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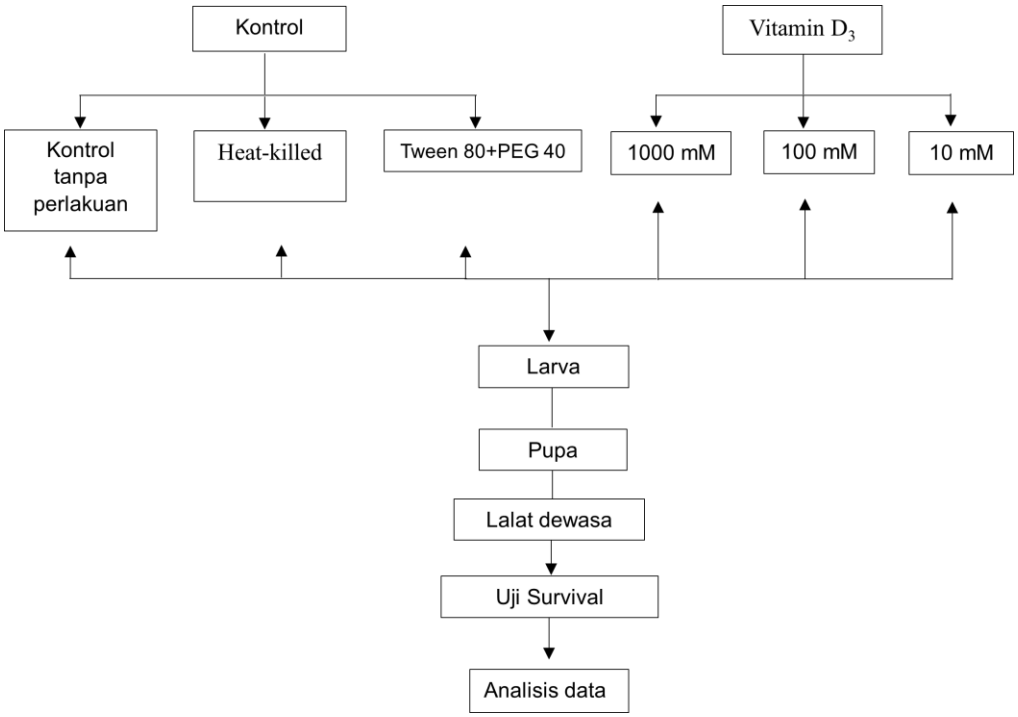
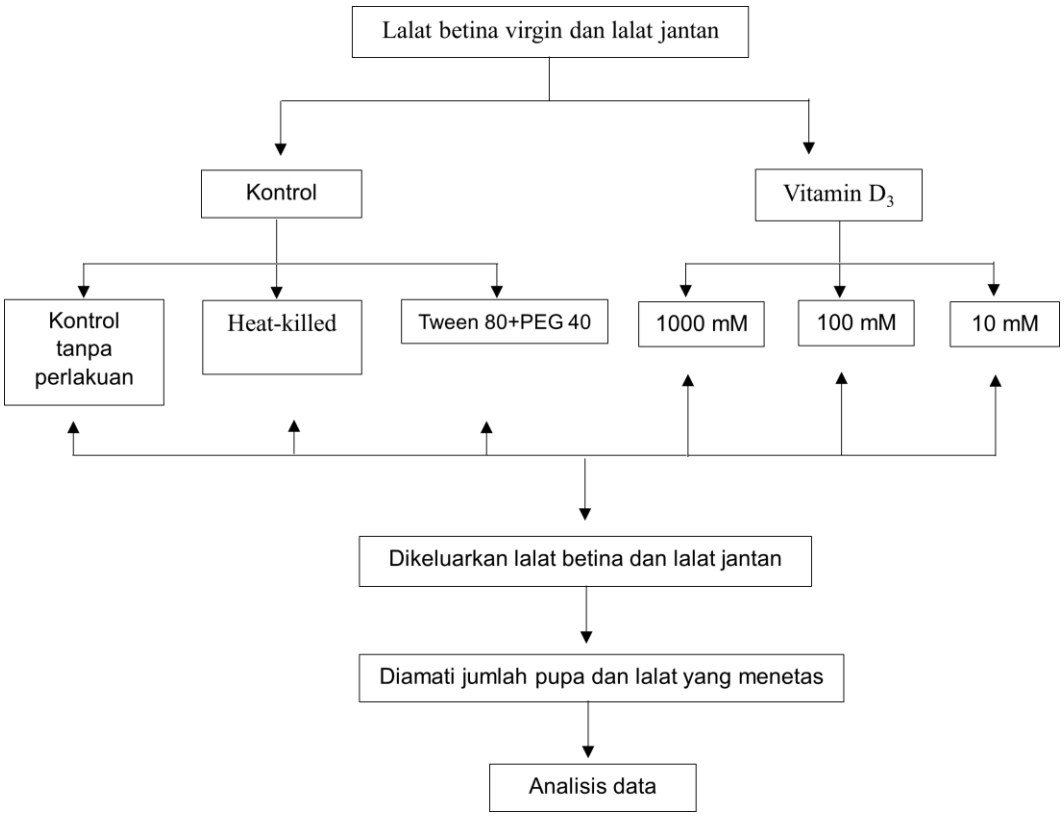
LAMPIRAN

Lampiran 1. Skema kerja penelitian



Lampiran 2. Skema kerja penelitian uji reproduksi



Lampiran 3. Perhitungan konsentrasi vitamin D_3

- **Vitamin D_3 5000 mM = 5 M**

  M $= \frac{g}{Mr} \times \frac{1000}{V}$

  5 M $= \frac{g}{384,64} \times \frac{1000}{10\ mL}$

  5 M = 19,616 g

- **Vitamin D_3 1000 mM = 1 M**

  M $= \frac{g}{Mr} \times \frac{1000}{V}$

  1 M $= \frac{g}{384,64} \times \frac{1000}{5\ mL}$

  1 M = 1,923 g

- **Vitamin D_3 100 mM = 0.1 M**

  M $= \frac{g}{Mr} \times \frac{1000}{V}$

  0.1 M $= \frac{g}{384,64} \times \frac{1000}{5\ mL}$

  0.1 M = 0,192 g

- **Vitamin D_3 10 mM = 0.01 M**

  M $= \frac{g}{Mr} \times \frac{1000}{V}$

  0.01 M $= \frac{g}{384,64} \times \frac{1000}{5\ mL}$

  0.01 M = 0,0192 g

Konversi g ke IU Vitamin D3

1 g = 40.000.000 IU

1,923 g = 76.920.000 IU

0.192 g = 7.680.000 IU

0.0192 g = 768.000 IU

Lampiran 4. Perhitungan konsentrasi vitamin D_3 pada pakan drosophila

Perhitungan konsentrasi Vitamin D_3 dalam pakan drosophila

- **Dibuat pengenceran untuk konsentrasi 1000 mM**

  V1×N1 = V2×N2

  V1× 5000 mM = 5 mL × 1000 mM

  V1 = $\frac{5\ \text{mL} \times 1000\ mM}{5000\ \text{m}M} = 1\ \text{mL}$

- **Dibuat pengenceran untuk konsentrasi 100 mM**

  V1×N1 = V2×N2

  V1× 1000 mM = 5 mL × 100 mM

  V1 = $\frac{5\ \text{mL} \times 100\ mM}{1000\ mM} = 0.5\ \text{mL}$

- **Dibuat pengenceran untuk konsentrasi 10 mM**

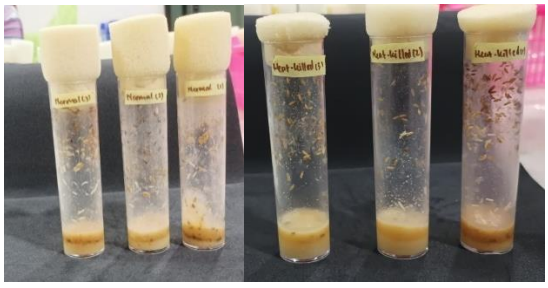
  V1×N1 = V2×N2

  V1× 100 mM = 5 mL × 10 mM

  V1 = $\frac{5\ \text{mL} \times 10\ mM}{100\ mM} = 0.5\ \text{mL}$

Lampiran 5. Gambar Penelitian

Gambar 7. Uji perkembangan larva Drosophila menjadi pupa hingga menjadi lalat dewasa, dilanjutkan pengujian survival pada lalat dewasa



Gambar 8. Uji Reproduksi

Gambar 9. Pengukuran total RNA

Lampiran 6. Analysis Of Variance (ANOVA)

Tabel 2. Hasil perbandingan tukey uji survival larva ke pupa setelah terpapar vitamin D_3 + Heat-killed *E. coli*

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Summary	Adjusted P Value
Kontrol Tanpa Perlakuan vs. Heat-killed *E. coli* (HKE)	16.67	-3.594 to 36.93	ns	0.1392
Kontrol Tanpa Perlakuan vs. HKE + Tween 80+ PEG 40	2.5	-16.45 to 21.45	ns	0.9978
Kontrol Tanpa Perlakuan vs. HKE + Vitamin D_3 1000 mM	13.33	-6.928 to 33.59	ns	0.3202
Kontrol Tanpa Perlakuan vs. HKE + Vitamin D_3 100 mM	6.667	-13.59 to 26.93	ns	0.8861
Kontrol Tanpa Perlakuan vs. HKE + Vitamin D_3 10 mM	0	-18.12 to 18.12	ns	>0.9999
Heat-killed *E. coli* (HKE) vs. HKE + Tween 80+ PEG 40	-14.17	-33.12 to 4.786	ns	0.2074
Heat-killed *E. coli* (HKE) vs. HKE + Vitamin D_3 1000 mM	-3.333	-23.59 to 16.93	ns	0.9937
Heat-killed *E. coli* (HKE) vs. HKE + Vitamin D_3 100 mM	-10	-30.26 to 10.26	ns	0.6086
Heat-killed *E. coli* (HKE) vs. HKE + Vitamin D_3 10 mM	-16.67	-34.79 to 1.455	ns	0.0804
HKE + Tween 80+ PEG 40 vs. HKE + Vitamin D_3 1000 mM	10.83	-8.119 to 29.79	ns	0.462
HKE + Tween 80+ PEG 40 vs. HKE + Vitamin D_3 100 mM	4.167	-14.79 to 23.12	ns	0.9772
HKE + Tween 80+ PEG 40 vs. HKE + Vitamin D_3 10 mM	-2.5	-19.15 to 14.15	ns	0.9959
HKE + Vitamin D_3 1000 mM vs. HKE + Vitamin D_3 100 mM	-6.667	-26.93 to 13.59	ns	0.8861
HKE + Vitamin D_3 1000 mM vs. HKE + Vitamin D_3 10 mM	-13.33	-31.46 to 4.789	ns	0.2202
HKE + Vitamin D_3 100 mM vs. HKE + Vitamin D_3 10 mM	-6.667	-24.79 to 11.46	ns	0.8325

Tabel 3. Hasil perbandingan tukey uji survival pupa ke lalat dewasa setelah terpapar vitamin D_3 + Heat-killed *E. coli*

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Summary	Adjusted P Value
Kontrol Tanpa Perlakuan vs. Heat-killed *E. coli* (HKE)	16.67	-4.404 to 37.74	ns	0.1626
Kontrol Tanpa Perlakuan vs. Tween 80 + PEG40	3.333	-17.74 to 24.40	ns	0.9945
Kontrol Tanpa Perlakuan vs. HKE + Vitamin D_3 1000 mM	13.33	-7.738 to 34.40	ns	0.3522
Kontrol Tanpa Perlakuan vs. HKE + Vitamin D_3 100 mM	6.667	-14.40 to 27.74	ns	0.8973
Kontrol Tanpa Perlakuan vs. HKE + Vitamin D_3 10 mM	0	-18.85 to 18.85	ns	>0.9999
Heat-killed *E. coli* (HKE) vs. Tween 80 + PEG40	-13.33	-34.40 to 7.738	ns	0.3522
Heat-killed *E. coli* (HKE) vs. HKE + Vitamin D_3 1000 mM	-3.333	-24.40 to 17.74	ns	0.9945
Heat-killed *E. coli* (HKE) vs. HKE + Vitamin D_3 100 mM	-10	-31.07 to 11.07	ns	0.6369
Heat-killed *E. coli* (HKE) vs. HKE + Vitamin D_3 10 mM	-16.67	-35.51 to 2.180	ns	0.0975
Tween 80 + PEG40 vs. HKE + Vitamin D_3 1000 mM	10	-11.07 to 31.07	ns	0.6369
Tween 80 + PEG40 vs. HKE + Vitamin D_3 100 mM	3.333	-17.74 to 24.40	ns	0.9945
Tween 80 + PEG40 vs. HKE + Vitamin D_3 10 mM	-3.333	-22.18 to 15.51	ns	0.9908
HKE + Vitamin D_3 1000 mM vs. HKE + Vitamin D_3 100 mM	-6.667	-27.74 to 14.40	ns	0.8973
HKE + Vitamin D_3 1000 mM vs. HKE + Vitamin D_3 10 mM	-13.33	-32.18 to 5.513	ns	0.2489
HKE + Vitamin D_3 100 mM vs. HKE + Vitamin D_3 10 mM	-6.667	-25.51 to 12.18	ns	0.8479

Tabel 4. Hasil perbandingan tukey uji survival lalat dewasa kontrol setelah terpapar vitamin D_3 + Heat-killed *E. coli*

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Summary	Adjusted P Value
Kontrol Tanpa Perlakuan vs. Heat-killed *E. coli* (HKE)	35	25.84 to 44.16	****	<0.0001
Kontrol Tanpa Perlakuan vs. HKE + Tween 80 + PEG 40	40.71	31.55 to 49.88	****	<0.0001
Heat-killed *E. coli* (HKE) vs. HKE + Tween 80 + PEG 40	5.714	-4.865 to 16.29	ns	0.3842

Tabel 5. Hasil perbandingan tukey uji survival lalat dewasa dihari ke 7 setelah terpapar vitamin D_3 + Heat-killed *E. coli*

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Summary	Adjusted P Value
Heat-killed *E. coli* (HKE) vs. HKE + Vitamin D_3 1000 mM	26.43	13.41 to 39.45	****	<0.0001
Heat-killed *E. coli* (HKE) vs. HKE + Vitamin D_3 100 mM	-15.24	-28.26 to -2.220	*	0.0174
Heat-killed *E. coli* (HKE) vs. HKE + Vitamin D_3 10 mM	-24.57	-36.10 to -13.04	****	<0.0001
HKE + Vitamin D_3 1000 mM vs. HKE + Vitamin D_3 100 mM	-41.67	-55.18 to -28.16	****	<0.0001
HKE + Vitamin D_3 1000 mM vs. HKE + Vitamin D_3 10 mM	-51	-63.08 to -38.92	****	<0.0001
HKE + Vitamin D_3 100 mM vs. HKE + Vitamin D_3 10 mM	-9.333	-21.42 to 2.750	ns	0.1729

Tabel 6. Hasil perbandingan tukey ekspresi gen *dpt*

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Summary	Adjusted P Value
Heat-killed *E. coli* vs. 1000 mM	6.816	4.944 to 8.687	***	0.0004
Heat-killed *E. coli* vs. 100 mM	4.435	2.563 to 6.307	**	0.0023
Heat-killed *E. coli* vs. 10 mM	2.73	0.8584 to 4.602	*	0.0138
1000 mM vs. 100 mM	-2.381	-4.252 to -0.5089	*	0.0223
1000 mM vs. 10 mM	-4.086	-5.957 to -2.214	**	0.0031
100 mM vs. 10 mM	-1.705	-3.577 to 0.1666	ns	0.067

Tabel 7. Hasil perbandingan tukey ekspresi gen *totA*

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Summary	Adjusted P Value
Heat-killed *E. coli* vs. 1000 mM	2.334	1.516 to 3.151	**	0.0011
Heat-killed *E. coli* vs. 100 mM	1.55	0.7330 to 2.367	**	0.0052
Heat-killed *E. coli* vs. 10 mM	1.79	0.9730 to 2.607	**	0.003
1000 mM vs. 100 mM	-0.7835	-1.601 to 0.03350	ns	0.0571
1000 mM vs. 10 mM	-0.5435	-1.361 to 0.2735	ns	0.1631
100 mM vs. 10 mM	0.24	-0.5770 to 1.057	ns	0.6602

Tabel 8. Hasil perbandingan tukey ekspresi gen *sod2*

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Summary	Adjusted P Value
Heat-killed *E. coli* vs. 1000 mM	4085	3092 to 5078	***	0.0003
Heat-killed *E. coli* vs. 100 mM	4088	3095 to 5081	***	0.0003
Heat-killed *E. coli* vs. 10 mM	4088	3095 to 5081	***	0.0003
1000 mM vs. 100 mM	3.555	-989.5 to 996.7	ns	>0.9999
1000 Mm vs. 10 mM	3.47	-989.6 to 996.6	ns	>0.9999
100 mM vs. 10 mM	-0.085	-993.2 to 993.0	ns	>0.9999

Tabel 9. Hasil perbandingan tukey ekspresi gen *sod1*

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Summary	Adjusted P Value
Heat-killed *E. coli* vs. 1000 mM	5495	3739 to 7251	***	0.0008
Heat-killed *E. coli* vs. 100 mM	5495	3739 to 7251	***	0.0008
Heat-killed *E. coli* vs. 10 mM	5495	3739 to 7251	***	0.0008
1000 mM vs. 100 mM	0.25	-1756 to 1756	ns	>0.9999
1000 mM vs. 10 mM	0.025	-1756 to 1756	ns	>0.9999
100 mM vs. 10 mM	-0.225	-1756 to 1756	ns	>0.9999

Tabel 10. Hasil perbandingan tukey uji reproduksi pupa setelah terpapar vitamin D_3 + Heat-killed *E. coli*

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Summary	Adjusted P Value
Heat-killed E.coli vs. 1000 mM	24.5	-6.180 to 55.18	ns	0.1383
Heat-killed E.coli vs. 100 mM	-18.5	-49.18 to 12.18	ns	0.33
Heat-killed E.coli vs. 10 mM	-41.2	-70.31 to -12.09	**	0.0054
1000 mM vs. 100 mM	-43	-73.68 to -12.32	**	0.0059
1000 mM vs. 10 mM	-65.7	-94.81 to -36.59	****	<0.0001
100 mM vs. 10 mM	-22.7	-51.81 to 6.406	ns	0.1512

Tabel 11. Hasil perbandingan tukey uji reproduksi lalat dewasa setelah terpapar vitamin D_3 + Heat-killed *E. coli*

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Summary	Adjusted P Value
Heat-killed E.coli vs. 1000 mM	22.25	-5.789 to 50.29	ns	0.1417
Heat-killed E.coli vs. 100 mM	-18.75	-46.79 to 9.289	ns	0.2509
Heat-killed E.coli vs. 10 mM	-38.75	-65.35 to -12.15	**	0.0044
1000 mM vs. 100 mM	-41	-69.04 to -12.96	**	0.0043
1000 mM vs. 10 mM	-61	-87.60 to -34.40	****	<0.0001
100 mM vs. 10 mM	-20	-46.60 to 6.600	ns	0.1726