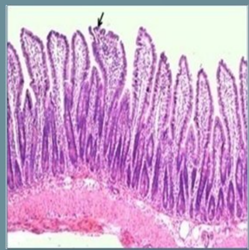  

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The Potential Immunomodulatory Effect of *L. rhamnosus* TW2 & *L. plantarum* TW14 Isolated from Local Goat Milk and Their Incorporation in Cheese

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I N D O N E S I A

Contents

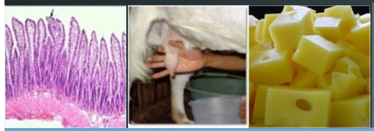
Introduction

- *Salmonella* is a causative agent of food poisoning outbreaks in Indonesia, ranging from 12.5 to 25% between 2008 and 2010.
- Infection of *S.* Typhimurium in the digestive tract is preceded by the invasion of the *Peyer's Patches* and M-cell, with ileum as the main target.
- Probiotic is alive, non-pathogenic microorganism that give beneficial effects on health when they are administered in adequate number.
- One role of probiotic bacteria is to maintain the balance of intestinal microflora in humans or animals, by means of reducing the incidence of gastrointestinal infections by pathogenic bacteria.

Introduction

4 ways probiotic defense against pathogens (Casey 2007)

- The antagonistic properties resulted from antimicrobial substances
- The ability to compete against pathogenic bacteria during attachment to intestinal mucosa
- The ability to compete against pathogenic bacteria for nutrients
- The improvement of host's immune system



Objectives

- To evaluate the influence *L. rhamnosus* TW2 and *L. plantarum* TW14 isolates, as well as cheese containing the LAB on:
  - the profiles of microflora
  - the morphological profile of ileum and caecum
  - the potency as immunomodulator
    - lymphocyte proliferation and
    - sIgA levels

Methodology

Testing was carried out with 5 treatment groups, each group consisted of 9 SD rats.

↓

LAB isolates were given orally as much as 1 mL/rat with a concentration of log 8 cfu mL^{-1}, while LAB-containing cheese was given as much as 1 g/rat, which contained log 8 cfu g^{-1} LAB.

↓

Infection of *S. Typhimurium* ATCC 14028 was done with concentration of log 8 cfu mL^{-1}.

↓

All rats were maintained for a total of 23 days

Treatment groups and stages of the experiment

Treatment Groups	Stages of the experiment		
	Initial Stage (day 1 to 10)	Infection Stage (day 11 to 13)	Final Stage (day 14 to 23)
Pro-typ-pro	Probiotic (pro)	*S.* Typhimurium *(Typ)*	Probiotic (pro)
Pro-typ-std	Probiotic (pro)	*S.* Typhimurium *(Typ)*	Control (std)
Che-typ-che	Cheese (che)	*S.* Typhimurium *(Typ)*	Cheese (che)
Che-typ-std	Cheese (che)	*S.* Typhimurium *(Typ)*	Control (std)
Std-typ-std	Control (std)	*S.* Typhimurium *(Typ)*	Control (std)

- ***Pro***: the rats were given isolate containing log 8 cfu mL^{-1} LAB;
- ***Che***: the rats were given fresh cheese containing log 8 cfu g^{-1} LAB;
- **Typ**: the rats were infected with *S.* Typhimurium ATCC 14028 as many as log 8 cfu mL^{-1}.
- ***Std***: the rats were given standard feed only.

The rats in each treatment were dissected after the initial, infection, and final stage

Methodology

The measured variables were:

1. PROFILES OF INTESTINE MICROFLORA
   - Quantitative Test of Lactic Acid Bacteria
   - Quantitative Test for Salmonella

2. POTENTIAL IMMUNOMODULATORY
   - Measuring of lymphocyte cells
   - Test for Secretory Immunoglobulin A (SIgA)

3. PROFILES OF INTESTINE MORPHOLOGY
   - Test for Hematoxylin-Eosin (HE) Staining

All treatment protocols have been approved by the Committee of Ethics, Department of Health, Republic of Indonesia No: KE.01.02/EC/06H/2011.

Result and Discussion

Number of LAB (log cfu g^{-1}) in ileum and caecum

Sampel	Sampling time (day)	pro-typ-pro	pro-typ-std	che-typ-kej	che-typ-std	std-typ-std (control)
Ileum	10	6.22±0,62	6,78±0,2	5,58±0,21	5,47±1,34	5,75±0,68
	13	6,05±0,14	6,30±0,38	5,45±0.05	5,10±0,47	4,34±0,27
	23	6,23±0,80	5,45±0,37	6,12±0,71	5,57±0,63	4,98±0,92
Caecum	10	5,56±0,17	6,32±0,56	6,07±0,49	5,97±0,77	5,36±0,17
	13	6,30±0,61	6,69±0,04	6,24±0,24	6,10±0,67	4,60±0,44
	23	6,29±0,62	6,04±0,21	6,33±0,40	5,74±0,48	5,32±0,31

- Day 10, the number of LAB in the ileum & caecum was higher than control.
- During the infection (day 11-13), the number of LAB was decreased in all treatments, both in ileum and caecum.
- Giving more LAB isolates and cheese after infection until day-23, increased the number of LAB.

Result and Discussion

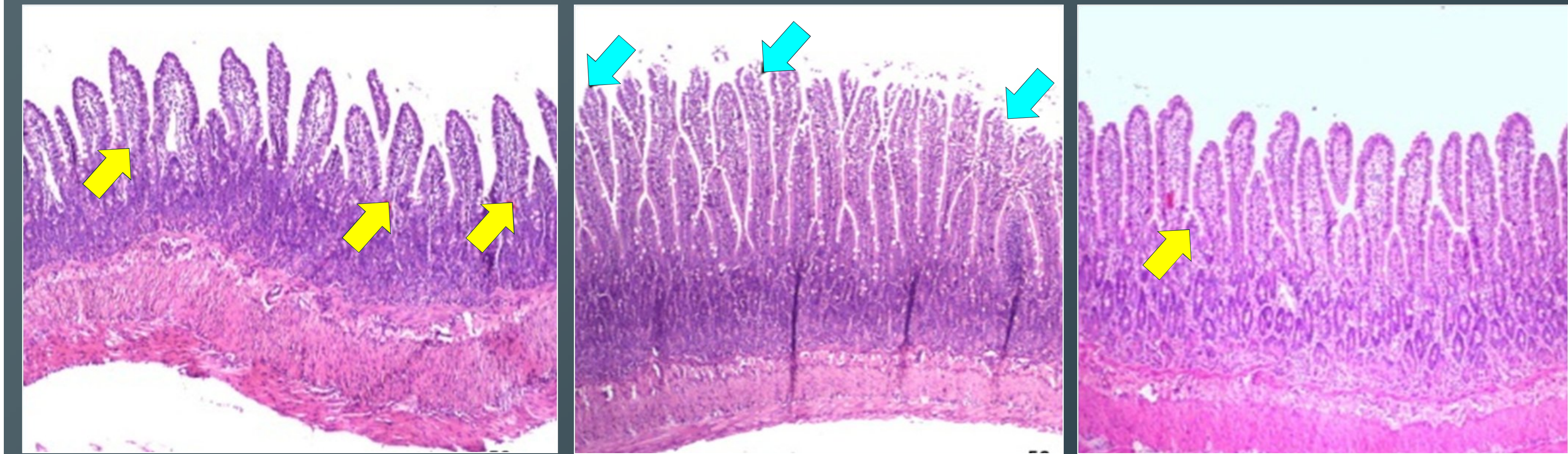
Number of *S.* Typhimurium (log CFU g^{-1}) in ileum and caecum

Sample	Sampling period	pro-typ-pro	pro-typ-std	che-typ-che	che-typ-std	std-typ-std (control)
Ileum	10	Nd	Nd	Nd	Nd	Nd
	13	3.22±0.29	3.10±0.01	3.68±0.21	3.60±0.17	4.59±0.20
	23	Nd	3.07±0.06	1.45±1.02	3.13±0.73	4.48±0.07
Caecum	10	Nd	Nd	Nd	Nd	Nd
	13	3.80±0.05	3.61±0.25	3.47±0.03	3.53±0.13	4.81±0.22
	23	Nd	0.8±1.38	Nd	2.94±0.07	4.92±0.62

- Before infection, the *S.* Typhimurium was not detected in both ileum & caecum.
- The number of *S.* Typhimurium in treatment groups was lower than control group ⇨ presence of LAB ⇨ inhibit the growth of the patogenic bacteria.
- The growth of *S.* Typhimurium was completely inhibited when the rat were continuously given LAB, during the post-infection.

Result and Discussion

Morphological profile of ileal villi after LAB feeding



Before infection | **During infection** | **After infection**

- *Blue arrows*: the damage ileal villi due to the infection of *S.* Typhimurium is indicated by the release of intestinal epithelial cells
- *Yellow arrow*: the goblet cells proliferation in the ileal villi before and after infection

Result and Discussion

Number of lymphocytes in the lymph (x 10^6 cell mL^{-1})

Sampling period (days)	Treatment				
	pro-typ-pro	pro-typ-std	che-typ-che	che-typ-std	std-typ-std (control)
10	99 ± 47	57 ±5.7	140±93	74± 6.4	57 ± 13
13	230 ±8.5	45± 34	27± 21	18± 8.1	6.8±1.6
23	370 ± 28	79 ± 59	190±15	120±71	15 ±2.1

- At day-10, the number of lymphocytes in all treatment was higher than the control.
- Treatment of LAB isolates and cheese containing LAB was able to improve the number of lymphocyte cells during the first 10 days, infection with *S.* Typhimurium, and post infection.

Result and Discussion

Absorbance value of secretory immunoglobulin A (SIgA)

Sample	Sampling period (days)	Absorbance value of SIgA OD 450 nm				
		pro-typ-pro	pro-typ-std	che-typ-che	che-typ-std	std-typ-std (control)
ileum	10	0.089±0.00^{a}	0.096±0.00^{a}	0.112±0.00^{b}	0.102±0.00^{ab}	0.101±0.00^{a}
	13	0.114±0.00^{b}	0.119±0.00^{b}	0.086±0.00^{a}	0.090±0.00^{a}	0.095±0.00^{a}
	23	0.102±0.00^{b}	0.093±0.00^{a}	0.078±0.00^{a}	0.090±0.00^{a}	0.085±0.00^{a}
caecum	10	0.097±0.00^{a}	0.112±0.00^{b}	0.101±0.01^{a}	0.085±0.00^{a}	0.104±0.01^{a}
	13	0.087±0.01^{a}	0.083±0.00^{a}	0.089±0.00^{a}	0.101±0.00^{a}	0.096±0.00^{a}
	23	0.97±0.00^{a}	0.092±0.00^{a}	0.079±0.00^{a}	0.084±0.00^{a}	0.086±0.00^{a}

- At the 10th days initial treatment, the absorbance of sIgA in ileum of che-typ-che isolates group, was increasing compared to the control.
- At the infection stage of *S.* Typhimurium, the LAB isolate treatment (pro-typ-pro) was able to increase sIgA absorbance in ileum.
- There is no influence of LAB isolates administration to sIgA in caecum.

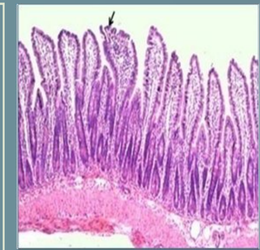
Conclusion

Mixed isolates of
L. rhamnosus TW2 & L. plantarum TW14 and
cheese contaning the LAB

Were able to show preventive functions during *S.* Typhimurium ATCC 14028 infection

Were able to show remedial functions during *S.* Typhimurium ATCC 14028 infection

Demonstrate potential immunomodulatory



Thank You !