Validation of *E. coli* O157:H7 intervention strategies for multi-needle injected whole muscle, non-intact beef

A. Ponrajan[1,2], M.A. Harrison[2], T.D. Pringle[1], J.R. Segers[1], B.K. Lowe[1], R.O. McKeith[1], R.M. Pitzer[1], A.M. Stelzleni[1],

[1]University of Georgia Meat Science and Technology Center, Athens.

[2]University of Georgia Department of Food Science and Technology, Athens

Abstract

Objective

- Study the effects of two antimicrobials, MOstatin™ and IONAL® (World Technology Ingredients, Inc, Jefferson, GA), on psychrotropic organisms in enhanced top round IMPS 169A and top sirloin IMPS 184B (FPL Foods LLC, Augusta, GA) from cull cows.
- Validate the use of these antimicrobials against *Escherichia coli* O157:H7 for multi-needle injected top round IMPS 169A from cull cows.

Brief Methods

Psychrotropic organisms

- Whole muscles were procured 3 d after slaughter, and injected on d 4 to achieve 10% pickup with 0.5% NaCl and 0.4% sodium tripolyphosphate (CNT) plus 2% MOstatin™ (MO) or 1% IONAL® (IN) in the final product.
- Ten muscles were used for each treatment x muscle combination.
- After injection, muscles were vacuum sealed and rested at 0±1°C for 10 d. After 10 d, 2.5cm steaks were fabricated into simulated retail packages (PVC overwrap) and stored under luminescence at 4±1°C for 21 d. A 25cm^2 sample was taken from the top surface of each steak on d 1, 7, 14 and 21 and enumerated for psychrotropic organisms (PSY)

***E. coli* O157:H7 Challenge**

- 10 top rounds were used for each treatment. The muscles were surface inoculated with ampicillin resistant, green fluorescent pigment expressing *E.coli* O157:H7 (6.40 log CFU/cm^2).
- After injection, muscles were vacuum sealed and rested at 4±1°C for 10 d.
- After 10 d, the muscles were cut in half. One half was sampled raw and other half was cooked to a core temperature of 60°C and held at 60°C for 12 minutes following Appendix A (USDA) before sampling.
- A meat block with a 25cm^2 surface area was aseptically excised from the center of the whole muscle sample, sliced in 1/3 increments (top, middle and bottom) and each third was enumerated and enriched for *E. coli* O157:H7 for translocation effects

Results

- For PSY, there was significant treatment x time interaction for both the muscles (P<0.05). For top rounds, there was no significant difference for PSY between the treatments on d 1 (P>0.05); however, with the increase in time (d 7, 14, 21) CNT had a higher outgrowth of PSY compared to IN and MO (P<0.05). For top sirloins, there was a significant treatment x time interaction with CNT having higher PSY counts compared to IN and MO on all days (P<0.05).
- For the raw samples of the *E. coli* study, in the middle and bottom thirds, there was a significant difference between CNT and IN or CNT and MO treatments (P<0.05), but no significant difference between IN and MO treatments (P>0.05). In the top third, there was a significant difference between CNT and IN treatments (P<0.05), but no significant difference between CNT and MO or IN and MO treatments (P>0.05). For all the treatments, there was a significant difference between the top and middle thirds (P<0.05). There was also a significant difference between the top and bottom thirds (P<0.05), but no significant difference between middle and bottom thirds (P>0.05).
- In the case of the cooked samples, no *E. coli* was detected.

E. coli Challenge

- Injections were performed in a Biosafety Level 2 Laboratory, with containment of the pathogen given the highest priority.
- The muscles were inoculated by dotting the inoculum on two 8x8 cm^2 surfaces on the lean face and rested for at least 20 minutes before injection, to allow for attachment of the pathogen.
- Brine pickup, purge and pH changes were recorded for each muscle as shown in Table 1.
- The brine was contaminated with *E. coli* by inoculating the calibration muscles and passing them twice through the injector before each run.
- The changes in *E. coli* counts in the brine during injection were recorded as shown in Table 2.
- The sampling was done in a laminar flow chamber under aseptic conditions.
- The cooked meat was enriched in Modified EC broth and after 24h incubation at 37°C it was streaked on to Tryptic Soy Agar with 50µg/ml ampicillin and suspected colonies were confirmed with latex agglutination kits for *E.coli* O157.

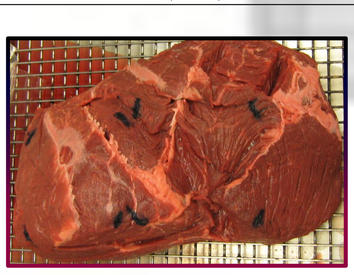
Table 1: E. coli O157:H7 Challenge - Pickup, Purge and pH changes

Treatment	Control	Ional®	MOstatin™
Pickup (%)	11.4 ± 0.55^a	10.28 ± 0.81^a	11.96 ± 0.62^a
Purge (%)	1.76 ± 0.12^b	2.89 ± 0.27^a	2.84 ± 0.39^{ab}
pH before injection	5.71 ± 0.03^a	5.72 ± 0.05^a	5.70 ± 0.04^a
pH after injection	6.11 ± 0.10^a	5.79 ± 0.04^b	5.92 ± 0.05^b
pH after 10d storage	5.71 ± 0.05^b	5.89 ± 0.05^a	5.83 ± 0.05^{ab}

Means within a row followed by the same letters are not significantly different (p > 0.05)

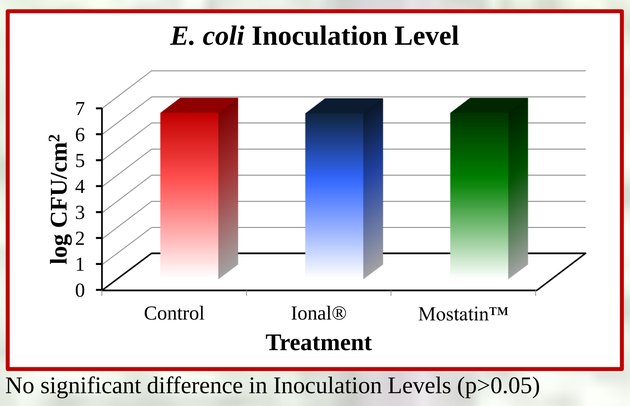
Table 2: *E.coli* Levels in Brine (CFU/ml)

Treatment	Control	Ional®	MOstatin™
Initial (detection limit)	< 0.60	< 0.60	< 0.60
After Calibration	4.23	4.38	4.34
After 5th muscle	4.71	4.78	4.81
After 10th muscle (End)	5.04	5.04	5.15

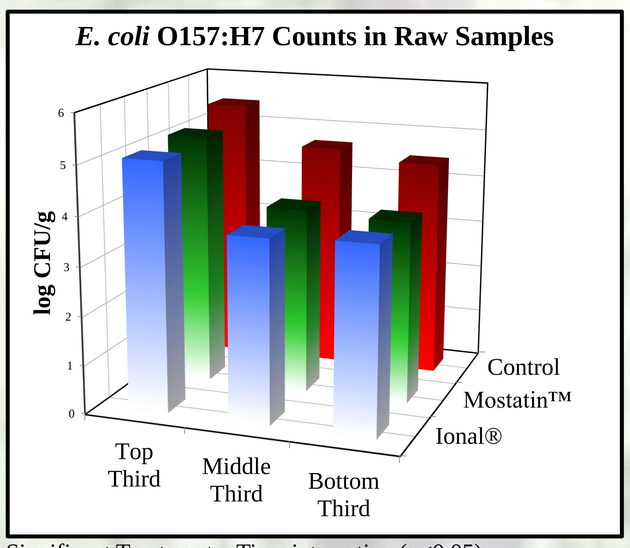


Muscle showing inoculation sites marked with dye

Muscle cut into two halves along the inoculation marking, on sampling day



No significant difference in Inoculation Levels (p>0.05)



Significant Treatment x Time interaction (p<0.05)

Multi-needle Injector prepared for injecting inoculated muscles

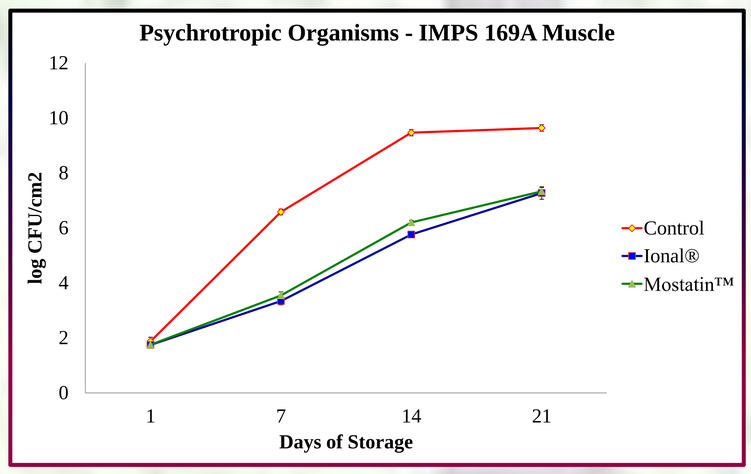
Smokehouse for processing according to USDA Appendix A

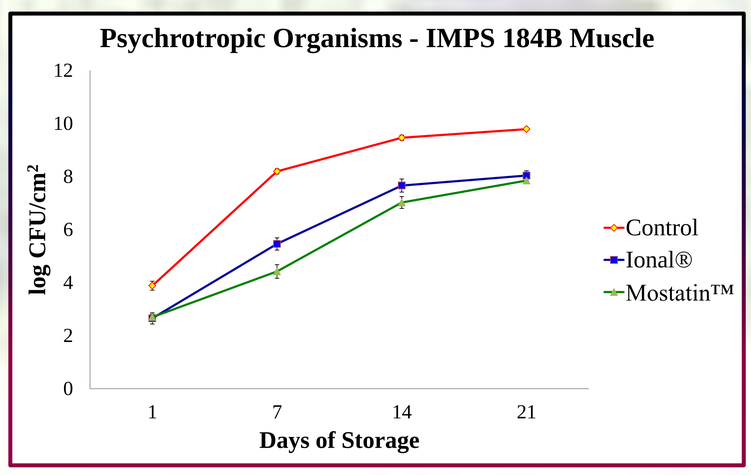
Psychrotropic Organisms

Day 14 Control Sample

Day 14 Ional ® Sample

Day 14 MOstatin™ Sample





Conclusion

- The inclusion of MOstatin™ and IONAL® in enhanced beef products controls psychrotropic organisms and may be an effective *E.coli* O157:H7 hurdle strategy.
- Further research is currently being performed to investigate possible improvements in the effectiveness of these antimicrobials by studying their antimicrobial activity in laboratory simulated food system model.