CHAPTER 3

Objective

The objective of the study is divided into three specific aims as the followings.

Specific aim # 1

To investigate the role of the novel non-human isolated strain, *Lactobacillus paracasei* ST11 (HP4), in the alteration of representative gut microbiota composition in the genus/family levels in the diet-induced obese rats.

Specific aim # 2

To elucidate the effect of the probiotic *Lactobacillus paracasei* ST11 (HP4) consumption on the attenuation of metabolic endotoxemia, an increase in serum lipopolysaccharide (LPS) levels causing low-grade systemic inflammation, in the diet-induced obese rats.

Specific aim # 3

To determine the influence of the probiotic *Lactobacillus paracasei* ST11 (HP4) consumption on the gut inflammation by attenuation of gut pro-inflammatory cytokine gene expressions of diet-induced obese rats.

Hypothesis of the Study

I hypothesized that administration of *Lactobacillus paracasei* ST11 (HP4), a novel non-human origin isolated probiotic strain, (10^8 colony forming units (cfu) per day) by orally gavage to diet-induced obese rats for 12 weeks improves obese rat metabolic parameters by improving gut dysbiosis (decreased LPS-containing Gram-negative bacteria, Enterobacteriaceae, while increased beneficial bacteria, Bifidobacteria and/or Lactobacilli), attenuating gut inflammation and metabolic endotoxemia.

Scope of Study

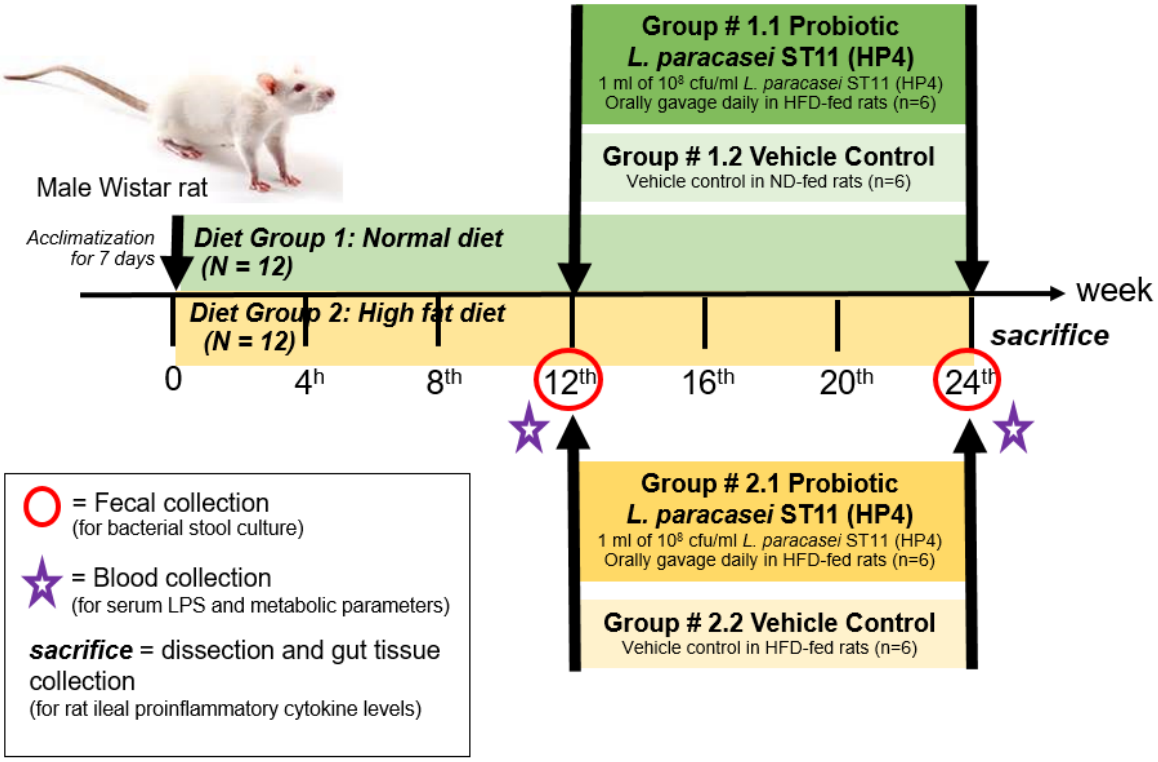

Figure 2 Experimental design After 7-day of acclimatization, male Wistar rats (n=24) were divided into two groups and fed *ad libitum* with normal or high-fat diet (12 rats per group) for 12 weeks. All rats in each group were further subdivided into two subgroups and orally gavaged with 10^8 cells of probiotic *Lactobacillus paracasei* ST11 (HP4) defined as an experimental group (Diet Group#1.1 and 2.1) or 1 ml of sterile phosphate buffered saline (PBS) as a vehicle control group (Diet Group#1.2 and 2.2) daily for 12 weeks. Then, rat stool was cultured using the appropriate selective media for Bifidobacteria, Lactobacilli and Enterobacteriaceae, at the 12th week (pre-treatment). Serum lipopolysaccharide (LPS) levels and metabolic parameters were measured at the 12th and 24th weeks. At the end of study (24th week), all rats were euthanized and colon content was collected for bacterial stool culture (post-treatment). Rat ileums were collected by immediate snap frozen in liquid nitrogen or submerged in RNA preservative solution for tissue RNA extraction and pro-inflammatory gene expression analysis.

Expecting Benefits

I aimed to evaluate the effects of probiotic bacteria *Lactobacillus paracasei* ST11 (HP4) on the alteration of gut microbiota composition, metabolic endotoxemia, and gut inflammation in the diet-induced obese rats. In this study, male Wistar rats (*Rattus norvegicus*) were used as an animal model. I expected that the study could provide the benefit and new findings to obesity and its related disease therapy, including

(1) The changes in gut microbiota composition in the high-fat diet-induced obese rats, *Rattus norvegicus* strain, when compared to vehicle control rats because the most previous accumulating studies performed in the diet-induced obese mice. This new study may show the different outcomes in different host background.

(2) The gut inflammation in the diet-induced obese *Rattus norvegicus* rats compared with normal diet-fed control rats.

(3) The induction of metabolic endotoxemia in long-term high-fat diet-consumed obese *Rattus norvegicus* rats compared to the lean counterparts.

(4) The roles of strain-specific effects of a novel non-human original isolated probiotic lactic acid bacteria, *L. paracasei* ST11 (HP4), on the gut microbiota composition, metabolic endotoxemia and gut inflammation in diet-induced obese rat model.

(5) These findings would provide some data of the pre-clinical study before the applications in the clinical study will be conducted.