

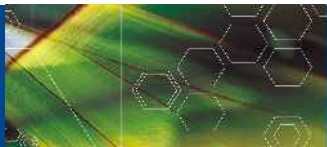


Massey University

Institute of Food, Nutrition and Human Health

Modulation of the intestinal microbiota: The ability of AOCE “food product containing antioxidants with curing effects” and CAM30 “blackcurrant extract powder” to positively modulate key markers of gastrointestinal function

Dr. Abdul L. Molan
Senior Research Scientist,
Human Nutrition and Physiology Division



Te Kunenga
ki Pūrehuroa

Introduction

- **The human colon is a movable bioreactor that harbours a large and complex microbial flora. Essentially, the role of the colonic organisms is the fermentation of various substrates that escaped digestion in the upper GI tract.**
- **Colon bacteria are often classified as potentially harmful or potentially health promoting based on their fermentative features. The saccharolytic fermentation of carbohydrates leads to the production of SCFA that provide additional energy to the host while the end products of proteolytic (protein) fermentation include various toxic substances.**



Definition of ' A Healthy or Balanced Microflora'

Cummings et al (2004) defined a healthy or balanced microflora as;

' A healthy, or balanced microflora is one that is predominantly saccharolytic and comprises significant numbers of bifidobacteria and lactobacilli'



The balance between the good and bad bacteria is very delicate

Although the human body is created with a proper ratio of good to bad bacteria, this ratio is frequently altered as a result of today's modern lifestyle. The delicate balance between good and bad bacteria can be disturbed by:

- The use of antibiotics,
- The use of oral contraceptives and steroids,
- Exposure to radiation through X-rays and radiation therapies,
- The consumption of refined sugars and other refined foods,
- Poor digestion and poor elimination of waste,
- Stress,
- Unhealthy diet.



How can the viability of probiotic organism enhanced?

- **Although probiotic bacteria can pass through the GI tract, some studies have shown that they do not colonise and grow even after prolonged feeding periods. Accordingly, there is a need for a strategy that enhances viability and promotes growth of probiotic bacteria in the colon.**
- **Subsequently, modification of the human intestinal microbiota has become an important objective in nutritional science. This goal can be achieved in three ways:**
 - 1. By inclusion in the diet of a significant proportion of beneficial bacteria, (probiotics), [with the expectation that they will grow and colonize the gut]**
 - 2. By giving non-digestible carbohydrates (prebiotics), like fructo-oligosaccharides, which have shown an ability to promote the growth of desirable bacteria,**
 - 3. Or by giving a mixture of both probiotics and prebiotics (synbiotics).**



What are Probiotics ?

- **A probiotic is an organism which contributes to the health and balance of the intestinal tract. A probiotic is also called "friendly", "beneficial", or "good" organism which when ingested acts to maintain a healthy intestinal tract and help fight illness and disease.**
- **The list of positive effects that have been attributed to bifidobacteria and lactobacilli includes:**
 1. **Stimulation of the immune system,**
 2. **Production of vitamins,**
 3. **Inhibition of intestinal pathogens,**
 4. **Reduction of constipation.**
 5. **Relief of vaginitis**



Definition of “Prebiotics”

‘Prebiotics are non digestible food ingredients that selectively stimulate the growth of one or a limited number of beneficial bacteria in the colon, to improve host health’.



Why do we need new prebiotics?

- Currently available prebiotics (such as FOS and inulin) can help the survival and proliferation of probiotic bacteria, but are limited by drawbacks such as side effects and allergies.
- Therefore, alternative prebiotics with fewer or no side effects are needed that could either be incorporated in a probiotic food matrix, or used as a stand-alone prebiotic to enhance the growth of lactic acid bacteria naturally present in the intestine.
- Essentially, the aims of pro- and prebiotic supplementation are very similar (i.e., to improve the host's health via modulation of the intestinal microbiota) but are achieved in different ways, namely, by introducing exogenous species (probiotics) or by stimulating indigenous bacteria (prebiotics).



Is there any link between bacterial species and the risk of colon cancer?

- Some species of bacteria are capable of generating carcinogens, mutagens, and tumour promoting agents in the large bowel.
- β -glucuronidase, for example, is synthesised by colonic bacteria, and it is known to produce mutagens, carcinogens, various tumour promoters, and deconjugates with many endogenous and exogenous compounds.
- Increased incidence of colon-rectal tumors in experimental studies was associated with high levels of β -glucuronidase activity.



Blackcurrant

Blackcurrant berries are rich sources of various phenolic substances with antioxidative, antibacterial, antiviral and anti-inflammatory properties including flavonoids and anthocyanins as well as vitamin C.



Objectives

- To assess the effect of aqueous extracts from CAM30 and AOCE* on the population size of bacteria (good and bad) in the ceca of rats orally gavaged with these extracts, inulin (positive control) or water (negative control) three times weekly for four weeks. Fluorescence *in situ* hybridization (FISH) method was used to assess these effects.
- To assess the effects of extracts on selected metabolic indexes potentially involved in colonic carcinogenesis.

* AOCE – food product containing antioxidants with curing effects.



Materials and Methods

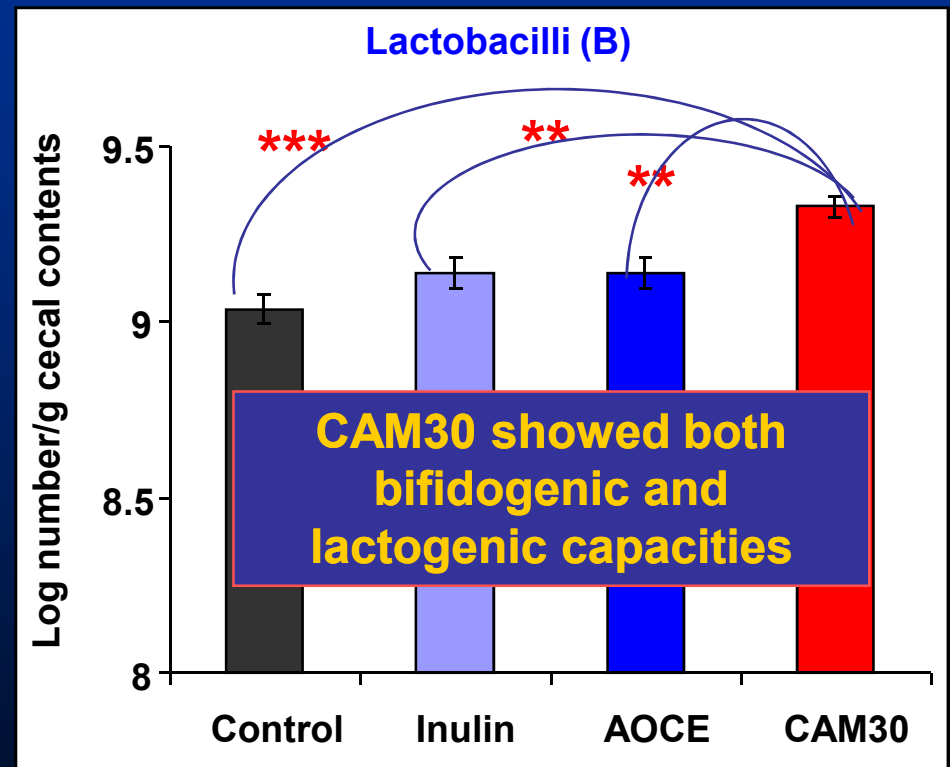
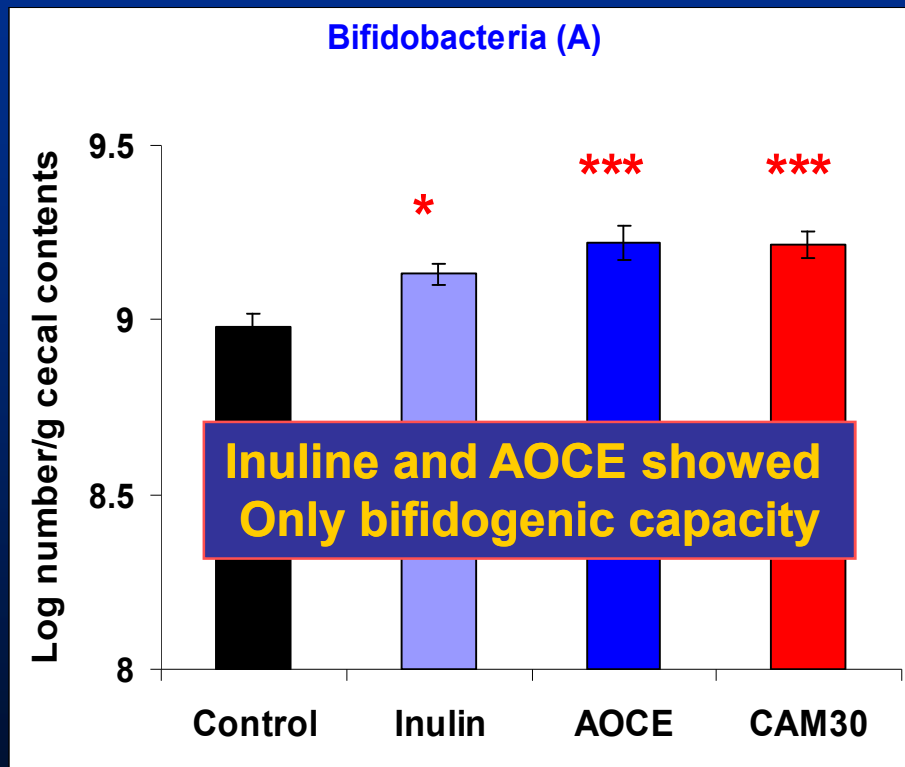
- Forty male rats were allocated randomly into 4 equal groups and gavaged orally with water (negative control), inulin (positive control), CAM30 and AOCE extracts, respectively, 3 times weekly for 4 weeks.
- The gavaging dose was based on a dose rate of 13.4 mg and 30 mg/kg body weight for CAM30 and AOCE, respectively. For inulin, the dose rate was equivalent to 1% of food intake. The dosage was adjusted weekly according to body weight.
- At the end of the experiment, rats were euthanized by CO₂ inhalation. After dissection, the secum with contents was removed. The cecal contents were stored at -80 °C until the numbers of bacteria were monitored. FISH technique was used to monitor the numbers of *good and bad bacteria* in the cecal samples using genus-specific oligoneucleotide probes.
- Fresh cecal samples were used to determine the activities of β -glucuronidase and β -glucosidase in the cecal samples.



Results



Numbers of *Bifidobacterium* species (A) and *Lactobacillus* species (B) recovered from cecal samples hybridized with genus-specific oligoneucleotide probes (Bif164 and Lab158) in FISH analysis.

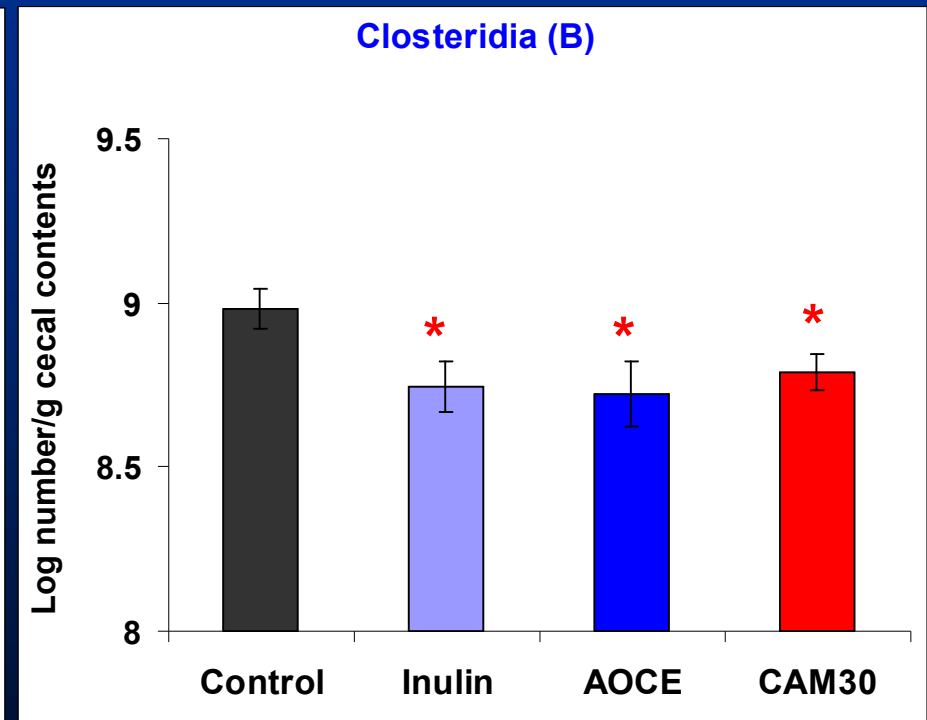
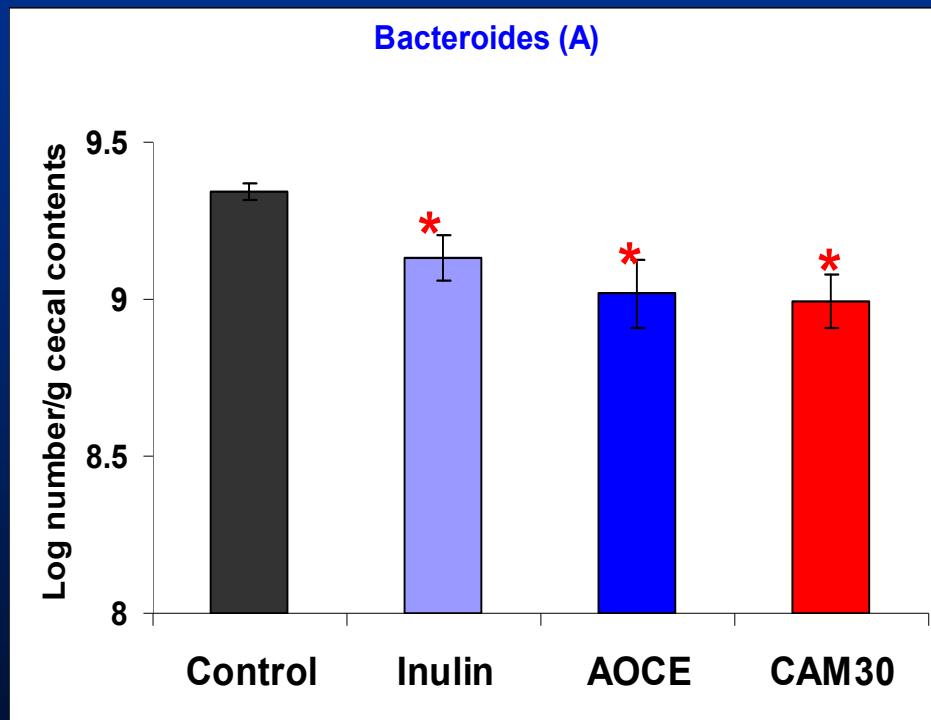


* AOCE – food product containing antioxidants with curing effects.



Massey University

Numbers of *Bacteroides* species (A) and *Clostridium* species (B) from cecal samples hybridized with genus-specific oligoneucleotide probes (Bac203 and His150) in FISH analysis.

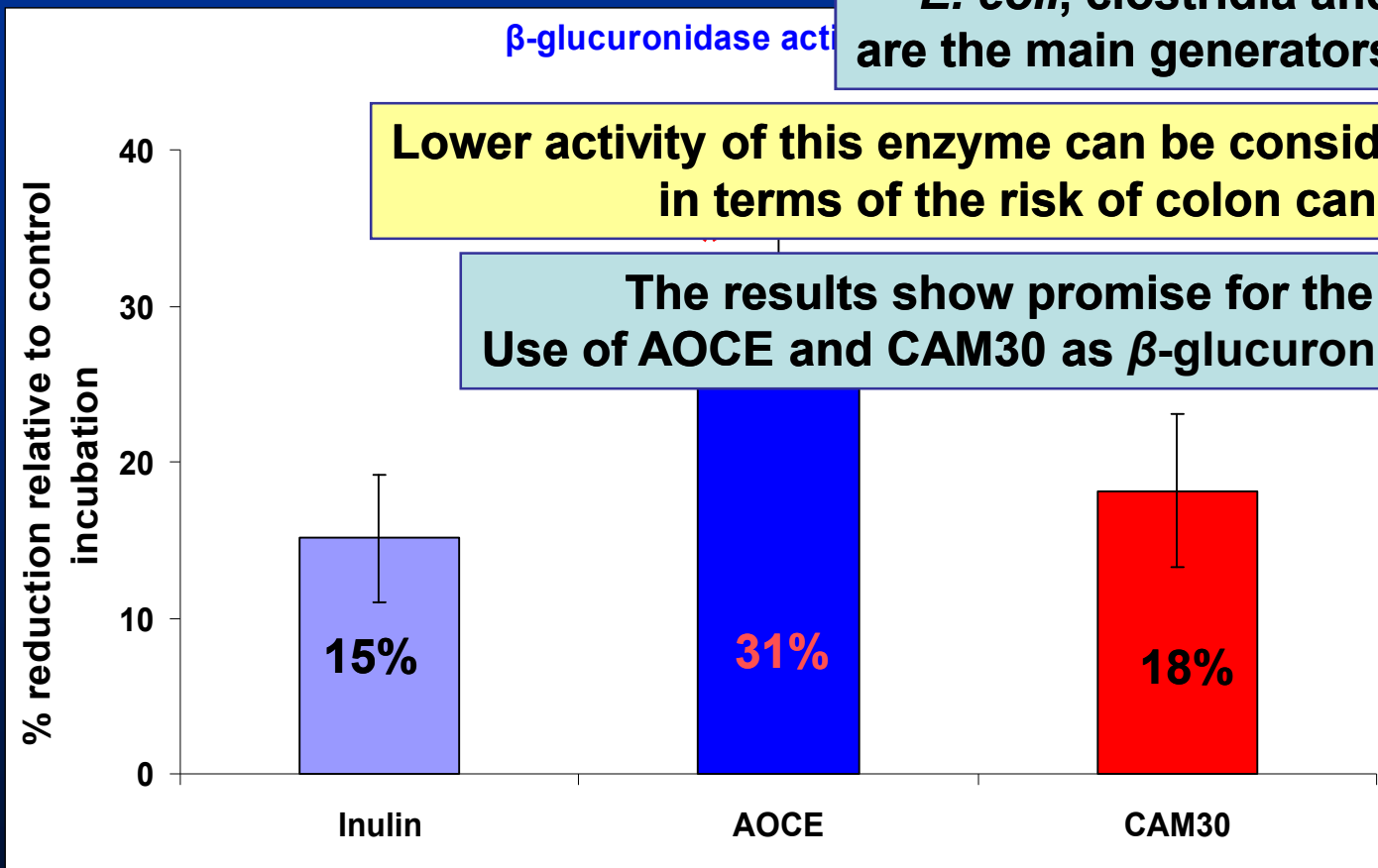


*** AOCE – food product containing antioxidants with curing effects.**



Massey University

% reduction in the activity of β -glucuronidase (mg/g wet cecal contents/h) in cecal contents of rats gavaged with AOCE, CAM30 extracts and inulin relative to the control group gavaged with water.



***E. coli*, clostridia and bacteroides are the main generators of this enzyme**

Lower activity of this enzyme can be considered beneficial in terms of the risk of colon cancer

The results show promise for the potential Use of AOCE and CAM30 as β -glucuronidase inhibitors

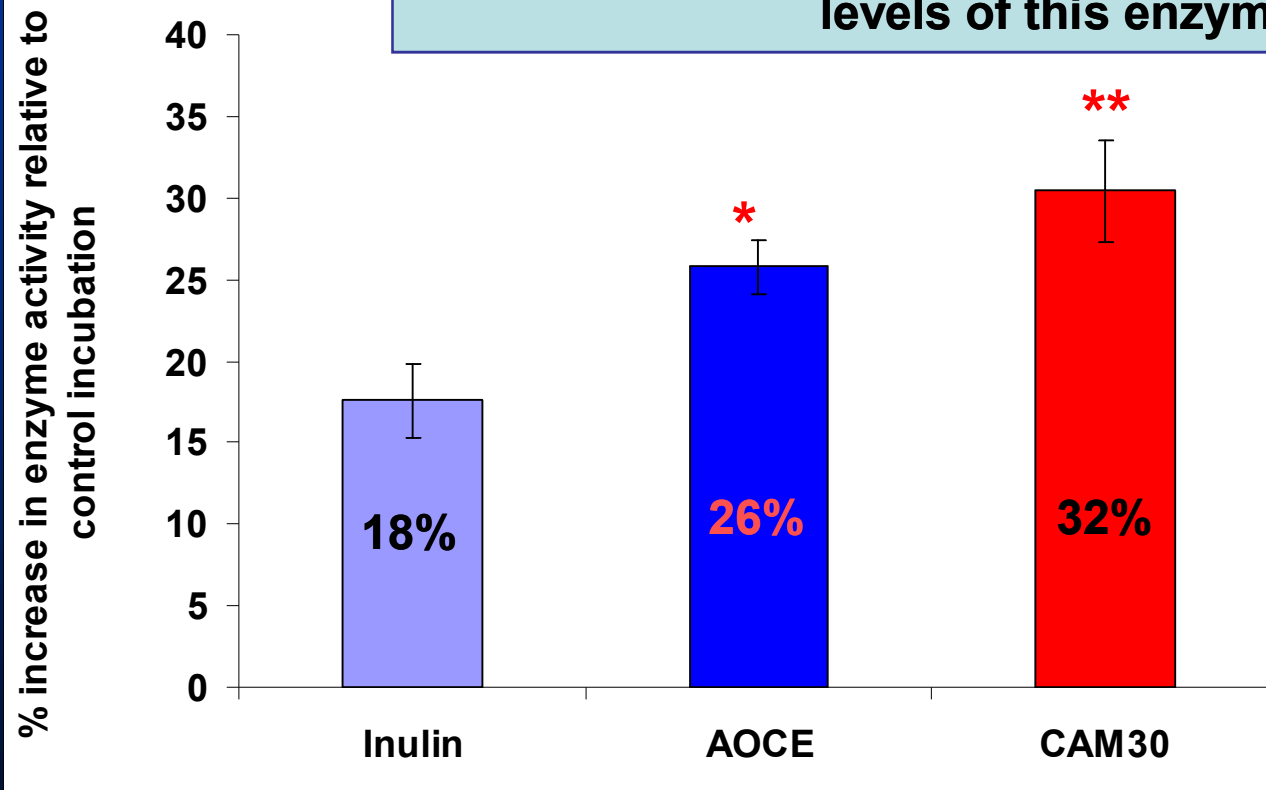
*** AOCE – food product containing antioxidants with curing effects.**



Massey University

% increase in the activity of β -glucosidase (mg/h/g) in cecal contents of rats gavaged with AOCE, CAM30 extracts and inulin relative to the control group gavaged with water.

This increase may be due to the increase in the numbers of bifidobacteria and lactobacilli which possess higher levels of this enzyme.



*** AOCE – food product containing antioxidants with curing effects.**



Massey University

Conclusions and Recommendations (1)

- This study has identified CAM30 and AOCE as good prebiotics that can significantly promote the growth of friendly bacteria and lower the numbers of undesirable bacteria in the cecum of rats.
- Under the present experimental conditions, this study showed that CAM30 and AOCE can decrease the activity of β -glucuronidase and increase the activity of β -glucosidase, which could be considered as potentially beneficial for the host.
- The importance of this finding is generated from the fact that the bacterial enzyme β -glucuronidase is considered to be one of the enzymes that increases risk for colon cancer.

*** AOCE – food product containing antioxidants with curing effects.**



Conclusions and Recommendations (2)

- The results suggest that CAM30 and AOCE might have the potential to promote the survival, colonization and activity of friendly bacteria in the GI tract, thus potentially improving their beneficial effects.
- There were no significant differences in food intake, water intake and body weight of rats gavaged with CAM30, AOCE and inulin when compared with rats in the control group.
- This study has shown excellent results that need to be confirmed in human studies involving both healthy volunteers and subjects with clinical conditions that have a supposed microbial aetiology (e.g. ulcerative colitis, bowel cancer and irritable bowel syndrome).

* AOCE – food product containing antioxidants with curing effects.

