



**Massey University**

Institute of Food, Nutrition and Human Health

# **Consumption of First Leaf and CAM30 by healthy humans can beneficially affect bowel function and fecal parameters related to colon cancer**

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**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.



# 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 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 positively 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 diseases.**



# What are 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 growth of probiotic bacteria, but are limited by some side effects.
- Therefore, alternative prebiotics with fewer or no side effects are needed to enhance the growth of lactic acid bacteria naturally present in the intestine.



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

- Some species of bacteria are capable of generating carcinogens and tumour promoting agents in the large intestine.
- $\beta$ -glucuronidase, for example, is generated by some colonic bacteria, and it is known to produce various tumour promoters.
- Some studies have shown that increased incidence of colorectal tumors in experimental studies was associated with high levels of  $\beta$ -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.**
- **The majority of the blackcurrant anthocyanin is retained in the small and large intestines during the digestion and excretion processes.**



# Objectives

- To assess the effect of First Leaf (FL) and CAM30 on the population size of bacteria (good and bad) in the gut of healthy humans given these products. Fluorescent *in situ* hybridization (FISH) method was used to assess these effects.
- To assess the effects of FL and CAM30 on selected metabolic indexes potentially involved in colonic carcinogenesis.
- To assess the effect of FL and CAM30 on the fecal pH.

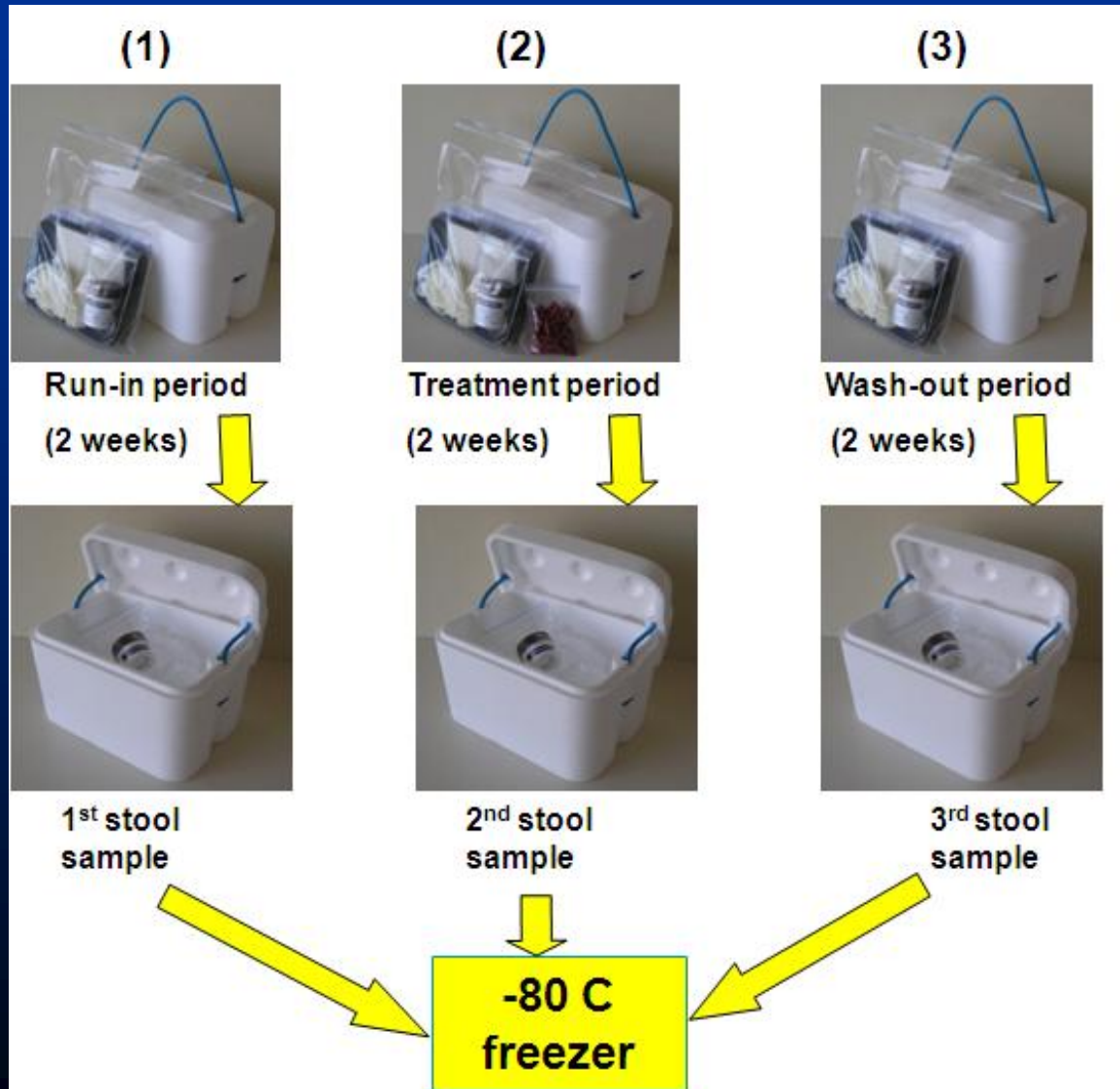


# Subjects and Methods

- Thirty healthy subjects were divided randomly into 2 equal groups and given orally First Leaf [4 capsules (1500 mg)/day] and CAM30 [4 capsules (672 mg)/day], 3 times daily for 14 consecutive days.
- Three stool samples from each subject were collected; at baseline (d0; period 1), after 2 (d14; period 2) weeks of products ingestion, followed by samples at week 4 (d28; washout, period 3). The samples 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 fecal samples using genus-specific oligoneucleotide probes.
- The fecal pH and the activities of some bacterial enzymes in the fecal samples were also determined.



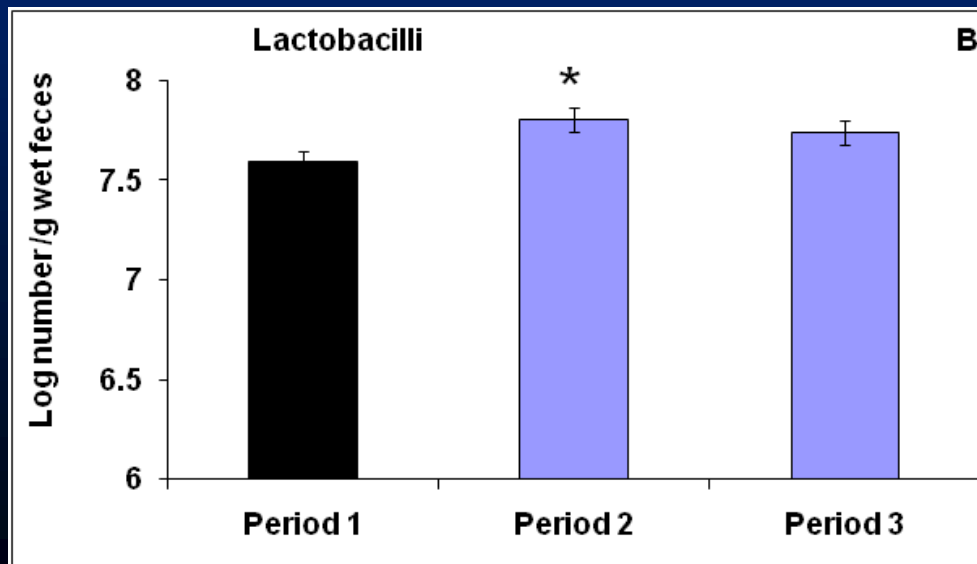
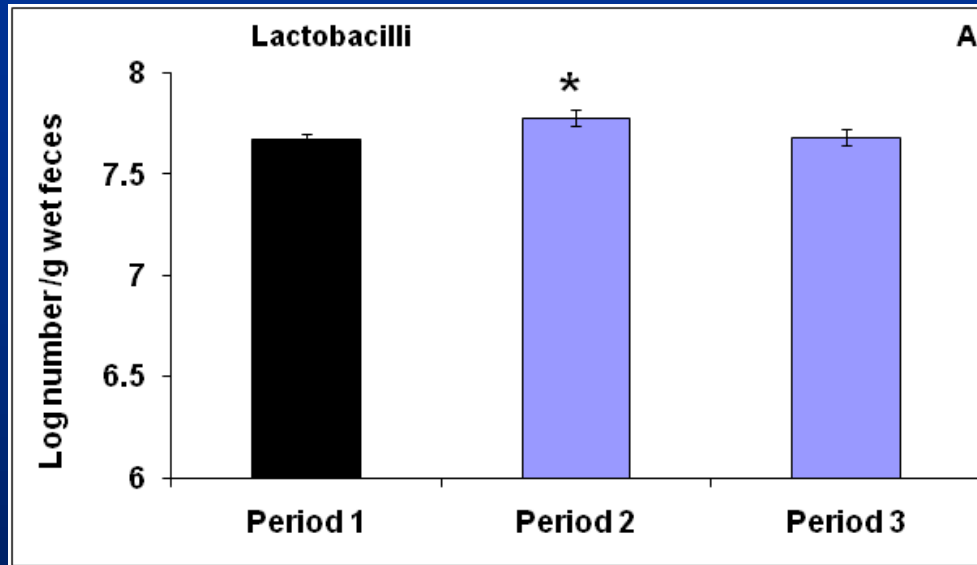
# Collection of stool samples



# Results



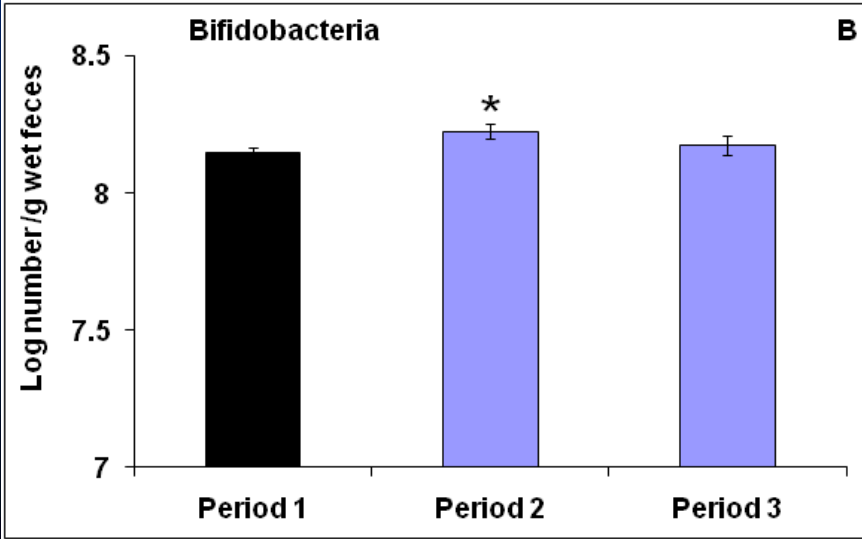
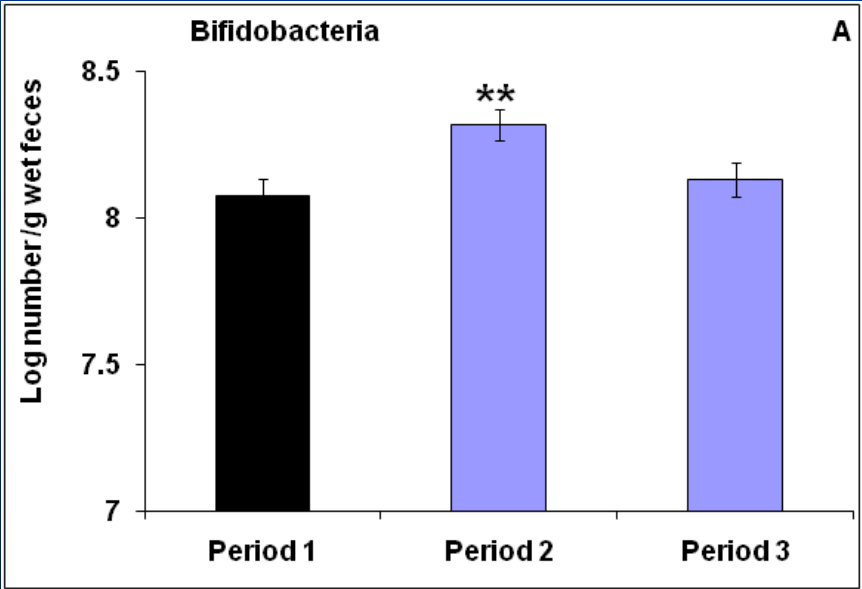
Numbers of *Lactobacillus* species recovered from the fecal samples hybridized with genus-specific oligoneucleotide probes (Lab158) in FISH analysis. The volunteer groups were given either First Leaf (panel A) or CAM30 (panel B) daily for 14 days.



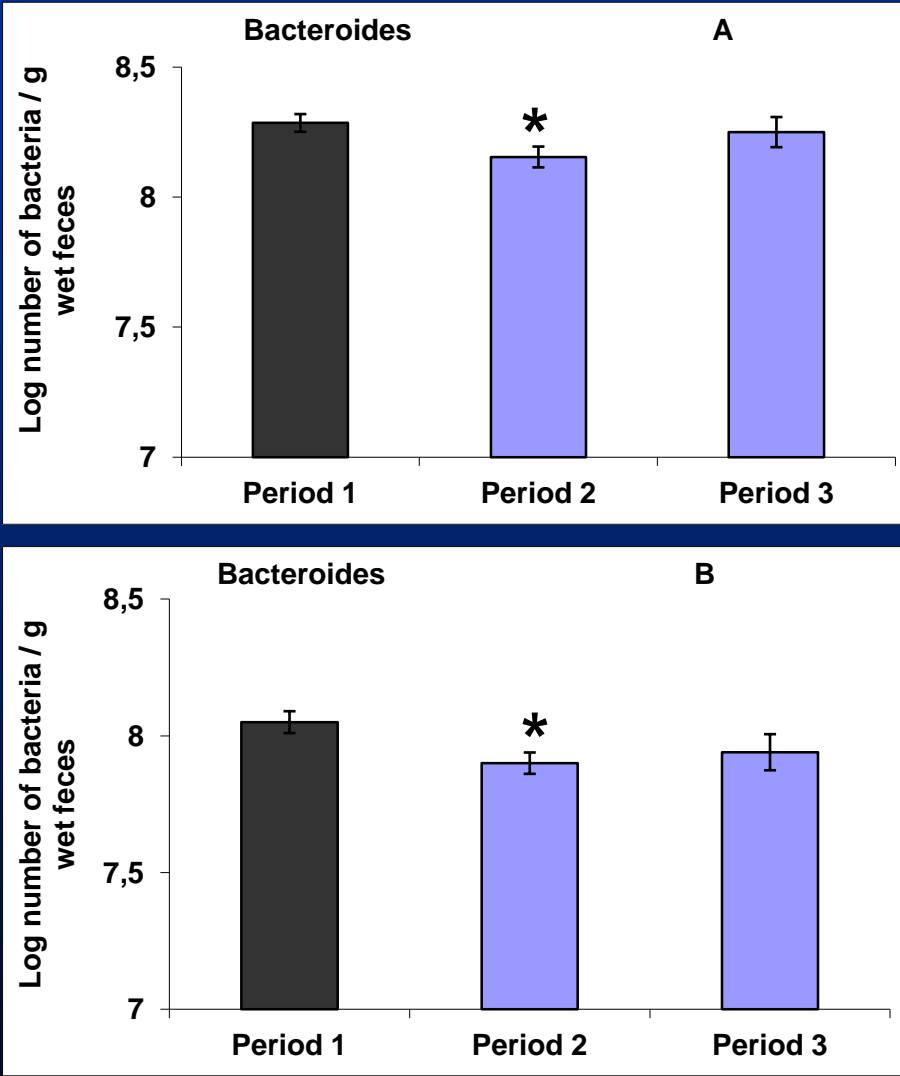
The data are expressed as Means  $\pm$  standard errors of the means ( $n = 15$  subjects/group). \*  $P \leq 0.05$  by analysis of variance versus the baseline (period 1).



Numbers of *Bifidobacterium* species recovered from the fecal samples hybridized with genus-specific oligoneucleotide probes (Bif164) in FISH analysis. The volunteer groups were given **either** First Leaf (panel A) or CAM30 (panel B) daily for 14 days.

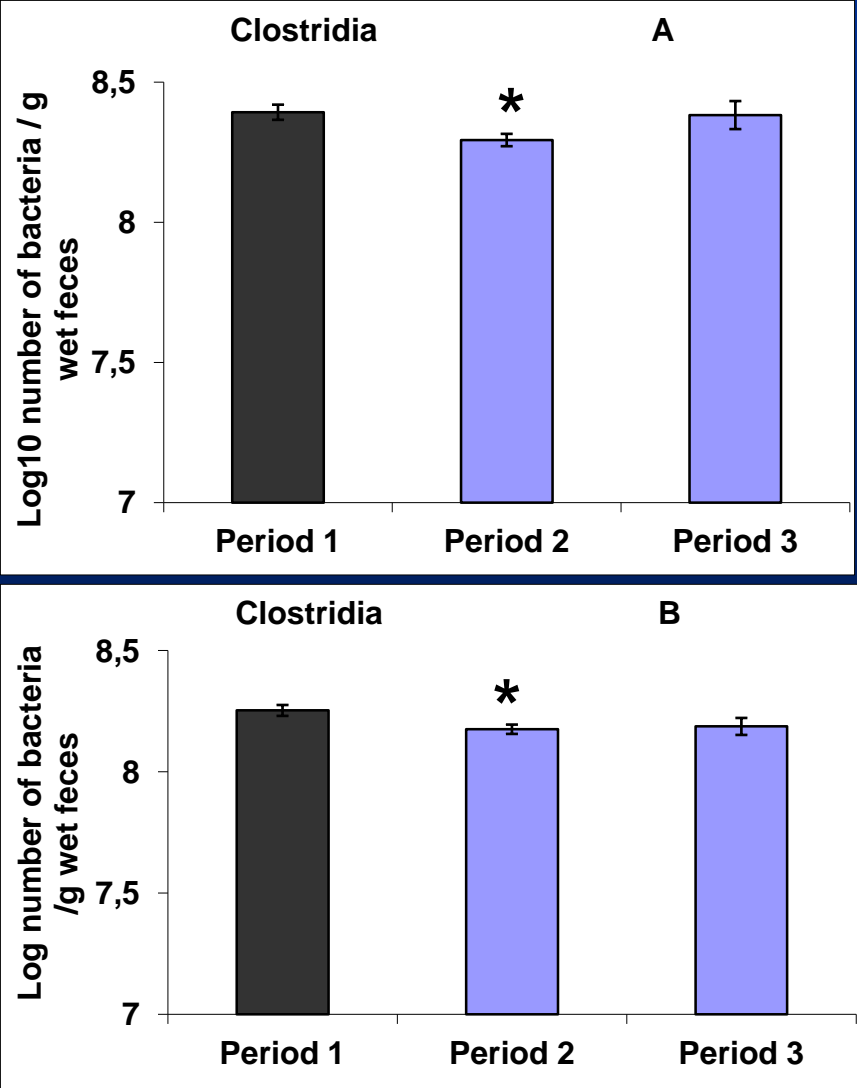


Numbers of *Bacteroides* species from fecal samples collected before and after consumption of First Leaf (panel A) or CAM30 (panel B). The samples were hybridized with genus-specific oligoneucleotide probes (Bac203) in FISH analysis.



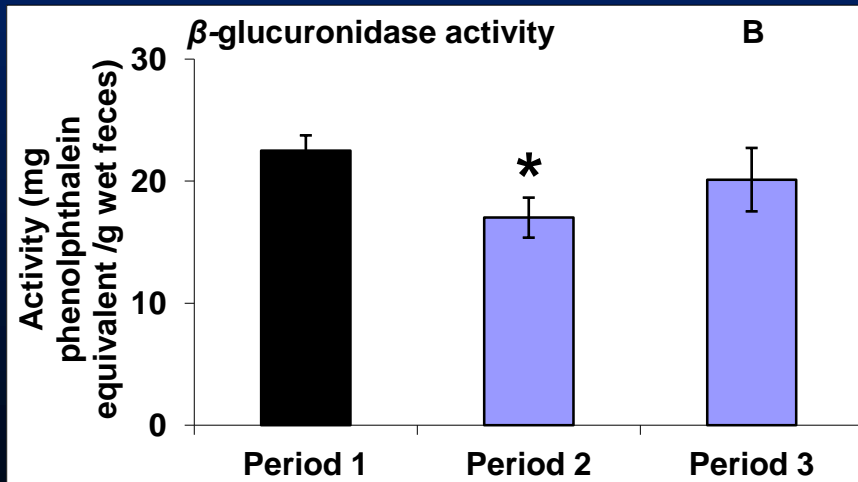
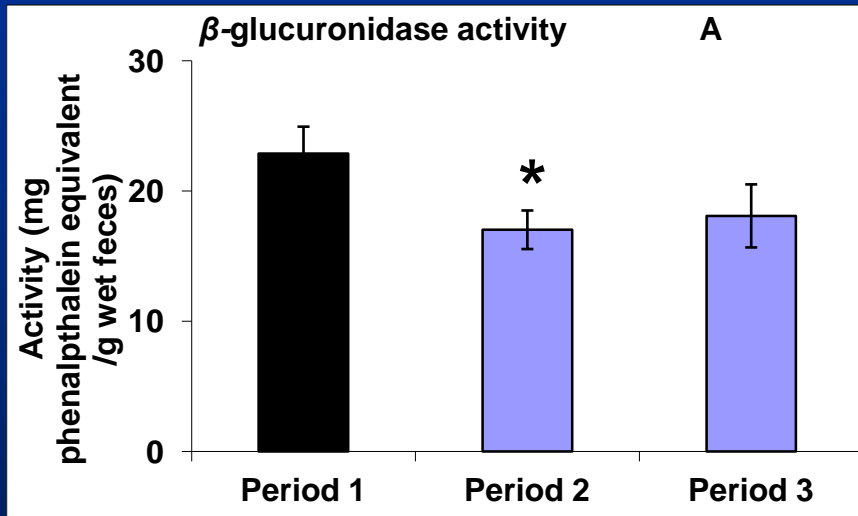


Numbers of *Clostridium* species from fecal samples collected before and after consumption of First Leaf (panel A) or CAM30 (panel B). The samples were hybridized with genus-specific oligoneucleotide probes (His150) in FISH analysis.



# Activity of fecal $\beta$ -glucuronidase before and after consumption of First Leaf (FL; panel A) or CAM30 (panel B).

The results show promise for the potential use of FL and CAM30 as  $\beta$ -glucuronidase inhibitors



*E. coli*, *Clostridium* spp. and *Bacteroides* spp. are the main generators of this enzyme

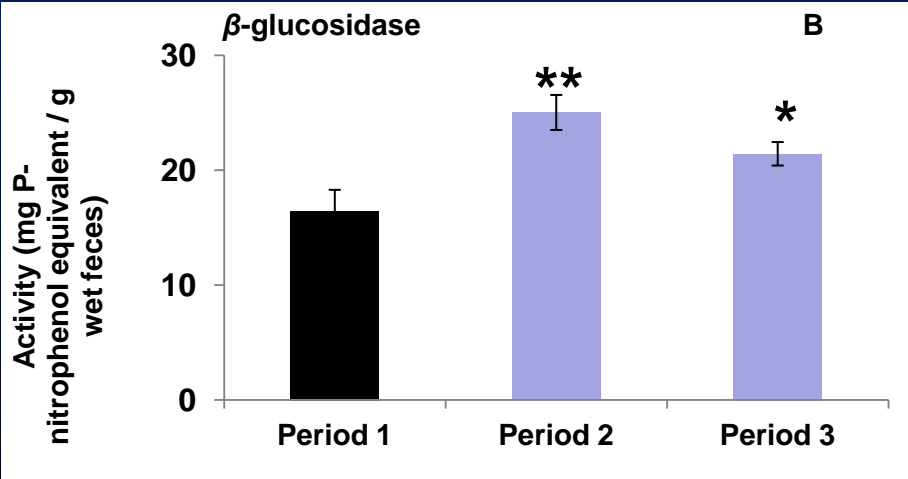
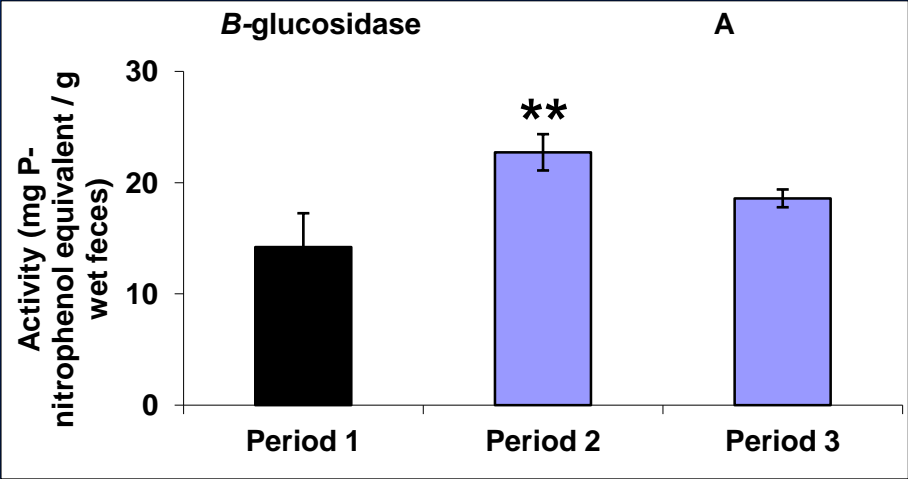
Lower activity of this enzyme can be considered beneficial in terms of the risk of colon cancer

Treat.	% reduction relative to period 1:	
	Period 2	Period 3
FL	26	21
CAM30	24	11



Activity of fecal  $\beta$ -glucosidase before and after consumption of either First Leaf (FL; panel A) or CAM30 (panel B).

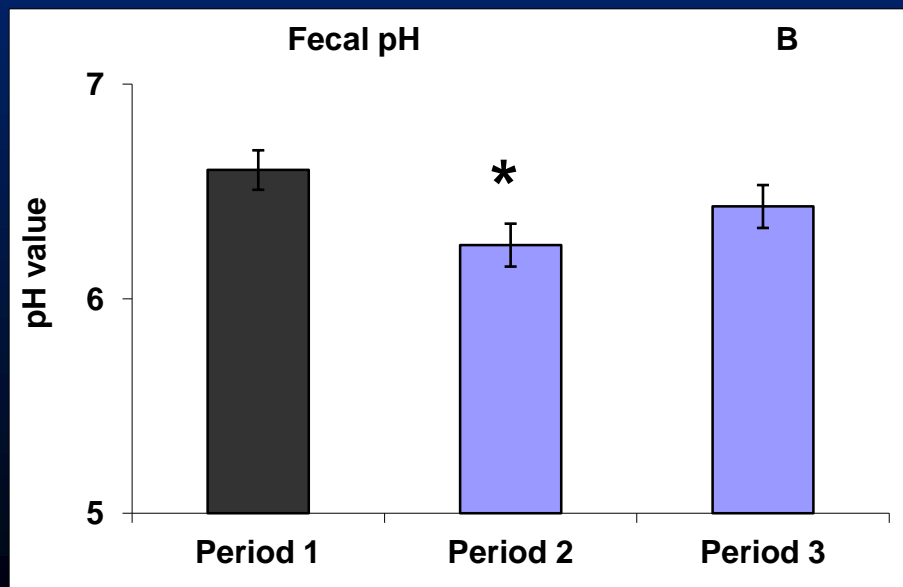
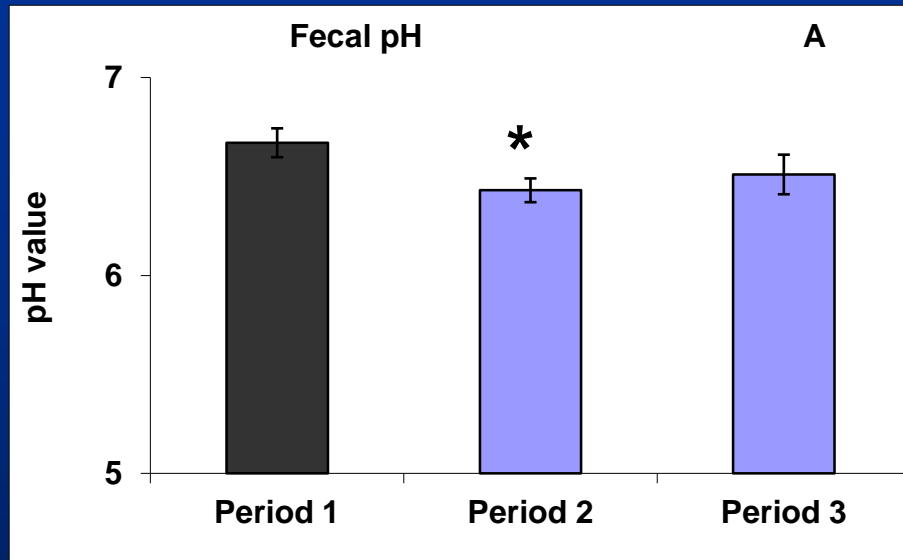
This increase may be due to the increase in the numbers of bifidobacteria and lactobacilli which possess higher levels of this enzyme.



Treat.	% increase relative to period 1:	
	Period 2	Period 3
FL	33	21
CAM30	30	21



## Fecal pH before and after consumption of either First Leaf (panel A) or CAM30 (panel B).



It has been reported that a high colonic pH promotes co-carcinogen formation (responsible for the development of colorectal cancer) from bacterially degraded bile acids and that acidification of the colon either by dietary fibre or milk may prevent this process.



# Conclusions and Recommendations (1)

- FISH analysis showed that consumption of First Leaf and CAM30 led to significant increases in the population sizes of both lactobacilli and bifidobacteria when compared to the baseline period.
- Consumption of First Leaf and CAM30 led to a significant reduction in the activity of  $\beta$ -glucuronidase, a proposed marker for colon cancer. This finding may be explained by the significant reduction in the populations of *Bacteroides* spp. and *Clostridium* spp.



# Conclusions and Recommendations (2)

- Consumption of First Leaf and CAM30 led to a significant increment in the activity of  $\beta$ -glucosidase which is mainly generated by lactic acid bacteria. This may be explained by the ability of these products to enhance the growth of lactobacilli and bifidobacteria.
- Consumption of First Leaf and CAM30 led to a significant reduction in the population sizes of undesirable bacteria such as *Bacteroides* spp. and *Clostridium* spp.
- The results showed that First Leaf and CAM30 supplementation significantly decreased the fecal pH value.



## Conclusions and Recommendations (3)

The results of this study open up the possibility that consumption of First Leaf and CAM30 can offer significant benefits to human health through acting as novel prebiotic agents (via increasing the numbers of lactobacilli and bifidobacteria in the gut) and novel inhibitors of  $\beta$ -glucuronidase which is one of the enzymes that increases risk for colon cancer.

