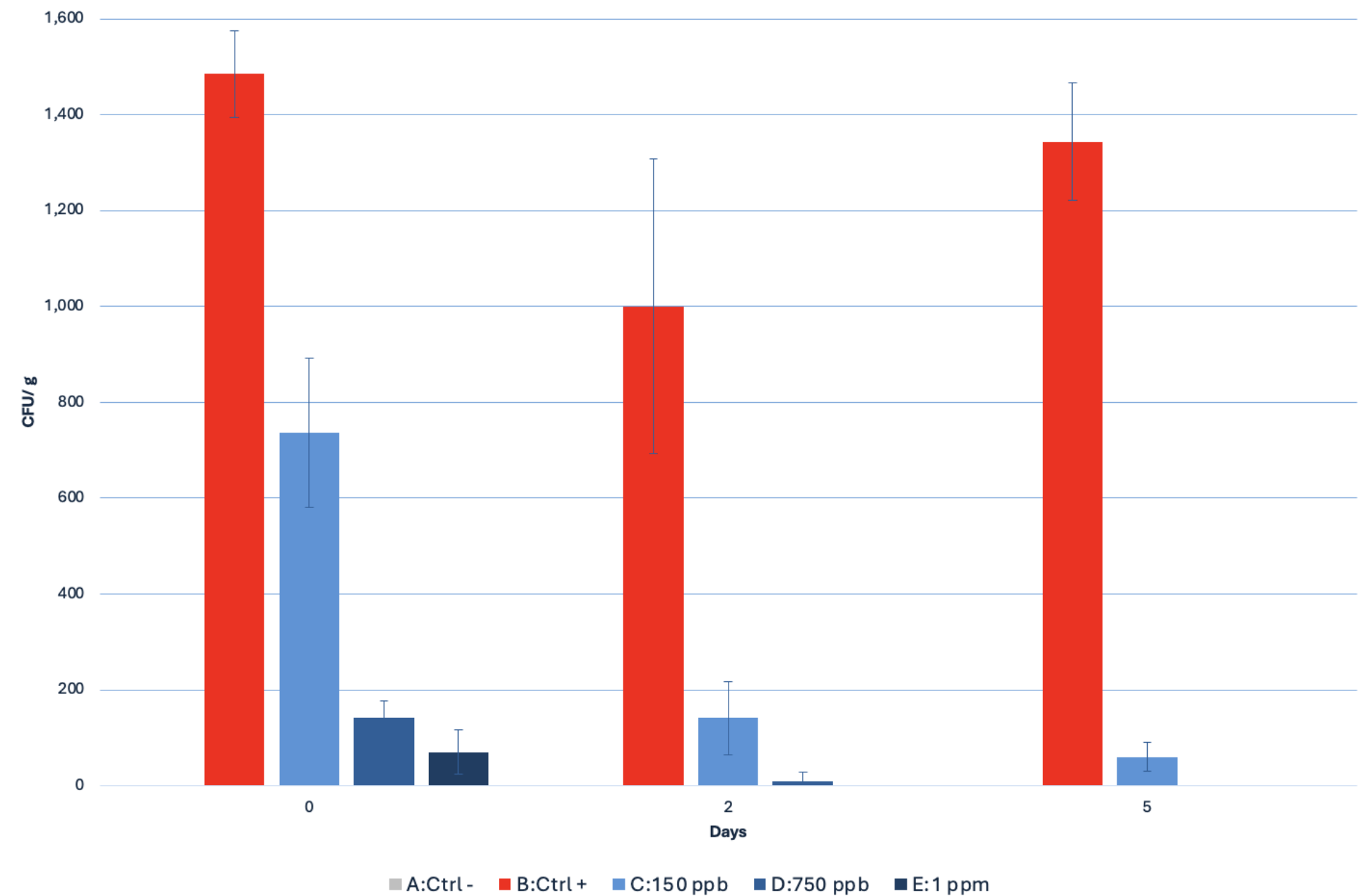


Figure 2: Enumeration of *Listeria monocytogenes* on diced pork tenderloin over 7 days at 4°C. Batch 1: negative control, batch 2: positive control, batch 3: 150 ng/g of Inneo treatment and batch 4: 50 ng/g of Inneo treatment. **** = p-value < 0.0001.

Proof of concept

INNEO's efficacy on beef

INNEO has been shown to be effective against ***Listeria monocytogenes*** in beef. While Inneo is active for up to 24 hours, the positive effects persist for 7 days.

- **Food matrix:** Diced beef
- **Inoculation level cfu/g :** 10^3
- **Expiration date :** 4 days
- **1.5x Expiration date :** 7 days
- **Storage conditions :** 4°C, aerobically

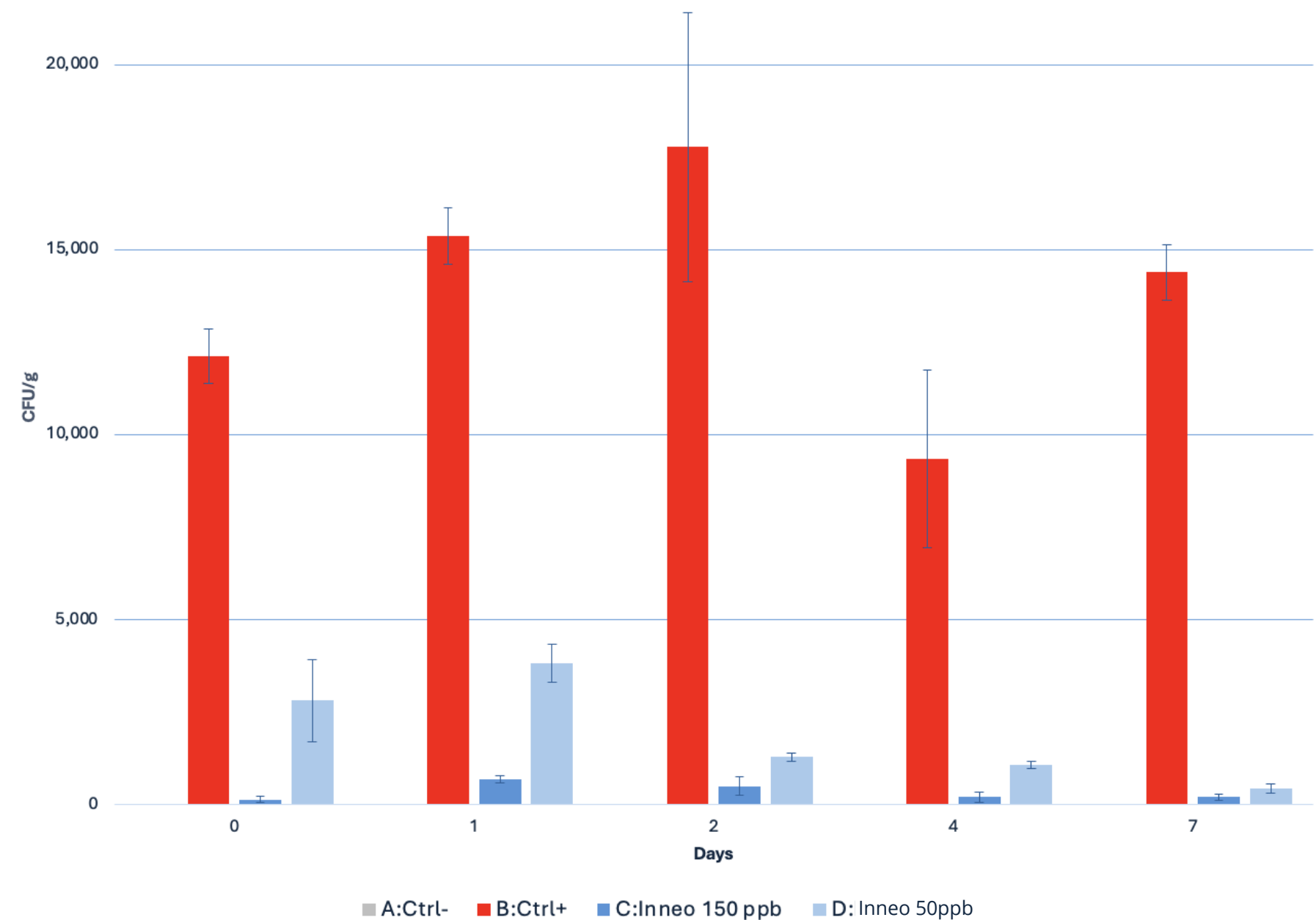


Figure 3: Enumeration of *Listeria monocytogenes* on diced beef eye round over 7 days at 4°C. Batch 1: negative control, batch 2: positive control, batch 3: 150 ng/g of Inneo treatment and batch 4: 50 ng/g of Inneo treatment. *** = p-value < 0.0001.

Proof of concept

INNEO's efficacy on salmon filet

INNEO has been shown to be effective against ***Listeria monocytogenes*** in fresh salmon filet. While Inneo is active for up to 24 hours, the positive effects persist for 7 days.

- **Food matrix:** Diced salmon file
- **Inoculation level cfu/g :** 10^3
- **Expiration date :** 4 days
- **1.5x Expiration date :** 7 days
- **Storage conditions :** 4°C, aerobically

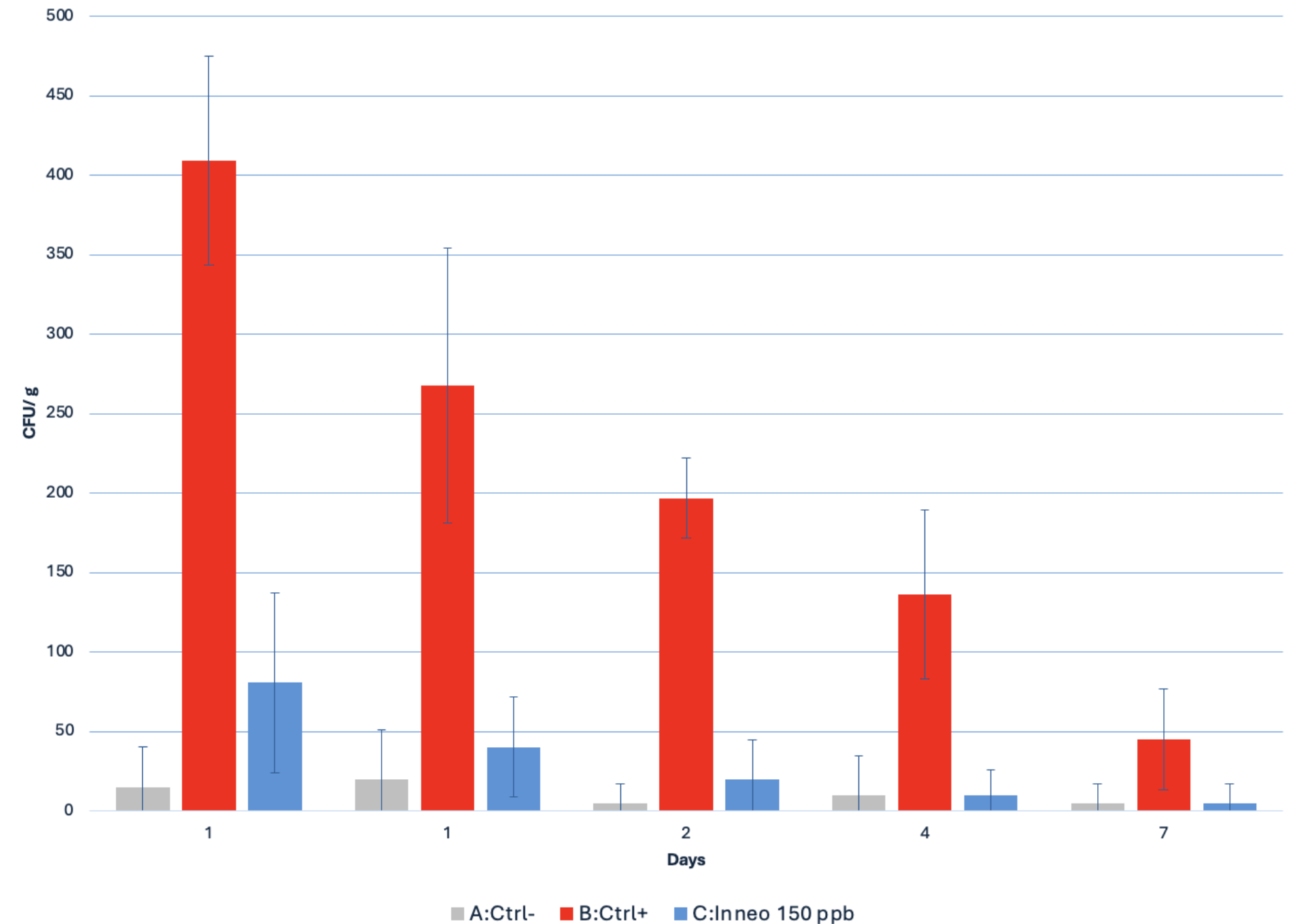


Figure 4: Enumeration of *Listeria monocytogenes* on diced fresh salmon filet over 7 days at 4°C. Batch 1: negative control, batch 2: positive control, batch 3: 150 ng/g of Inneo treatment and batch 4: 50 ng/g of Pediocin PA-1M31L treatment. **** = p-value < 0.0001.

Proof of concept

INNEO's efficacy on cold-smoked salmon

INNEO has demonstrated its effectiveness against ***Listeria monocytogenes*** on cold-smoked salmon. While Inneo is active for up to 24 hours, the positive effects persist for 21 days.

- **Food matrix:** Cold-smoked salmon
- **Inoculation level cfu/g :** 10^3
- **Expiration date :** 14 days
- **1.5x Expiration date :** 21 days
- **Storage conditions :** 4°C, vacuumed-packed

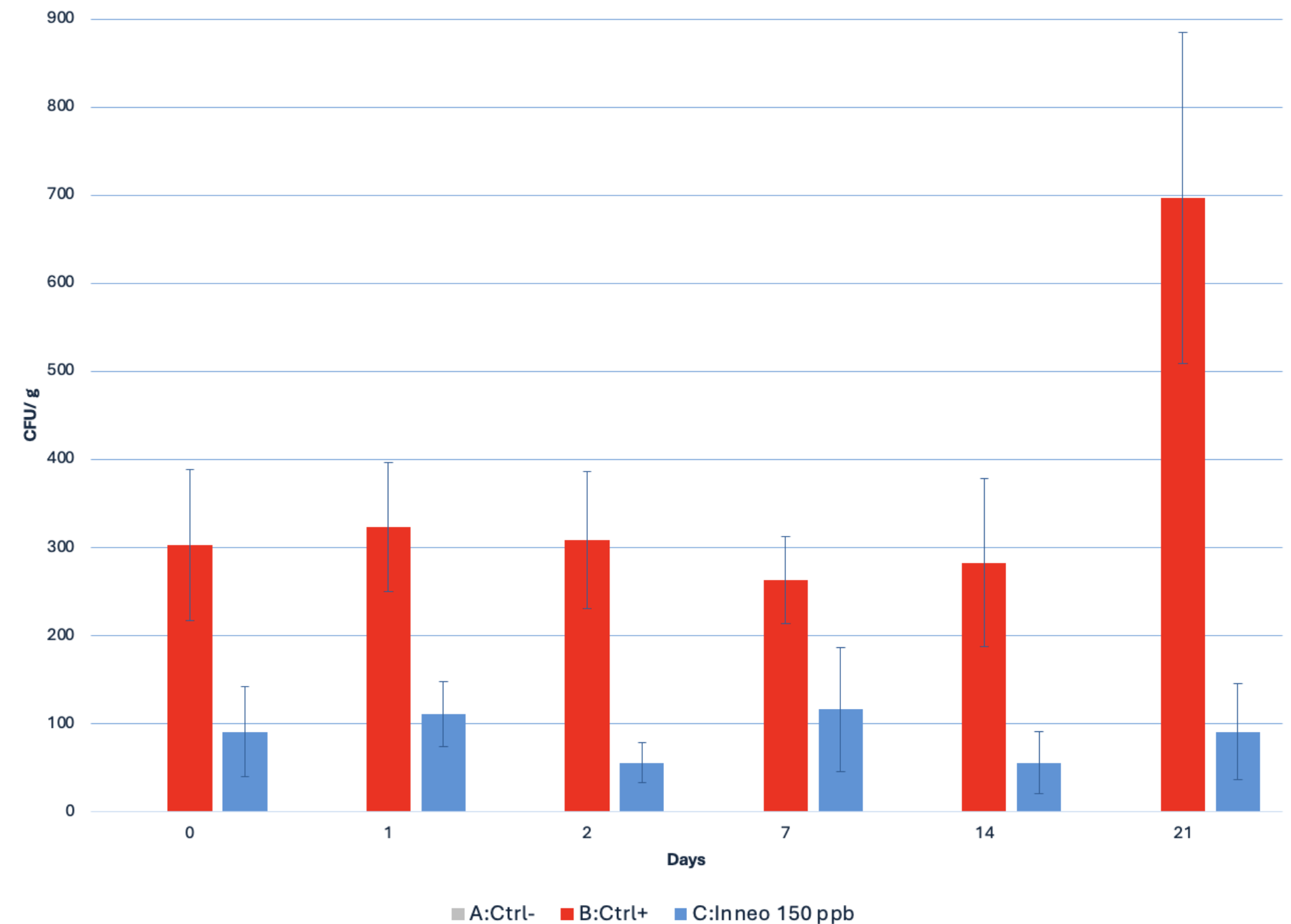


Figure 4: Enumeration of *Listeria monocytogenes* on diced fresh salmon filet over 7 days at 4°C. Batch 1: negative control, batch 2: positive control, batch 3: 150 ng/g of Inneo treatment and batch 4: 50 ng/g of Pediocin PA-1M31L treatment. **** = p-value < 0.0001.

Proof of concept

INNEO's efficacy on cheese curds

INNEO has demonstrated its effectiveness against *Listeria monocytogenes* on cheese. While Inneo is active for up to 24 hours, the positive effects persist for 4 days.

- **Food matrix:** Cheese curds
- **Inoculation level cfu/g :** 10^5
- **Expiration date :** 3 days
- **1.5x Expiration date :** 4 days
- **Storage conditions :** 4°C, aerobically

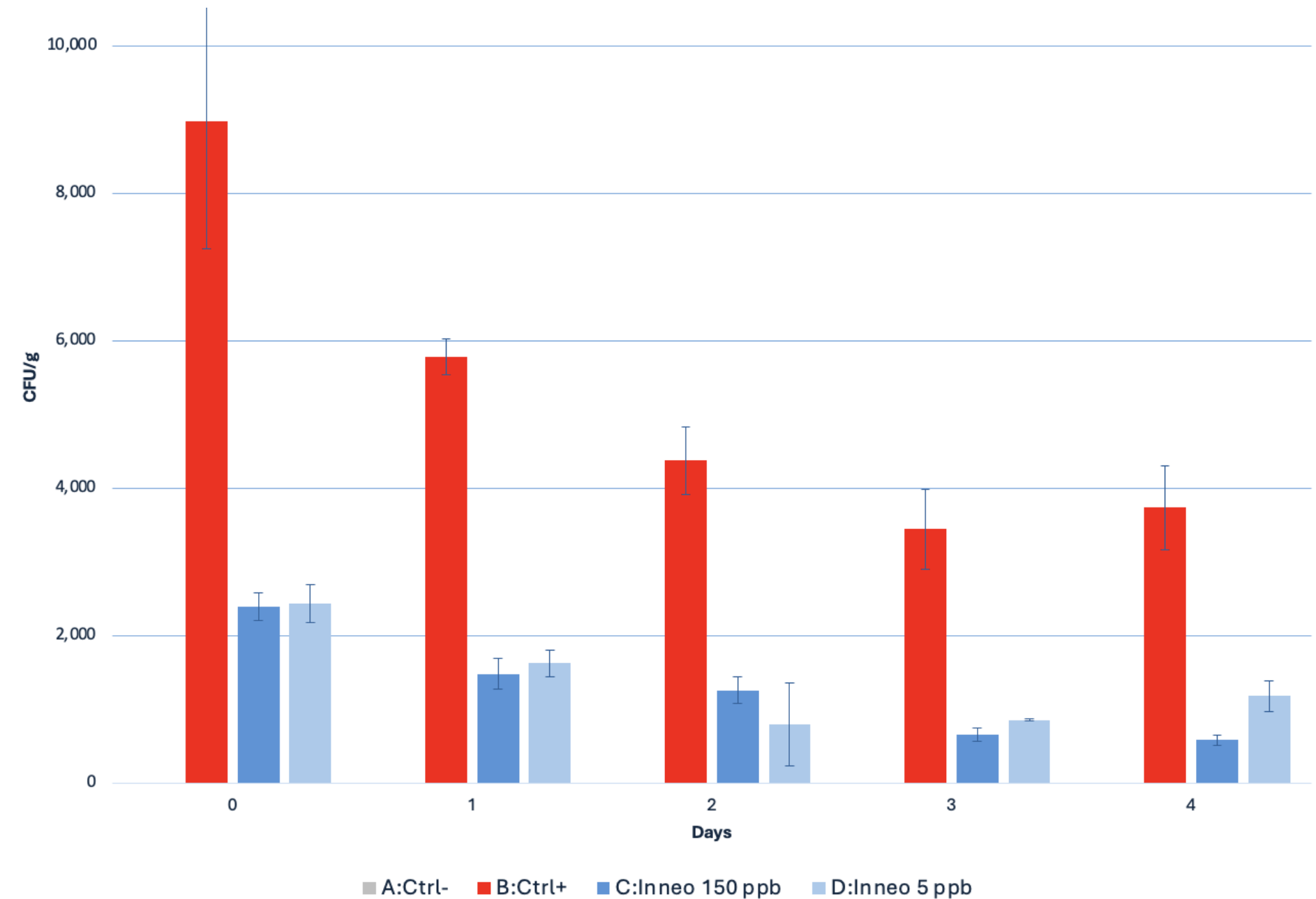


Figure 7: Enumeration of *Listeria monocytogenes* on cheddar cheese curds over 21 days at 4°C, vacuum-packed. Batch 1: negative control, batch 2: positive control, batch 3: 150 ng/g of Inneo treatment and batch 4: 50 ng/g of Inneo treatment. **** = p-value < 0.0001.

Proof of concept

INNEO's efficacy on mixed cut vegetables

INNEO has demonstrated its effectiveness against ***Listeria monocytogenes*** on mixed cut vegetables. While Inneo is active for up to 24 hours, the positive effects persist for 7 days.

- **Food matrix:** Mixed cut vegetables
- **Inoculation level cfu/g :** 10^5
- **Expiration date :** 7 days
- 1.5x Expiration date : 10 days
- **Storage conditions :** 4°C, aerobically

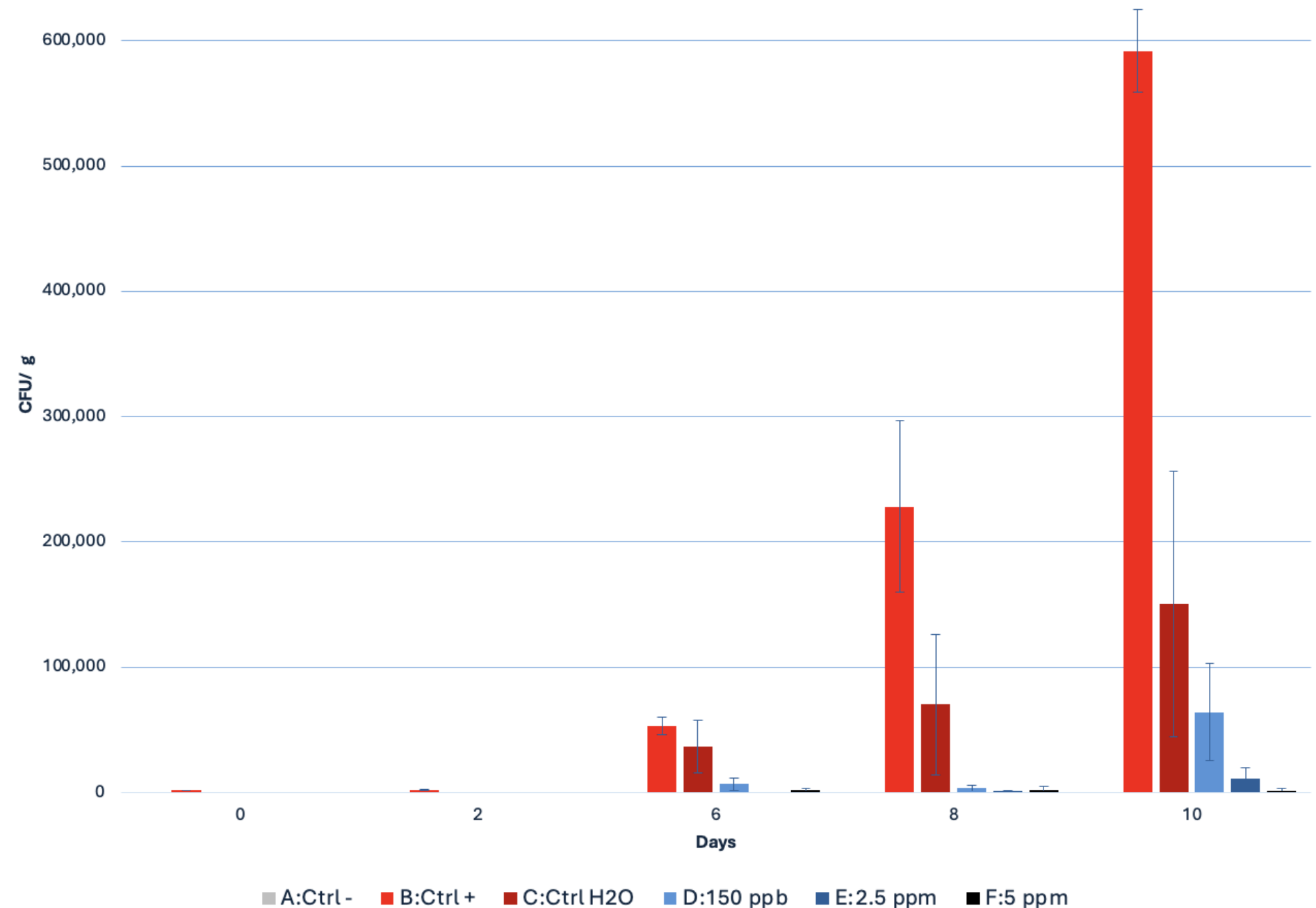


Figure 8: Enumeration of *Listeria monocytogenes* on mixed cut vegetables over 7 days incubation at 4°C. Batch 1: negative control, batch 2: positive control, batch 3: 150 ng/g of Inneo treatment and batch 4: 50 ng/g of Inneo treatment. **** = p-value < 0.0001.

Proof of concept

Supplementary data

This section contains supplementary data generated with Inneo through challenge tests. The results show that Inneo can reduce and control the amount of *Listeria monocytogenes* present on RTE chicken sausages. This highlights the potential of this technology to deliver safe products while utilizing a clean label alternative.

- **Food matrix:** RTE chicken wieners
- **Inoculation level cfu/g :** 10^3
- **Expiration date :** 2 days
- **1.5x Expiration date :** 3 days
- **Storage conditions :** 4°C, aerobically

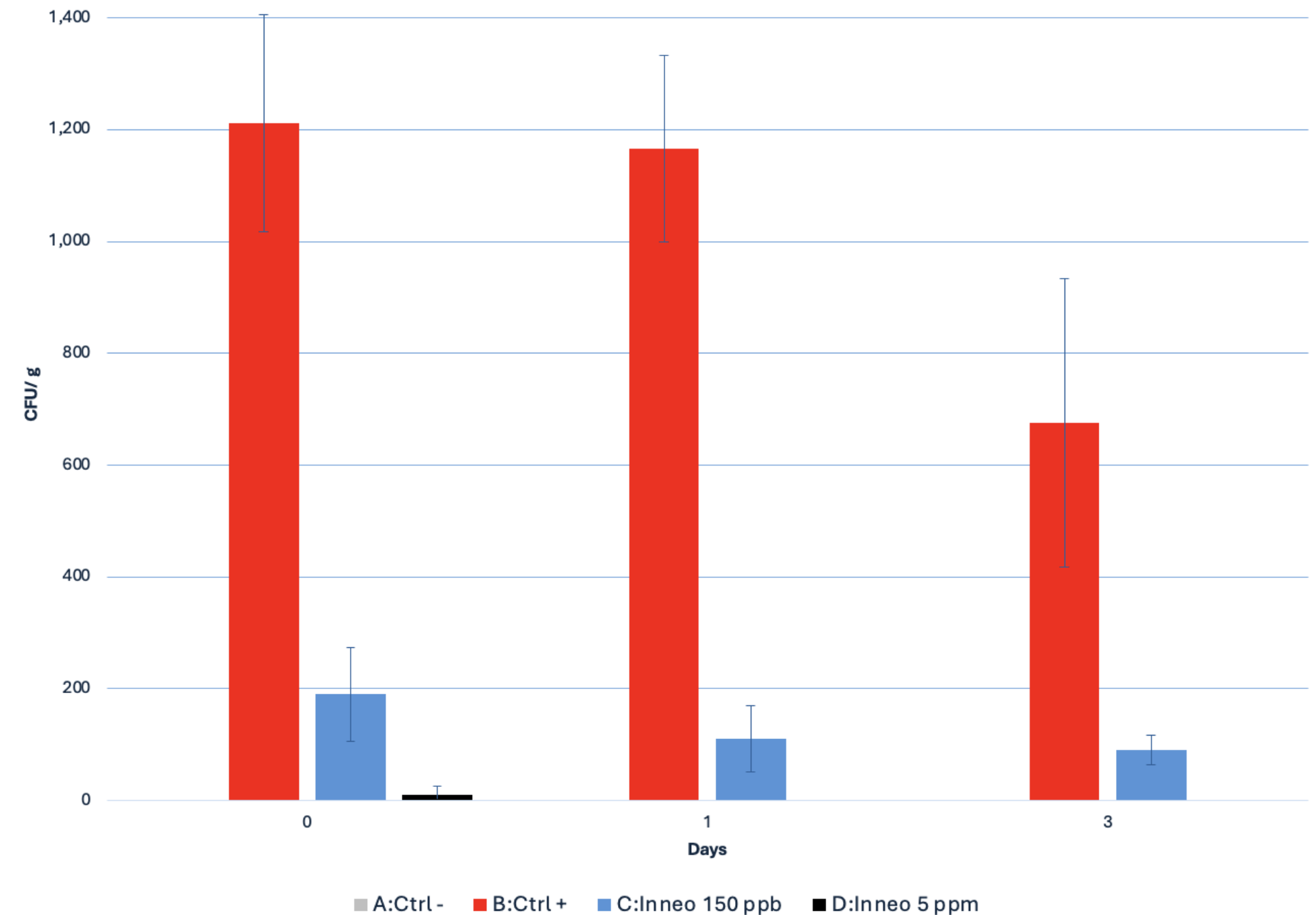


Figure 9: Enumeration of *Listeria monocytogenes* on chicken sausages for each experimental group in cfu/g at each analysis day. Statistical values **** show that using Inneo at 150 ppb significantly reduces the amount of *Listeria monocytogenes* compared to the positive control with a p value < 0.0001.

Proof of concept

Supplementary data

This experiment is designed to demonstrate the antimicrobial activity of Inneo against foodborne pathogen *Listeria monocytogenes* in sliced mushrooms and to monitor its growth during the shelf life. Overall, Inneo showed a great potential to reduce and control the growth of *Listeria monocytogenes* for the time of the experiment, without any change to the organoleptic characteristics of sliced mushrooms.

- **Food matrix:** Sliced mushrooms
- **Inoculation level cfu/g :** $10^{1-1.5}$
- **Expiration date :** 5 days
- **1.5x Expiration date :** 10 days
- **Storage conditions :** 7°C, aerobically

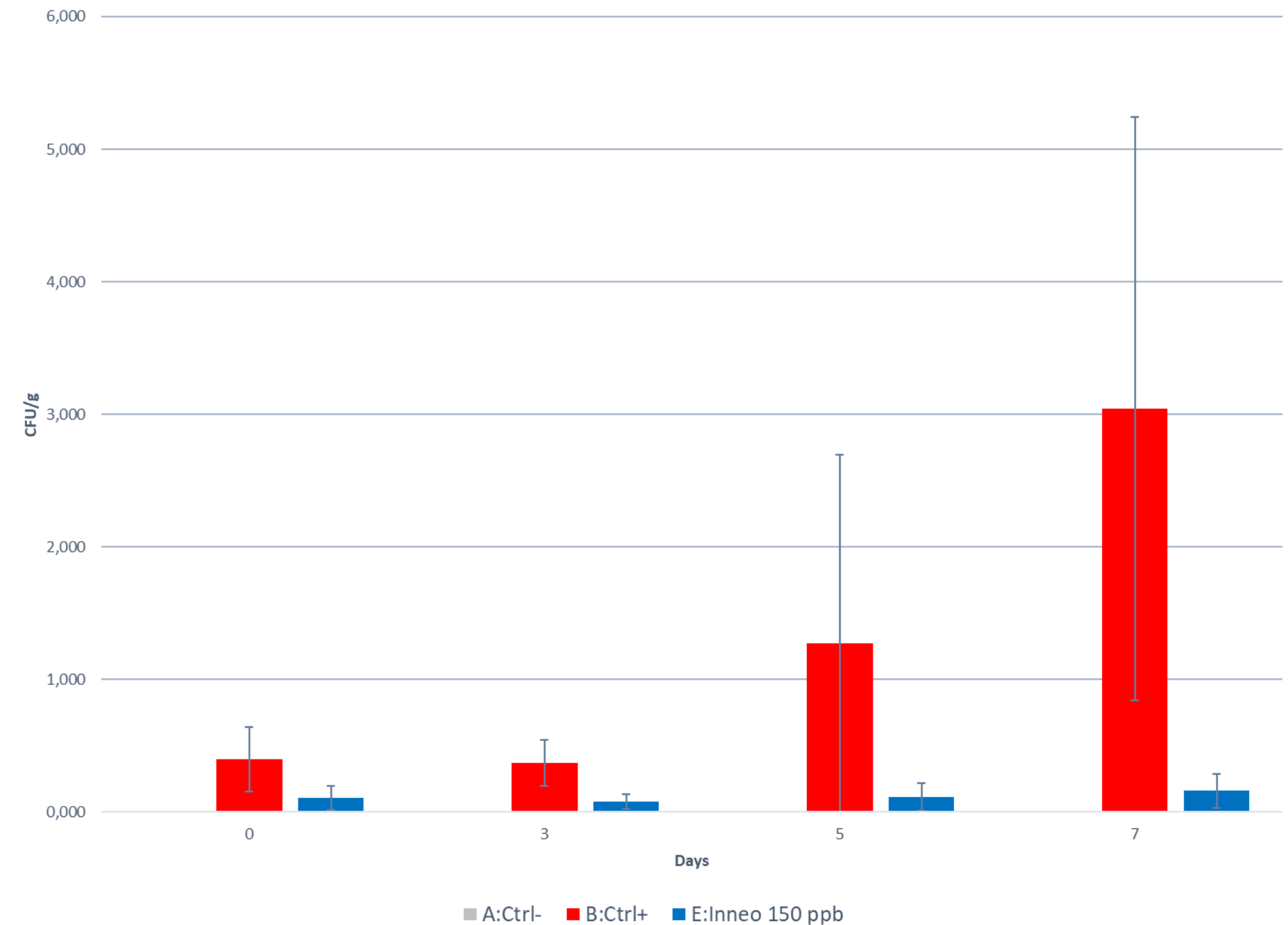


Figure 10: Enumeration of *Listeria monocytogenes* on mushrooms for each experimental group in cfu/g at each analysis day

Proof of concept

Analysis of challenge test results

All graphics demonstrated an immediate effect of INNEO against the strains as soon as day 0 (approximately 4 hours after the treatment). Also, in most of the matrices, INNEO's treatment allows to reach less than 100 cfu/g of ***Listeria monocytogenes*** for ready-to-eat food. This limit is reached for the following matrices: salmon, smoked salmon, shrimps, poultry and partially beef. Some matrices show a higher level of the pathogen: cheese curds and pork, however these matrices were inoculated with a higher inoculum (10^5 cfu/g).

Interestingly, even if these matrices were inoculated with a higher inoculum, the reduction in ***Listeria*** has an average of 2 Log₁₀. In conclusion, it is possible to notice the technical effect of INNEO against ***Listeria monocytogenes*** is significant on every food. These results prove INNEO's anti-***Listeria*** activity.

Our data shows a rapid effect of INNEO over ***Listeria monocytogenes*** and a very low regrowth rate after its application. Moreover, INNEO has no lasting effect on the food and if a contamination by ***L. monocytogenes*** occurs hours/days after application of INNEO on the food, efficacy cannot be guaranteed due to the degradation of the peptide. Application is therefore recommended as close to packaging as possible to avoid any risk of recontamination

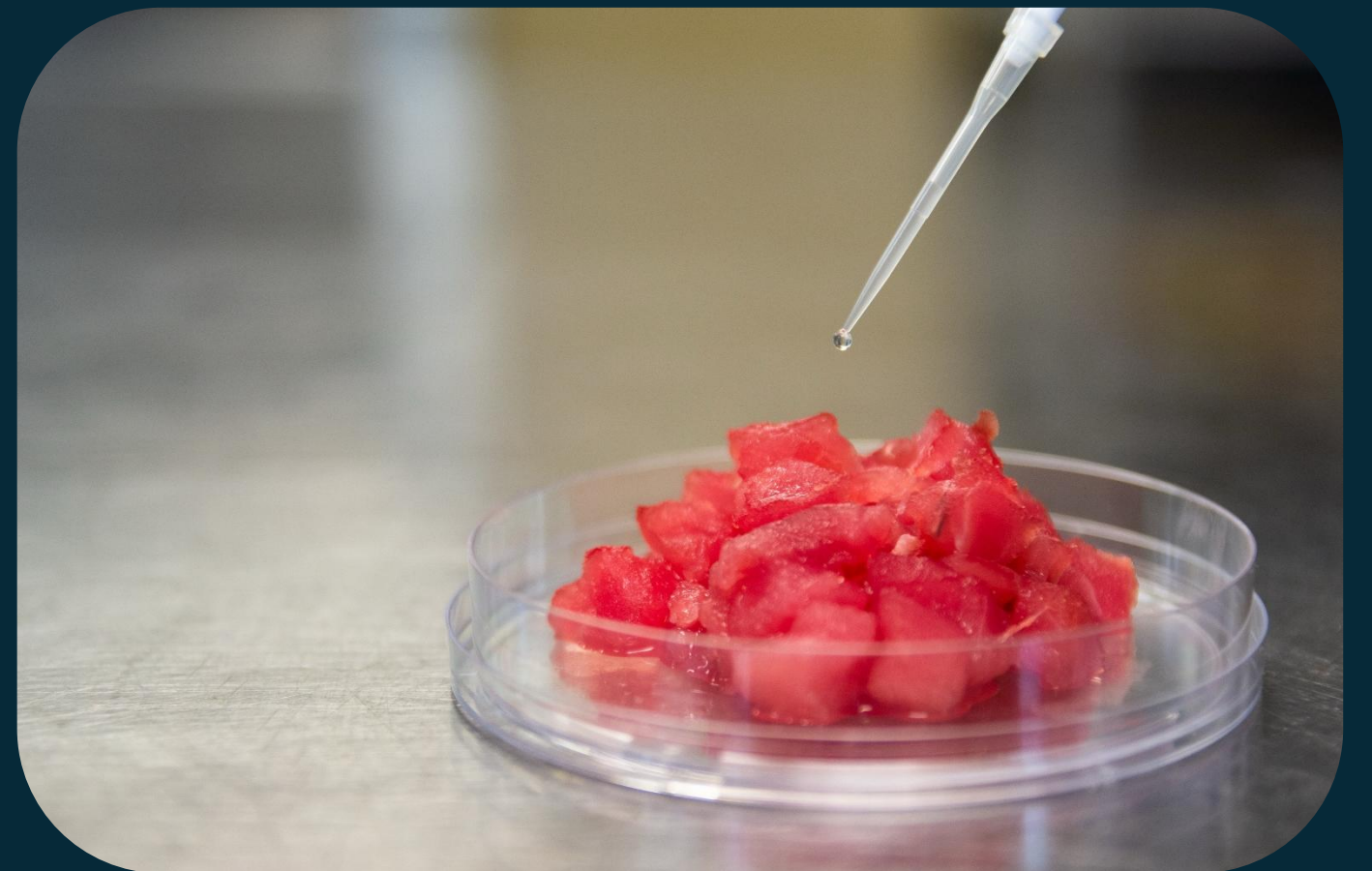
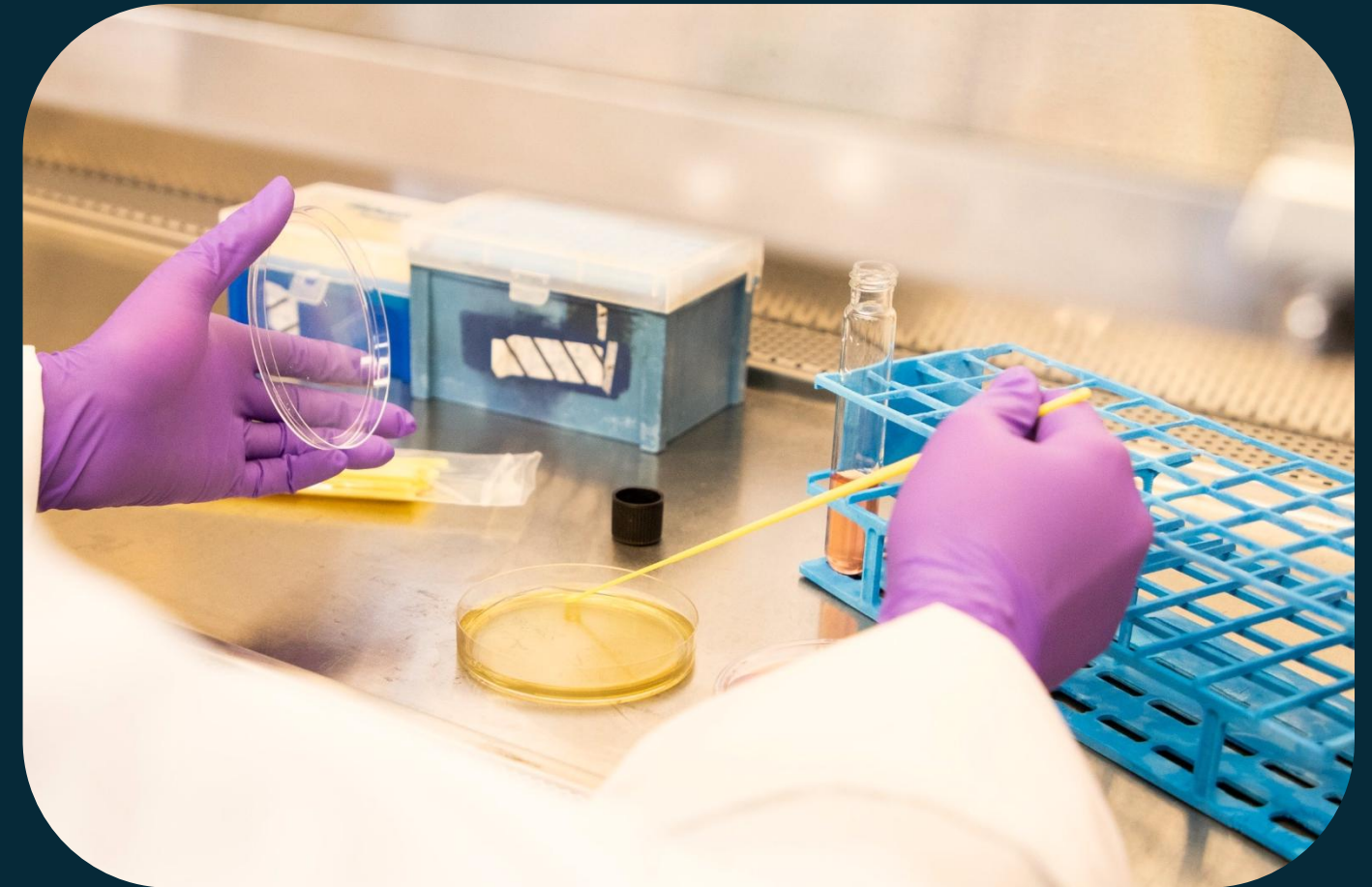


Support and implementation

Validation

Before implementation, our laboratory technicians conduct complimentary compatibility tests to ensure there is no interaction between Inneo and your food products. If needed, additional services such as shelf-life studies, challenge testing, and microbial testing can be performed at Innodal's **ISO 17025-certified** laboratory.

Additionally, Innodal provides on-site support for implementation, including equipment installation if required, and validation processes. Following implementation, our technicians offer ongoing technical support, including spray validation and regular site visits to ensure optimal performance.



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Innovation - Trust - Collaboration

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