CHAPTER 5

Results

Probiotic *Lactobacillus paracasei* ST11 (HP4) improves metabolic parameter in obese rat by decreasing serum total cholesterol levels

First, I would like to investigate whether the new strain of probiotic *Lactobacillus* could improve some metabolic parameters in the obese rats. After 12 weeks on the high fat diet, rats demonstrated significantly increases in their body weight, serum total cholesterol, triglyceride, low density lipoprotein (LDL) and very-low density lipoprotein (VLDL) levels but not high density lipoprotein (HDL) compared to normal diet-fed rat (Table 4)**.** Then, to investigate whether the new strain of probiotic *L. paracasei* ST11 (HP4) could ameliorate these obese rat metabolic abnormalities. Daily administration of 10^8 cells of *L. paracasei* ST11 (HP4) for 12 weeks was performed and I found that obese rats fed with this probiotic have significantly decreased plasma total cholesterol levels (approximately 50% reduction) compared to non-treated obese rats fed with vehicle control (normal PBS) (Table 4). However, the consumption of *L. paracasei* ST11 (HP4) could not decrease rat body weight, visceral fat weight, serum glucose, triglyceride, LDL and VLDL levels (Table 5). These results suggested that probiotic *L. paracasei* ST11 (HP4) confers the cholesterol-lowering effect *in vivo* and tends to be able to decrease plasma triglyceride levels even the result is not yet statistically significant (p-value = 0.07). However, the other metabolic abnormalities including gaining weight, visceral fat accumulation, plasma glucose, LDL and VLDL could not altered by consumption of 10^8 cfu daily of this new probiotic strain.

Table 4 Metabolic parameters at the 12th week of the male Wistar rats fed by normal or high fat diets compared to the baselines

Metabolic parameters	Baseline	Normal diet-fed	High-fat diet-fed
Body weight (g)	224.3 ± 4.1	422.5 ± 4.8	**570 ± 17.3*** **
Plasma glucose (mg/dL)	131.6 ± 4.5	137.3 ± 2.2	146.3 ± 4.7
Plasma total cholesterol (mg/dL)	54.4 ± 2.6	50.18 ± 2.3	**89.9 ± 5.7*** **
Plasma triglyceride (mg/dL)	61.5 ± 2.7	59.7 ± 3	**81.1 ± 7***
Plasma HDL cholesterol (mg/dL)	22.3 ± 0.4	25.5 ± 0.9	24.8 ± 1.5
Plasma LDL and VLDL cholesterol (mg/dL)	30.9 ± 3	31.2 ± 2.4	**47.5 ± 3.6****

High fat diet consumption for 12 weeks significantly increases rat body weight, plasma total cholesterol, triglyceride and LDL and VLDL cholesterol levels (HDL = high density lipoprotein, LDL = low density lipoprotein, VLDL = very-low density lipoprotein, *, **, *** = $P < 0.05$, 0.01 and 0.001 compared to the normal diet-fed group, respectively)

Table 5 Metabolic parameters of the lean and obese male Wistar rats daily fed by 10^8 colony forming units of probiotic *Lactobacillus paracasei* ST11 (HP4)

Metabolic parameters	Lean (normal-diet fed) rat		Obese (high-diet fed) rat	
	Vehicle	*L. paracasei* (ST11) HP4	Vehicle	*L. paracasei* (ST11) HP4
Body weight (g)	505 ± 15	506.2 ± 13	**734 ± 21*** **	650 ± 44
Visceral fat weight (g)	25.3 ± 3	24.7 ± 1.4	**64.4 ± 5****	71.9 ± 2.3
Plasma glucose (mg/dL)	130.1± 8	141.7 ± 7.6	150 ± 9.4	148.9
Plasma glucose AUC ($mg/dLxminx10^4$)	1.79 ± 0.1	2.45 ± 0.32	**3.8 ± 0.58***	2.78 ± 0.1
Plasma total cholesterol (mg/dL)	66.1 ± 5	57.6 ± 2.3	**114.4 ± 9.9***	**69 ± 3.1**$^{\#}$
Plasma triglyceride (mg/dL)	61.5 ± 4.8	58.7 ± 6.5	**83.3 ± 6.2***	**68.9 ± 6.9** (p=0.07)
Plasma HDL cholesterol (mg/dL)	29.5 ± 1.9	30.8 ± 1.5	30.1 ± 1.5	30.4 ± 2.9
Plasma LDL/VLDL cholesterol (mg/dL)	28.8 ± 4.2	22.4 ± 5.6	**50.5 ± 10****	36.6 ± 5.7 (p=0.12)

Daily consumption of *L. paracasei* ST11 (HP4) for 12 weeks promotes significantly decreases rat plasma total cholesterol (# $p < 0.05$) and tends to decrease plasma triglyceride levels ($p = 0.07$) in obese rat compared to the non-treated (vehicle control) obese group. However, the consumption of *L. paracasei* ST11 (HP4) shows no effect on rat body weight, visceral fat weight, plasma glucose, glucose tolerance, HDL, LDL and VLDL cholesterol levels. (HDL = high density lipoprotein, LDL = low density lipoprotein, VLDL = very-low density lipoprotein, *, **, *** = $p < 0.05$, 0.01 and 0.001 compared to the normal diet-fed group respectively.)

Attenuation of systemic and local low-grade inflammations in diet-induced obese rat by probiotic *Lactobacillus paracasei* ST11 (HP4)

Next, I investigated whether the long-term consumption of high fat diet leads to systemic (blood circulation) and local (gut tissue) inflammations in rat by detecting serum LPS levels and ileal pro-inflammatory cytokine gene expressions, respectively. By using the Pierce® LAL Chromogenic Endotoxin Quantitation Kit (Thermo Fisher Scientific, USA), I found that obese rats fed with high fat diet for 12 weeks have higher serum LPS levels than normal diet fed rats (Figure 3A). This result supported the previous study (35) that prolonged consumption of high fat diet could significantly induce metabolic endotoxemia in Wistar rat. Then, to determine the role of probiotic *L. paracasei* ST11 (HP4) in the attenuation of metabolic endotoxemia, obese rats were orally gavaged by 10^8 cfu of *L. paracasei* ST11 (HP4) in 2 ml of normal PBS as a vehicle daily for 12 weeks. Interestingly, I found that the obese rats fed with this probiotic strain show lower serum levels of LPS than non-treated and lean rats (Figure 3B). To elucidate the role of high fat diet consumption in low-grade gut inflammation, I quantified the pro-inflammatory cytokine gene (*Il-1b* and *Il-6*) expressions in rat ileum using real-time quantitative polymerase chain reaction (qRT-PCR) at the 12th week after the probiotic challenge. I found that high fat diet consumption increases the expressions of pro-inflammatory cytokine IL-1β and IL-6 in rat ileum and the consumption of probiotic *L. paracasei* ST11 (HP4) decreases these expressions significantly (Figure 4A and B, respectively). These results suggested that probiotic *L. paracasei* ST11 (HP4) could attenuate the low-grade inflammations in both systemic (blood circulation) and local (ileum) sites of the obese rats.

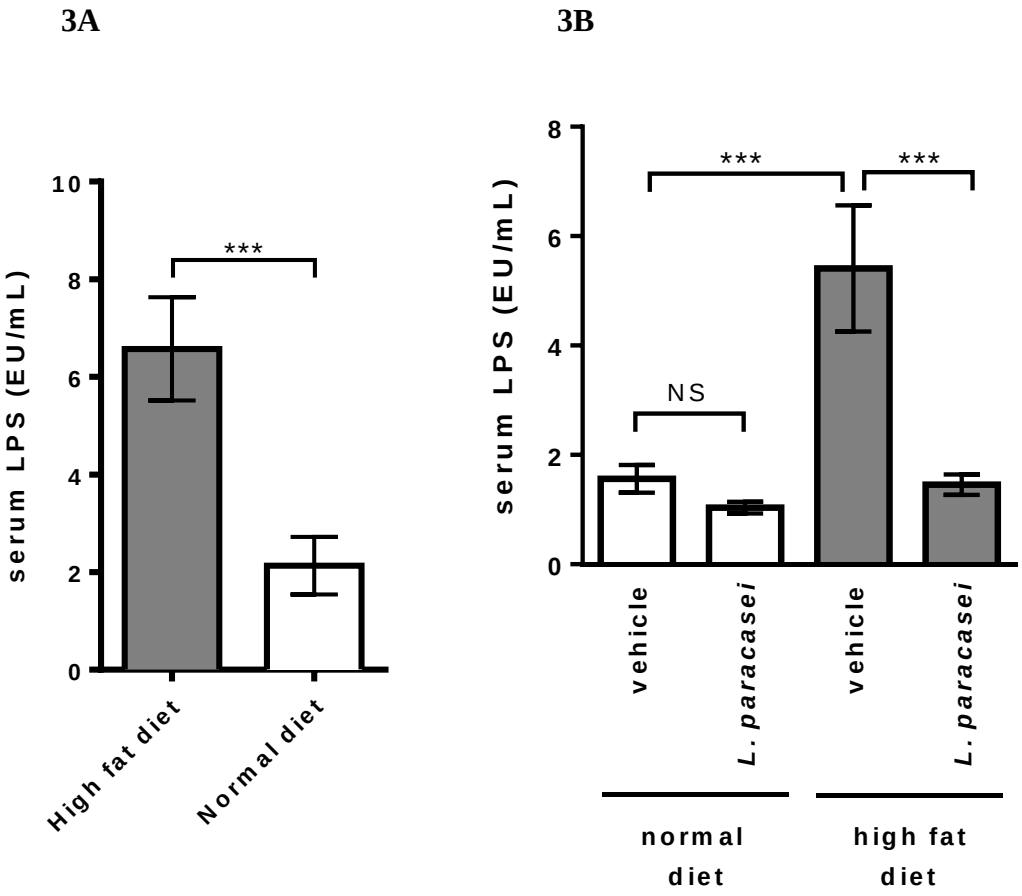


Figure 3 Rat developed metabolic endotoxemia, an increased serum lipopolysaccharide levels, after 12 weeks on high fat diet (grey bar) (A). 12 weeks of daily consumption of probiotic *L. paracasei* ST11 (HP4) treatment attenuates metabolic endotoxemia in the diet-induced obese rats (B). *(Bars represent mean +/- SEM, *** represents p-value < 0.001, SEM = Standard error of the mean and NS = non statistically significant)*

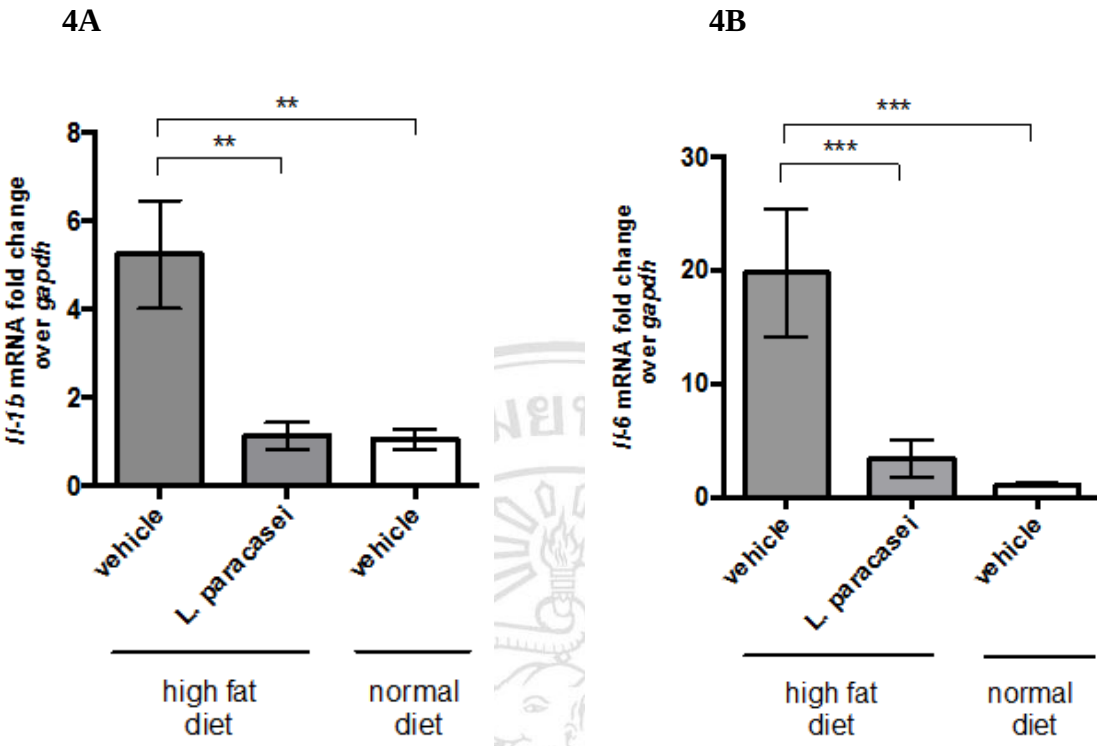


Figure 4 Long-term *L. paracasei* ST11 (HP4) feeding significantly decreases ileal pro-inflammatory cytokine interleukin (IL)-1β (A) and IL-6 (B) mRNA expressions in the diet-induced obese rats (grey bar) compared to the vehicle control group (white bar). *(Bars represent mean +/- SEM (standard error of the mean) and **, *** represent p-value < 0.01, 0.001 respectively)*

High fat diet consumption results in mild alterations of gut microbiota viable counts and independent of probiotic *L. paracasei* ST11 (HP4) administration

To determine whether high-fat diet consumption alters rat gut bacteria community. I examined the three large groups of rat gut microbiota including Gram negative LPS-containing Enterobacteriaceae and two of Gram positive gut mucosal beneficial microbiota, *Bifidobacterium* and *Lactobacillus*, by using the bacterial stool culture method as previously described (34). In brief, fresh rat stool or colon content were collected and immediately diluted with sterile normal phosphate buffered saline. Colony forming units per gram colon content were quantified by 10-fold serial dilution with separately plating on appropriate selective media for each bacterial groups (Enterobacteriaceae, *Bifidobacterium* and *Lactobacillus*). The results showed that long-term (12 weeks) on high fat diet results in enhanced growth of Gram negative LPS-containing Enterobacteriaceae in fecal pellets of obese rats but no significant changes in numbers of *Bifidobacterium* and *Lactobacillus* (Figure 5). Then, I investigated whether the administration of probiotic *L. paracasei* ST11 (HP4) could alter the viable population of these three groups of gut microbiota. Surprisingly, feeding both lean and obese rats with 10^8 cfu daily of probiotic *L. paracasei* ST11 (HP4) for 12 weeks shows no effect on the recovered numbers of Enterobacteriaceae, *Lactobacillus* and *Bifidobacterium* compared to the vehicle (normal phosphate buffered saline) control groups (Figure 6A, B and C, respectively). However, there was a higher in *Lactobacillus* cfu per gram of colon content of those obese rats fed with probiotic *L. paracasei* ST11 (HP4) than the lean counterparts (Figure 6B). Interestingly, the enhanced growth of Enterobacteriaceae that previously (at 12^{th} week) found in the obese rat's gut could not be observed at the 24^{th} week on the high fat diet challenge (Figure 6A). These data suggested that long term (12 weeks) consumptions of high fat diet could promote the blooming of Gram negative LPS-containing Enterobacteriaceae but not the prolonged consumption of high-fat fed as 24 weeks. However, the daily consumption of 10^8 cells of probiotic *L. paracasei* ST11 (HP4) for 12 weeks could not alter the viable counts of these three groups of gut microbiota.

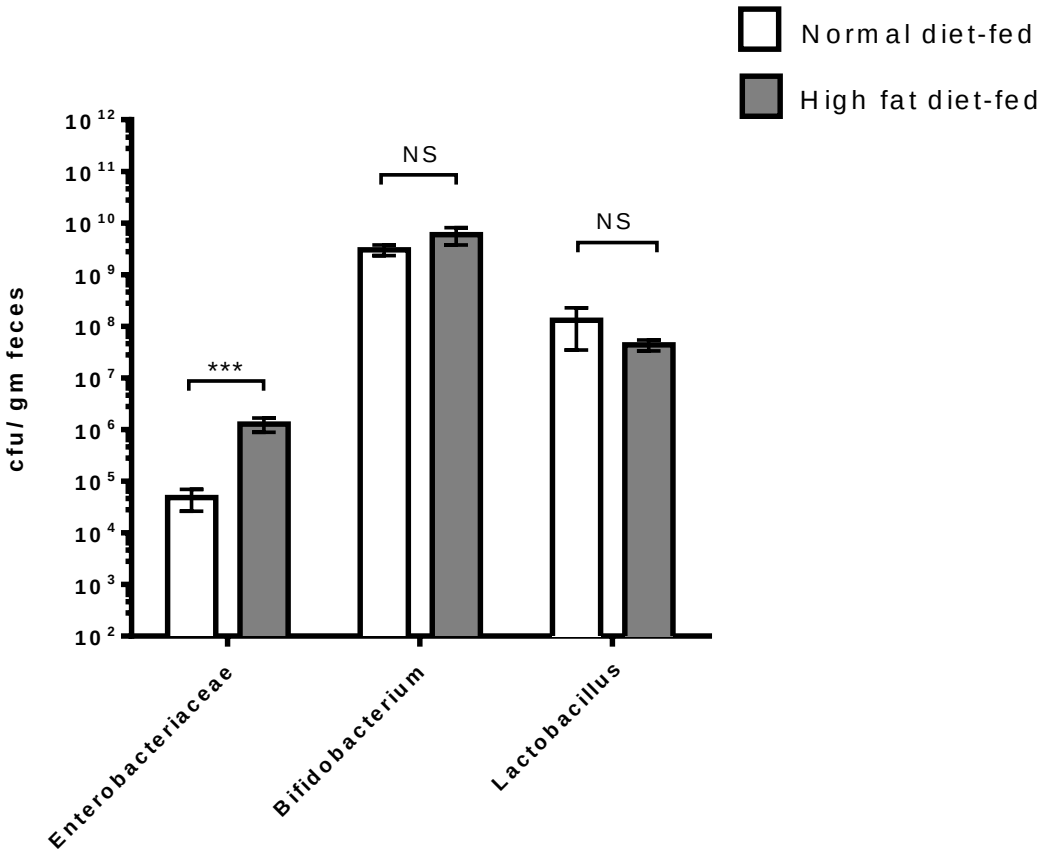
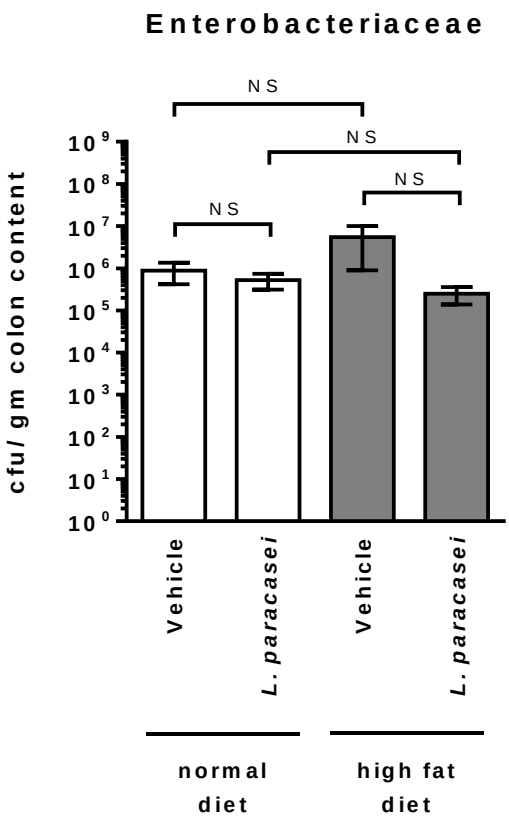
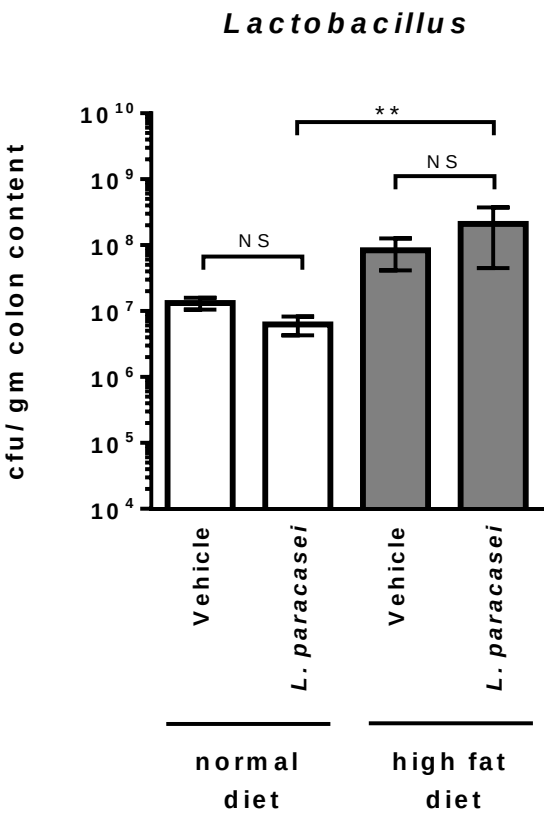


Figure 5 Long-term high-fat diet consumption promotes the growth of Enterobacteriaceae in rat compared to normal diet without the effects on the *Bifidobacterium* and *Lactobacillu*s burden *(Bars represent geometric mean +/- SEM (Standard error of the mean), *** represents p-value < 0.001, SEM = and NS = non statistically significant).*

6A



6B



6C

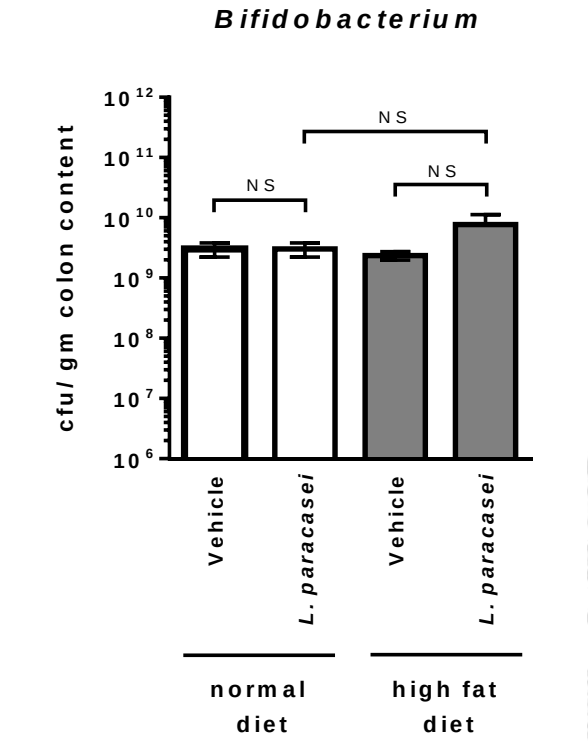


Figure 6 Treatment with probiotic *L. paracasei* ST11 (HP4) shows no effect on the Enterobacteriaceae burdens in the diet-induced obese rats (A). However, *L. paracasei* ST11 (HP4) consumption promotes the growth of *Lactobacillus* numbers in the diet-induced obese rats compared to their lean counterparts (** *p*-value < 0.01) (B). *L. paracasei* ST11 (HP4) treatment does not alter the *Bifidobacterium* numbers in the diet-induced obese group compared with the lean group *(Bars represent geometric mean +/- SEM (Standard error of the mean) and NS = non statistically significant).*