ASSESSING THE PROPORTION OF NUTRIENTS REMOVED FROM THE LARVAL DIET BY QUEENSLAND FRUIT FLY (*BACTROCERA TRYONI*) AT A MASS-REARING FACILITY AND POSSIBLE USES OF SPENT MEDIA.

Bernard C. Dominiak[1] and Benjamin G. Fanson[2]

[1] New South Wales Department of Primary Industries, Biosecurity and Food Safety, Locked Bag 21, Orange, NSW 2800 Australia
[2]Centre for Integrative Ecology, Deakin University, Waurn Ponds, Victoria, Australia
Email: bernie.dominiak@dpi.nsw.gov.au

Summary

Queensland fruit fly (Qfly) is a major horticultural pest in Australia. The Sterile Insect Technique (SIT) has been used to manage Qfly incursions for over two decades. As part of the SIT programme, millions of sterile Qfly are produced at a mass-rearing facility in Camden, NSW, resulting in the disposal of tonnes of spent larval diet each year. We analysed the nutritional composition of spent larval media to identify possible uses as a livestock feed. Nutrients remaining in spent media were compared with fresh, unused media. The largest change in diet quality was in water-soluble carbohydrates, which decreased significantly from 35% to 15%. Crude protein significantly increased from 17.6% to 18.2% and crude fat increased significantly from 1.0% to 1.3% possibly due to the presence of residual Qfly larvae in spent media. There was a significant decrease in dry matter, organic matter and digestibility of organic matter. Given its nutritional composition, spent fruit fly media has the potential to be used as a livestock feed, thereby reducing the current economic and environmental costs of spent media disposal.

Key words: By-product, SIT, tephritid, livestock

INTRODUCTION

Tephritid fruit flies are a major pest species across the world, causing direct losses to many fresh fruit and some vegetable industries. Consequently, fruit flies have major adverse impacts on the economies of many countries (Stephenson *et al.*, 2003) and effective management of fruit flies is of paramount economic importance. With the increasing globalisation of trade, fruit flies have become a major quarantine concern, triggering the implementation of regional surveillance and control programs (Stanaway *et al.*, 2001, IAEA, 2003, Reid and Malumphy, 2009).

Within Australia, the two main horticultural pest species are Queensland fruit fly (Qfly) (*Bactrocera tryoni* Froggatt) and Mediterranean fruit fly (Medfly) (*Ceratitis capitata* Weidemann). Both species have been pests for more than a century and traditional control and eradication programs have relied heavily on pesticides (Dominiak and Daniels, 2012). However, there is a growing concern about the impact of pesticide residues on human health and the range of approved use patterns continued to decline (Dominiak and Ekman, 2013). The use of the sterile insect technique (SIT) is a favoured option because it is pesticide free and species specific (Dominiak *et al.*, 2011a).

Following the major Qfly outbreaks in the late 1980's in south eastern Australia, a purpose built Qfly production facility was constructed in 1996 at Camden, New South Wales, Australia (Dominiak *et al.*, 2002). The original development of standard operating procedures was reported by Jessup and Cruickshank (1999). Since then, there has been research to optimise quality parameters (Dominiak *et al.*, 2002, Weldon, 2005, Dominiak *et al.*, 2008, Campbell *et al.*, 2009, Reynolds and Orchard, 2011), irradiation (Collins *et al.*, 2008, 2009, Collins and Taylor, 2011, Bloomfield *et al.*, 2017) and some quality control tests (Dominiak *et al.*, 2011b, Dominiak *et al.*, 2014). Such improvements have increased the effectiveness of the SIT programme and hence reduced operating costs.

Larval nutrient mixtures have been reported for several fruit fly species (Niino-DuPonte *et al.* 1997, Mastrangelo *et al.* 2010). However, nutrient use or removal has rarely been studied. The nutrient use by Qfly has never been analysed or reported. Hence the understanding of nutrient demand could be improved by this study to optimise production volume and economics.

The current Qfly larval media is a mixture of white sugar, yeast, preservatives and lucerne chaff (Fanson *et al.*, 2014). About 2.5 tonnes of spent fruit fly media is produced for every 10 million pupae generated at the Camden facility. Annual production varies due to demand; however annual production exceeded 400 million pupae in at least half of the production years resulting in a considerable disposal problem. Initially the spent media was taken to land fill. Subsequently, it was used a compost on garden beds and shrubbery at the Camden facility.

One potential use of spent Qfly larval media is as an alternative livestock feed. Several studies have explored the use of spent fruit fly media from Medfly facilities for agricultural animal production and found

that the nutritional composition of spent fruit fly media could be used in ruminant and monogastric livestock (Niino-DuPonte *et al.*, 1997, Mastrangelo *et al.*, 2010) and in pacific white shrimp (Forster *et al.*, 2010). Here, we analysed the nutritional composition of both fresh and spent media as this represents the extremes of larval waste (e.g. unused extra feed and fully seeded feed) from the Camden Queensland Fruit Fly Production Facility. This analysis allowed us to determine the nutrients removed by Qfly larvae and available for livestock.

MATERIALS AND METHODS

Rearing process and diet composition

The Queensland Fruit Fly Mass-Rearing facility is located at the NSW Department of Primary Industry's (DPI) Elizabeth Macarthur Agricultural Institute in Camden, New South Wales, Australia. Detailed descriptions of factory processes and procedures can be found in Terras *et al.* (1997), Jiang *et al.* (2000), and Jiang *et al.* (2001). Production procedures change periodically but at the time of this study, the larval diet was created by mixing lucerne (20kg), torula yeast (5.6kg), white sugar (11.3kg), citric acid (588g), sodium benzoate (307g), and methyl paraben (250g) with hot water (100L). The diet was then divided into 4.5kg portions and spread across metal trays (550 x 550 x 30 mm high). Prior to adding the media, trays were placed within a plastic bag to minimise washing and for easy disposal of the spent media. Each tray of larval media was then seeded with 2-3ml of Qfly eggs (approximately 40,000 eggs).

Twenty-eight trays were placed in each rearing tower. Initially, towers were placed in the first larval rearing room at 26°C with a perspex lid on top of the tower to retain heat. Thermometers were used to monitor the temperature of the media and the perspex cover was removed if the temperature became too high. On day five after egg application, the medium trays were watered to cool the media and maintain moisture. If the medium was not watered, the media became sticky and interfered with hopping of late stage larvae. When towers became consistently too hot, the towers were moved in the second larval rearing room held at 21°C and 80% RH. Larvae began to egress from the larval diet and started to hop from day five but the main hopping period was from day seven to day 10. Larvae were collected on ripple cardboard in a tray at the bottom of the tower and allowed to pupate. After 10 days from egging, the diet and any remaining larvae were disposed of without treatment. As Qfly is endemic to the Sydney area (Dominiak and Daniels, 2012), any survivors from the spent media were deemed unlikely to significantly add to local populations.

Sampling and nutritional analysis

Sampling commenced on 27 February 2012 and the final sample was submitted on 25 May 2012. A subsample weighed over one kilogram and was a composite of ten subsamples taken from randomly selected trays in one tower. Towers were selected at random. A subsample was placed in a plastic bag supplied by the Wagga Wagga facility and the air removed. Samples were sent by overnight courier to Wagga Wagga where they were stored at 4ºC until analysed. The same procedure was used to sample towers 10 days later from the used diet. Sampling took place in ten different weeks.

Samples were transferred to the feed quality testing laboratory at the Wagga Wagga Agricultural Institute (DPI) for nutritional analysis. Samples were subjected to the following standard assays used for stock feed (AFIA, 2011): acid deter fibre (ADF), neutral deter fibre (NDF), digestible organic matter (DOM), dry matter (DM), dry matter digestibility (DMD), inorganic ash (ASH), organic matter (OM), metabolisable energy (ME), crude fat (CF), crude protein (CP), and water soluble carbohydrates (WSC) (Table 1).

Data analysis

All statistical analyses were conducted using Statistical Analysis Software (SAS) v9.2 (Cary, NC). We conducted a separate linear mixed model analysis for each component measured. The experimental design was a complete randomised block with subsampling. We included diet status (fresh vs. used) as the fixed factor, and batch id and subsample (nested in batch by diet status) as random effects. All analyses satisfied homoscedasticity and normality assumptions. Means are presented as least-squares mean ± one standard error.

RESULTS AND DISCUSSION

Overall, all nutritional components differed significantly between the fresh and used diets. There were varying levels of batch variability in the nutrient estimates, though this variability was small compared to the mean changes in nutrient composition (Table 1). After 10 days, the used diets had on average a 4.1 ± 0.7% ($p<0.001$) decrease in dry matter (DM). Of the remaining dry matter, there was a very slight decrease (0.2 ± 0.08%; $p=0.02$) of organic matter (OM), and a decrease (4.9 ± 1.0%; $p<0.001$) in the digestibility of that organic matter (DOM). Both the

percentage of acid deter fibre ($p<0.001$) and neutral deter fibre ($p<0.001$) increased (Table 1).

Table 1. The effect of Qfly larval feeding on the nutritional composition of larval medium. Values are least-square means with ± one standard error. Batch repeatability provides a measure of how variability the batches were (higher repeatability indicates more batch variability).

Nutritional Component	Fresh Diet	Used Diet	F-Value	P-Value	Batch Repeatability
Acid Deter Fibre (ADF)	17.0 ± 0.43	20.7 ± 0.43	45.42	<0.001	0.35
Crude Fat (CF)	1.0 ± 0.04	1.3 ± 0.04	31.99	<0.001	0.52
Crude Protein (CP)	17.6 ± 0.42	18.2 ± 0.42	9.95	0.008	0.92
Digestible Organic Matter (DOM)	78.4 ± 0.60	73.1 ± 0.60	39.90	<0.001	0.31
Dry Matter (DM)	26.8 ± 0.31	23.2 ± 0.31	69.36	<0.001	0.23
Dry Matter Digestibility (DMD)	79.4 ± 0.61	73.9 ± 0.61	40.95	<0.001	0.30
Inorganic Ash (ASH)	5.8 ± 0.11	6.0 ± 0.11	6.10	0.017	0.58
Metabolisable Energy (ME)	11.9 ± 0.09	11.3 ± 0.09	26.36	<0.001	0.36
Neutral Deter Fibre (NDF)	24.8 ± 0.54	32.5 ± 0.54	100.18	<0.001	0.05
Organic Matter (OM)	94.2 ± 0.11	94.0 ± 0.11	6.10	0.017	0.58
Water Soluble Carbohydrates (WSC)	34.7 ± 0.54	13.7 ± 0.54	1057.96	<0.001	0.30

The reduction in digestibility was likely due to the large drop (21.2 ± 0.7%) in water soluble carbohydrates (WSC). Simple carbohydrates are a major source of energy in many fruit fly factory diets (Chang *et al.*, 2001, Chang *et al.*, 2006, Chang and Vargas, 2007, Khan, 2013). Decreasing levels of simple carbohydrates has been shown to strongly affect larval development in flies (Gray, 2014). Though the percentage decrease in WSC was large, the overall change in metabolisable energy was minimal (decrease of 0.6% ± 0.09%; $p<0.001$).

The slight increase the percentages of crude fat (CF) and crude protein (CP) (0.3 ± 0.05% ($p<0.001$) and 0.5 ± 0.2%; ($p=0.008$) respectively) offset the decrease WSC. The increase in protein and crude fat may be due to multiple reasons. First, the large decrease in WSC, partly due to the high metabolism of larval development, would lead to decreased amount of overall diet and hence CP and CF would increase relative to WSC. Second, the used larval media still contained some Qfly larvae which had not completed development before disposal. As the spent media is untreated, these larvae may complete their life cycle after disposal if not consumed immediately by livestock. Since fruit fly larva store large quantities of lipids and sugar is essential to lipid production (Nestel *et al.*, 2003), WSC was likely converted to CF, leading to an increase in CF. Similar patterns of CP and CF increasing have been found in other fruit fly diet studies (Niino-DuPonte *et al.*, 1997, Mastrangelo *et al.*, 2010).

For rearing Qfly, larvae consume large quantities of WSC. In the current diet, this is nominally provided by white sugar. One question we pose now – is white sugar the optimum carbohydrate source for Qfly larvae? Possibly fructose should be used as this is more aligned with carbohydrate sources in fruit. More research is required to optimise the larval diet.

Regarding alternative uses of spent media, the nutritional composition of spent fruit fly media suggests that it contains a source of nutrients. The protein of the spent medium was higher (Niino-DuPonte *et al.*, 1997) or similar (Forster *et al.*, 2010, Mastrangelo *et al.*, 2010) to other studies finding that spent fruit fly medium could be used for livestock feed. We found that crude fat (1.3%) was similar to

Mastrangelo *et al.* (2010) but lower than Forster *et al.* (2010).

Miller (2004) reviewed the needs of livestock. The optimum energy density varies with species. For young, fast growing animals and high yielding animals, high-energy diets maximise production potential of animal protein. In older or less productive animals, lower energy diets achieve maximum protein deposition. Fish require higher protein diets and the fresh or spent media cannot supply the required level. The larval diets may be suitable for pregnant but not lactating pigs. For turkeys, larval diets may be suitable for finishers but not starters or growers (Miller 2004). While there is potential for the Qfly spent media to be used as livestock feed, further assessments need to be done to ensure other aspects, such as palatability or economics, of the fruit fly diet are actually suitable for animal diets.

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