

Fly Lifecycle

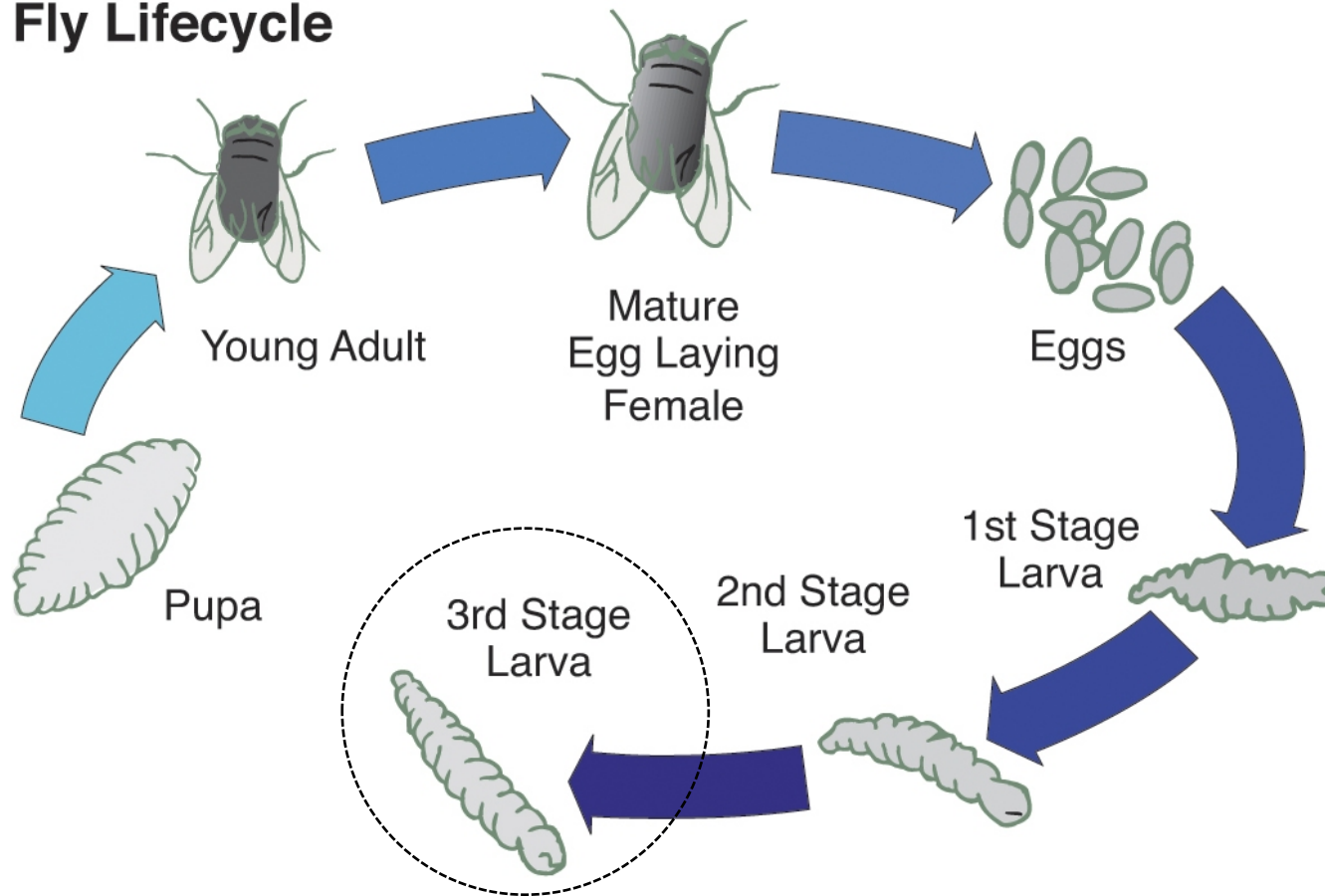


Figure 1: Life cycle of the Housefly (*Musca domestica*). LM for this study was produced from 3rd stage larvae (circled).

Table 1: Nutritional comparison of Housefly Larva Meal with common aquaculture feed ingredients.

| | Larva Meal ^a | Soy Protein Concentrate ^b | Fishmeal ^b |
|--|-------------------------|--------------------------------------|-----------------------|
| Dry Matter (%) | 91.5 | 94.3 | 93.7 |
| Crude Protein (%) | 56.39 | 67.4 | 67.8 |
| Fat (%) | 16.78 | 2.1 | 9.0 |
| Digestible Energy (Mcal/lb) | 1.66 | 2.23 | 2.27 |
| Calcium (%) | 0.68 | 0.4 | 5.4 |
| Phosphorus (%) | 1.08 | 0.8 | 1.5 |
| ^a This study: analysis performed by Brookside Labs (New Bremen, OH), ^b (Barrows et. al 2015) | | | |

Table 2: Diet Design for this study

| Ingredients | Control Diet | 5% Larva Meal | 30% Larva Meal |
|---|--------------|---------------|----------------|
| Fish meal | 10 | 10 | 10 |
| Larva meal | 0 | 5 | 30 |
| Soy protein concentrate | 20.7 | 15.8 | 0 |
| Corn gluten meal | 20.7 | 20.7 | 12.29 |
| Wheat gluten | 5 | 5 | 5 |
| Wheat flour | 25.4 | 26.1 | 29.7 |
| Fish oil | 11 | 11 | 11 |
| Soybean Oil | 5.8 | 4.9 | 0.5 |
| Mineral/Vitamin Mix | 1.5 | 1.5 | 1.5 |
| *Formulated using WinFeed 2.8 software | | | |

Table 3: Proximate Analysis of Experimental Diets

| Diet | Fat (%) | Crude Protein (%) | Calcium (%) | Phosphorous (%) | Potassium (%) | Magnesium (%) | Sodium (%) | Iron (ppm) | Manganese (ppm) | Copper (ppm) | Zinc (ppm) |
|----------------|--------------|-------------------|---------------|-----------------|---------------|----------------|----------------|---------------|-----------------|--------------|--------------|
| Control | 21.03 ± 0.77 | 41.2 ± 0.60 | 0.589 ± 0.021 | 0.705 ± 0.025 | 0.753 ± 0.023 | 0.145 ± 0.0035 | 0.101 ± 0.0038 | 169.7 ± 8.33 | 91.6 ± 2.45 | 61.9 ± 0.92 | 96.1 ± 8.60 |
| 5% LM | 19.5 ± 0.70 | 42.1 ± 1.20 | 0.734 ± 0.071 | 0.796 ± 0.030 | 0.745 ± 0.025 | 0.139 ± 0.0031 | 0.136 ± 0.0050 | 222.0 ± 8.66 | 100.8 ± 6.37 | 63.1 ± 1.44 | 98.0 ± 1.76 |
| 30% LM | 19.4 ± 0.15 | 41.1 ± 0.86 | 0.743 ± 0.054 | 0.871 ± 0.020 | 0.776 ± 0.015 | 0.136 ± 0.0026 | 0.278 ± 0.0036 | 515.7 ± 20.55 | 166.3 ± 4.73 | 69.6 ± 0.67 | 147.7 ± 3.79 |

Figure 2: Schematic depiction of treatment groups

| | Week | | | | | | | |
|----------------|---|---|---|---|---|---|-------------|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| Group 1 | Standard diet | | | | | | | |
| Group 2 | 5% LM diet | | | | | | | |
| Group 3 | 30% LM diet | | | | | | | |
| Group 4 | Standard diet | | | | | | 5% LM diet | |
| Group 5 | Standard diet | | | | | | 30% LM diet | |
| n | 6 aquaria per group, 14 fish per aquaria, 84 fish per group | | | | | | | |

Figure 3: Survival curve for the growth trial phase of the experiment. Higher mortality was observed in the 5% LM diet group, particularly during the first two weeks of feeding.

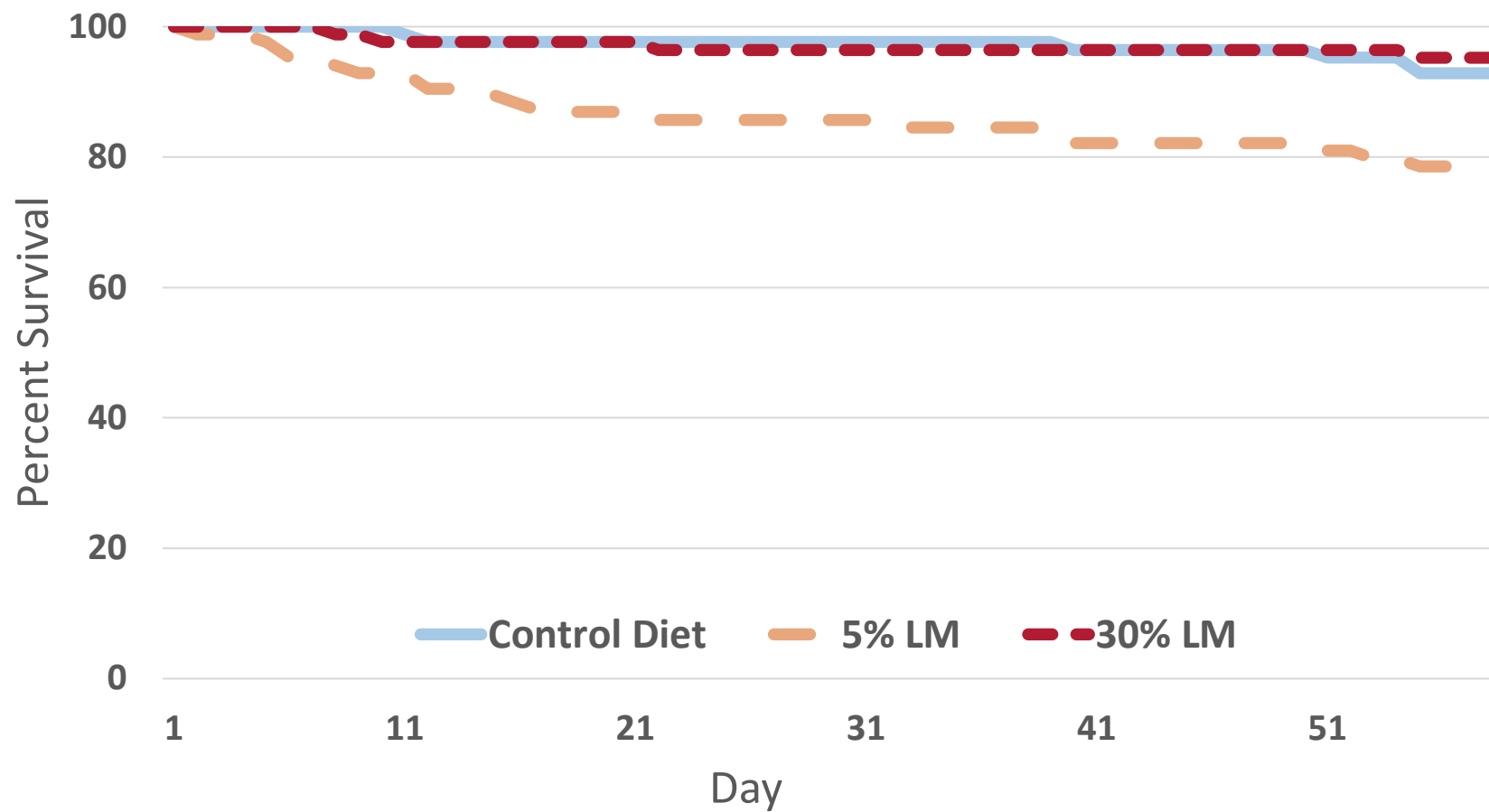


Figure 4: Average growth over the course of the feeding trial. The 30% LM diet performed best, followed by the 5% diet. However, due to high mortality in the 5% diet group, care should be taken in interpreting growth results, as these represent only surviving fish.

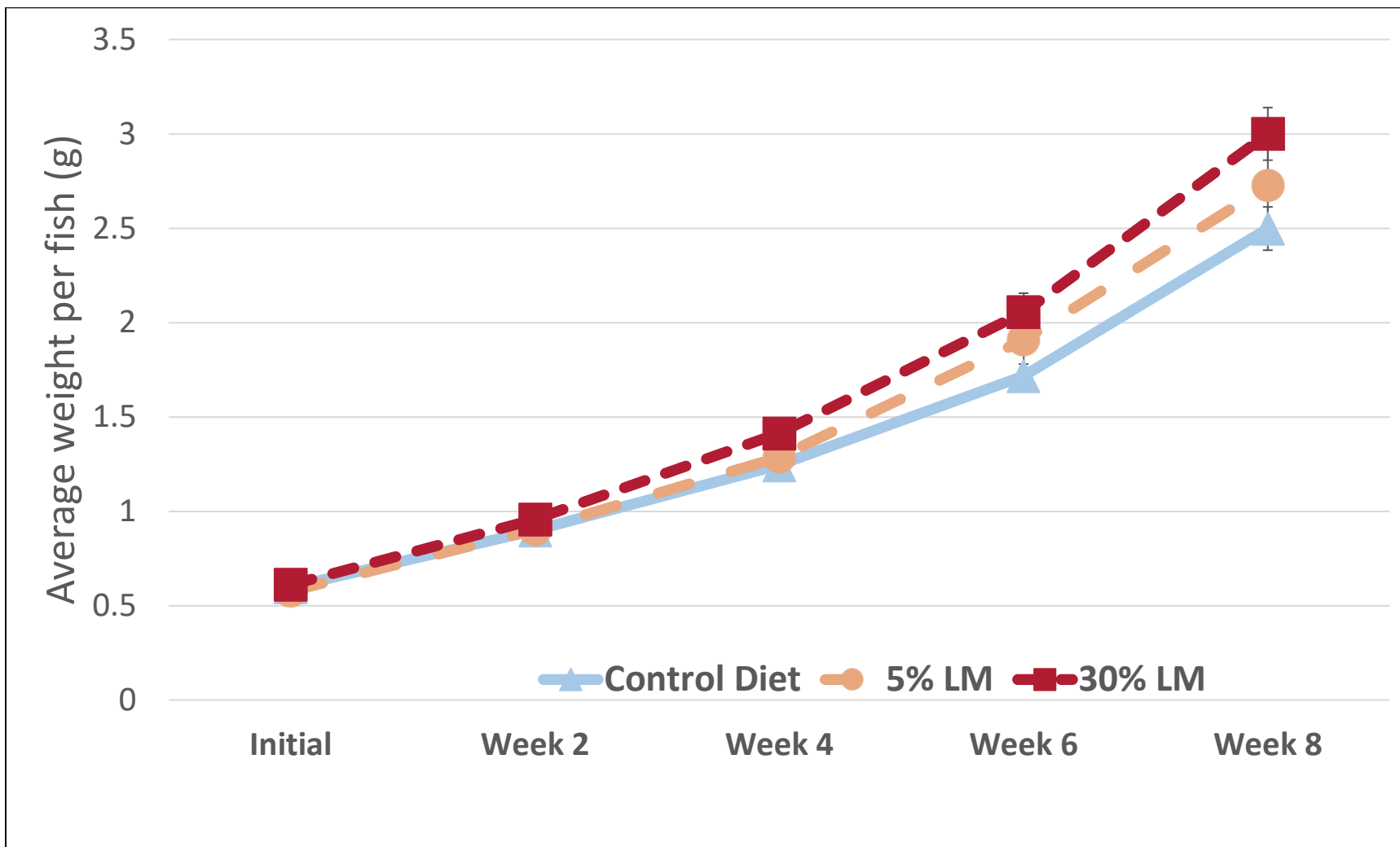


Table 4: Feed Conversion ratio over the course of the feeding trial. High mortality in the 5% LM diet group reduced FCR.

| Diet Group | Feed Intake per tank (g) | Total Weight Gain per tank (g) | Feed Conversion Ratio (FCR) |
|------------|--------------------------|--------------------------------|-----------------------------|
| Control | 36.12 | 23.28 | 1.55 |
| 5% LM | 36.12 | 21.32 | 1.69 |
| 30% LM | 36.12 | 31.32 | 1.15 |

Figure 5: Serum lysozyme activity. Activity was elevated in fish fed LM diets for 2 weeks.

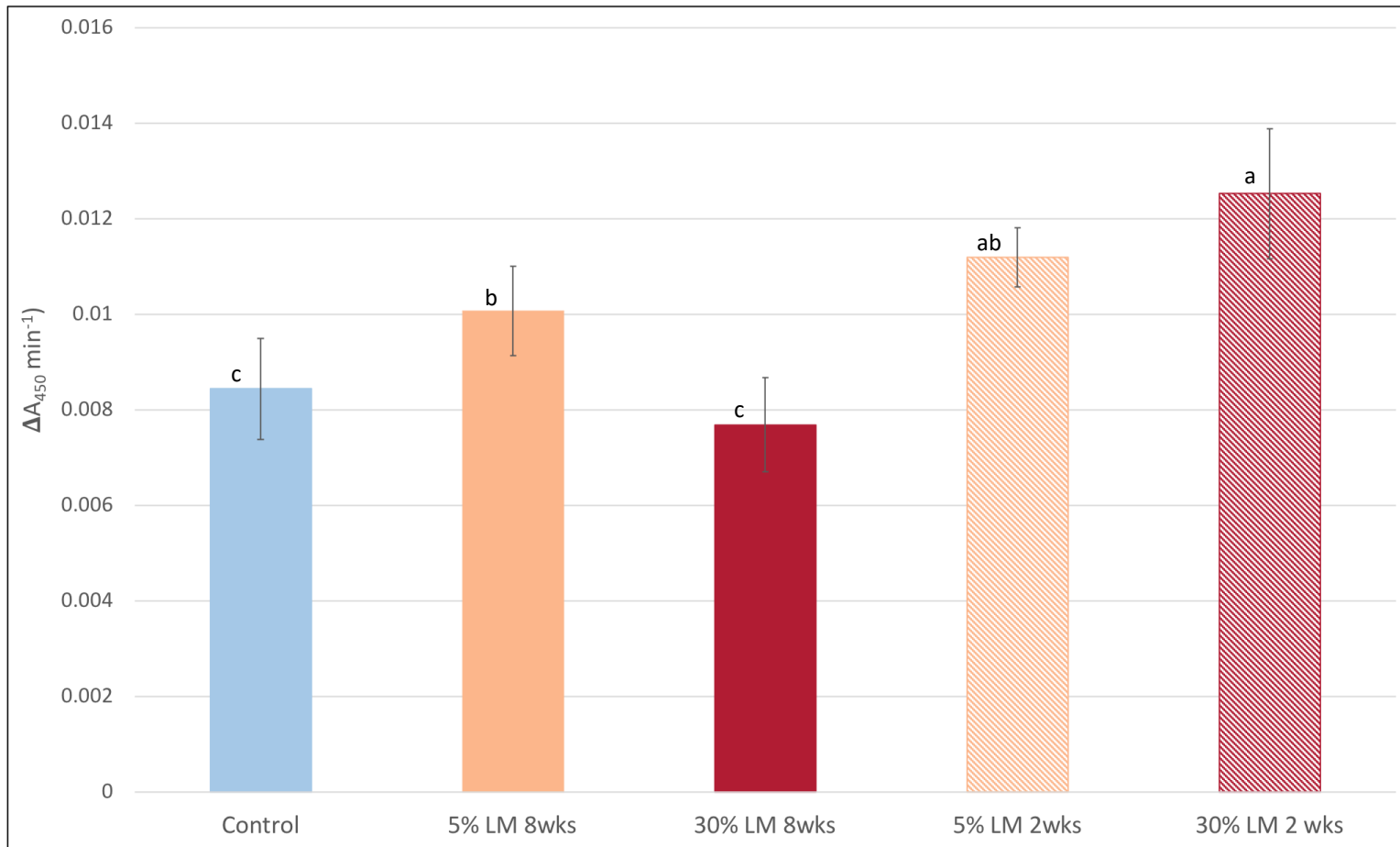


Figure 6: Survival curve for the infection challenge. Low mortality across all treatments was observed, making it impossible to draw conclusions about the presence of absence of a protective effect of LM.

