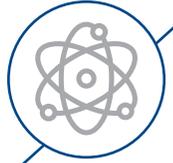


Research Notes

ARM & HAMMER



***In-vitro* assay confirms ability of CELMANAX to bind harmful bacteria**

CELMANAX™ Refined Functional Carbohydrates™ (RFC™) is a multicomponent, all-natural feed supplement that has Generally Recognized as Safe (GRAS) status as a feed ingredient.

STUDY OVERVIEW

This study was designed to test the ability of CELMANAX to bind *E. coli* and *Salmonella* sp. *in vitro* using a quantitative assay. Since yeast mannans have been reported to bind certain bacteria, the study also compared the correlation between mannan content and binding ability of competitive yeast products.

Experiment 1¹ – Quantitative assay for binding ability:

- 1.0 mL of overnight grown cell culture was centrifuged and pellet was resuspended in either sterile saline (control) or in 20 or 40 mg/mL of CELMANAX*
- The tubes were left undisturbed for 30 minutes to allow bound cells to settle
- Unbound cells were removed from the solution and plated
- The assay was conducted in quadruplet and standard deviation was calculated

Experiment 2 – Quantitative assay for binding ability and mannan content:

- A competitive analysis was conducted to compare binding capabilities of CELMANAX SCP* and A-MAX Ultra™ against competitive yeast products using recommended feeding rates (Bio-Mos®, OmniGen-AF®, Diamond V® XPC and Nutritek®).
- Mannan content of all tested products was determined using an enzymatic assay

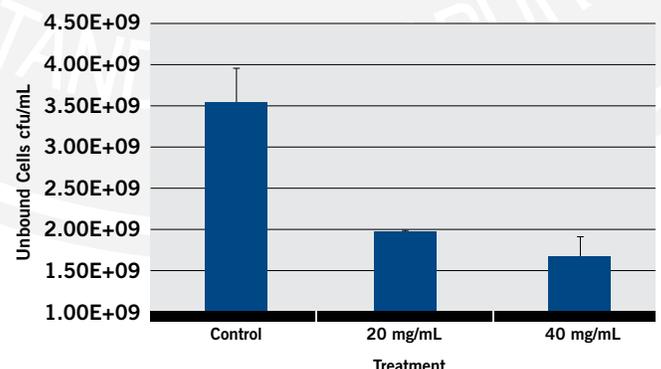
PRODUCT	RECOMMENDED FEEDING RATE FROM PRODUCT SHEET, g/hd/d	PROPOSED QUANTITATIVE AGGLUTINATION CONCENTRATION IN ASSAY, mg/mL**
CELMANAX SCP	3	4
A-MAX Ultra	14	20
Product A	14	20
Product B	19	27
Product C	2 or 4	10
Product D	56	80

RESULTS

Experiment 1:

- Light green clumps of bound cells were observed in the binding test when *E. coli* and different concentrations of CELMANAX were mixed.
- CELMANAX at 20 and 40 mg/mL bound 47% and 53% of *E. coli* F18, respectively (Fig. 1).

FIGURE 1: Effect of CELMANAX on *E. coli* F18



- Binding was also demonstrated when *Salmonella* cells were mixed with 20 and 40 mg/mL of CELMANAX™ (Fig. 2).
- CELMANAX at 40 mg/mL bound different *Salmonella* species by 80 – 98% (Fig. 2).

Experiment 2:

- Percentages of successfully bound bacteria were analyzed and compared. CELMANAX SCP had the highest rate of binding at 90% (Fig. 3).
- Mannan content had little relationship to binding ability.

CONCLUSIONS

- CELMANAX bound enteropathogenic bacteria like *E. coli* and *Salmonella in vitro*. This interaction could potentially prevent infections by these bacteria by binding them and preventing them from binding to the host tissue.
- Although mannans and mannan-oligosaccharide (MOS) are associated with agglutination, the amount of mannans or MOS within a product does not always correlate with the degree of agglutination.

FIGURE 2: Effect of CELMANAX on *Salmonella*

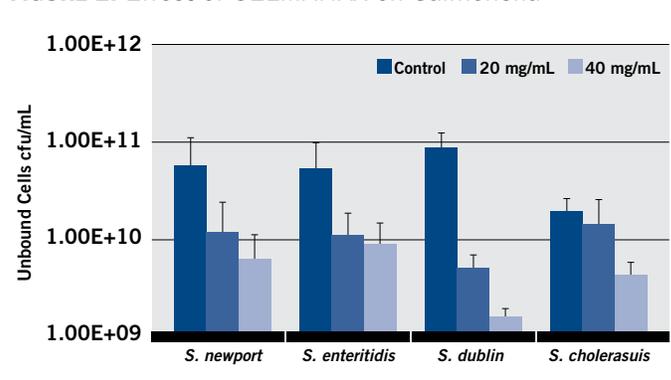
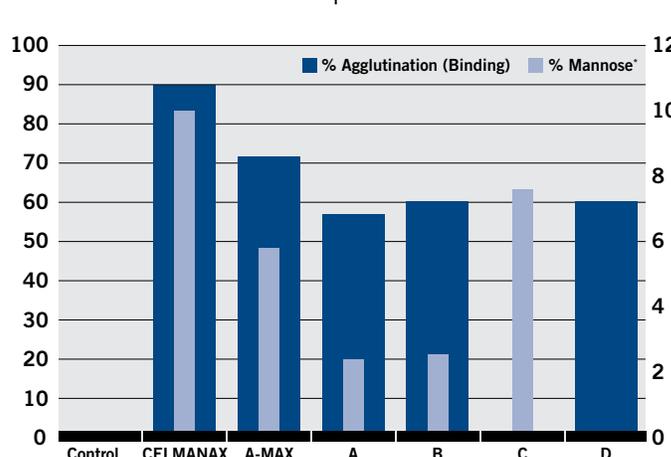


FIGURE 3: Relationship between pathogen binding and mannose content in various products



*Mannose determined at Complex Carbohydrate Research Center, University of Georgia



* CELMANAX and CELMANAX SCP contain different concentrations of the same active ingredients. The feeding rates are, therefore, different but deliver the same quantity of active ingredients per recommended dose.

** Quantitative agglutination is the actual amount of bacteria bound by the different products. Products were used at 2x the qualitative concentration to assure meaningful quantitative assay values.

1 Jalukar S, Oppy J, Holt M. *In-vitro* assay to evaluate ability of enzymatically hydrolyzed yeast containing MOS to bind enteropathogenic bacteria. Presented as an abstract at the Midwest Animal Science Meeting, Des Moines, Iowa, March 2009, Abstract #228. Research Bulletin 31 and Research Bulletin 39.

