The development of a synthetic diet for investigating the effects of macronutrients on the development of *Plodia interpunctella*

Joanne Littlefair*, Katherine A. Nunn & Robert J. Knell

School of Biological and Chemical Sciences, Queen Mary University of London, Fogg Building, Mile End Road, London E1 4NS, UK

*Correspondence: E-mail: j.littlefair@qmul.ac.uk

1 **Abstract**

2

3 The use of chemically defined artificial diets has allowed researchers to examine questions

4 within nutritional ecology about how macronutrients affect life history traits and resource-

5 based trade-offs. Using a chemically defined diet, it is possible to manipulate both the total

6 nutritional content and the ratio of macronutrients (i.e., protein, carbohydrate, or lipids)

7 within the diet. Studies using the geometric framework have made use of these diets to

8 examine lifespan, fecundity, and immune responses. Here we develop an artificial diet

9 suitable for rearing Lepidopteran larvae. We created diets with three different proportions of

10 non-nutritive material (30, 50, and 70% indigestible cellulose) relative to protein and

11 carbohydrate macronutrients, and compared these to standard wheat bran laboratory diet. We

12 were then able to examine the effects of variable nutrient content on lifespan and

13 development time in *Plodia interpunctella* Hübner (Lepidoptera: Pyralidae).

14

15 The diet was able to support development that is comparable to bran-based laboratory diets.

16 Total nutrient content affected development time: females that fed on the diet with the highest

17 nutrient content took the longest time to reach eclosion. We also found evidence to support

18 dietary restriction, with larvae receiving the fewest nutrients having the longest lifespan as

19 adults.

20 These findings are indicative of the usefulness of this diet as a tool to further investigate the

21 effects of nutrient content and macronutrient imbalance on resource based trade-offs and life-

22 history traits.

23

24 **Introduction**

25 An essential part of many mass-rearing programmes, artificial diets maximise cost- and time-

26 effectiveness by, for example, eliminating the need for the maintenance of multiple trophic

27 levels (e.g., artificial diets can replace host plants and prey as a food source for herbivorous

28 insects and predators, respectively). Commercial examples of artificial diets include their use

29 in mass rearing insects for sterile insect technique or predatory insects for pest control

30 (Robinson & Hendricks, 2005; Riddick, 2008). On a smaller scale, artificial diets can be used

31 within an academic research setting to rear insects as model organisms to answer a number of

32 evolutionary and ecological questions (Vanderzant, 1974). The development of these diets is

33 often complex and time-consuming, however, because they must be tailored to the individual

34 nutrient requirements or feeding behaviour of each species.

1   If diets are chemically defined then the ratio of macronutrients (i.e., protein,

2 carbohydrate, or lipid) is known and can be adjusted, which has facilitated recent research in

3 nutritional ecology on the importance of various components of nutrition on different aspects

4 of fitness such as lifespan, total egg production, or immune responses (Simpson & Abisgold,

5 1985; Lee et al., 2008; Povey et al., 2009; Cotter et al., 2011; Harrison et al., 2014). Known

6 as the geometric framework, the work of Simpson and Raubenheimer in creating a

7 visualisation and space-state modelling tool has allowed us to model life-history traits over a

8 response surface of different intake quantities of macronutrients (Simpson & Raubenheimer,

9 1995). This is only possible when using multiple artificial diets containing different ratios of

10 macronutrients while also varying the total available nutrition. Using the geometric

11 framework, it has been possible to visualise the relationship between different macronutrients

12 and the performance consequences of consuming them, even locating the individual

13 nutritional optima for traits such as reproduction or longevity, facilitating our knowledge of

14 resource-based life-history trade-offs (Lee et al., 2004; Povey et al., 2009; Simpson &

15 Raubenheimer, 2009).

16   Here, we describe a synthetic diet suitable for rearing larvae of Indian meal moth,

17 *Plodia interpunctella* Hübner (Lepidoptera: Pyralidae). *Plodia interpunctella* is an

18 economically important pest of stored products and is commonly found in post-harvest

19 settings such as freight containers, warehouses, and grain mills. The larvae are dietary

20 generalists, infesting various food sources including grain, dried products (e.g., fruit, nuts,

21 and seeds), and animal feed. Major research topics using *P. interpunctella* as an insect model

22 include sexual selection (Gage, 1998; Lewis et al., 2011), host-parasite dynamics (Sait et al.,

23 1994; Knell et al., 1996), and their status as agricultural pests. The creation of this artificial

24 diet, and the examination of its effects on development will facilitate the use of *P.*

25 *interpunctella* in nutritional ecology. Artificial diets for *P. interpunctella* have been created in

26 the past, as it is a well-studied insect model organism. However, these have not been

27 chemically defined, and were predominantly created for ease of rearing *P. interpunctella* in

28 laboratory conditions (Fraenkel & Blewett, 1946; Silhacek & Miller, 1972). We also

29 determined how total nutrient availability of the new artificial diet affected length of the

30 developmental stages of *P. interpunctella*, their longevity as adults, and whether this differs

31 from the length of life stages in moths reared on standard wheat bran-based laboratory diet.

32

33 **Material and methods**

1 *Plodia interpunctella* **culturing**A stock population of *P. interpunctella* has been maintained

2 at Queen Mary University since December 2011, when it was started using animals from an

3 outbred population at the University of Leeds. The population is maintained on ad-libitum

4 standard lab food [organic wheat bran (Mount Pleasant Mill, Kirton-in-Lindsey, UK),

5 brewers' yeast (MPBio, Cambridge, UK), and glycerol (Alfa Aesar, Heysham, UK) in a

6 10:1:1 ratio] at 27 °C on an L12:D12 h cycle. To create the next generation, over 200 mixed

7 adults are placed in a funnel with both ends secured with net, and allowed to mate. The

8 resulting eggs are collected and placed on standard laboratory food, and the larvae are

9 allowed to grow until adulthood.

10

11 **Creating artificial diet suitable for rearing *Plodia interpunctella***

12 The diet constructed is agar based (Lee et al., 2004; Povey et al., 2009; Cotter et al., 2011), as

13 multiple pilot experiments showed that powder-based diets are not suitable to support the

14 growth of *P. interpunctella* (Simpson & Abisgold, 1985). Three artificial diets were tested

15 with different amounts of non-nutritive material (indigestible cellulose) relative to protein

16 and carbohydrate macronutrients (30, 50, and 70% cellulose). These percentages of cellulose

17 were selected based on pilot experiments to give a broad range of total nutritional content

18 while still being able to support development.

19   The cellulose (Sigma-Aldrich, New Road Gillingham, UK), sucrose (Sigma-Aldrich),

20 cholesterol (Fisher Scientific, Loughborough, UK), linseed oil (MPBio), glycerol, and

21 Wesson's salts (MPBio) (Table 1) were weighed and added to an autoclavable pot, totalling

22 75 g per diet. A 1% solution of agar was added in a 4:1 ratio to the dry ingredients (300 ml of

23 agar per diet). The mixture was thoroughly blended with a magnetic stirrer and the solution

24 was left to cool to 37 °C. The casein (Sigma-Aldrich), peptone (Sigma-Aldrich), albumen

25 (Fisher Scientific), methyl hydroxybenzoate preservative (1 g l^{-1} diet dissolved in 2 ml

26 ethanol; VWR, Lutterworth, UK) and Vanderzant vitamin mix (Sigma-Aldrich) were added.

27 The solution was mixed again and set in the refrigerator at 4 °C overnight. Using this

28 artificial diet formulation, both the total quantity of nutrients and the ratio of individual

29 nutritional components can be varied.

30   Fresh standard laboratory diet was made by mixing organic wheat bran, brewer's

31 yeast, and glycerol in a 10:1:1 ratio using a food mixer. Third instars were collected from the

32 stock population, and each larva was added to an individual 55-mm Petri dish with 0.85 ±

33 0.05 g of one of the four diets (n = 50 per treatment). Third instars were used because,

34 although the diet is capable of supporting *P. interpunctella* from egg to adulthood, very small

1 larvae sometimes drown in the diet, making it difficult to obtain a large enough sample size

2 to perform the experiment. The diet was changed every 2-3 days to prevent desiccation.

3 Larvae were weighed as wandering fifth instars 23 days after egg laying, but as many of the

4 larvae consuming the bran-based diet were already pupating, the sample sizes for this

5 treatment are uneven (30% cellulose: n = 43; 50% cellulose: n = 44; 70% cellulose: n = 44;

6 bran-based diet: n = 7). The larvae were checked daily, and time to pupation, eclosion, and

7 death as adults was recorded.

8

9 **Statistical analysis**

10 Development time, adult lifespan, and fifth instar weight data were analysed in R v.3.0.1 (R

11 Development Core Team, 2013) using ANOVA and post-hoc Tukey tests. Development time

12 was defined as the amount of time until eclosion (i.e., as larvae and pupae), and any larvae

13 that did not eclose were removed from this analysis. The percentage of larvae that survived

14 until eclosion was analysed with a generalised linear model with binomial errors and a logit

15 link. Likelihood-ratio tests were used to compare models with and without specific

16 explanatory variables and non-significant terms were dropped until minimal adequate models

17 were reached (Zuur et al., 2009). Following the examination of diagnostic plots from the

18 fitted models, the data in the analysis of development time were square-root transformed to

19 reduce heteroscedasticity.

20

21 **Results**

22 There was a significant interaction between sex and diet affecting development time

23 (measured as number of days spent in the larval and pupal stages) (ANOVA: $F_{3,137} = 4.12$, P

24 = 0.0078; Figure 1). The post-hoc Tukey test indicated that females consuming the 30%

25 cellulose diet took significantly longer than those given the other diets to reach eclosion,

26 taking 41.9 days. In comparison, females consuming the 50% cellulose diet took 35.3 days,

27 females consuming the 70% cellulose diet took 36.2 days and females consuming the bran-

28 based diet took 31.6 days. The variance in development time for females on the 30%

29 cellulose diet was much greater than all of the other sex*diet combinations (Figure 1). In

30 males, none of the diet treatments led to significant differences in development time.

31   Larval diet treatment had a significant effect on adult lifespan (ANOVA: $F_{3,137} =$

32 16.99, P<0.0001; Figure 2). Figure 2 indicates that larvae consuming the 30% cellulose diet

33 had the shortest adult lifespan (7.27 days), and as the nutrient content of the diet decreased,

34 adult lifespan increased (50% cellulose: 8.45 days, 70% cellulose: 9.43 days). Larvae

1   consuming the bran-based diet had the longest adult lifespan (10.2 days). A post-hoc Tukey

2   test confirmed this, showing that the 30% cellulose diet was different from the 70% cellulose

3   diet and the bran-based diet. The 50% cellulose diet was different from the bran-based diet.

4   Also sex had a significant main effect: females lived longer than males ($F_{1,137} = 7.01$, P =

5   0.0090). The interaction of sex and diet treatment was not significant ($F_{3,137} = 2.095$, P =

6   0.10). Diet had no effect on fifth instar weight ($F_{3,131} = 1.34$, P = 0.27). Only sex affected

7   weight, with females weighing 21.8% more than males ($F_{1,134} = 77.9$, P<0.0001).

8   The diet consumed had a weakly significant effect on the percentage of larvae

9   surviving to eclosion (i.e., dying in the larval or pupal stages) (likelihood ratio test statistic =

10   8.38, d.f. = 3,192, P = 0.039). Table 2 illustrates the survival for each diet treatment. Sex had

11   no effect (likelihood ratio test statistic = 0.345, d.f. = 1,185, P = 0.56), nor any interaction

12   between sex and diet (likelihood ratio test statistic = 1.30, d.f. = 3,185, P = 0.73).

13

14   **Discussion**

15   Using gel-based formulations, we successfully created artificial diets that produced similar

16   survival, longevity, and development times when compared with the grain-based diet that *P.*

17   *interpunctella* usually consume in the laboratory. The larval and pupal development time on

18   the 50% cellulose diet was not significantly different from the bran diet in females. In males,

19   none of the diets were significantly different from each other in terms of effects on

20   development time. For both sexes, the 70% cellulose diet allowed *P. interpunctella* to achieve

21   an adult lifespan comparable to the bran-based diet. Finally, diet did not affect the weight at

22   fifth instar. Therefore, the artificial diets created are sufficiently similar to the grain-based

23   diet, allowing us to infer realistic facts about the biology of *P. interpunctella* when we

24   artificially manipulate their nutrition.

25   These similarities with the standard laboratory diet notwithstanding, there were

26   several interesting effects produced by the range of artificial diets examined here, which

27   bodes well for experiments using *P. interpunctella* as a model organism to examine the

28   effects of macronutrient manipulation. For example, increasing the ratio of cellulose to

29   macronutrients decreased the adult lifespan. Individuals reared on the bran-based diet had the

30   longest adult lifespan, and the artificial diet with the least available nutrition (70% cellulose)

31   was comparable to this. This is surprising, given that a greater ratio of nutritional to inert

32   components would increase the amount of resources available for acquisition during the

33   larval stage, and therefore could intensify investment in traits such as longevity during the

34   adult stage, potentially leaving more time to find a mate.

Several possible explanations for this are gaining traction within the nutritional ecology literature. Dietary restriction experiments have shown that limiting a component of nutrition can increase the organism's lifespan, sometimes with dramatic effects. This mechanism is highly conserved, with taxa as diverse as yeasts, rotifers, insects, rats, and primates showing similar effects of lifespan extension (Yu et al., 1982; Partridge et al., 2005; Weithoff, 2007; Colman et al., 2009). There is still debate as to the precise nature of the limitation needed, e.g., caloric restriction or limitation of one particular macronutrient or even amino acid (Mair et al., 2005; Masoro, 2005; Lee et al., 2008; Grandison et al., 2009; Piper et al., 2011). Although essential for life, certain macronutrients may in fact be toxic when consumed in excessive quantities. Current evidence suggests excessive amounts of protein can be detrimental to lifespan, possibly due to higher levels of reactive oxygen species or increased DNA damage (Simpson & Raubenheimer, 2009; Solon-Biet et al., 2014).

Another interesting effect of the high macronutrient diet is the dramatic extension of the development time required by females to reach eclosion, which is longer than on any other diet. This effect is particularly increased by four individuals that took longer than 50 days in the development period, possibly entering a diapause state induced by sub-optimal diet quality. *Plodia interpunctella* larvae are able to enter a facultative pre-pupal diapause which can be induced by photoperiod, temperature, strain of origin, or diet (Williams, 1964; Bell, 1994; Wijayaratne & Fields, 2012). Diapause seems to be an important life stage for most stored-product Lepidoptera (Bell, 1994), which are able to undergo diapause in crevices of warehouses undisturbed. Termination of diapause then occurs when more favourable conditions resume. Finally, a lower proportion of larvae survived to eclosion on the artificial diets with higher macronutrient content, although this was possibly because slightly more larvae drowned in the moist texture of these two diets.

To conclude, a synthetic artificial diet was created to support the development of *P. interpunctella* larvae. The ratio of available macronutrients within it can be manipulated, increasing the tools available to us to use *P. interpunctella* as a model organism for the study of resource-based life-history trade-offs and the effects of macronutrient imbalance.

References

Bell CH (1994) A review of diapause in stored product insects. Journal of Stored Products

1   Research 30: 99–120.

2   Colman RJ, Anderson RM, Johnson SC, Kastman EK, Kosmatka KJ et al. (2009) Caloric

3   restriction delays disease onset and mortality in rhesus monkeys. Science 325: 201–204.

4   Cotter SC, Simpson SJ, Raubenheimer D & Wilson K (2011) Macronutrient balance mediates

5   trade-offs between immune function and life history traits. Functional Ecology 25: 186–

6   198.

7   Fraenkel G & Blewett M (1946) The dietetics of the caterpillars of three *Ephestia* species, *E.*

8   *kuehniella*, *E. elutella*, and *E. cautella*, and of a closely related species, *Plodia*

9   *interpunctella*. Journal of Experimental Biology 22: 162–171.

10 Gage MJG (1998) Influences of sex, size, and symmetry on ejaculate expenditure in a moth.

11   Behavioral Ecology 9: 592–597.

12 Grandison RC, Piper MDW & Partridge L (2009) Amino-acid imbalance explains extension

13   of lifespan by dietary restriction in *Drosophila*. Nature 462: 1061–1064.

14 Harrison SJ, Raubenheimer D, Simpson SJ, Godin JJ, Susan M & Bertram SM (2014)

15   Towards a synthesis of frameworks in nutritional ecology: interacting effects of protein,

16   carbohydrate and phosphorus on field cricket fitness. Proceedings of the Royal Society

17   of London B 281: 20140539.

18 Knell RJ, Begon M & Thompson DJ (1996) Transmission dynamics of *Bacillus thuringiensis*

19   infecting *Plodia interpunctella*: a test of the mass action assumption with an insect

20   pathogen. Proceedings of the Royal Society of London B 263: 75–81.

21 Lee KP, Raubenheimer D & Simpson SJ (2004) The effects of nutritional imbalance on

22   compensatory feeding for cellulose-mediated dietary dilution in a generalist caterpillar.

23   Physiological Entomology 29: 108–117.

24 Lee KP, Simpson SJ, Clissold FJ, Brooks R, Ballard JWO et al. (2008) Lifespan and

25   reproduction in *Drosophila*: new insights from nutritional geometry. Proceedings of the

26   National Academy of Sciences of the USA 105: 2498–2503.

27 Lewis Z, Wedell N & Hunt J (2011) Evidence for strong intralocus sexual conflict in the

28   Indian meal moth, *Plodia interpunctella*. Evolution 65: 2085–2097.

29 Mair W, Piper MDW & Partridge L (2005) Calories do not explain extension of life span by

30   dietary restriction in *Drosophila*. PLoS Biology 3: 1305–1311.

31 Masoro EJ (2005) Overview of caloric restriction and ageing. Mechanisms of Ageing and

32   Development 126: 913–922.

33 Partridge L, Piper MDW & Mair W (2005) Dietary restriction in *Drosophila*. Mechanisms of

34   Ageing and Development 126: 938–950.

1 Piper MDW, Partridge L, Raubenheimer D & Simpson SJ (2011) Dietary restriction and
2   aging: A unifying perspective. Cell Metabolism 14: 154–160.
3 Povey S, Cotter SC, Simpson SJ, Lee KP & Wilson K (2009) Can the protein costs of
4   bacterial resistance be offset by altered feeding behaviour? Journal of Animal Ecology
5   78: 437–446.
6 R Development Core Team (2013) R: A Language and Environment for Statistical
7   Computing. R Foundation for Statistical Computing, Vienna, Austria.
8 Riddick EW (2008) Benefits and limitations of factitious prey and artificial diets on life
9   parameters of predatory beetles, bugs, and lacewings: a mini-review. BioControl 54:
10   325–339.
11 Robinson AS & Hendricks J (2005) Prospects for the future development and application of
12   the sterile insect technique. Sterile Insect Technique: Principles and Practice in Area-
13   Wide Integrated Pest Management (ed by VA Dyck, J Hendricks & AS Robinson), pp.
14   727-760. Springer, Dordrecht, The Netherlands.
15 Sait SM, Begon M & Thompson DJ (1994) The effects of a sublethal baculovirus infection in
16   the Indian meal moth, *Plodia interpunctella*. Journal of Animal Ecology 63: 541–550.
17 Silhacek DL & Miller GL (1972) Growth and development of the Indian meal moth, *Plodia*
18   *interpunctella* (Lepidoptera: Phycitidae), under laboratory mass-rearing conditions.
19   Annals of the Entomological Society of America 65: 1084–1087.
20 Simpson SJ & Abisgold JD (1985) Compensation by locusts for changes in dietary nutrients:
21   behavioural mechanisms. Physiological Entomology 10: 443–452.
22 Simpson SJ & Raubenheimer D (1995) The geometric analysis of feeding and nutrition: a
23   user's guide. Journal of Insect Physiology 41: 545–553.
24 Simpson SJ & Raubenheimer D (2009) Macronutrient balance and lifespan. Aging 1: 875–
25   880.
26 Solon-Biet SM, McMahon AC, Ballard JWO, Ruohonen K, Wu LE et al. (2014) The ratio of
27   macronutrients, not caloric intake, dictates cardiometabolic health, aging, and longevity
28   in ad libitum-fed mice. Cell Metabolism 19: 418–430.
29 Vanderzant ES (1974) Development significance, and application of artificial diets for
30   insects. Annual Review of Entomology 19: 139–160.
31 Weithoff G (2007) Dietary restriction in two rotifer species: the effect of the length of food
32   deprivation on life span and reproduction. Oecologia 153: 303–308.
33 Wijayaratne LKW & Fields PG (2012) Effects of rearing conditions, geographical origin, and
34   selection on larval diapause in the Indianmeal moth, *Plodia interpunctella*. Journal of

1   Insect Science 12: 1–19.

2   Williams G (1964) The life-history of the Indian meal-moth, *Plodia interpunctella* (Hübner)

3   (Lep. Phycitidae) in a warehouse in Britain and on different foods. Annals of Applied

4   Biology 53: 459–475.

5   Yu B, Masoro EJ, Murata I, Bertrand H & Lynd F (1982) Life span study of SPF Fischer 344

6   male rats fed ad libitum or restricted diets: longevity, growth, lean body mass and

7   disease. Journal of Gerontology 37: 130–141.

8   Zuur AF, Ieno EN, Walker NJ, Saveliev AA & Smith GM (2009) Mixed Effects Models and

9   Extensions in Ecology with R. Springer, New York, NY, USA.

10

11

12   **Figure captions**

13   **Figure 1** Development time (mean number of days spent in the larval and pupal stage ± 95%

14   confidence interval) of *Plodia interpunctella* males (Panel A) and females (Panel B) on diets

15   containing 30, 50, or 70% cellulose and a control bran diet. Means within a panel capped

16   with different letters are significantly different (Tukey test: P<0.05). Comparisons between

17   sexes indicated a significant difference only in the 30% cellulose treatment (Tukey test:

18   P<0.05).

19

20   **Figure 2** Lifespan (mean number of days as adults ± 95% confidence interval) of *Plodia*

21   *interpunctella* as influenced by (A) diets containing 30, 50, or 70% cellulose and a control

22   bran diet, and (B) sex. Means in A capped with different letters are significantly different

23   (Tukey test: P<0.05). The asterisk indicates a significant difference between sexes (ANOVA:

24   P<0.05).

25

1   **Table 1** Formulation for the three final artificial diets for the rearing of *Plodia interpunctella*,

2   based on the percentage cellulose (i.e., non-nutritive inert material). Quantities are given in

3   percentages (wt/wt) and for convenience also in g (or ml, for glycerol and linseed oil)

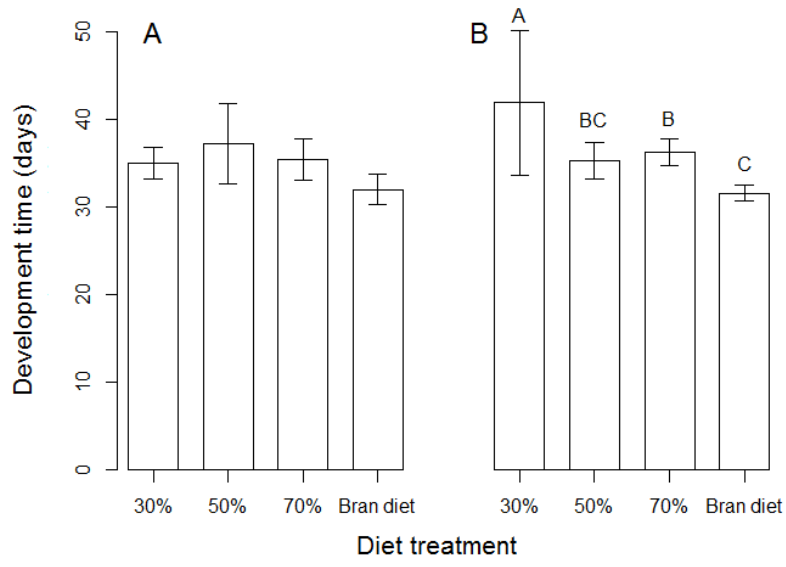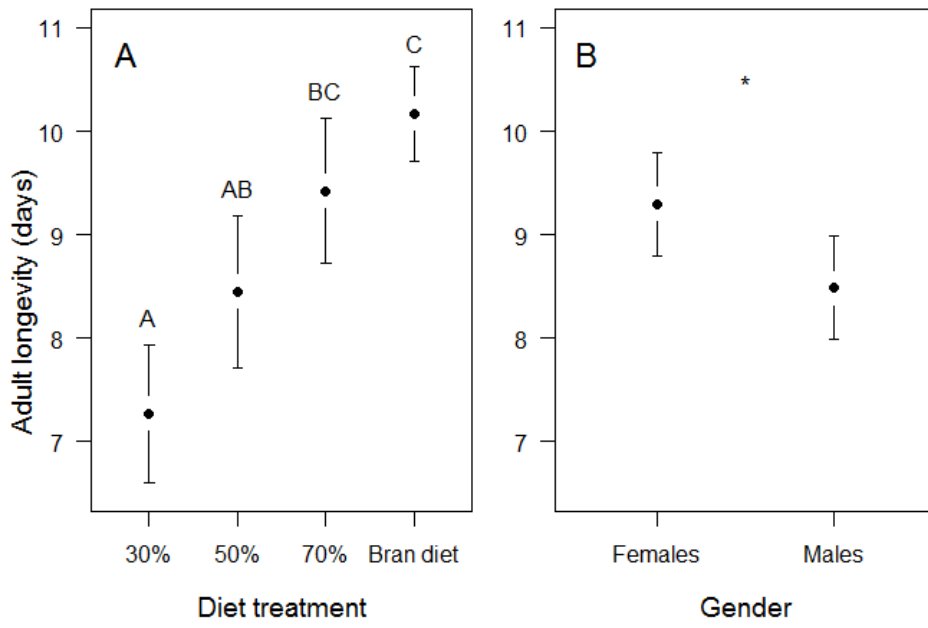| Ingredients	30% cellulose		50% cellulose		70% cellulose	
	%	g	%	g	%	g
Cellulose	30	22.5	50	37.5	70	52.5
Casein	13.11	9.83	8.67	6.50	4.22	3.17
Peptone	13.11	9.83	8.67	6.50	4.22	3.17
Albumen	13.11	9.83	8.67	6.50	4.22	3.17
Sucrose	19.67	14.75	13	9.75	6.33	4.75
Cholesterol	1	0.75	1	0.75	1	0.75
Wesson's salts	1	0.75	1	0.75	1	0.75
Glycerol (ml)	4	3	4	3	4	3
Linseed oil (ml)	4	3	4	3	4	3
Vitamin mix	1	0.75	1	0.75	1	0.75

4

5

1 **Table 2** Survival (%) of *Plodia interpunctella* larvae until eclosion for each diet treatment

2 (see Table 1 for diet description)

Larval diet treatment	Survival (%)
30% cellulose	66a
50% cellulose	67.4a
70% cellulose	80ab
Bran-based diet	87.2b

3 Means followed by different letters are significantly different (Wald tests: $P<0.05$)

1

2   Fig 1

3

1

2   Fig 2

3