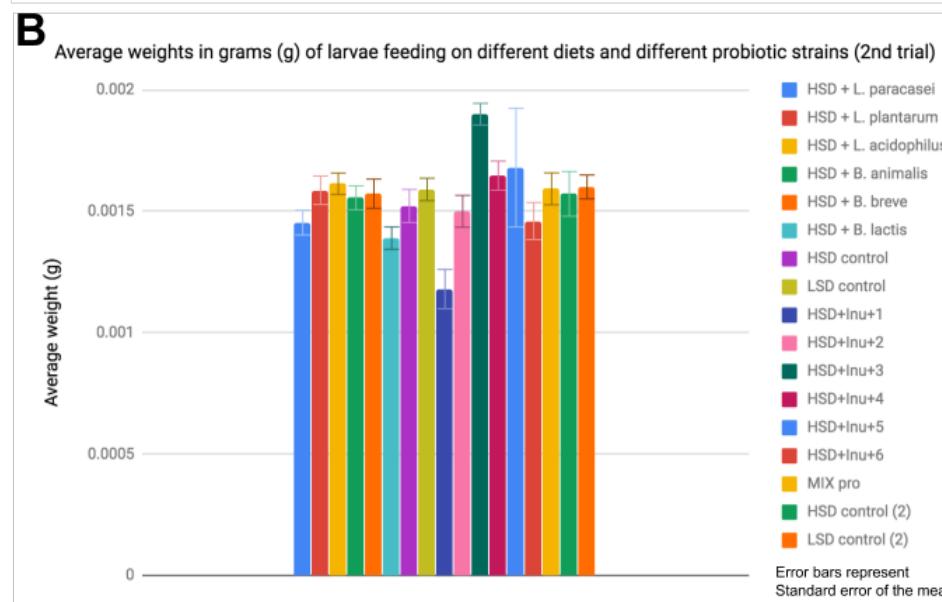
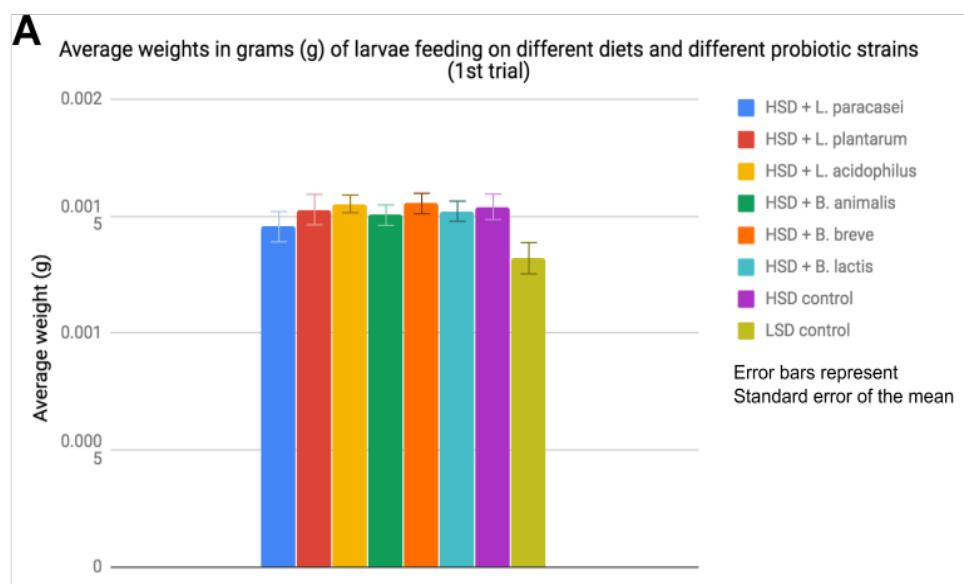
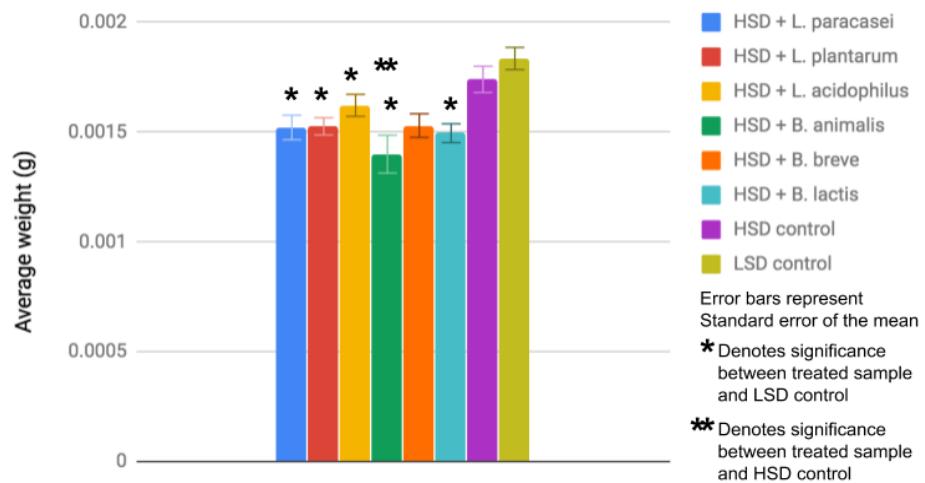


Appendix A

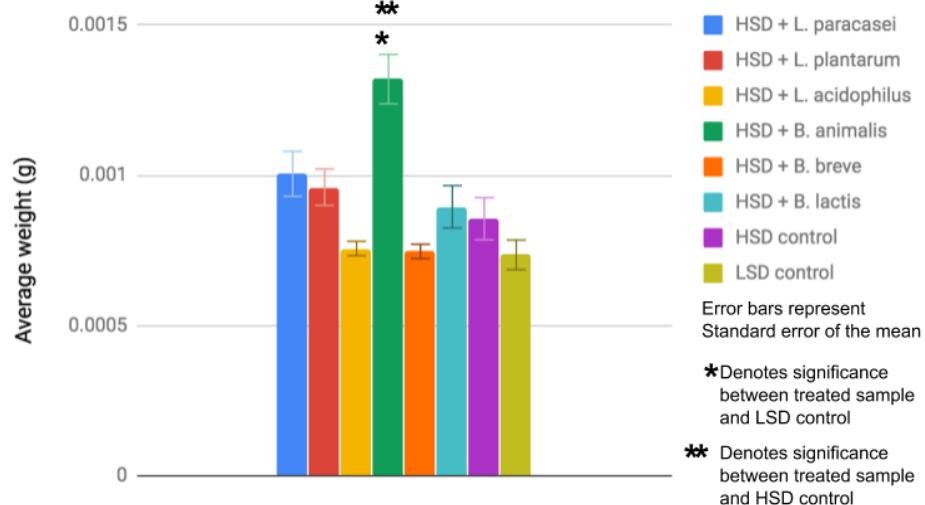


C

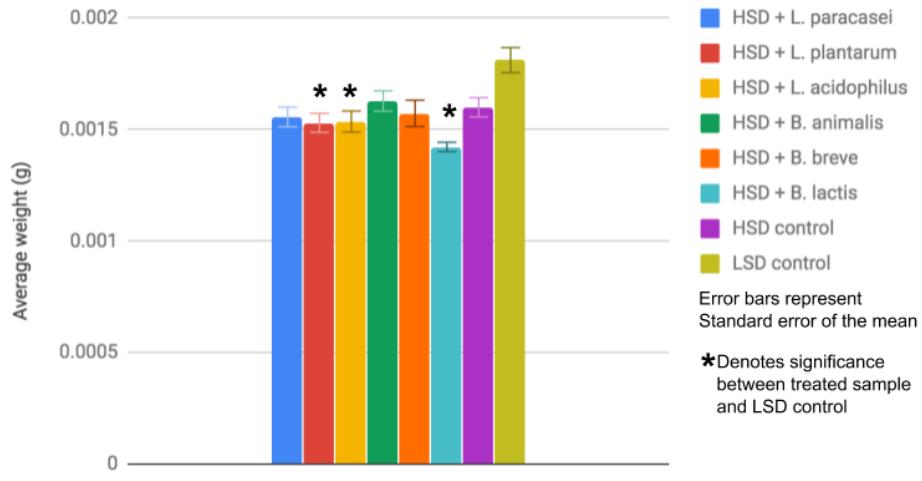
Average weights in grams (g) of 3rd instar larvae feeding on different diets and different probiotic strains (3rd trial)

**D**

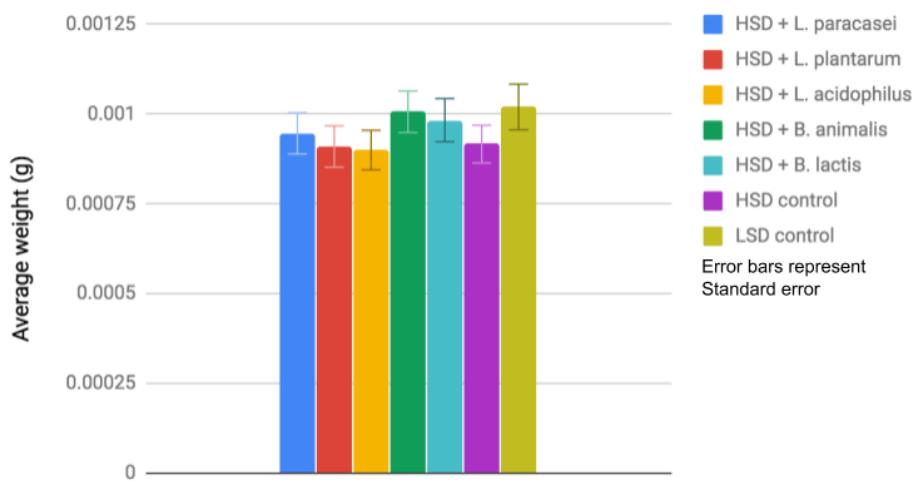
Average weights in grams (g) of adult flies feeding on different diets and different probiotic strains (3rd trial)

**E**

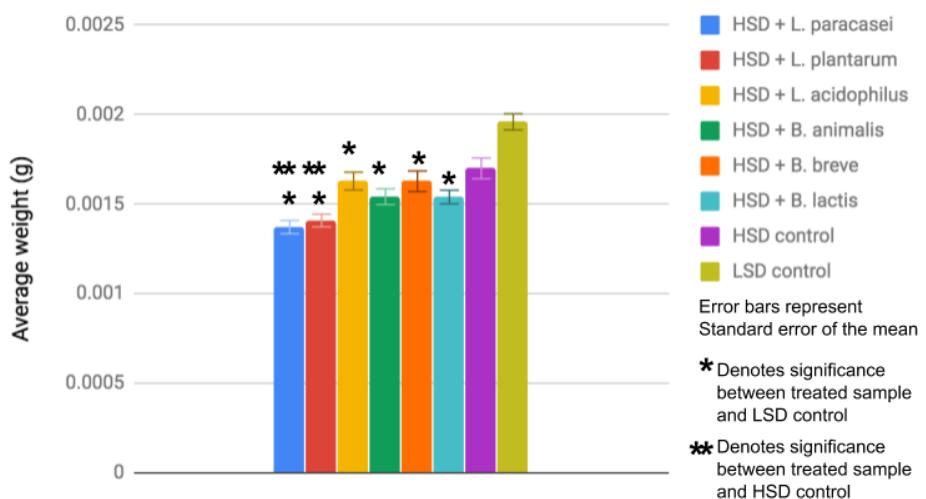
Average weights in grams (g) of 3rd instar larvae feeding on different diets and different probiotic strains (4th trial)



F Average weights in grams (g) of adult flies feeding on different diets and different probiotic strains (4th trial)



G Average weights in grams (g) of 3rd instar larvae feeding on different diets and different probiotic strains (5th trial)



H

Average weights in grams (g) of adult flies feeding on different diets and different probiotic strains (5th trial)

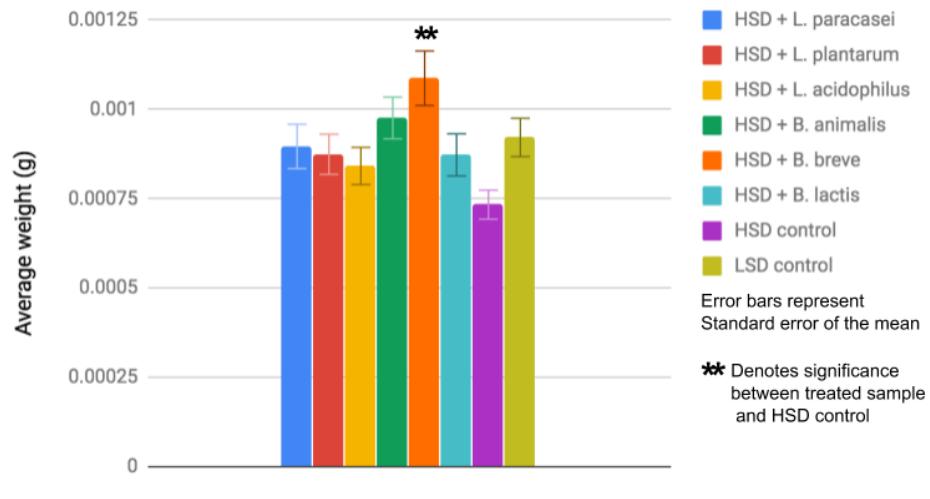
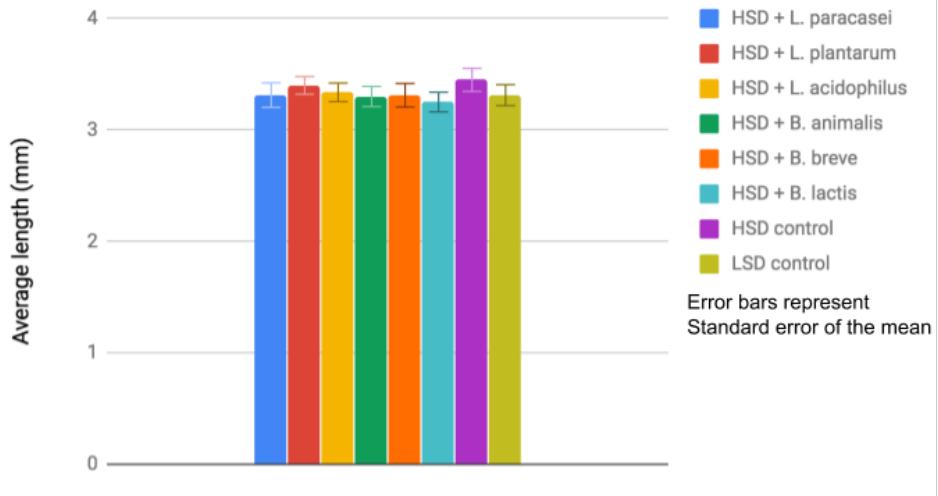


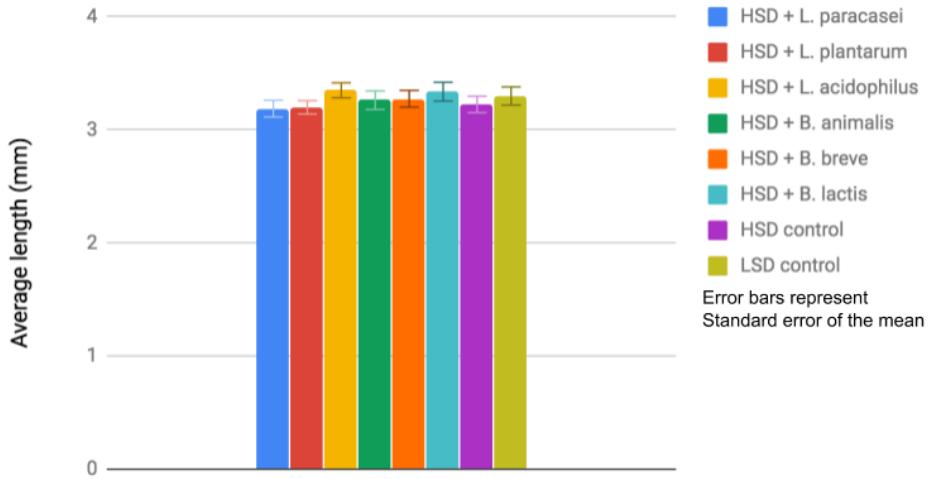
Figure A1. Average weights in grams (g) of: A, B, C, E, and G: larvae feeding on HSD, LSD and HSD with treatments. D, F, and H: adult flies feeding on HSD, LSD and HSD with treatments. * Asterisk denotes significance ($p<0.05$), and is determined using (One-Way ANOVA, $p>0.05$ and Kruskal-Wallis, $p>0.05$).

A

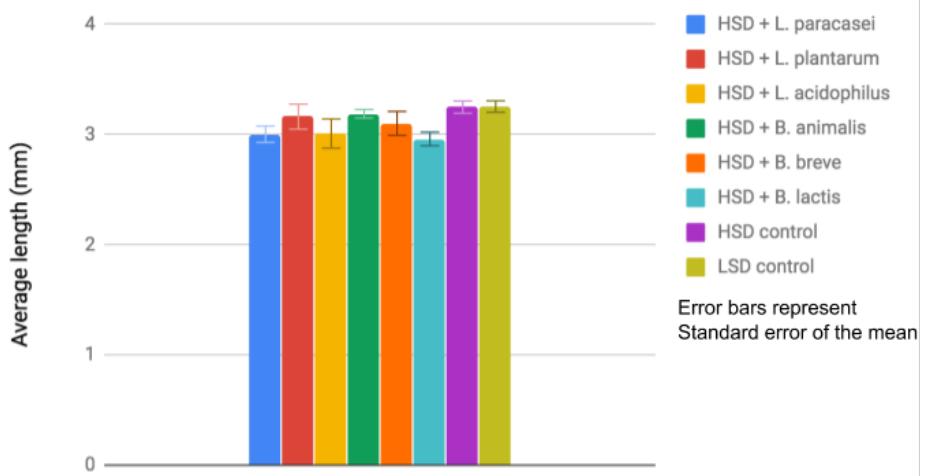
Average length in millimeter (mm) of larvae feeding on different diets and different probiotic strains (1st trial)

**B**

Average length in millimeter (mm) of larvae feeding on different diets and different probiotic strains (2nd trial)

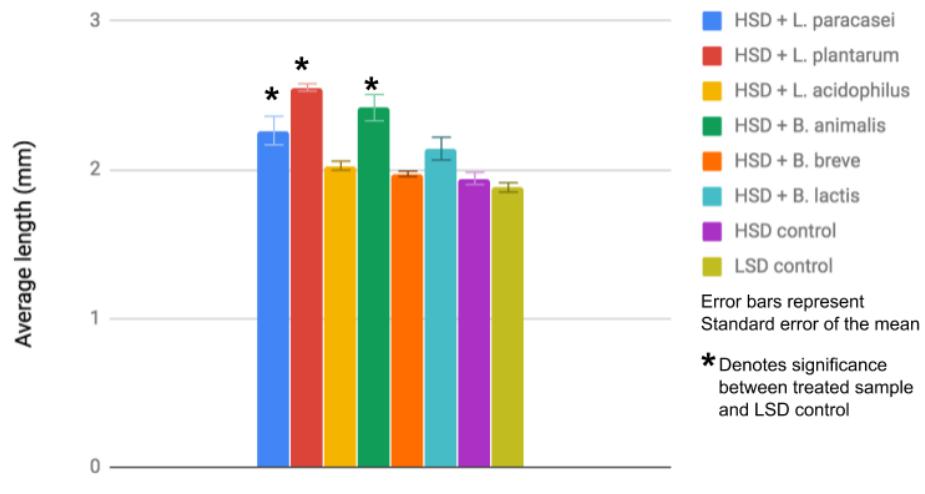
**C**

Average length in millimeter (mm) of 3rd instar larvae feeding on different diets and different probiotic strains (3rd trial)

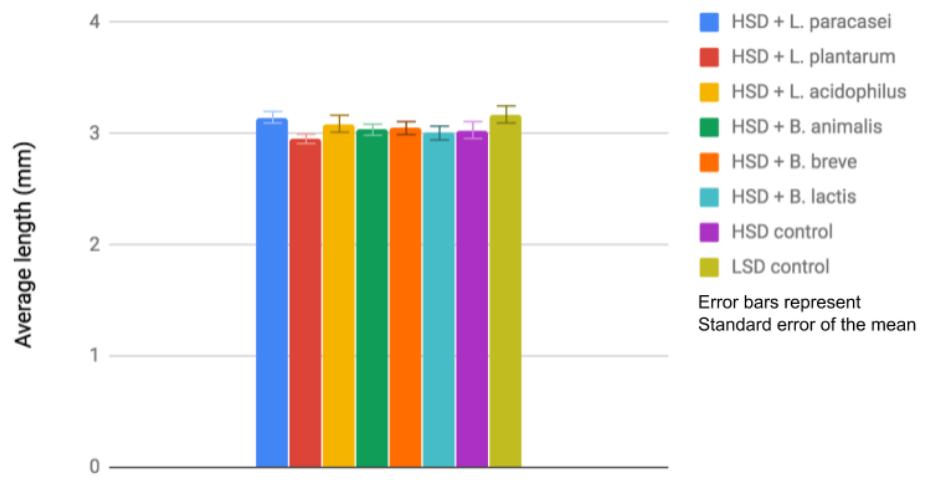


D

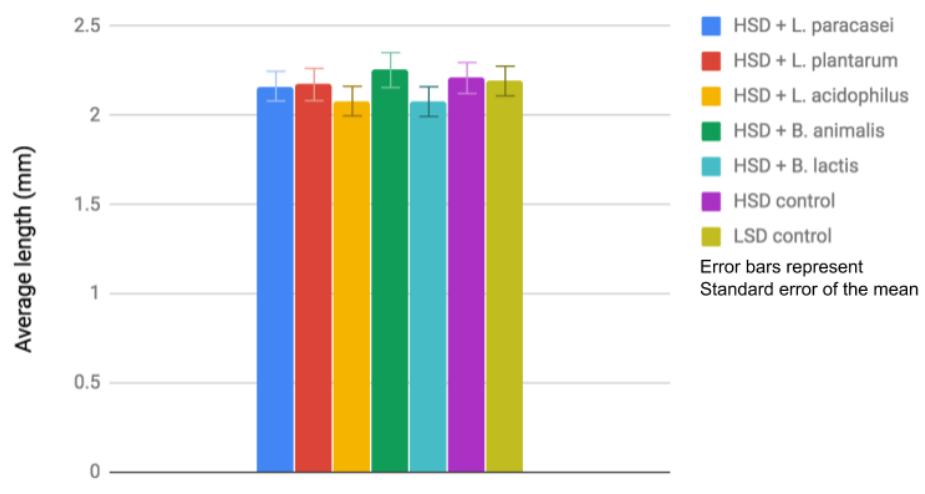
Average length in millimeter (mm) of adult flies feeding on different diets and different probiotic strains (3rd trial)

**E**

Average length in millimeter (mm) of 3rd instar larvae feeding on different diets and different probiotic strains (4th trial)

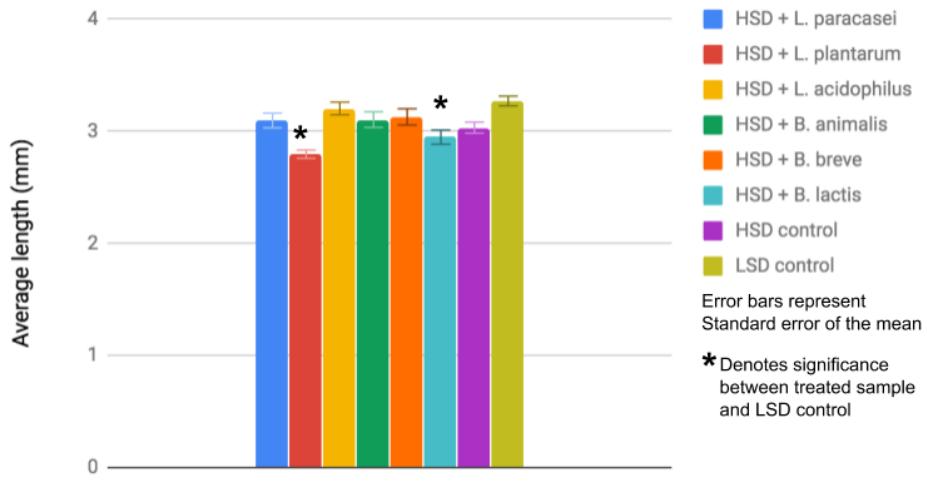
**F**

Average length in millimeter (mm) of adult flies feeding on different diets and different probiotic strains (4th trial)



G

Average length in millimeter (mm) of 3rd instar larvae feeding on different diets and different probiotic strains (5th trial)

**H**

Average length in millimeter (mm) of adult flies feeding on different diets and different probiotic strains (5th trial)

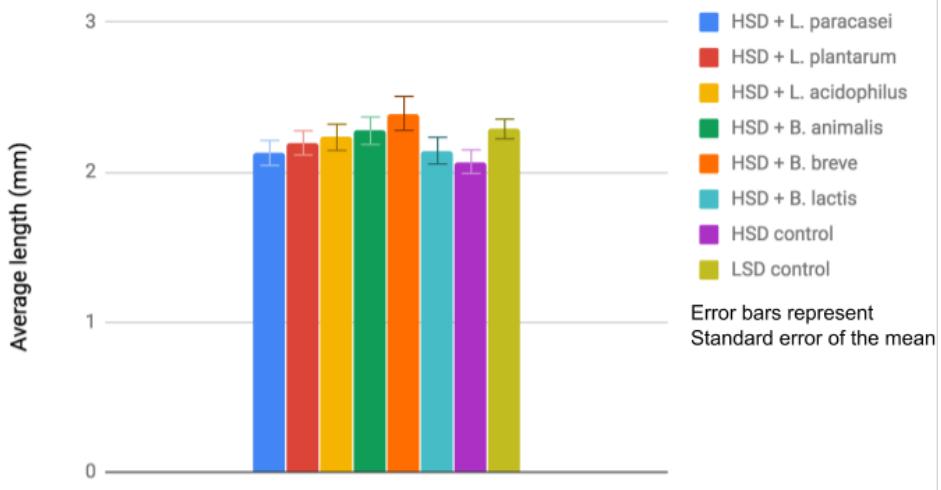
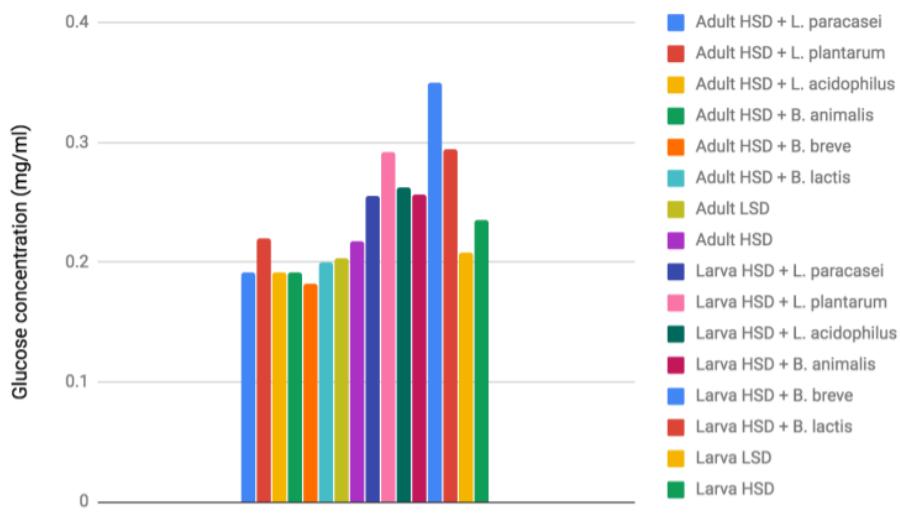


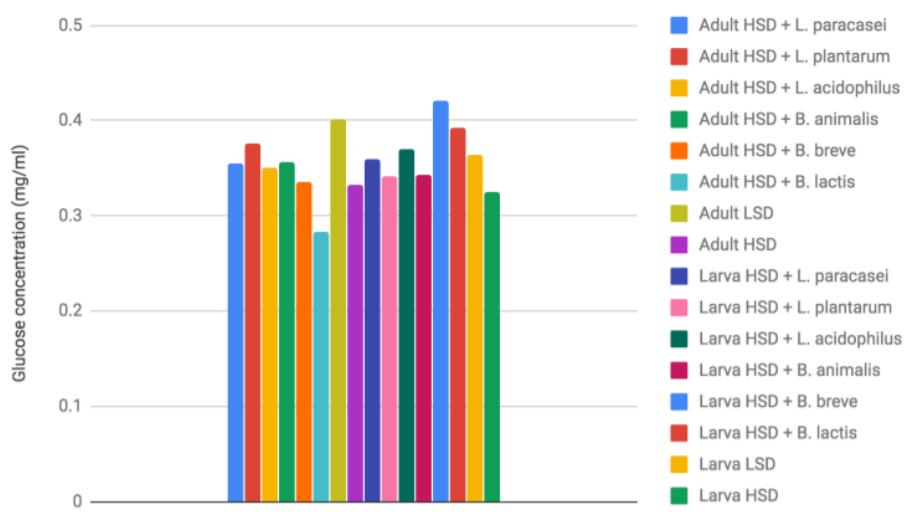
Figure A2. Average lengths in millimetres (mm) of: A, B, C, E, and G: larvae feeding on HSD, LSD and HSD with treatments. D, F, and H: adult flies feeding on HSD, LSD and HSD with treatments. * Asterisk denotes significance ($p<0.05$), and is determined using (One-Way ANOVA, $p>0.05$ and Kruskal-Wallis, $p>0.05$).

A

Glucose concentration in mg/ml in adult flies and 3rd instar larvae feeding on different diets and different probiotic strains (Trial 1) (15 minutes incubation)

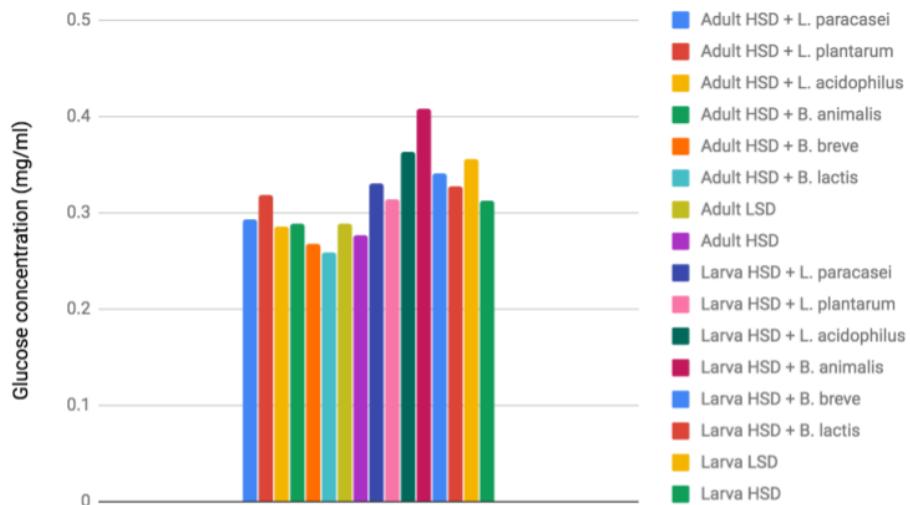
**B**

Glucose concentration in ml/ml in whole adult flies and 3rd instar larvae feeding on different diets and different probiotic strains (110 minutes incubation)

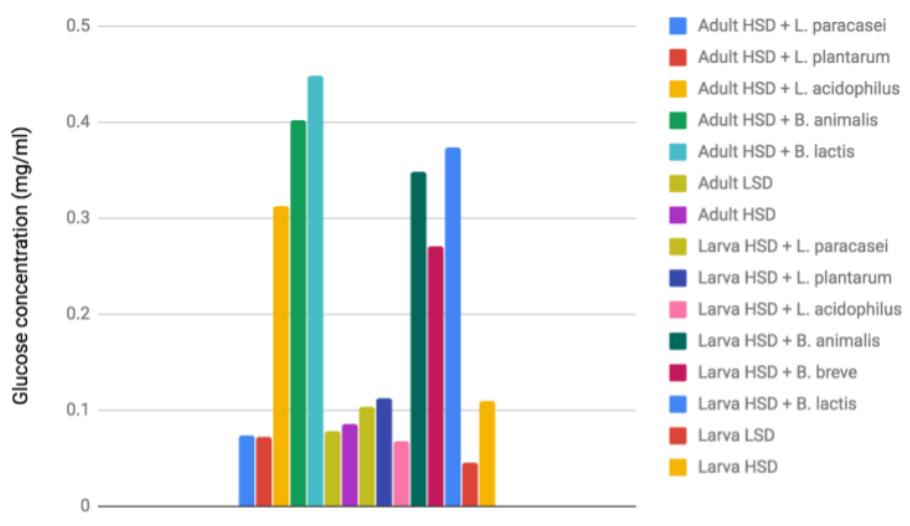


C

Glucose concentration in mg/ml in whole adult flies and 3rd instar larvae feeding on different diets and different probiotic strains (160 minutes incubation)

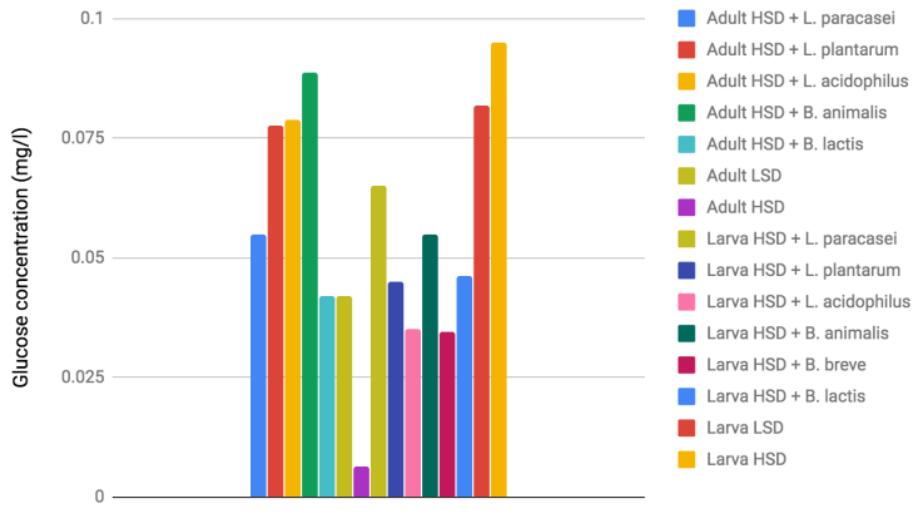
**D**

Glucose concentration in mg/ml in whole adult flies and 3rd instar larvae feeding on different diets and different probiotic strains (Trial 2) (15 minutes incubation)

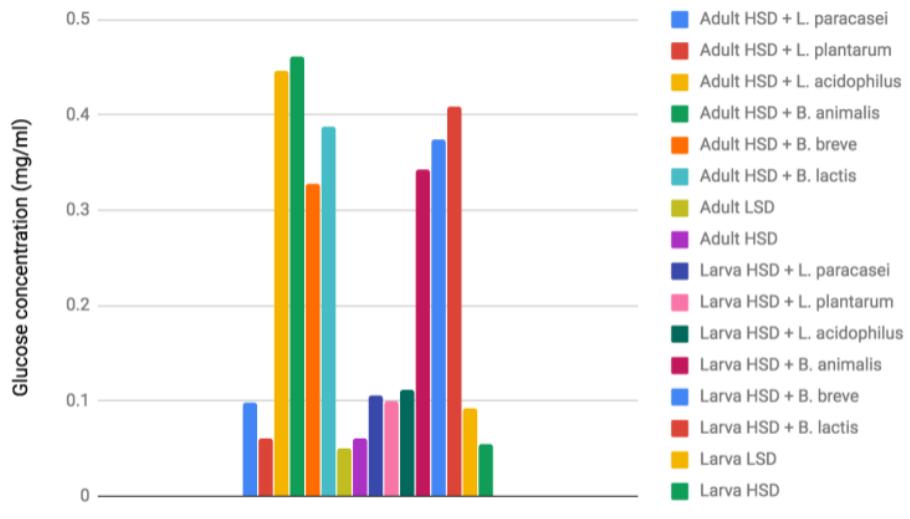


E

Glucose concentration in mg/ml in whole adult flies and 3rd instar larvae feeding on different diets and different probiotic strains (Trial 2) (24 hours incubation)

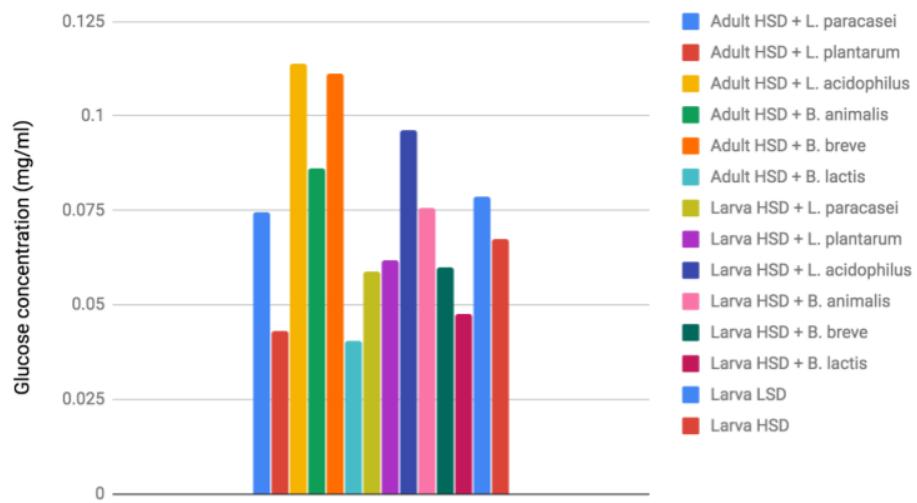
**F**

Glucose concentration in mg/ml in whole adult flies and 3rd instar larvae feeding on different diets and different probiotic strains (Trial 3) (15 minutes incubation)

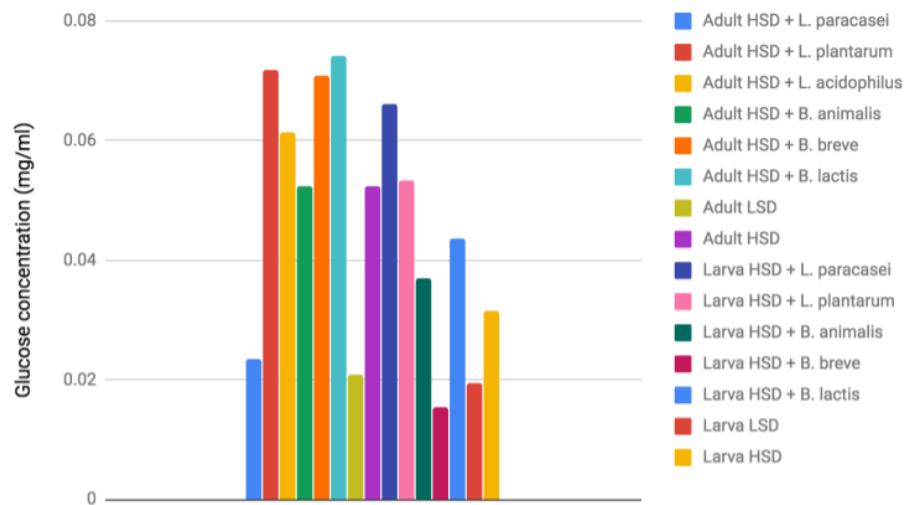


G

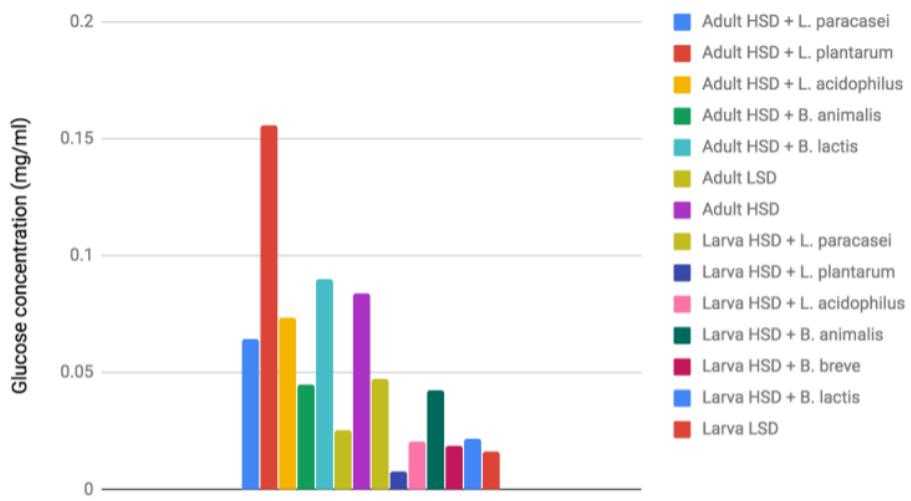
Glucose concentration in mg/ml in whole adult flies and 3rd instar larvae feeding on different diets and different probiotic strains (Trial 3) (24 hours incubation)

**H**

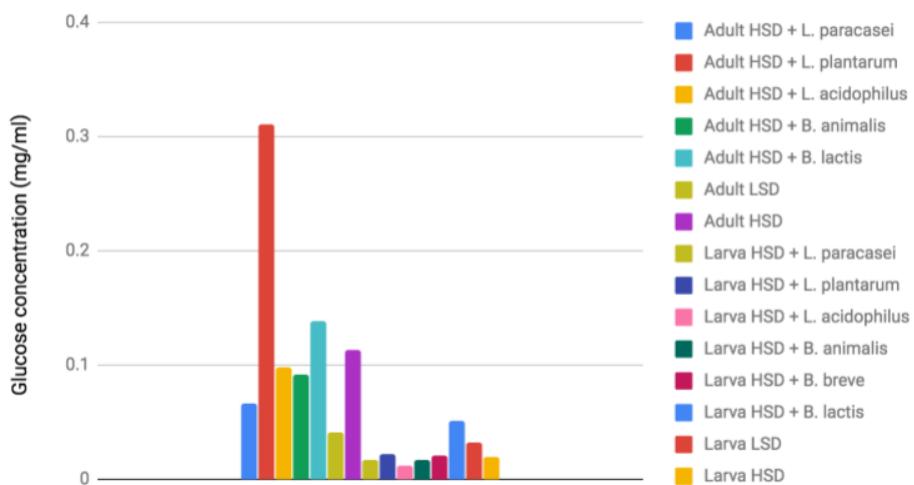
Glucose concentration in mg/ml in adult flies and 3rd instar larvae feeding on different diets and different probiotic strains (Trial 1) Gycogen protocol

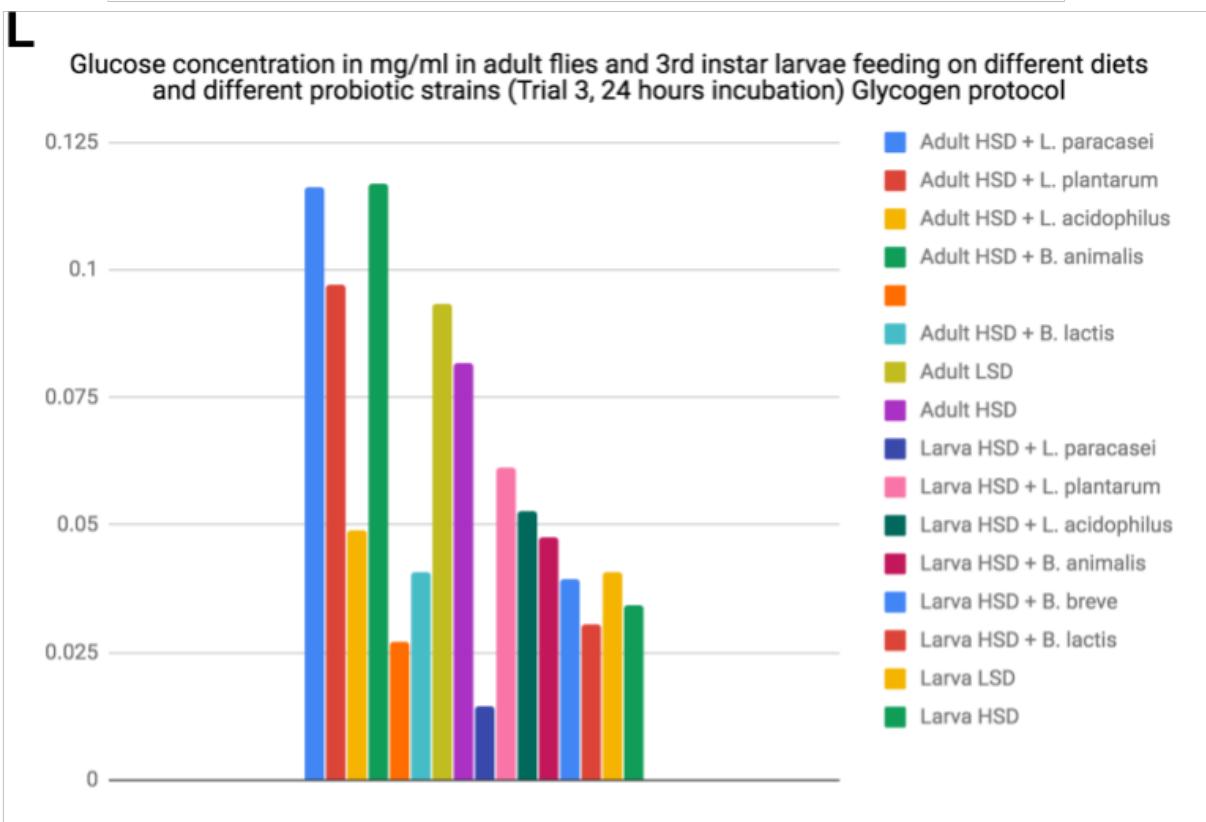
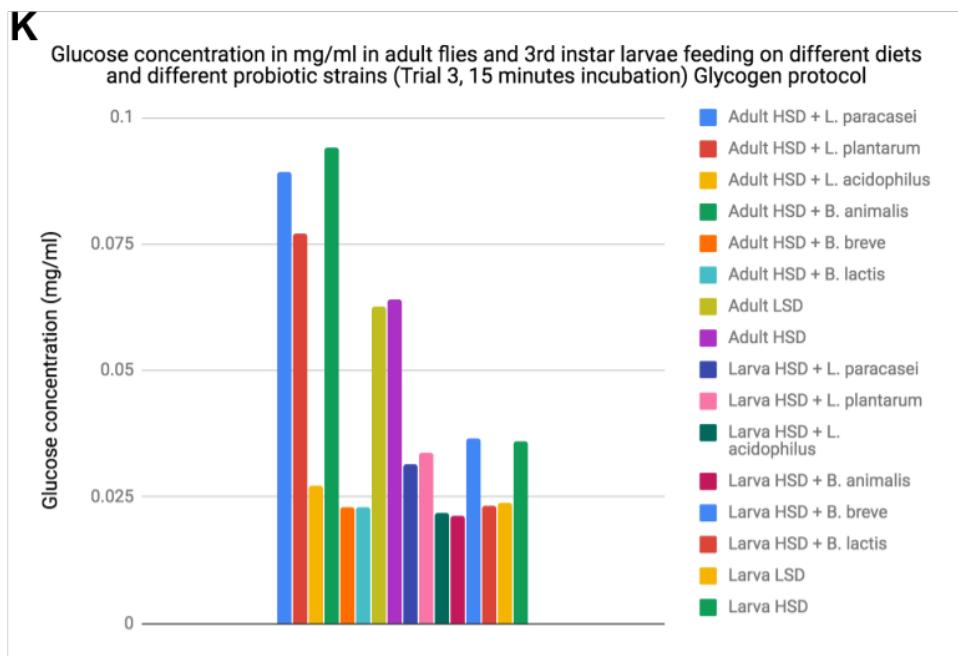


I Glucose concentration in mg/ml in adult flies and 3rd instar larvae feeding on different diets and different probiotic strains (Trial 2, 15 minutes incubation) Glycogen protocol



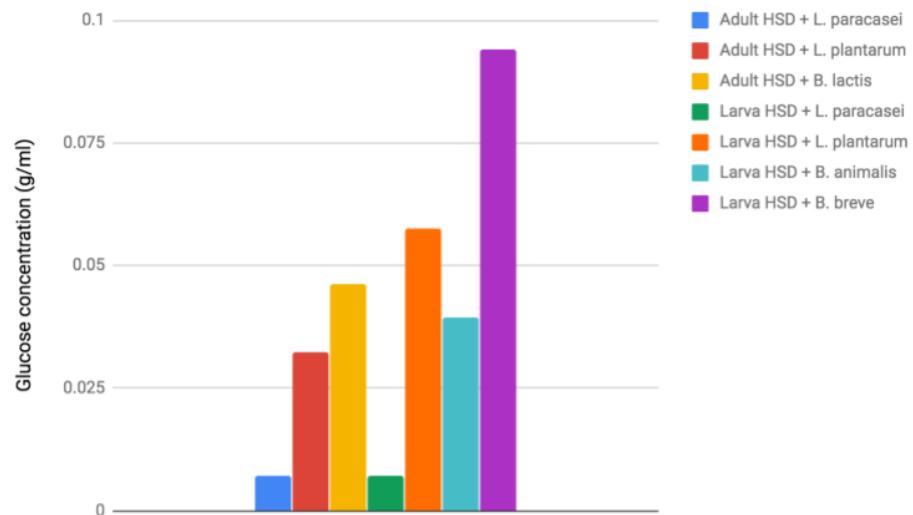
J Glucose concentration in mg/ml in adult flies and 3rd instar larvae feeding on different diets and different probiotic strains (Trial 2, 24 hours incubation) Glycogen protocol



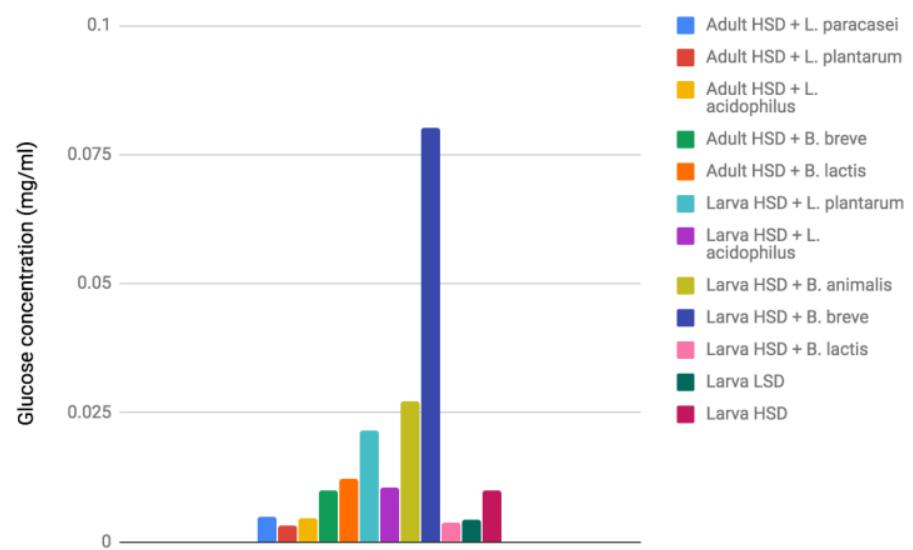


M

Glucose concentration in mg/ml in adult flies and 3rd instar larvae feeding on different diets and different probiotic strains (Trial 1, 15 minutes incubation) Trehalose protocol

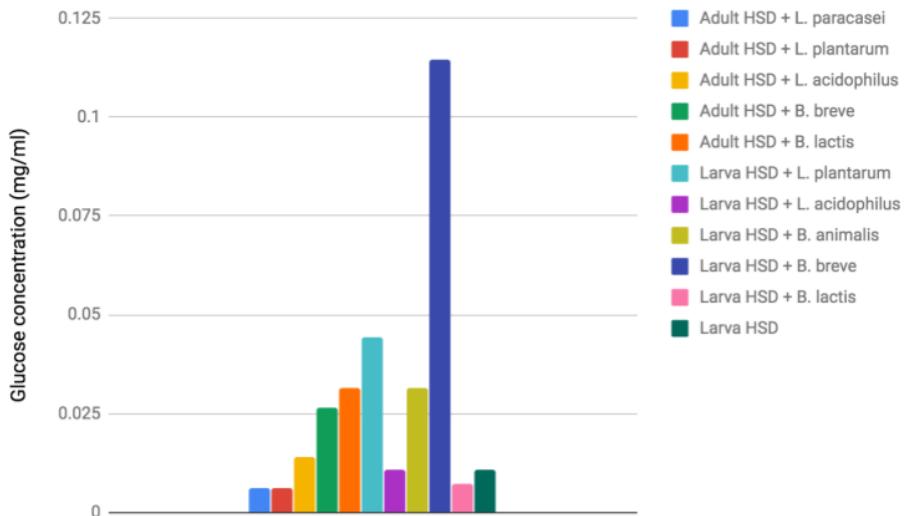
**N**

Glucose concentration in mg/ml in adult flies and 3rd instar larvae feeding on different diets and different probiotic strains (Trial 1, 24 hours incubation) Trehalose protocol

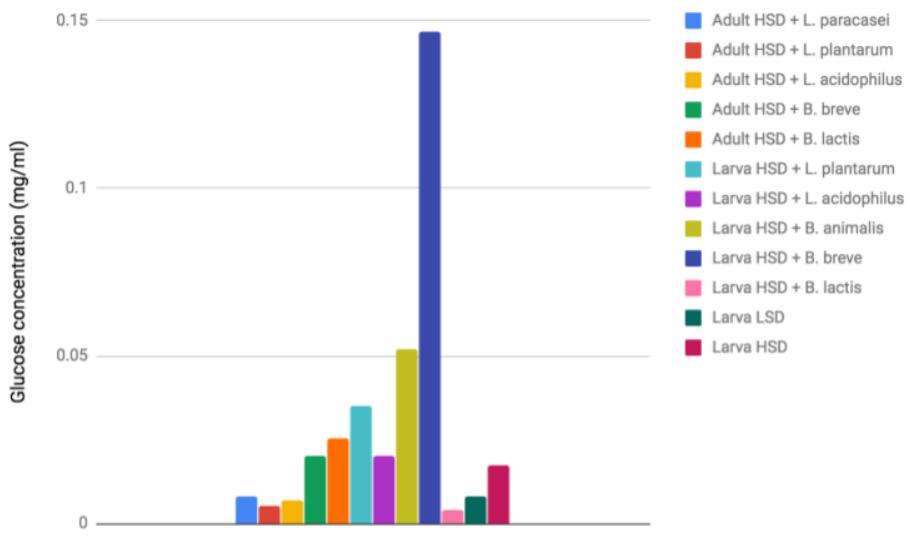


O

Glucose concentration in mg/ml in adult flies and 3rd instar larvae feeding on different diets and different probiotic strains (Trial 1, 48 hours incubation) Trehalose protocol

**P**

Glucose concentration in mg/ml in adult flies and 3rd instar larvae feeding on different diets and different probiotic strains (Trial 1, 72 hours incubation) Trehalose protocol



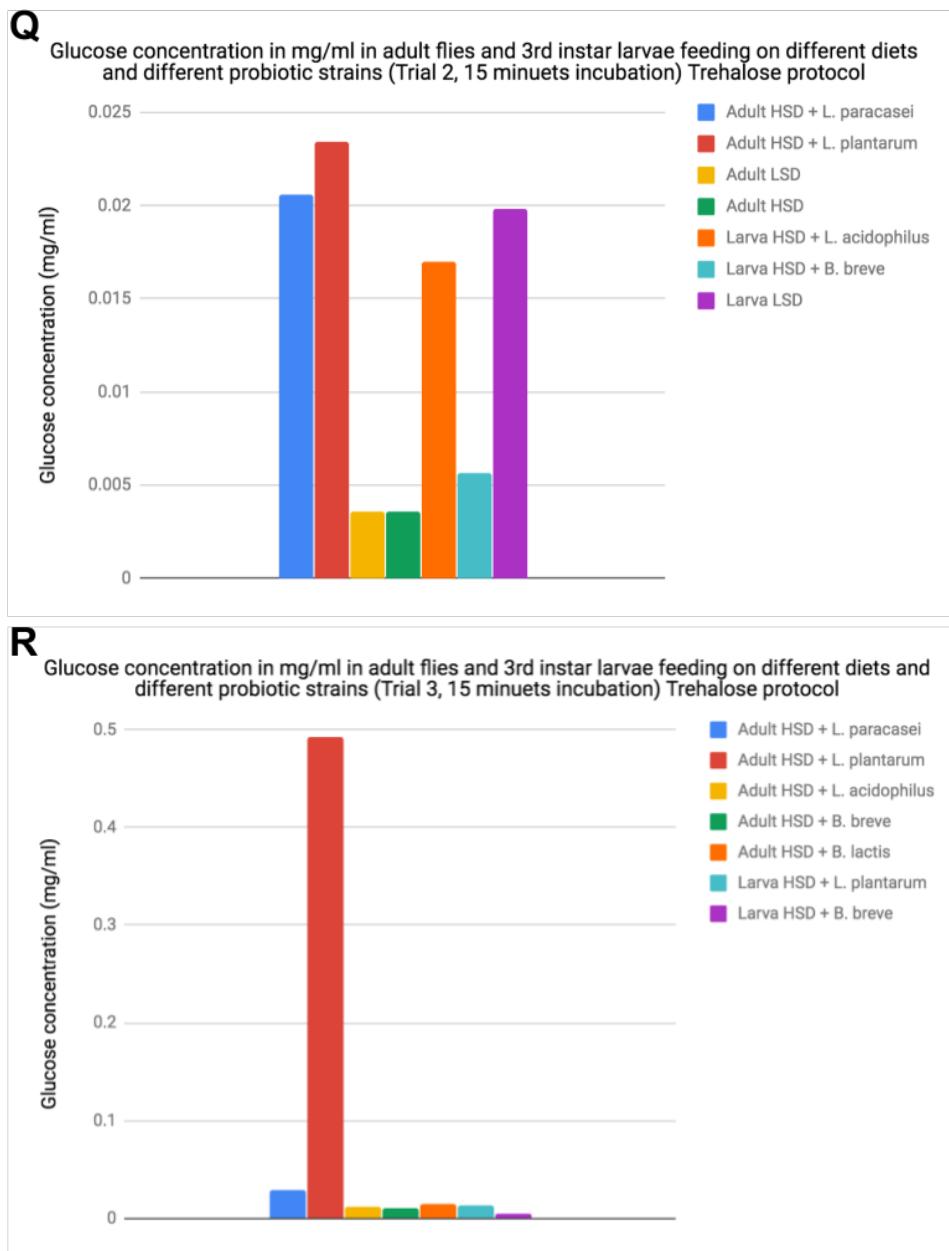


Figure A3. Carbohydrate concentrations in milligram per millilitre (mg/ml). A-G: Glucose concentrations in adult flies and larvae feeding on HSD, LSD and HSD with treatments. H-L: Glucose concentrations (Glycogen protocol) in adult flies and larvae feeding on HSD, LSD and HSD with treatments. M-R: Glucose concentrations (Trehalose protocol) in adult flies and larvae feeding on HSD, LSD and HSD with treatments. Since these protocols were carried out using only 3rd, 4th and 5th trials samples. These trials were reassigned the order as trials 1, 2 and 3 respectively (i.e 3rd trial is represented above as trial 1 ...etc). No statistical tests were carried out using these data. The combined data on the other hand were tested for significance (Figures 4, 5 and 6).

Appendix B

Kaplan-Meier survival plot of flies feeding on different diets and probiotic strains

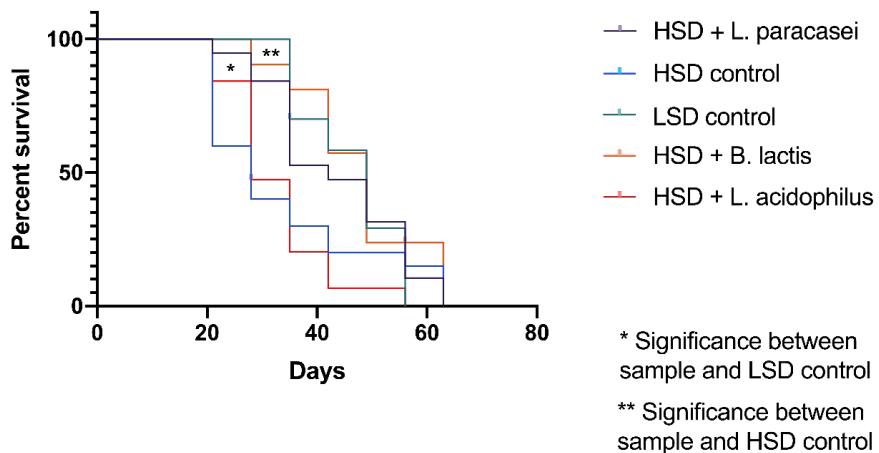


Figure B1. Kaplan-Meier survival plot of flies feeding on HSD and LSD (control samples) and HSD with probiotics.
 * Asterisk denote significance ($p<0.05$), and is determined using Log-rank (mantel-Cox) test.

Appendix C

Table C1. Raw Cq data of *PEPCK*, *Fbp*, *Hex-t1* and *Zw* obtained from qPCR.

Sample	Gene	Cq
Adult HSD	<i>PEPCK</i>	33.59
Adult HSD	<i>PEPCK</i>	30.51
Adult HSD	<i>PEPCK</i>	30.8
Adult HSD	<i>Fbp</i>	29.43
Adult HSD	<i>Fbp</i>	29.89
Adult HSD	<i>Fbp</i>	30.23
Adult HSD	<i>Hex-t1</i>	31.67
Adult HSD	<i>Hex-t1</i>	32.11
Adult HSD	<i>Hex-t1</i>	31.46
Adult HSD	<i>Zw</i>	30.65
Adult HSD	<i>Zw</i>	31.23
Adult HSD	<i>Zw</i>	29.94
Adult HSD		NaN
Adult HSD	<i>RPL32</i>	24.08
Adult HSD	<i>RPL32</i>	23.9
Adult HSD	<i>RPL32</i>	23.88
Adult LSD	<i>PEPCK</i>	30.6
Adult LSD	<i>PEPCK</i>	30.06
Adult LSD	<i>PEPCK</i>	30.49
Adult LSD	<i>Fbp</i>	30.18
Adult LSD	<i>Fbp</i>	30.23
Adult LSD		NaN

Adult LSD	<i>Hex-t1</i>	19.65
Adult LSD	<i>Hex-t1</i>	32.85
Adult LSD	<i>Hex-t1</i>	33.26
Adult LSD	<i>Zw</i>	32.14
Adult LSD	<i>Zw</i>	38.91
Adult LSD	<i>Zw</i>	32.58
Adult LSD	NaN	
Adult LSD	<i>RPL32</i>	25.2
Adult LSD	<i>RPL32</i>	26.84
Adult LSD	<i>RPL32</i>	26.28
Adult HSD + <i>L. paracasei</i>	<i>PEPCK</i>	30.85
Adult HSD + <i>L. paracasei</i>	<i>PEPCK</i>	31.4
Adult HSD + <i>L. paracasei</i>	<i>PEPCK</i>	31.7
Adult HSD + <i>L. paracasei</i>	<i>Fbp</i>	29.68
Adult HSD + <i>L. paracasei</i>	<i>Fbp</i>	30.03
Adult HSD + <i>L. paracasei</i>	<i>Fbp</i>	29.96
Adult HSD + <i>L. paracasei</i>	<i>Hex-t1</i>	38.36
Adult HSD + <i>L. paracasei</i>	<i>Hex-t1</i>	37.44
Adult HSD + <i>L. paracasei</i>	<i>Hex-t1</i>	NaN
Adult HSD + <i>L. paracasei</i>	<i>Zw</i>	31.77
Adult HSD + <i>L. paracasei</i>	<i>Zw</i>	32.34
Adult HSD + <i>L. paracasei</i>	<i>Zw</i>	31.62
Adult HSD + <i>L. paracasei</i>	NaN	
Adult HSD + <i>L. paracasei</i>	<i>RPL32</i>	NaN
Adult HSD + <i>L. paracasei</i>	<i>RPL32</i>	26.58
Adult HSD + <i>L. paracasei</i>	<i>RPL32</i>	27.33
Adult HSD + <i>L. plantarum</i>	<i>PEPCK</i>	31.43
Adult HSD + <i>L. plantarum</i>	<i>PEPCK</i>	32.37
Adult HSD + <i>L. plantarum</i>	<i>PEPCK</i>	31.54
Adult HSD + <i>L. plantarum</i>	<i>Fbp</i>	28.65
Adult HSD + <i>L. plantarum</i>	<i>Fbp</i>	29.36
Adult HSD + <i>L. plantarum</i>	<i>Fbp</i>	29.05
Adult HSD + <i>L. plantarum</i>	<i>Hex-t1</i>	36.51
Adult HSD + <i>L. plantarum</i>	<i>Hex-t1</i>	35.56
Adult HSD + <i>L. plantarum</i>	<i>Hex-t1</i>	NaN
Adult HSD + <i>L. plantarum</i>	<i>Zw</i>	30.86
Adult HSD + <i>L. plantarum</i>	<i>Zw</i>	34.31
Adult HSD + <i>L. plantarum</i>	<i>Zw</i>	31.51
Adult HSD + <i>L. plantarum</i>	NaN	
Adult HSD + <i>L. plantarum</i>	<i>RPL32</i>	24.92

Adult HSD + <i>L. plantarum</i>	<i>RPL32</i>	25.14
Adult HSD + <i>L. plantarum</i>	<i>RPL32</i>	24.59
Adult HSD + <i>L. acidophilus</i>	<i>PEPCK</i>	31.05
Adult HSD + <i>L. acidophilus</i>	<i>PEPCK</i>	31.45
Adult HSD + <i>L. acidophilus</i>	<i>PEPCK</i>	31.27
Adult HSD + <i>L. acidophilus</i>	<i>Fbp</i>	29.75
Adult HSD + <i>L. acidophilus</i>	<i>Fbp</i>	29.61
Adult HSD + <i>L. acidophilus</i>	<i>Fbp</i>	30.4
Adult HSD + <i>L. acidophilus</i>	<i>Hex-t1</i>	37.72
Adult HSD + <i>L. acidophilus</i>	<i>Hex-t1</i>	34.62
Adult HSD + <i>L. acidophilus</i>	<i>Hex-t1</i>	33.3
Adult HSD + <i>L. acidophilus</i>	<i>Zw</i>	31.72
Adult HSD + <i>L. acidophilus</i>	<i>Zw</i>	31.01
Adult HSD + <i>L. acidophilus</i>	<i>Zw</i>	NaN
Adult HSD + <i>L. acidophilus</i>		NaN
Adult HSD + <i>L. acidophilus</i>	<i>RPL32</i>	26.12
Adult HSD + <i>L. acidophilus</i>	<i>RPL32</i>	25.96
Adult HSD + <i>L. acidophilus</i>	<i>RPL32</i>	25.75
Adult HSD + <i>B. animalis</i>	<i>PEPCK</i>	29.39
Adult HSD + <i>B. animalis</i>	<i>PEPCK</i>	31.13
Adult HSD + <i>B. animalis</i>	<i>PEPCK</i>	31.71
Adult HSD + <i>B. animalis</i>	<i>Fbp</i>	30.01
Adult HSD + <i>B. animalis</i>	<i>Fbp</i>	29.48
Adult HSD + <i>B. animalis</i>	<i>Fbp</i>	29.26
Adult HSD + <i>B. animalis</i>	<i>Hex-t1</i>	35.14
Adult HSD + <i>B. animalis</i>	<i>Hex-t1</i>	35.96
Adult HSD + <i>B. animalis</i>	<i>Hex-t1</i>	35.74
Adult HSD + <i>B. animalis</i>	<i>Zw</i>	30.89
Adult HSD + <i>B. animalis</i>	<i>Zw</i>	30.4
Adult HSD + <i>B. animalis</i>	<i>Zw</i>	22.96
Adult HSD + <i>B. animalis</i>		NaN
Adult HSD + <i>B. animalis</i>	<i>RPL32</i>	24.33
Adult HSD + <i>B. animalis</i>	<i>RPL32</i>	24.51
Adult HSD + <i>B. animalis</i>	<i>RPL32</i>	24.28
Adult HSD + <i>B. breve</i>	<i>PEPCK</i>	35.69
Adult HSD + <i>B. breve</i>	<i>PEPCK</i>	35.1
Adult HSD + <i>B. breve</i>	<i>PEPCK</i>	35.56
Adult HSD + <i>B. breve</i>	<i>Fbp</i>	32.05
Adult HSD + <i>B. breve</i>	<i>Fbp</i>	32.78
Adult HSD + <i>B. breve</i>	<i>Fbp</i>	32.63

Adult HSD + <i>B. breve</i>	<i>Hex-t1</i>	NaN
Adult HSD + <i>B. breve</i>	<i>Hex-t1</i>	NaN
Adult HSD + <i>B. breve</i>	<i>Hex-t1</i>	38.8
Adult HSD + <i>B. breve</i>	<i>Zw</i>	35.06
Adult HSD + <i>B. breve</i>	<i>Zw</i>	33.87
Adult HSD + <i>B. breve</i>	<i>Zw</i>	35.08
Adult HSD + <i>B. breve</i>		NaN
Adult HSD + <i>B. breve</i>	<i>RPL32</i>	26.98
Adult HSD + <i>B. breve</i>	<i>RPL32</i>	27.05
Adult HSD + <i>B. breve</i>	<i>RPL32</i>	26.93
Adult HSD + <i>B. lactis</i>	<i>PEPCK</i>	33.59
Adult HSD + <i>B. lactis</i>	<i>PEPCK</i>	31.8
Adult HSD + <i>B. lactis</i>	<i>PEPCK</i>	31.45
Adult HSD + <i>B. lactis</i>	<i>Fbp</i>	30.22
Adult HSD + <i>B. lactis</i>	<i>Fbp</i>	30.36
Adult HSD + <i>B. lactis</i>	<i>Fbp</i>	29.9
Adult HSD + <i>B. lactis</i>	<i>Hex-t1</i>	33.93
Adult HSD + <i>B. lactis</i>	<i>Hex-t1</i>	34.22
Adult HSD + <i>B. lactis</i>	<i>Hex-t1</i>	35.79
Adult HSD + <i>B. lactis</i>	<i>Zw</i>	31.57
Adult HSD + <i>B. lactis</i>	<i>Zw</i>	30.71
Adult HSD + <i>B. lactis</i>	<i>Zw</i>	31.19
Adult HSD + <i>B. lactis</i>		NaN
Adult HSD + <i>B. lactis</i>	<i>RPL32</i>	26.2
Adult HSD + <i>B. lactis</i>	<i>RPL32</i>	26.05
Adult HSD + <i>B. lactis</i>	<i>RPL32</i>	26.31
Larva HSD	<i>PEPCK</i>	30.87
Larva HSD	<i>PEPCK</i>	30.6
Larva HSD	<i>PEPCK</i>	30.39
Larva HSD	<i>Fbp</i>	31.19
Larva HSD	<i>Fbp</i>	31.18
Larva HSD	<i>Fbp</i>	30.53
Larva HSD	<i>Hex-t1</i>	35.84
Larva HSD	<i>Hex-t1</i>	35.86
Larva HSD	<i>Hex-t1</i>	35.78
Larva HSD	<i>Zw</i>	34.1
Larva HSD	<i>Zw</i>	34.07
Larva HSD	<i>Zw</i>	32.67
Larva HSD		NaN
Larva HSD	<i>RPL32</i>	27.32
Larva HSD	<i>RPL32</i>	26.82
Larva HSD	<i>RPL32</i>	27.36

Larva LSD	<i>PEPCK</i>	33.65
Larva LSD	<i>PEPCK</i>	33.73
Larva LSD	<i>PEPCK</i>	33.72
Larva LSD	<i>Fbp</i>	31.47
Larva LSD	<i>Fbp</i>	31.4
Larva LSD	<i>Fbp</i>	31.08
Larva LSD	<i>Hex-t1</i>	35.76
Larva LSD	<i>Hex-t1</i>	36.51
Larva LSD	<i>Hex-t1</i>	37.76
Larva LSD	<i>Zw</i>	32.28
Larva LSD	<i>Zw</i>	34.27
Larva LSD	<i>Zw</i>	33.28
Larva LSD		NaN
Larva LSD	<i>RPL32</i>	27.24
Larva LSD	<i>RPL32</i>	27.68
Larva LSD	<i>RPL32</i>	27.45
Larva HSD + <i>L. paracasei</i>	<i>PEPCK</i>	29.57
Larva HSD + <i>L. paracasei</i>	<i>PEPCK</i>	30.5
Larva HSD + <i>L. paracasei</i>	<i>PEPCK</i>	29.05
Larva HSD + <i>L. paracasei</i>	<i>Fbp</i>	30.29
Larva HSD + <i>L. paracasei</i>	<i>Fbp</i>	30.58
Larva HSD + <i>L. paracasei</i>	<i>Fbp</i>	30.61
Larva HSD + <i>L. paracasei</i>	<i>Hex-t1</i>	35.18
Larva HSD + <i>L. paracasei</i>	<i>Hex-t1</i>	35.22
Larva HSD + <i>L. paracasei</i>	<i>Hex-t1</i>	34.24
Larva HSD + <i>L. paracasei</i>	<i>Zw</i>	33.05
Larva HSD + <i>L. paracasei</i>	<i>Zw</i>	32.08
Larva HSD + <i>L. paracasei</i>	<i>Zw</i>	32.32
Larva HSD + <i>L. paracasei</i>		NaN
Larva HSD + <i>L. paracasei</i>	<i>RPL32</i>	26.41
Larva HSD + <i>L. paracasei</i>	<i>RPL32</i>	25.94
Larva HSD + <i>L. paracasei</i>	<i>RPL32</i>	26.32
Larva HSD + <i>L. plantarum</i>	<i>PEPCK</i>	NaN
Larva HSD + <i>L. plantarum</i>	<i>PEPCK</i>	26.17
Larva HSD + <i>L. plantarum</i>	<i>PEPCK</i>	25.02
Larva HSD + <i>L. plantarum</i>	<i>Fbp</i>	NaN
Larva HSD + <i>L. plantarum</i>	<i>Fbp</i>	NaN
Larva HSD + <i>L. plantarum</i>	<i>Fbp</i>	NaN
Larva HSD + <i>L. plantarum</i>	<i>Hex-t1</i>	NaN
Larva HSD + <i>L. plantarum</i>	<i>Hex-t1</i>	NaN
Larva HSD + <i>L. plantarum</i>	<i>Hex-t1</i>	NaN
Larva HSD + <i>L. plantarum</i>	<i>Zw</i>	35.25

Larva HSD + <i>L. plantarum</i>	<i>Zw</i>	33.36
Larva HSD + <i>L. plantarum</i>	<i>Zw</i>	34.91
Larva HSD + <i>L. plantarum</i>		NaN
Larva HSD + <i>L. plantarum</i>	<i>RPL32</i>	NaN
Larva HSD + <i>L. plantarum</i>	<i>RPL32</i>	NaN
Larva HSD + <i>L. plantarum</i>	<i>RPL32</i>	NaN
Larva HSD + <i>L. acidophilus</i>	<i>PEPCK</i>	NaN
Larva HSD + <i>L. acidophilus</i>	<i>PEPCK</i>	31.35
Larva HSD + <i>L. acidophilus</i>	<i>PEPCK</i>	31.52
Larva HSD + <i>L. acidophilus</i>	<i>Fbp</i>	31.43
Larva HSD + <i>L. acidophilus</i>	<i>Fbp</i>	31.4
Larva HSD + <i>L. acidophilus</i>	<i>Fbp</i>	31.54
Larva HSD + <i>L. acidophilus</i>	<i>Hex-t1</i>	34.85
Larva HSD + <i>L. acidophilus</i>	<i>Hex-t1</i>	35.12
Larva HSD + <i>L. acidophilus</i>	<i>Hex-t1</i>	35.54
Larva HSD + <i>L. acidophilus</i>	<i>Zw</i>	34.85
Larva HSD + <i>L. acidophilus</i>	<i>Zw</i>	35.93
Larva HSD + <i>L. acidophilus</i>	<i>Zw</i>	37.98
Larva HSD + <i>L. acidophilus</i>		NaN
Larva HSD + <i>L. acidophilus</i>	<i>RPL32</i>	28.66
Larva HSD + <i>L. acidophilus</i>	<i>RPL32</i>	28.2
Larva HSD + <i>L. acidophilus</i>	<i>RPL32</i>	28.03
Larva HSD + <i>B. animalis</i>	<i>PEPCK</i>	30.33
Larva HSD + <i>B. animalis</i>	<i>PEPCK</i>	29.55
Larva HSD + <i>B. animalis</i>	<i>PEPCK</i>	30.52
Larva HSD + <i>B. animalis</i>	<i>Fbp</i>	30.8
Larva HSD + <i>B. animalis</i>	<i>Fbp</i>	31.01
Larva HSD + <i>B. animalis</i>	<i>Fbp</i>	31.05
Larva HSD + <i>B. animalis</i>	<i>Hex-t1</i>	36.1
Larva HSD + <i>B. animalis</i>	<i>Hex-t1</i>	34.43
Larva HSD + <i>B. animalis</i>	<i>Hex-t1</i>	34.88
Larva HSD + <i>B. animalis</i>	<i>Zw</i>	38.4
Larva HSD + <i>B. animalis</i>	<i>Zw</i>	NaN
Larva HSD + <i>B. animalis</i>	<i>Zw</i>	NaN
Larva HSD + <i>B. animalis</i>		NaN
Larva HSD + <i>B. animalis</i>	<i>RPL32</i>	28.72
Larva HSD + <i>B. animalis</i>	<i>RPL32</i>	28.89
Larva HSD + <i>B. animalis</i>	<i>RPL32</i>	28.54
Larva HSD + <i>B. breve</i>	<i>PEPCK</i>	31.74
Larva HSD + <i>B. breve</i>	<i>PEPCK</i>	30.54
Larva HSD + <i>B. breve</i>	<i>PEPCK</i>	30.35
Larva HSD + <i>B. breve</i>	<i>Fbp</i>	30.52

Larva HSD + <i>B. breve</i>	<i>Fbp</i>	30.56
Larva HSD + <i>B. breve</i>	<i>Fbp</i>	30.85
Larva HSD + <i>B. breve</i>	<i>Hex-t1</i>	NaN
Larva HSD + <i>B. breve</i>	<i>Hex-t1</i>	37.73
Larva HSD + <i>B. breve</i>	<i>Hex-t1</i>	NaN
Larva HSD + <i>B. breve</i>	<i>Zw</i>	NaN
Larva HSD + <i>B. breve</i>	<i>Zw</i>	NaN
Larva HSD + <i>B. breve</i>	<i>Zw</i>	39.39
Larva HSD + <i>B. breve</i>		NaN
Larva HSD + <i>B. breve</i>	<i>RPL32</i>	29.55
Larva HSD + <i>B. breve</i>	<i>RPL32</i>	28.43
Larva HSD + <i>B. breve</i>	<i>RPL32</i>	29.94
Larva HSD + <i>B. lactis</i>	<i>PEPCK</i>	31.48
Larva HSD + <i>B. lactis</i>	<i>PEPCK</i>	31.14
Larva HSD + <i>B. lactis</i>	<i>PEPCK</i>	31.08
Larva HSD + <i>B. lactis</i>	<i>Fbp</i>	31
Larva HSD + <i>B. lactis</i>	<i>Fbp</i>	30.9
Larva HSD + <i>B. lactis</i>	<i>Fbp</i>	31.51
Larva HSD + <i>B. lactis</i>	<i>Hex-t1</i>	35.28
Larva HSD + <i>B. lactis</i>	<i>Hex-t1</i>	36.04
Larva HSD + <i>B. lactis</i>	<i>Hex-t1</i>	35.19
Larva HSD + <i>B. lactis</i>	<i>Zw</i>	NaN
Larva HSD + <i>B. lactis</i>	<i>Zw</i>	NaN
Larva HSD + <i>B. lactis</i>	<i>Zw</i>	NaN
Larva HSD + <i>B. lactis</i>		NaN
Larva HSD + <i>B. lactis</i>	<i>RPL32</i>	30.49
Larva HSD + <i>B. lactis</i>	<i>RPL32</i>	29.58
Larva HSD + <i>B. lactis</i>	<i>RPL32</i>	29.96
RT- control	<i>PEPCK</i>	NaN
RT- control	<i>PEPCK</i>	NaN
RT- control	<i>PEPCK</i>	NaN
RT- control	<i>Fbp</i>	NaN
RT- control	<i>Fbp</i>	NaN
RT- control	<i>Fbp</i>	NaN
RT- control	<i>Hex-t1</i>	NaN
RT- control	<i>Hex-t1</i>	NaN
RT- control	<i>Hex-t1</i>	NaN
RT- control	<i>Zw</i>	NaN
RT- control	<i>Zw</i>	NaN
RT- control	<i>Zw</i>	NaN
RT- control		NaN
RT- control	<i>RPL32</i>	39.94

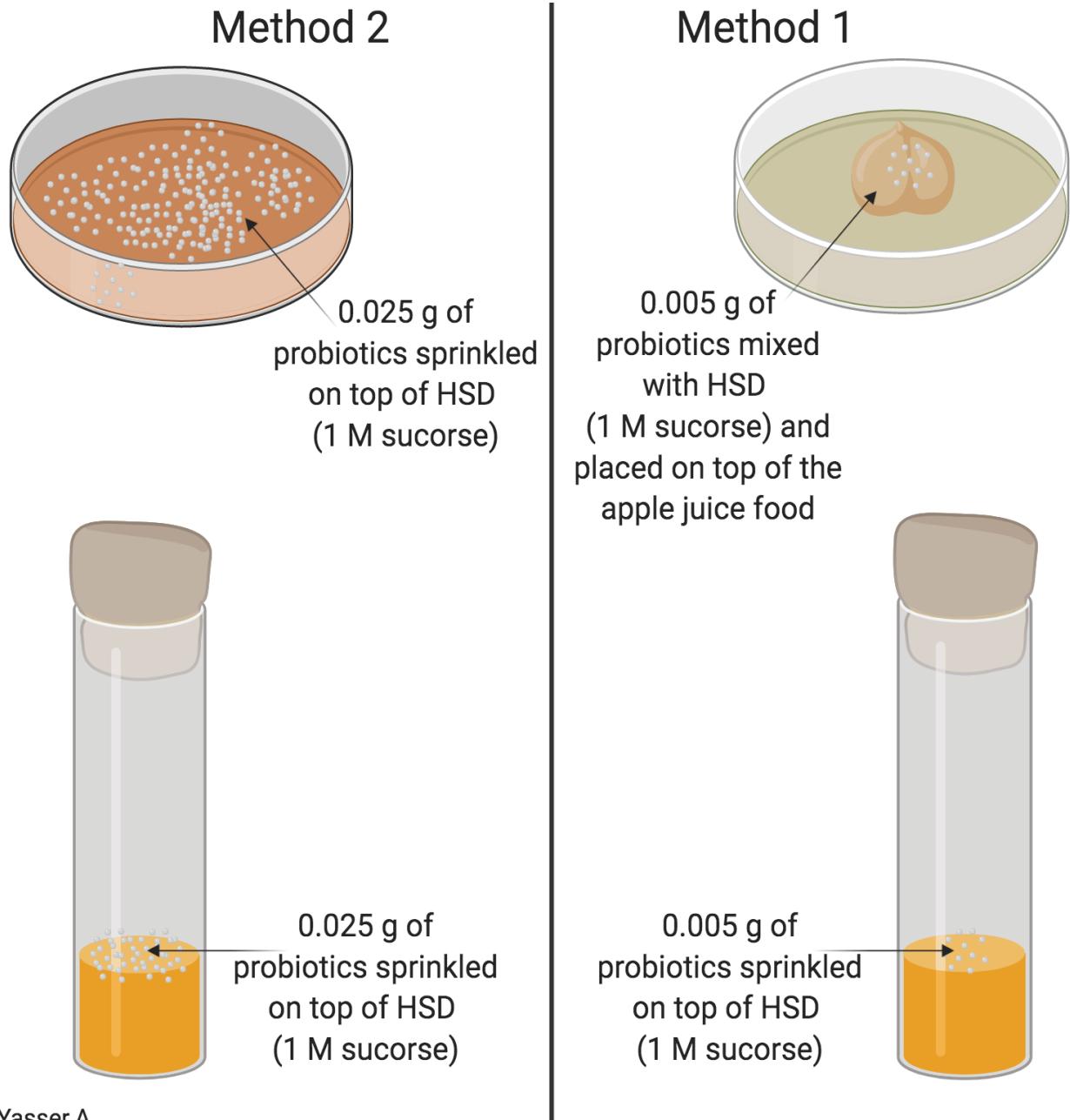
RT- control	<i>RPL32</i>	NaN
RT- control	<i>RPL32</i>	NaN
Water control	<i>PEPCK</i>	28.44
Water control	<i>PEPCK</i>	NaN
Water control	<i>PEPCK</i>	29.08
Water control	<i>Fbp</i>	NaN
Water control	<i>Fbp</i>	39.56
Water control	<i>Fbp</i>	NaN
Water control	<i>Hex-t1</i>	NaN
Water control	<i>Hex-t1</i>	NaN
Water control	<i>Hex-t1</i>	NaN
Water control	<i>Zw</i>	NaN
Water control	<i>Zw</i>	NaN
Water control	<i>Zw</i>	NaN
Water control		NaN
Water control	<i>RPL32</i>	NaN
Water control	<i>RPL32</i>	NaN
Water control	<i>RPL32</i>	NaN

Appendix D

sp P35568 IRS1_HUMAN	MASPPESDGFSVORKVGYLRKPKSMHKRFFVLRAASEAGGPARLEYENEKKWRHKSSAP	60
sp Q9XTN2 IRS1_DROME	MAS---ISDDGMALSGYLKKLKTMKKKFVLYET-STSAARLEYYDTEKKFLQR-AEP	54
	*** * . * . *** : * : * : *** : : : * : * : * : * : * : * : * :	
sp P35568 IRS1_HUMAN	KRSIPL ESCFNINKRADSKNKHVALYTRDEHFAIAADSEAEQDSWYQALLQLHNRAKGH	120
sp Q9XTN2 IRS1_DROME	KRVIYLKNCFPNIRRLDTKHFVIVLSSRDGGFGIVLENENDLRKWLDKLLVLQRNIANS	114
	*** * : * . *** : * : * : * : * : * : * : * : * : * : * : * : * :	
sp P35568 IRS1_HUMAN	HDGAALGAGGGGGSCGSSGLGEAGEDLSYGDVPPGPAFKEVWQVILKPKGLGQTKNLI	180
sp Q9XTN2 IRS1_DROME	N-----GTAHSPYDHVWQVVIQKKGISEKVGIT	142
	: :	
sp P35568 IRS1_HUMAN	GIYRLCLTSKTISPVKLNSEA-----AAVVIQLMNRIRRCHSE--NFFFIEVGRSAV	230
sp Q9XTN2 IRS1_DROME	GTYHCCLTSKS LTFCVIGPEKTPNGEDRVASIEILLTTIRRCGHASPQCIFYVELGRQSV	202
	* : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * :	
sp P35568 IRS1_HUMAN	TGPGEFWMQVDDDSVVAQNMHETILEAMRAMSDEFRPRS-----KSQSSS	274
sp Q9XTN2 IRS1_DROME	LGSGLDWMETDNAAIATNMHNTIISAMSAKTESNTNLINVYQNRPDLSHEPMRKRSSSAN	262
	* : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * :	
sp P35568 IRS1_HUMAN	NCSNPISVPLRRHHLNNPPPSQVCLTRRSRTESITATSPASMVCGKPGSFRVRASSDGE	334
sp Q9XTN2 IRS1_DROME	EASKPINVNVIQNSQN-----SLELR-----SCSSPHNYGFGR---ERCDSLPTRNG	306
	: : : : * : : : * : * : * : * : * : * : * : * : * : * : * : * : * :	
sp P35568 IRS1_HUMAN	TMSRPASVDGSPVSPSTNRTHAHRHRGS--ARLHPPLNHS--RSIPMPASRCSPSATSPV	390
sp Q9XTN2 IRS1_DROME	TLSESSNQ-----TYFGSNHGLRSNTISGIRPHSTNKHSNSPFTM-PLRCSESEESSI	359
	* : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * :	
sp P35568 IRS1_HUMAN	SLSSSSTSGHGSTS DCLFPRRSSASVSGSPSDGGFISSDEYGSSPCDFRSSFRSVTPDSL	450
sp Q9XTN2 IRS1_DROME	SVIDESDD--NGSFSHYRLNTRSSETAIPEENIDDFASALEF-----SKVTEQN-	405
	* : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * :	
sp P35568 IRS1_HUMAN	GHTPPAR GEEELSNYICMGGKGPSTLTAP-----NGHYI L S-----	486
sp Q9XTN2 IRS1_DROME	-----VS DENYIPMNPVNPTDAIHEKEKADMQRLEDASLHFNFPEHASEKLAKDFD	456
	* : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * :	
sp P35568 IRS1_HUMAN	RGGNGHRC TPGTGLGTSPALAGDEAA SAADLDNRF-----RKRTHSAGTSPIT	535
sp Q9XTN2 IRS1_DROME	LDSDNQCCRPI RAYSIGNKV-----EHLKFKNRKLGHLN D TGQNP N RV RAY SV GS K SKIP	510
	* : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * :	
sp P35568 IRS1_HUMAN	HQKTPQS QSSVASIEEYTEMMPA-----YP-PGGGSGGRL-----PG	570
sp Q9XTN2 IRS1_DROME	RCDL-----Q-RVVLVEDN KHEFTANRSQSSITKEGTSYGSANRQKKSTSAPLLSLKNQINS	567
	: : : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * :	
sp P35568 IRS1_HUMAN	H-----RHS A F V P T R S Y P -----EEGLEMHP LERRG--GH	598
sp Q9XTN2 IRS1_DROME	DRMSDLMEIDFSQATN LEKQKF I K N N E I P K Y I E N V F P K A P R T D S S S L T L H A T S O K D I F N G	627
	* : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * :	
sp P35568 IRS1_HUMAN	HRPD SSTLHTDDGYMPMSPGVAPVPSGRKGSGDYMPMSPKS V SAPQQI -INPI RRHP Q RV	657
sp Q9XTN2 IRS1_DROME	TKLNNTAITSEDGYLEMKPV-----GNGYT PSSNCLPMKVEKLKLSDYQTAPP TATAAPV	683
	: : : : : : : * : * : * : * : * : * : * : * : * : * : * : * : * :	
sp P35568 IRS1_HUMAN	DPNGYMMMSPSGGCSPD I GGGPSSSSSSNAVP SGTSYKLWTNGVGHHSHVLPHPKPP	717
sp Q9XTN2 IRS1_DROME	H-----DLINKISTYN I SAEK WRE QPS RSEE KK S-----NS-	713
	* : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * :	
sp P35568 IRS1_HUMAN	VESSGGKLLPC TGDY MN M SPVG-----DSNTSSPSDCYYGPEDPQHKPVLSYYSLPR	769
sp Q9XTN2 IRS1_DROME	-----PLNDNTFSSKP TNV E STSKSHDV H S ANQIDCEK VCA QSSDK--LNN-HLAD	761
	* : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * :	
sp P35568 IRS1_HUMAN	SFKHTQR PGE PEE GARHQH LRL STSS GR LLY AAT ADD SSS S-----	810
sp Q9XTN2 IRS1_DROME	KIVE-----NNNL DIG-GHEEK KL VHS ISSE DYTQIKD KNS NDFTKFNEAGYKILQIKSDSSL	817
	: : : : : : : * : * : * : * : * : * : * : * : * : * : * : * : * :	
sp P35568 IRS1_HUMAN	TSSDSLGGGYCGARLEPSLPHPHQV LQPHPL PRKV D TAA QTNS RLARP TRLS LGDP KAST	870
sp Q9XTN2 IRS1_DROME	I SS KLYQ KG I HKDN LER S QRL TES---VNTIPDNATATAVSSSS---LTKFNINSAK PAA	871
	* : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * :	
sp P35568 IRS1_HUMAN	LPRAREQQQQQQPLLHPPPEPKSPG EYVNIEFGSDQSGYLSGPVAF-----	915
sp Q9XTN2 IRS1_DROME	AADSR-----STGTD P STPQN ILQIKD LNFP S RSSS RIS QP E LHY AS LD LPH CSG QN PA	925
	: * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * :	
sp P35568 IRS1_HUMAN	-----HSSPSVRCP SQL QPAP REEET GT E EY M KMDL LG P G R R A A W Q E S T G V E M G R L G P	967
sp Q9XTN2 IRS1_DROME	KYLKRG S R E S P P V S A C P E D-----GNTYAKIDFD Q S D S S S S-----	961
	* : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * :	
sp P35568 IRS1_HUMAN	APPGAASICRPT RAVPSSRG D YMTQM SC P R Q S Y V D T S P A A P V S Y A D M R T G I A A E E V S L P	1027
sp Q9XTN2 IRS1_DROME	-----	961

Figure D1. Comparison of conserved proteins (Insulin receptor substrate 1 (IRS1) and Fox O transcription factor (FOXO)) involved in insulin signalling, in *Drosophila* (DROME) and *Homosapeins* (HUMAN). An * (asterisk) indicates positions which have a single, fully conserved residue. A : (colon) indicates conservation between groups of strongly similar properties - scoring > 0.5 in the Gonnet PAM 250 matrix. A . (period) indicates conservation between groups of weakly similar properties - scoring <= 0.5 in the Gonnet PAM 250 matrix (Uniport 2019).

Appendix E



Yasser.A

Figure E1. The doses of probiotics used and their application sites in methods 1 and 2.

Appendix 1

The diet recipes used for vials, Petri dishes and apple plates are:

Apple juice plate:

- 800 ml of cold tap water mixed with 18 g bacto agar-agar (the mix boiled in a microwave and cooled down for few minutes afterwards)
- 40 ml of concentrated apple juice was mixed with 20 g of sucrose and diluted with 160 ml of water
- The diluted mix was then added to the 800 ml water-agar mix.
- 12 ml of 10% ethanolic Nipagin

Added to empty Petri-dishes

Low sugar diet (LSD) for fly vials:

1,4 g agar + 200 ml water (from the tap) – brought to boil
13 g inactive yeast is then added
+ 6 g cornflour
+ 10,3 g sugar (sucrose) (= 0,15 M)
Cooled to app. 60 °C then 1,5 ml Nipagen is added
Divided into fly vials app. 3-4 cm thick layer.

High sugar diet (HSD) for fly vials:

1,4 g agar + 200 ml water (from the tap) – brought to boil
13 g inactive yeast is then added
+ 6 g cornflour
+ 68,4 g sugar (sucrose) (= 1 M)
Cooled to app. 60 °C then 1,5 ml Nipagen is added
Divided into fly vials app. 3-4 cm thick layer.

app. 0,005 g (or 0.025 g) freeze-dried bacteria (probiotics) are added to the top of the food in one vial.

Base: Low sugar diet (LSD) for adding to apple plates (larvae studies):

13 g inactive yeast is mixed with:
+ 6 g cornflour
+ 2 g sugar (sucrose)
+ 40 ml water (heated in the microwave)
Added to the top of apple plates

Base: High sugar diet (HSD) for adding to apple plates (larvae studies):

13 g inactive yeast is mixed with:
+ 6 g corn flour
+ 14 g sugar (sucrose)
+ 40 ml water (heated in the microwave)
Added to the top of apple plates

app. 0,005 g (or 0.025 g) freeze-dried bacteria (probiotics) are mixed with 2 g of Base-food.

The probiotic strains used: *L. paracasei*, *L. plantarum*, *L. acidophilus*, *B. animalis subsp lactis*, *B. breve*, and *B. lactis HN019*.

Multi probiotics contained: *L. paracasei*, *L. plantarum*, *L. acidophilus*, *B. animalis subsp lactis*, *B. breve*, and *B. lactis HN019 + Inulin*.

Appendix 2

Table 2.1. The concentrations and purities of RNA in Larva and adult fly samples feeding on HSD, LSD and HSD with treatments.

Sample	RNA conc. ng/ul	A _{260/230}	A _{260/280}	RNA conc. ng/ul	A _{260/230}	A _{260/280}
	Adult		Larva			
HSD + <i>L. paracasei</i>	142.64	0.71	1.64	524.01	2.18	2.23
HSD + <i>L. plantarum</i>	81.68	1.92	2.01	360.71	1.06	2.24
HSD + <i>L. acidophilus</i>	113.36	0.76	2.23	464.58	0.72	2.17
HSD + <i>B. animalis</i>	107.88	1.29	2.25	513.94	2.3	2.21
HSD + <i>B. breve</i>	76.24	1.07	1.14	643.26	2.26	2.28
HSD + <i>B. lactis</i>	108.66	1.67	2.19	386.16	0.87	2.2
LSD control	134.97	0.47	2.19	416.9	0.82	2.2
HSD control	88.86	1.26	2.2	595.41	2.49	2.27

Appendix 3

Table 3.1. TaqMan Probes used and their descriptions.

Gene	Gene symbol in humans	Gene symbol in <i>Drosophila</i>	TaqMan assay	Catalog number	Relevance
Fructose bisphosphatase	<i>FBP1</i>	<i>Fbp</i> CG31692	<u>Dm01811104_g1</u>	4448892	Gluconeogenesis
Glucose-6-phosphate dehydrogenase	G6PD	Zw	<u>Dm01813970_g1</u>	4448892	Glucose metabolism
Phosphoenolpyruvate carboxykinase 1	PCK1	<i>PEPCK</i>	<u>Dm01816546_s1</u>	4448892	Gluconeogenesis
Hexokinase	HK 1	<i>Hex-t1</i>	<u>Dm02153984_s1</u>	4448892	Glucose metabolism
Ribosomal protein L32	<i>RPL32</i>	<i>RPL32</i>	<u>Dm02151827_g1</u>	4331182	Reference gene