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**PROBIOTIC *BIFIDOBACTERIUM LACTIS* HN019  
ENHANCES THE RESISTANCE AND IMMUNITY  
AGAINST ENTERIC PATHOGENS**

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## ABSTRACT

Probiotics are defined as a group of live microorganisms, including some microbial stimulants that exert health promoting effects, such as the maintenance of a normal intestinal microbiota, increased nutritional value of foods, anticarcinogenic activity, reduction of serum cholesterol levels, alleviation of lactose intolerance and stimulation of the immune system. Some of strains of lactic acid bacteria (LAB) are representative probiotics. The objective of this study was to examine the immunomodulatory and antiinfection properties of a new identified LAB strain- *Bifidobacterium lactis* using two animal models.

Two experiments were conducted and reported in this thesis. In the first experiment, a piglet weaning diarrhoea model was used to test the efficacy of *Bifidobacterium lactis* HN019 protecting against diarrhoea associated with Rotavirus and *E. coli*. 17 three-week-old piglets were allocated into two groups balanced for liveweight and litter of origin. The first group (n=8) was orally administered *B. lactis* HN019 ( $10^9$  cfu/piglet/day) through the experiment; the second group (n=9) was not given *B. lactis* HN019 (control). After one week, the animals were penned individually and weaned onto a weaner diet. Blood samples were taken to measure the antibody responses, cell proliferation, and phagocytic activity of leukocytes (monocytes and neutrocytes). Also the effect of *B. lactis* HN019 on weaning diarrhoea was assessed by monitoring the severity of diarrhoea, feed intake and liveweight gain of the piglets on the weaner diet. Compared to the controls, piglets receiving *B. lactis* HN019 had lower severity of weaning diarrhoea, higher survival rate and feed conversion efficiency (or liveweight gain). The protection was associated with lower levels of faecal rotavirus and *E. coli* shedding, higher phagocytic activities and cell proliferative response to mitogens, and higher specific antibody titers. These results suggest that dietary *B. lactis* can reduce the severity of weaning diarrhoea associated with rotavirus and *E. coli*, and the probiotic is associated with enhanced immune responsiveness.

In the second experiment, the protective effects of *Bifidobacterium lactis* HN019 against *E. coli* O157:H7 and associated immunological parameters were investigated using murine models. After one week acclimatisation on a skim milk powder (SMP)-based diet, eighty-six

BALB/c and C57 mice were selected and randomised to two treatment groups. One group was fed on the SMP-based diet until the end of the experiment, while the other group was fed the SMP-based diet supplemented with *B. lactis* HN019 ( $3 \times 10^8$  cfu/g). After one week on these diets, mice were intragastrically inoculated with 0.1 ml *E. coli* O157:H7 suspension ( $10^9$  cfu/ml). Protection against *E. coli* O157:H7 infection was assessed by monitoring the morbidity, feed intake, bacterial translocation to visceral tissues (spleen and liver) and immune responsiveness. Phagocytic activities of blood and peritoneal cells, and antibody titres against *E. coli* O157:H7 in intestinal content were also measured. The results showed that *B. lactis* HN019-fed mice conferred a significant degree of protection against *E. coli* O157:H7 challenge in comparison to the control mice that did not receive *B. lactis* HN019. Protection included lower morbidity and higher post-challenge feed intake, reduced pathogen translocation to blood, spleen and liver, as well as significantly higher phagocytic activities of blood and peritoneal cells and anti-*E. coli* IgA level in gut content. These results suggest that *B. lactis* HN019 can enhance the host resistance to *E. coli* O157:H7 and that the protection is associated with enhanced immune functions.

In summary, potential immune enhancing effects of *B. lactis* HN019 were investigated in one pig trial and one mice trial. The results showed that supplement of *B. lactis* HN019 relieved diarrhoea associated with rotavirus and *E. coli* infection in piglets and enhance the host resistance to *E. coli* O157:H7 challenge in mice. Immunological measurements indicated *B. lactis* HN019 fed groups had significant higher phagocytosis and anti-*E. coli* IgA levels. And the pathogen shedding was also reduced in *B. lactis* HN019 fed groups. As concluded, *B. lactis* HN019 can provide a protective role against special enteric pathogen infection by its immunomodulatory effects.

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## ABBREVIATIONS

<i>B. lactis</i> HN019	<i>Bifidobacterium lactis</i> HN019 (DR10™)
BHI	brain heart infusion broth
ConA	concanavalin A
DTH	delayed-type hypersensitivity
EaggEC	enteroaggregative <i>E. coli</i> .
EHEC	enterohaemorrhagic <i>E coli</i>
ELISPOT	enzyme-linked immunospot assay.
EPEC	enteropathogenic <i>E coli</i> .
ETEC	enterotoxigenic <i>E.coli</i>
FACS	fluorescence-activated cell sorting
FITC	fluorescein isothiocyanate
FMLP	N-formyl-methionyl-lircyl-phenylalamine).
GALT	gut associated lymphoid tissue
GIT	gastrointestinal tract
GRAS	generally recognized as safe
HLA	human leukocyte antigens
ICAM-1	intercellular adhesion molecule-1
IFNs	interferons
IFU	infectious forming units
IgA	immunoglobulin A
IL	interleukin
LAB	lactic acid bacteria
LGG	<i>L. casei subsp. casei</i> strain GG
LPS	lipopolysaccharide
M cells	membranous cells
M-CSF	monocyte colony-stimulating factor
NK cells	natural killer cells
NZDRI	New Zealand Dairy Research Institute
PBL	peripheral blood lymphocytes
PBMCs	human peripheral blood mononuclear cells

PBS	phosphate buffered saline
PFC	plaque-forming cells
PHA.	phytohaemoagglutinin
PMNs	polymorphonuclear cells
PP	Peyer's patches
RA	rheumatoid arthritis
SCFA	short chain fatty acids
SI	stimulation index
SIgA	secretory IgA
SLE	systemic lupus erythematosus
SMP	skim milk powder
SRBC	sheep red blood cells
TNF	tumour necrosis factor
TNF- $\alpha$	tumour necrosis factor- $\alpha$
WHO	World Health Organization