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Nutritional requirements for reproduction and survival in the blowfly, *Lucilia sericata*

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ABSTRACT

Insects with access to finite energy resources must allocate these between maintenance and reproduction in a way that maximises fitness. This will be influenced by a range of life-history characteristics and the environment in which any particular insect species lives. Here females of the blowfly, *Lucilia sericata* (Diptera: Calliphoridae), were fed diets differing in protein and carbohydrate (sucrose) content, and the allocation of lipid to reproduction was quantified using a spectrophotometric method of analysis. Immediately after adult emergence, total body lipid, scaled for differences in body size, showed an initial decline as it was utilised to meet the metabolic demands of cuticle deposition, muscle maturation and then flight. When flies were denied access to sucrose, stored lipid then continued to decrease until flies died, usually within four days of emergence. However, flies given access to sucrose were able to increase body lipid content, demonstrating that carbohydrate is essential for homeostasis and that it can be used to synthesise lipid. Nevertheless, female flies fed sucrose only were unable to synthesise egg yolk. Only flies provided with protein were able to mature eggs. However, the rate of egg maturation and number and size of eggs matured were greater for female flies given liver compared to flies provided with pure whey protein powder. The results demonstrate the importance of different dietary components for different elements of the life-history of *L. sericata*, namely survival and reproduction.

Key Words: blowfly, carbohydrate, lipid, protein, resource allocation.

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Introduction

Insects must partition resources between competing physiological processes, including homeostasis, development (particularly ecdysis and metamorphosis), growth, reproduction and locomotion (particularly flight). The precise patterns seen in resource allocation are of interest in terms of understanding insect adaptive life-history responses to environmental constraints and will be expected to maximise fitness, ultimately through greater reproductive success (Stearns, 1989). Resources must be derived from the dietary intake obtained from the environment, as juveniles, adults or both, and are stored in the body prior to use (Brown *et al.,* 2004). In many insects, particularly holometabolous taxa, the juvenile stage is the primary period of resource accumulation, allowing it to meet both the requirements of growth, development and metamorphosis (Downer and Matthews, 1976; Lease and Wolf, 2011) but also to provide resource required by the newly emerged adult (Boggs, 1981, 2009; Muntzer *et al*., 2015).

Reserves are stored in the body of most insects in two main forms: as carbohydrate, in the form of glycogen, and as lipid, including triglycerides (TAG) (Beenakkers *et al.,* 1985; Clements, 1992; Lorenz and Anand, 2004). These storage products may have specific uses. Carbohydrate is utilized as a short-term energy supply for operations that require large quantities of energy, such as the pupal moult (Siegert, 1995), emergence from the puparium and flight immediately following pupal ecdysis (Mayer and Candy, 1969; Jutsum and Goldsworthy, 1976; Visser and Ellers, 2008). Some insects also have the ability to oxidize the amino acid proline as metabolic fuel for flight (Teulier *et al.*, 2016). In contrast, lipid provides a longer-term energy source for metamorphosis, embryogenesis, and reproduction (Troy *et al.,* 1975; Kawooya and Law, 1988; Lorenz and Anand, 2004). Carbohydrates and lipid may be acquired from the diet, but many arthropods are also able to synthesize lipid *de novo* from carbohydrates (Beenakkers *et al.,* 1985; Downer, 1985; Ziegler and Van Antwerpen, 2006; Blanckenhorn *et al.,* 2007). Variations in the rate of lipid accumulation in insects may be modulated by a range of environmental factors, particularly temperature (Muntzer *et al.,* 2015; Sinclair and Marshall, 2018). Changes in the rate of lipid accumulation may also be an important part of the physiological response to environmental challenge. For example, increased lipid accumulation triggered by falling temperatures is an important contributor to increasing cold tolerance in *Drosophila suzukii* (Diptera: Drosophilidae)

(Enriquez and Colinet, 2019). Lipids also provide metabolic reserves to facilitate overwintering. Female insects may have a higher stored lipid reserves than males to facilitate egg production (Lease and Wolf, 2011), with higher lipid contents in both ovaries and haemolymph (Sayah, 2008). Protein is also an important dietary component in many insect species, particularly in relation to egg production (Wall *et al.,* 2002), and the ratio of carbohydrate to protein in the diet may be important in determining reproductive output and lifespan, with lifespan being maximized at a high carbohydrate:protein ratio in other dipterans, including *Drosophila melanogaster* (Lee *et al*., 2008; Jensen *et al*., 2015) and Queensland fruit flies (Fanson *et al*., 2009).

Here lipid accumulation by the female blowfly, *Lucilia sericata* (Diptera: Calliphoridae), was quantified and the allocation of lipid to egg production or stored in the body was examined in relation to different feeding regimes. The blowfly, *L. sericata,* is used as a model because it is easy to rear under laboratory conditions and it has been the subject of extensive previous research (Wall *et al.,* 2002; Muntzer *et al.,* 2015). It is also of economic significance in animal husbandry (Hall and Wall, 1995) and forensic science (Gassberger and Reiter, 2001; Clark *et al.,* 2006). The importance of protein for vitellogenesis in *L. sericata* has been demonstrated previously and a highly flexible physiological response to protein limitation has been highlighted (Wall *et al*., 2002). In this species egg development has been shown to be a two-step process, in which females fed an inadequate level of protein initiate vitellogenesis in all proximal oocytes, but then subsequently selectively withdraw yolk to mature only a proportion of the available oocytes, the number of which is dependent on the amount of protein available. Similar findings have been reported in *Drosophila* (Lee *et al.,* 2008). However, previous studies of *L. sericata* have focussed largely on the consequences of protein limitation alone. The aim of the present study was to expand on this and to gain a more complete picture of the consequences of resource accumulation and differential allocation in *L. sericata*, by determining the interaction and physiological consequences of both protein and carbohydrate availability.

Methods and materials

A breeding colony of *L. sericata* was maintained at the University of Bristol in 32 × 32 × 32 cm plastic mesh cages (Bugdorm.com, Taiwan) in a cooled incubator at 25 °C and 60 %

relative humidity and provided with water and sucrose *ad libitum* and lamb liver as required to allow vitellogenesis and oviposition. Water was provided in the form a water-filled plastic cup (284 ml), inverted onto a 90 mm diameter filter paper in a 90 mm dimeter Petri-dish lid. As an index of body size, for each fly, the posterior cross vein, *dm-cu*, located between the fourth and fifth longitudinal veins of the right wing, was measured (mm) using an eyepiece graticule in a binocular microscope, as used in previous studies (Hayes *et al.,* 1998).

Lipid measurement

Van Handel (1985) first used spectrophotometry, based on a microquantity colorimetric sulfphovanillan method (SPV), to estimate the total lipid content of mosquitoes. The essential principle involved in this method is that sulphuric acid reacts with unsaturated lipids to produce a carbonium ion, while phosphoric acid reacts with vanillin to create an aromatic phosphate ester, and the activated carbonyl group of phosphor-vanillin reacts with the carbonium ion to manufacture a charged coloured complex that is constant by resonance and light absorption at 525 nm.

For lipid analysis of intact female *L. sericata*, adult flies were first dried in an oven at 70°C for 24 h and then weighed to the nearest microgram using an ultrasensitive microbalance (Sartorius-CPA26P, Germany). Each weighed fly was then placed individually into a clean glass test tube (16 ×150 mm), then thoroughly crushed using a 3 mm diameter glass rod. While keeping the glass rod inside the test tube, 5 ml of chloroform-methanol (1:1) was added. Then, after removing the glass rod, a further 5 ml of chloroform-methanol (1:1) was added. Next, 0.50 ml of supernatant was carefully transferred to a clean glass tube, using a 1000µl Pipette (StarLab, ErgoOne®, UK) and this tube was then placed in a dry bath (LSE single block digital, Corning Ltd., UK) at 100˚C, in a fume cupboard, to evaporate off the solvent. Once the solvent had evaporated, 0.2 ml of sulphuric acid was added, and the test tube placed again in the dry bath for around 10 min. The test tube was removed and left to cool for two min, after which 4.8 ml of vanillin/phosphoric acid regent was added. The latter was prepared by dissolving 600 mg of vanillin (Sigma Aldrich Ltd., UK) in 100 ml hot water and 400 ml 85% phosphoric acid (Sigma Aldrich Ltd., UK), and it was then stored in the dark. A vortex mixer (Bibby Scientific, UK) was then used to spin the sample

thoroughly following which 1 ml of the solution was transferred into a 1 ml cuvette and read immediately in a spectrophotometer (WPA Biowave UV/Vis Spectrophotometer, Biochrom Ltd., UK) at 525 nm, using a vanillin/phosphoric acid only blank.

Absolute lipid values were extrapolated from the spectrophotometer RFU (Relative Fluorescence Unit) reading using a standard curve. The standard curve was obtained by using eight dilutions of analytical standard soybean oil solution (0.917 g/ml) (Sigma Aldrich, UK). These dilutions were 0.0, 12.5, 25, 50, 100, 200, 400, and 800 µl, diluted in methanol: chloroform (1:1), following the procedure described above. To draw the calibration curve, all eight dilutions were analysed in triplicate, and the average of the three readings was used.

Effects of diet on lipid accumulation and egg production

Approximately 100 newly-emerged flies of both sexes were placed into a 32 × 32 × 32 cm cage and subjected to one of six feeding regimes, all of which included water provided *ad libitum*. These were: (1) water only, (2) 25 g of granulated sucrose, (3) 25 g of granulated sucrose plus 25g of lamb liver, (4) 25 g of pure whey protein powder (Gold standard Whey, Optimum Nutrition Ltd., UK), (5) 25g of sucrose plus 25g of whey protein powder and (6) soybean oil (Fisher Scientific Ltd., UK). Each food type was placed onto a square plastic weigh boat (8 × 8 × 2 cm), except for the soybean oil, where – to prevent the flies becoming stuck in the oil – an approximately 10x10 cm square of cotton wool was dipped into the pure oil and then placed into a 90 mm dimeter Petri-dish lid. For seven days after emergence, six female flies were removed from each cage each day, killed by chilling and then subjected to lipid analysis as described above. The study did not consider females older than seven days to minimise the risk that females were included that might have already oviposited or started yolk resorption in the absence of suitable oviposition sites (Wall *et al*., 1991). There were two replicates for each dietary treatment, giving twelve flies per day for data analysis.

In a second trial, newly emerged flies were provided with the six feeding regimes described above, but each day six adult females were removed for analysis of reproductive development. For this, the abdomen of each female was removed and placed on a

microscope slide in a small volume of Ringer's Insect solution (0.9% saline) (OXOID, Fisher Scientific Ltd). The ovaries were then carefully removed by dissection using forceps and eggs teased out under a dissection microscope (Leica S6E, Germany). The degree of oocyte maturation (yolk deposition) was assessed and, where yolk deposition was complete, the number of mature oocytes was counted. The average length of mature oocytes was measured to the nearest micrometre using a Leica eyepiece graticule. The ovaries plus oocytes and the remainder of the body were then dried separately for lipid analysis as described above. As above, females were dissected daily for up to seven days after emergence. There were two replicates for each dietary treatment, giving twelve flies per day for data analysis.

Data analysis

For each female analysed, the amount of lipid recorded was divided by its wing vein length to remove the effects of absolute variation in fly size (Hayes *et al.,* 1998). This is described as the normalised lipid content; normalised lipid values are expressed as µg/µm with all means presented ± their standard deviation (SD). The normalised lipid content of intact flies, their ovaries or their bodies minus their ovaries, when given different feeding regimes, were compared by one-way ANOVA followed by Tukey post-hoc multiple range tests following tests for normality and homogeneity of variances. The relationships between normalised lipid content and age by day were characterised by linear regression plotted over a subset of the data (usually from day 2); non-linear regression was unable improve the fit significantly. Where significant changes in lipid content over time were detected, differences between dietary groups were examined by ANCOVA with age as the covariate. All analyses were undertaken using SPSS for Windows (IBM, Version 24).

Results

Effects of diet on total lipid accumulation

For female flies given water only, water plus whey protein powder or water plus soybean oil, normalised lipid values declined rapidly and by five days after emergence all had died (Fig. 1).

Immediately after emergence, the mean normalised total lipid in the intact female flies was 0.89 µg/µm (± 0.36), 0.77 µg/µm (± 0.32), 0.92 µg/µm (± 0.23) in the groups provided with liver and sucrose, sucrose only and whey protein powder and sucrose, respectively (Fig. 2). These initial normalised lipid values were not significantly different from each other ($F_{2,33}$= 0.81, P= 0.46). Subsequently, after an initial decline between days 1 and 2 in two of the treatment groups, normalised lipid levels then increased significantly over time in all three groups (liver and sucrose $F_{1,70}$= 61.6, P= 0.001, sucrose only $F_{1,70}$= 47.2, P= 0.001 and whey powder and sucrose $F_{1,70}$= 12.8, P= 0.001)..

At day 7, the mean normalised lipid contents were 1.40 µg/µm (± 0.35), 0.99 µg/µm (± 0.26) and 1.25 µg/µm (± 0.25) in these three groups, respectively. These values were significantly different from each other ($F_{2,33}$= 6.28, P= 0.005), with the group provided with liver having significantly higher lipid values at day 7 than the group provided with sucrose only, but not significantly different from the group provided with whey powder and sucrose. Overall, the accumulation of lipid with age was not significantly different between the liver plus sucrose-fed and whey protein plus sucrose-fed groups, but both were significantly greater than the group fed sucrose only ($F_{2,212}$= 72.7, P= 0.001, Table 1).

Effects of diet on lipid allocation to reproduction

In the ovaries of females given access to liver and sucrose, there was an initial significant decline in normalised lipid between day 1 and day 2 ($F_{1,22}$=15.6, P= 0.001); vitellogenesis was observed from day 3 and lipid values in the ovary increased significantly from day 2 to day 7 ($F_{1,70}$=51.03, P= 0.001, Fig. 3A). At day 7, in females given liver plus sucrose, the mean lipid content of the ovaries was 0.72 µg/µm (±0.26) and the ovaries of all females contained mature eggs with a mean number of 236.3 (±41.4) eggs.

In females given sucrose only, flies survived throughout the experiment and their ovaries showed a slight, but significant increase in normalised lipid from day 2 to day 7 ($F_{1,70}$=22.1, P= 0.001), but none matured eggs (Fig. 3B).

The normalised lipid content in the ovaries of the group that had access to whey powder and sucrose showed a significant decrease in lipid between day 1 and day 4 ($F_{1,46}$=36.7, P= 0.001); vitellogenesis was observed only from day 4 and normalised lipid values subsequently rose significantly from day 4 to day 7 ($F_{1,46}$=40.8, P= 0.001, Fig. 3C). In

these females at day 7, the mean normalised lipid content was 0.57 µg/µm (±0.23) and only 8 of the 12 dissected females were gravid; the other four had not fully completed vitellogenesis. However, once lipid deposition had started in females in the sucrose and whey powder-fed group, comparison of the slopes of the regressions shows that the rate of accumulation was not significantly different from that in the sucrose and liver-fed group (t=0.69, P=0.5, Table 1), although the overall the amounts of lipid differed significantly between all three diet groups ($F_{2,188}$=110.8, P= 0.001, Table 1). Females that were gravid at day 7 in the group fed whey protein powder and sucrose, matured batches of 227.5 (±7.5) eggs, which was not significantly different from the egg batch size of the females provided with liver and sucrose ($F_{1,22}$=0.53, P= 0.48). However, for those eggs that were mature the mean length at day 7 in the whey powder treatment group was 1.18 mm (±0.16) which was significantly smaller than the mean egg length of 1.35 mm (±0.45) recorded in the liver-fed group ($F_{1,22}$=12.22, P= 0.002).

Effects of diet on residual lipid

In the residual body, after removal of the ovary, normalised lipid values declined initially in the liver with sucrose and sucrose only feeding regimes. Lipid values then increased significantly over time in these two groups (liver plus sucrose: $F_{1,70}$= 20.1, P= 0.001, Fig. 4A; sucrose only: $F_{1,70}$=38.2, P= 0.001, Fig. 4B). However, normalised lipid showed no significant consistent change in the females given whey protein powder and sucrose ($F_{1,70}$= 0.02, P= 0.88, Fig. 4C).

By day 7, the mean normalised lipid content in the body was 0.68 µg/µm (± 0.20), 0.78 µg/µm (± 0.20) and 0.67 µg/µm (± 0.08) in the females given liver plus sucrose, sucrose only and whey protein powder plus sucrose, respectively. These values were not significantly different from each other ($F_{2,33}$= 1.45, P= 0.25, P> 0.05). Similarly the rates of accumulation of lipid in the residual body with age were not significantly different in the females given liver plus sucrose and sucrose only (Table 1) but both were significantly higher than in females given whey protein powder plus sucrose ($F_{2,212}$= 30.3, P= 0.001, P< 0.05).

Discussion

This study has quantified the change in lipid content of female *L. sericata* following adult emergence; at seven days following emergence at 25 °C females would be expected to be ready to oviposit their first egg batch (Wall *et al*., 1992). At day seven, there were significant differences in the meant total normalised lipid content between three dietary groups. While females with access to sucrose survived throughout the experiment and showed significantly increased lipid content, none of these females matured eggs. In the two treatments with access to protein (liver plus sucrose, whey powder plus sucrose), females matured eggs although the eggs were significantly smaller and not all females became gravid in the whey compared to liver-diet treatment.

Immediately after emergence, the normalised total lipid content was relatively high. Similar observations have been made with the parasitoid species, *Macrocentrus grandii* (Hymenoptera: Braconida) and *Pseudacteon tricuspis* (Diptera: Phoridae) which showed high lipid contents at emergence (Olson *et al*., 2000; Fadamiro *et al*., 2005). Generally, the lipid content showed an initial decline, probably because lipid is utilised rapidly to meet the metabolic demands of cuticle deposition and muscle maturation and then for flight (Justsum and Goldsworthy, 1976; Canavoso *et al*., 2001). This decline in lipid is likely to vary between insect species depending on the energy reserves accumulated in the juvenile stage (Hahn, 2005), the duration and type of metamorphosis and the activity of the newly emerged adult (Brown *et al*., 2004). Female *L. sericata* are generally relatively inactive for at least 24 h after emergence and then show increasing levels of activity; after 24-48h adult female *L. sericata* begin to search for a protein meal to initiate vitellogenesis and then, after mating, the females locate an appropriate oviposition site (Wall *et al*., 1992). The decline in lipid values also suggests that any initial food ingested immediately after adult emergence takes at least 24 h to be metabolised into measurable reserves.

Adult insects replenish their lipid reserves from dietary intake from the environment when available (Warburg and Yuval, 1996; Briegel *et al*., 2001). Here, when flies were not given carbohydrate, lipid content decreased gradually, and flies died within four days. Even whey protein powder or vegetable oil alone were insufficient to sustain flies. Whey protein is derived from milk and is a mixture of beta-lactoglobulin, alpha lactalbumin, bovine serum albumin, and immunoglobins. However, flies given access to sucrose were able to increase

the lipid content of the body. Clearly therefore carbohydrate is essential for homeostasis and carbohydrate can be used to synthesise lipid by *L. sericata*. Many arthropods have been shown to be able to synthesise lipid *de novo* from stored carbohydrates and convert it into glycogen (Beenakkers *et al*., 1985; Downer, 1985; Ziegler and Van Antwerpen, 2006; Blanckenhorn *et al*., 2007). However, here, female flies fed sucrose only were unable to synthesise egg yolk. Evidently, while carbohydrate is essential for survival, protein is essential for vitellogenesis (Wall *et al*., 2002; Lorenz and Anand, 2004).

Lipid and protein are considered to be the main compounds required for development of the embryo (Beenakkers *et al*., 1981), for example in *Culex quinquefasciatus* (Diptera: Culicidae) (Van Handel, 1993). Lipid forms 30-40% of dry ovary weight in several insect species (Allais *et al*., 1964; Troy *et al*., 1975; Briegel, 1990; Kawooya and Law, 1988; Ziegler and Van Antwerpen, 2006). The synthesis of vitellins by the fat body and transfer and accumulation in the oocyte is an essential initial stage in insect reproduction (Hagedorn and Kunkel, 1979; Postlethwait and Giorgi, 1985). Here, female flies that were given whey protein powder plus sucrose, were able to mature eggs, but initiated vitellogenesis later than those fed liver, only 4 of the twelve females dissected at day 7 had fully mature egg batches and those that did contained fewer, smaller eggs than those given liver. The data suggest that the lower protein availability in whey powder, compared to liver, resulted in a slower accumulation of the requisite concentrations of protein required to initiate vitellogenesis, as demonstrated previously (Wall *et al*., 2002). Hence, protein is confirmed as the function-limiting metabolite in egg production in *L. sericata*, as demonstrated previously by (Wall *et al*., 2002). However, it may also be that there are other important nutritional components in liver that are less abundant in whey protein powder, that are important for vitellogenesis in female, *L. sericata* (Rueda *et al*., 2010), such as the amino acids: aspartic acid, leucine, arginine, proline, phenylalanine, tyrosine, glycine, threonine and tryptophan (Marconic *et al.,* 1989). In experimental choice experiments, the flesh fly, *Sarcophaga crassipalpis*, was shown to self-select a nutrient intake that was highly carbohydrate-biased which maximized both lifespan and lifetime egg production, suggesting that flesh flies were able to balance their intake of various macronutrients to maximise fitness (Hawley *et al*., 2016). Clearly, the dietary importance of carbohydrate and lipid

cannot be overlooked, particularly because vitellogenesis requires substantial lipid accumulation (Galois 1984).

In the body minus the ovaries in female fly, *L. sericata*, the results illustrate that lipid content increased significantly over time in flies fed liver plus sucrose and sucrose only. The data suggest therefore that, in addition to egg development, lipid is accumulated in the fat body or in the haemolymph. In some arthropods, there is an increase in lipid in the midgut diverticula during vitellogenesis (Teshima and Kanazawa 1983; Castill and Lawrence 1989).

The lack of a significant increase in lipid in the residual bodies of females given whey powder plus sucrose may initially appear anomalous, but this may have been associated with the delayed onset of vitellogenesis in these flies indicating that resources were being preferentially allocated to reproduction rather than accumulated in the body. As discussed above, this is likely to be due to the dietary inferiority of whey protein powder compared to liver for vitellogenesis. Different brands of whey powder, containing varying blends of amino acid could be used to investigate the importance of specific dietary components.

In summary, the data show that adult female *L. sericata* fed on diets without carbohydrate are unable to survive; those fed diets containing carbohydrate are able to survive and synthesise lipid but are unable to synthesise egg yolk. Vitellogenesis and yolk deposition in the oocyte is only possible when protein is present in the diet. However, the nature of the protein source available influences the ability to initiate vitellogenesis, the rate of yolk deposition and the number and size of eggs matured. The data suggest that females are able respond to resource availability with a high degree of flexibility, allocating resource between the ovary and residual body in response to dietary availability.

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Table 1. Regression coefficients: intercept (a), slope (b), the standard error of the slope, r^2 and significance value, describing the relationship between normalised weight of lipid (µg/µm) of female *Lucilia sericata* and age (days), when fed three different diets. The linear regressions describe relationships for the intact body, ovary only and residual body minus the ovaries. Overall differences in lipid accumulation with age as determined by ANCOVA are denoted by similar letters.

	Treatment (diet)	**Regression coefficients**			**r^2**	**P**	**ANCOVA**
		a	**b**	**±SE**			
Intact body	Liver, sucrose and water	0.16	0.46	±0.02	0.47	0.001	a
	Sucrose and water	0.10	0.28	±0.01	0.44	0.001	b
	Whey protein powder, sucrose and water	0.06	0.87	±0.02	0.15	0.001	a
Ovary only	Liver, sucrose and water	0.05	0.12	±0.24	0.42	0.001	a
	Sucrose and water	0.02	0.02	±0.07	0.24	0.001	b
	Whey protein powder, sucrose and water	-0.36	0.14	±0.16	0.47	0.001	c
Body only	Liver, sucrose and water	0.05	0.39	±0.01	0.22	0.001	a
	Sucrose and water	0.08	0.26	±0.02	0.35	0.001	a
	Whey protein powder, sucrose and water					NS	

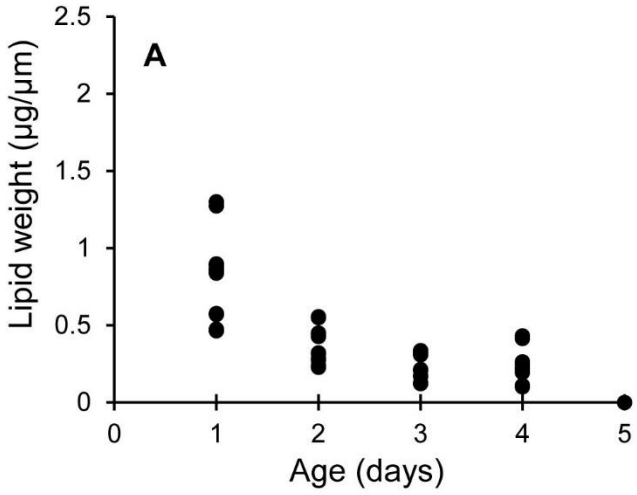
Figure Legends

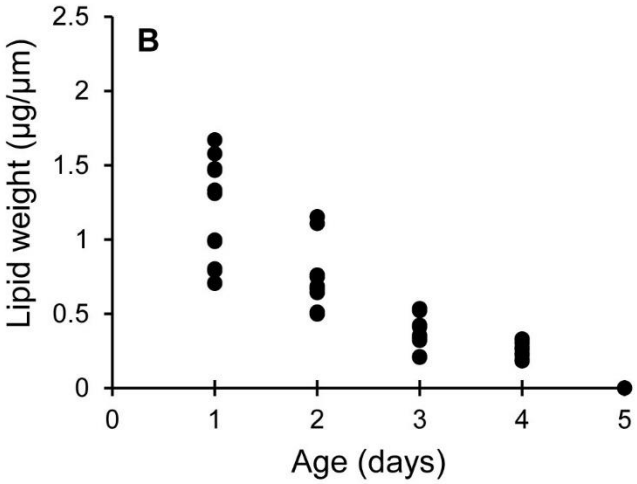
Figure 1. Normalised total lipid content (μg/μm) of intact female *Lucilia sericata* in relation to age (days). A: female flies given access to water only; B: female flies given access to whey protein powder and water; C: female flies given access to soybean oil and water.

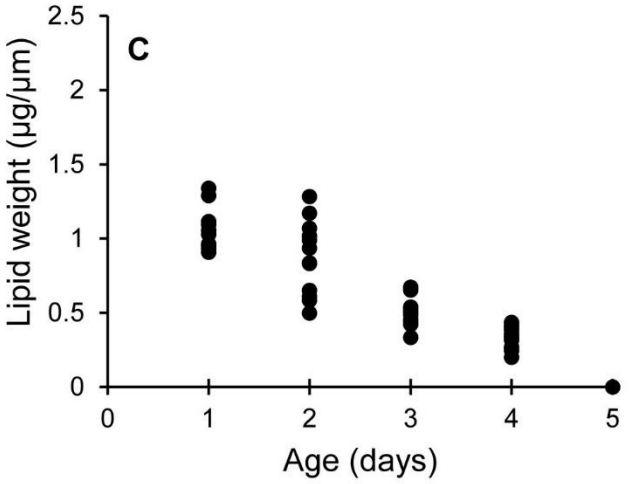
Figure 2. Normalised total lipid content (μg/μm) of whole female *Lucilia sericata* in relation to age (days). A: female flies given access to liver, sucrose and water; B: female flies given access to sucrose and water; C: female flies given access to whey protein powder, sucrose and water.

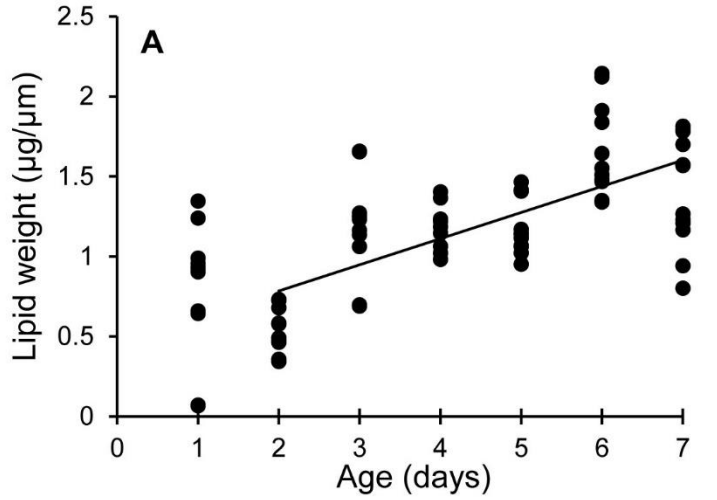
Figure 3. The normalised weight of lipid (μg/μm) of female *Lucilia sericata* in their ovary in relation to age (days). A: female flies given *ad lib.* access to liver, sucrose and water; B: female flies given *ad lib.* access to sucrose and water C: female flies given *ad lib.* access to whey protein powder, sucrose and water.

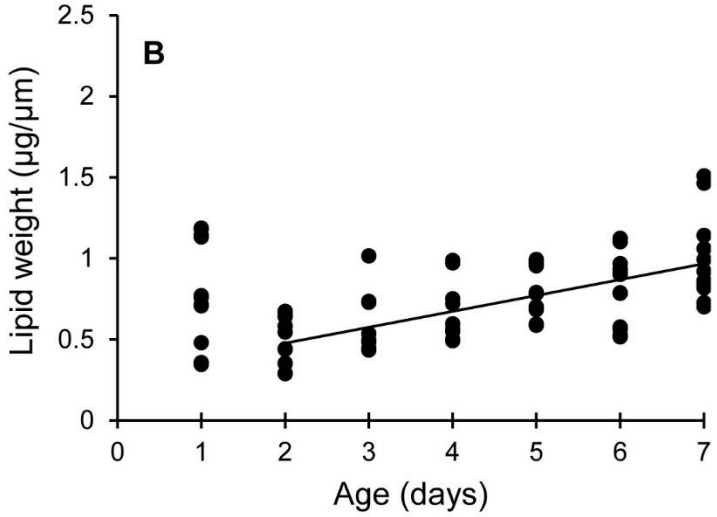
Figure 4. The normalised weight of lipid (μg/μm) of female *Lucilia sericata* in their body in relation to age (days). A: flies given *ad lib.* access to liver, sucrose and water; B: flies given *ad lib.* access to sucrose and water; C: flies given *ad lib.* access to whey protein powder, sucrose and water.


A
Lipid weight (µg/µm)
2.5
2
1.5
1
0.5
0
0
1
2
3
4
5
Age (days)


B
Lipid weight (µg/µm)
2.5
2
1.5
1
0.5
0
0
1
2
3
4
5
Age (days)


C
Lipid weight (µg/µm)
2.5
2
1.5
1
0.5
0
0
1
2
3
4
5
Age (days)


A
Lipid weight (µg/µm)
Age (days)
0
0.5
1
1.5
2
2.5
0
1
2
3
4
5
6
7


B
Lipid weight (µg/µm)
Age (days)
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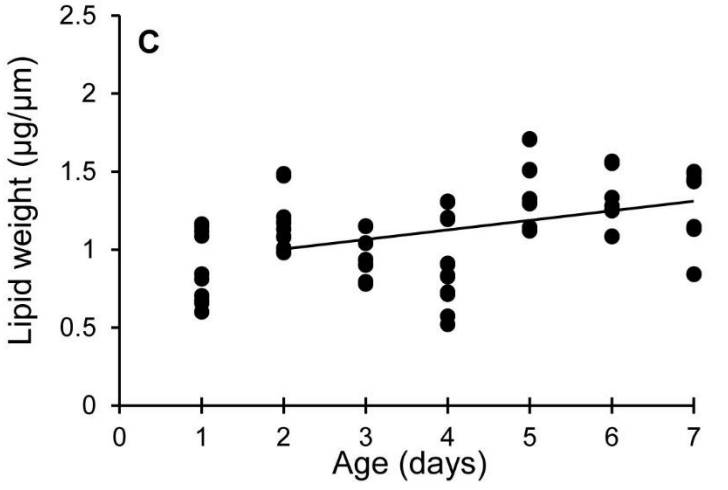
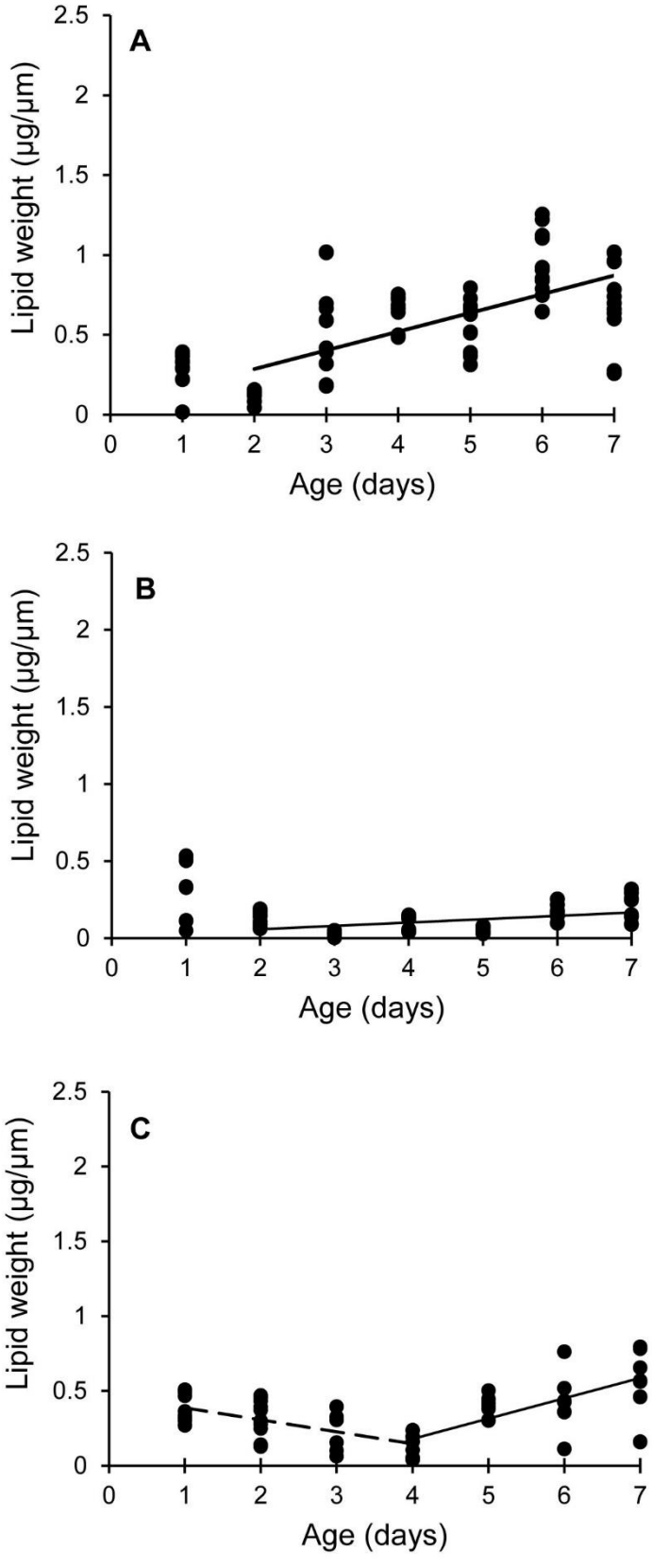
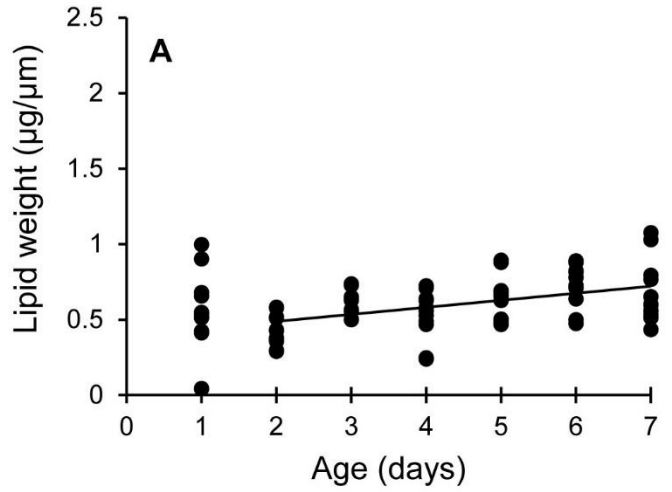
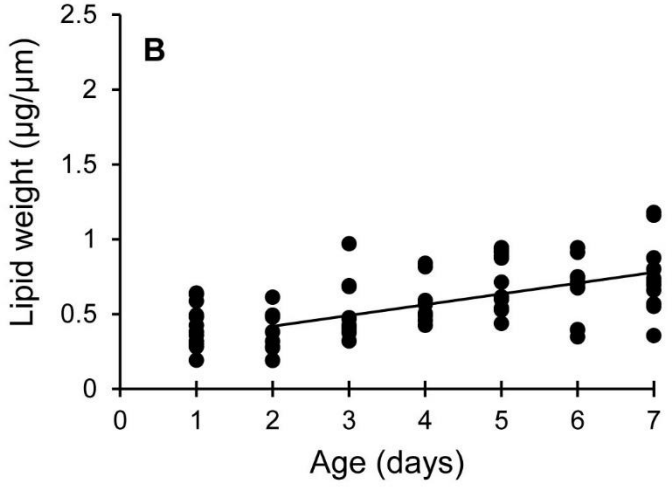
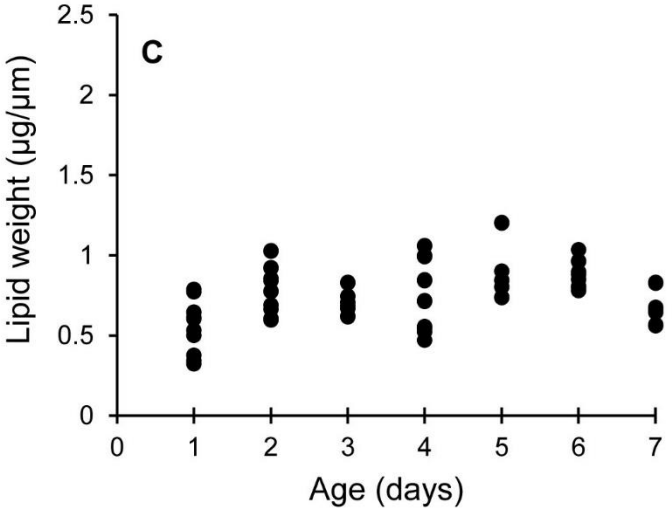
0.5
1
1.5
2
2.5
0
1
2
3
4
5
6
7


C
Lipid weight (µg/µm)
Age (days)
0
0.5
1
1.5
2
2.5
0
1
2
3
4
5
6
7


A
B
C
Lipid weight (µg/µm)
Age (days)
0
0.5
1
1.5
2
2.5
0
1
2
3
4
5
6
7


A
Lipid weight (µg/µm)
2.5
2
1.5
1
0.5
0
0
1
2
3
4
5
6
7
Age (days)


B
Lipid weight (µg/µm)
2.5
2
1.5
1
0.5
0
0
1
2
3
4
5
6
7
Age (days)


C
Lipid weight (µg/µm)
2.5
2
1.5
1
0.5
0
0
1
2
3
4
5
6
7
Age (days)