Final Report

Evaluation of black soldier fly larvae (BSFL) as an enrichment for laying hens

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1. GENERAL INFORMATION

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2. EXECUTIVE SUMMARY

Laying hens are high performing birds and face a number of challenges. In a year, they may lay about ten times their bodyweight in eggs with a feed conversion ratio (FCR) of about 2 kg feed for each 1 kg eggs. Their nutrition is therefore finely balanced, but they are also subject to stresses associated with living in large flocks with perceived competition for resources. After 16 weeks, 25% of flocks have over 20% of their birds showing feather loss. By the end of lay, more than 10% of birds show feather loss in 75% of flocks. One potential means of encouraging positive behaviours (and reducing negative behaviours such as injurious feather pecking) in the bird is by the provision of enrichments such as black soldier fly larvae (BSFL). The high protein and lipid content of the larvae also contribute to the nutrition of the bird, and the large number of antimicrobial compounds that have been identified in BSFL may help to control the proliferation of pathogenic (and potentially antimicrobial resistant, AMR) bacteria in the birds' gut.

A controlled study was conducted using 192 commercial (brown egg laying) point of lay pullets. Birds were blocked by liveweight and randomly allocated to one of 24 pens (eight birds per pen). Pens (1 x 1.3 m) were furnished with a perch and two nest boxes and bedded with shavings. A commercial layers' mash was provided ad lib to all birds via a single hopper in each pen. Clean, fresh water was freely available via two nipple drinkers in each pen. Treatments were then randomly allocated to each block of pens (three pens per block). There were two experimental phases. In the first phase (bird age 18-25 weeks), the treatments comprised: control (CON, the feed hopper was shaken when the other treatments were applied), live larvae (LL, 25 g live BSFL, ca 3 g/bird, were scattered from the pen roof each morning), dead larvae (DL, 25 g larvae were blended with 100 g feed and then poured over the feed in the hopper each morning). The purpose of the DL treatment was to make the provision of the larvae indistinguishable from the feed and determine whether any behavioural changes that were observed were a consequence of the LL enrichment rather than the nutritional composition of the BSFL. In the second phase (bird age 26-33 weeks), the amount of BSFL offered

was increased to 80 g/pen (ca 10 g/bird) and offered either as a single dose sprinkled from the roof of the pen as before, or placed in a hopper over the pen roof such that individual larvae dropped in to the pen over a 3 h period. Observations were made of birds' behaviour and performance. Samples of excreta were collected at the end of phase 2, the coliforms isolated from them and the proportion of those coliforms that were resistant to ampicillin or tetracycline determined by replicate plating.

There was no evidence of vice in any of the pens, and feather cover remained good for all birds. Providing live larvae increased foraging behaviour, and this was more evident when the larvae was provided as a single dose. Birds in the CON pens were more frequently observed preening. This change in behaviour may, in older birds, result in better feather cover in birds offered live BSFL. When the amount of BSFL offered was low (Phase 1), there was no effect of BSFL on bird performance. However, in Phase 2, BSFL reduced feed consumption on an approximately 1:1 substitution rate (fresh weight basis). Egg yield was not affected by treatment, but FCR (ignoring BSFL intake) was significantly (P=0.001) improved in birds offered BSFL (CON: 2.82, BSFL: 2.59). This difference could have a significant impact on flock profitability. BSFL did not affect coliform resistance to tetracycline (ca 42%) but resistance to ampicillin was >80% in CON birds and there was a tendency (P=0.149) for BSFL to reduce this (to 63%). This could be a major advantage of BSFL, if it could be used as a means of reducing the prevalence of AMR coliforms in the environment and food chain coming from laying hen enterprises.

3. INTRODUCTION AND OBJECTIVES

In the original proposal, free range laying hens were identified as a target for BSFL, as a means of addressing challenges associated with this type of production. Feather pecking can be a major challenge, causing extensive feather loss which can seriously adversely affect the welfare and performance of laying hens. Farmers may use beak trimming to prevent feather pecking, but this practice has a number of animal welfare issues and associated economic losses (Courtney *et.al,* 2015). In March 2018, DEFRA insisted that the Laying Hen Welfare Forum, "set out an action plan, with clear milestones for eliminating beak trimming as soon as possible." Live BSF larvae, as part of an overall stress reducing strategy, have been shown to reduce feather pecking, eliminating the need for beak trimming (Veldkamp and van Niekerk*,* 2019). Current remedies, aside from beak trimming, include lighting management practices to reduce stress, although success using this strategy is variable.

Farmers currently manage health issues by treating laying hens with antibiotics, which may contribute to the emerging issue of antibiotic resistance. The Responsible Use of Medicines in Agriculture (RUMA) set targets for the reduction and/or replacement of antibiotics in animal agriculture (RUMA, 2017). The use of BSFL in the production of laying hens may help to reduce the reliance on antibiotics for laying hens as Lee et al. (2018) reported that live BSF larvae can improve disease resistance and immunity, reducing the need for the use of prophylactic antibiotics (Lee *et al.*, 2018). Related to the use of BSFL as a means of reducing antibiotic use is the challenge of gut health in laying hens. Cost efficiencies are an on-going challenge for farmers to remain viable in a competitive environment. Gut health is critical to improving feed conversion efficiency, which farmers currently achieve using costly manufactured pre and probiotics. BSF larvae contain chitin, lauric acid and a wide range of amino-acids which have been demonstrated to support gut health (Gasco *et al,* 2018).

The objectives of this study were therefore to determine the effect of supplying laying hens with black soldier fly larvae on the performance, behaviour and health of the hens. The effect of BSFL on the prevalence of antibiotic resistant coliforms in freshly voided excreta was also determined. As effects of BSFL may be a consequence of

their chemical composition (rather than their value as an environmental enrichment), the effect of the means by which BSFL were administered to laying hens on their behaviour and performance was also determined.

4. MATERIAL AND METHODS

Hens and accommodation

The experiment was conducted at CEDAR, University of Reading, and was conducted in two phases. A total of 200 point of lay pullets (commercial, brown egg laying strain) were purchased from Humphreys Pullets (Winchester, Hampshire). The birds hatched on 24/9/20, were beak trimmed and then vaccinated against Salmonella on 2/10/20, 5/11/20 and 16/12/20. They were reared as part of a larger flock, and transferred to CEDAR on 11/1/21. They were place in a single, large pen bedded on straw and wood shavings. The pen was furnished with perches and platforms, and birds were fed a commercial layer's mash (Robinwood Mill, Todmorden) in hoppers suspended from the pen roof. Water was supplied via nipple drinkers.

On 18/1/21, birds were weighed and randomly allocated to one of 24 pens. They were labelled with a coloured and numbered leg ring. Pens (1x1.3 m) were arranged in blocks of three, and within each block, pens were allocated to one of three treatments. Hens that were allocated to the same treatment were labelled with the same colour leg ring. Pens were bedded with wood shavings (that were removed and replenished if they became damp), and furnished with two nest boxes (one on top of the other, each bedded with shavings), a perch, two nipple drinkers and a feed hopper. When in these pens, all birds were fed the same diet (the layer's mash from Robinwood Mill that they had been fed since their arrival at CEDAR). Each pen was allocated its own, labelled bag of feed. Feed was added to the hopper as needed, and when the bag was empty, another bag was labelled and placed in front of the pen. The dates when a new bag was allocated to each pen was recorded on a chart. A chart was affixed to each pen for recording when bags were allocated to the pen. Birds came into full lay three weeks after they were allocated to their pens.

Treatments

Phase 1

The study consisted of two phases. In the first phase (3/2/21-31/3/21, equivalent to weeks 19-27 of bird age), the availability of supply of BSFL was limited and so the amount offered to the birds was restricted to 25 g/pen/d (equivalent to *ca* 3 g/bird/d). The three treatments (eight replicate pens per treatment) were:

1. Control (CON): No BSFL were administered to the birds. When BSFL were administered to the other treatment pens, the person administering the larvae would go into the Control pen and shake the feed hopper briefly, to simulate the activity of feeding in the Dead Larvae treatment (see below).
2. Live Larvae (LL): 25 g live BSFL were sprinkled over the (mesh) roof of the pen onto the pen floor each morning. Larvae were weighed out once a week into plastic tubs, which were stored in a room (ca 18C). The tubs containing the larvae were taken out each morning and the larvae sprinkled into the pen.
3. Dead Larvae (DL): 25 g BSFL were blended with 100 g feed. This mixture was weighed (125 g) into brown paper bags and stored on trays next to the tubs containing LL. Each morning, a bag containing the feed/larvae mixture was sprinkled over the feed in the hopper of the DL pens. The purpose of blending the larvae into the feed was to make the larvae visually indistinguishable from the feed, so that the birds could not select the larvae in the hopper. As the larvae were mixed into the feed in the hopper, the foraging cue of live BSFL scattered on the floor (as with the LL treatment) was absent.

Phase 2

In the second phase of the study (1/4/21-26/5/21, equivalent to weeks 28-35 of bird age), the supply of BSFL increased. The amount fed to the birds increased to 80 g/pen/d (ca 10 g/bird/d, which was equivalent to the amount fed by Star et al., 2020 to white egg laying hens at end of lay). Data from Phase 1 had indicated that changes in behaviour associated with offering BSFL were a result of offering live BSFL. The effect of supplying live BSFL as a single dose or as a gradual release was therefore compared. The three treatments in this phase of the study were:

1. Control (CON): The same pens as the CON pens in Phase 1. In this phase, there was no stimulus from shaking the hopper when larvae were offered in other pens.
2. Rapid release (RR): The pens that were LL in Phase 1 received the RR treatment in Phase 2. Live BSFL were scattered from the roof of the pen in a single dose, as before. The only change from Phase 1 was the increase in the amount of BSFL offered.
3. Slow release (SR): A plastic milk bottle had its base removed. The lid had a hole cut in it (ca 2 cm diameter), and this was then fastened to the bottle. The bottle was upended and fixed to the roof of the pen. Live BSFL were poured into the bottle each day (80 g/d). The larvae crawled toward the hole in the lid, and then gradually dropped into the pen. It took between 1 and 4 h for the bottle to empty.

Live BSFL were procured by EcoInsect from other industrial suppliers for the purposes of this experiment.

Performance

Eggs were collected from each pen and placed in a tray above each pen. The number and weight of eggs collected from each pen was recorded on a daily basis. As mentioned above, the amount of feed offered to each pen was recorded by counting the number of bags (each containing 20 kg feed) that were allocated to each pen. At the end of both Phase 1 and Phase 2, the amount of feed remaining in the bag and hopper was recorded. From these data, the amount of feed consumed by each pen of birds was calculated. Feed conversion ratio (FCR) was calculated as the amount of feed consumed divided by the weight of eggs produced. Birds were weighed again at the end of Phase 2, and the change in bird liveweight calculated. FCR, taking account of change in bird weight and the weight of eggs produced was also calculated on a pen basis.

Behaviour

Bird behaviour was determined by on the dot recording (scan sampling) every minute for five minutes -1, 0, 1, 2 and (later in the study) 4 h post feeding of BSFL to the birds. This was done once a week every week throughout Phase 1 and Phase 2.

The number of birds in each pen engaged in different behaviours was recorded at each of these observation times. A Qualitative Behaviour Assessment (QBA) was recorded for each pen at the end of Phase 1, using the protocol described by Welfare Quality Network (2019). Continuous recording of two blocks of pens was done for 1 h at the time of feeding and 4 h post feeding to determine the effect of time and treatment on the birds' time budgets. The number of birds engaged in different behaviours throughout this recorded time was determined, and from this the proportion of time that birds spent engaged in these different activities was calculated.

Antimicrobial resistance (AMR)

A sample of freshly voided excreta was taken from each pen in Phase 2 (on 17/5/21). Samples (1 g) were serially diluted with sterile PBS in autoclaved tubes, and then a suspension (1 µl) was spread onto a plate containing MacConkey agar to select coliforms. The plates were incubated aerobically (37C) for 16 h. Replicate plates were then made by transferring the cultures to sterile velvet that was held on a block. Plates (containing MacConkey agar, MacConkey agar plus 20 µg/ml ampicillin, MacConkey agar plus 20 µg/ml tetracycline) were then placed on this velvet. The plates were then incubated aerobically (16 h, 37C) before being counted. The proportion of coliforms that were resistant to ampcilillin, and the proportion that were resistant to tetracycline, was then determined.

Data analysis

The effect of treatment, and the effect of offering BSFL, on the egg yield, feed intake and FCR was determined by ANOVA using the General Linear Model in Minitab (Minitab 19, Minitab Inc, PA). The proportion of observations of different behaviours (determined from both the spot sampling and continual recording observations) were calculated. The effect of treatment on behaviours observed by the spot sampling technique were determined by ANOVA (GLM, Minitab) using transformed data (proportion of counts within each behaviour, and proportion of counts of all behaviours relative to time of feeding) to determine the effect of treatment and period, and the effect of treatment on behaviour relative to time of feeding, respectively. Behaviours for QBA are recorded on a linear scale and were analysed

by principal component analysis. The effect of treatment and BSFL administration on the proportion of coliforms (isolated from the birds' excreta) that were resistant to ampicillin and tetracycline (separately) was determined by ANOVA.

5. RESULTS AND DISCUSSION

There was no evidence of feather pecking or other vice, or of any stereotyped behaviours throughout the study. Birds remained in good health and with good feather coverage.

Performance

These data are summarised in Table 1. The administration of larvae did not affect egg yield. In Phase 1, although there was no significant effect on FCR (P=0.071) there was a tendency for feed to be used less efficiently if BSFL were offered. However, in Phase 2 there was a significant improvement in FCR when BSFL were fed (if only the compound feed was considered). This was similar to the observations of Star et al. (2020), who also observed that feed intake was substituted at almost 1:1 on a freshweight basis by BSFL (ie 10 g larvae replaced 10 g feed). Depending on the relative price of BSFL and feed, this might be of considerable benefit to egg producers in terms of the profitability of production.

Table 1 *The effect of treatment on the performance of egg laying hens*

	Treatment			SEM	P
Phase 1	CON	DL	LL		
Eggs produced (number per pen)	409	400	406	7.7	0.689
Egg weight (kg/pen)	22.7	22.2	22.8	0.42	0.604
Feed intake (kg/pen)	75.1	73.8	77.3	1.51	0.273
FCR	3.32	3.64	3.47	0.092	0.071
Phase 2	CON	SR	RR		
Eggs produced (number per pen)	411	400	408	6.9	0.508
Egg weight (kg/pen)	24.8	24.5	25.0	0.46	0.714
Egg yield	91.8	90.8	91.4	1.34	0.864
Feed intake (kg/pen)	69.7	63.5	64.8	1.69	0.040
Bird liveweight change (kg/pen)	4.18	4.21	4.16	0.123	0.958
FCR (eggs only)	2.82	2.59	2.59	0.049	0.005
FCR (eggs plus liveweight change)	2.41	2.21	2.22	0.039	0.002

Behaviour

The spot sampling of behaviour identified some effects of treatment (Table 2). Birds offered small amounts of live larvae (treatment LL) were more likely to be observed foraging or exploring (walking, gently pecking another hen or allo-grooming, or pecking another object such as the bag of feed outside the pen). This differentiation between birds fed larvae (whether it be in one dose or more gradually through the day) and those that were not offered larvae was not evident (from spot sampling) as the birds got older. Exploratory behaviour is a positive behaviour (provided it does not develop into injurious pecking of other birds), and so the provision of even small amounts of BSFL (3 g/bird/d), when scattered on the pen floor, may be useful in encouraging these behaviours. This stimulation of exploratory behaviours does appear to be a function of live larvae as there was no such stimulation of exploratory behaviour in birds fed the same amount of BSFL but in a form that was mixed into the feed.

Preening was observed more frequently in CON birds (in phase 1 and phase 2) compared with birds fed a more generous amount of larvae (10 g/bird/d) that were gradually released into the pen (SR). Preening is a positive behaviour, but if excessive it can lead to feather loss. The reduction in preening observed (particularly with SR birds) may be associated (in the longer term) with an improvement in feather cover, which would have a positive impact on the welfare of the bird (and in the assessment of a unit's welfare status)

Table 2. *Effect of experimental phase and treatment on the proportional frequency within each behaviour observed from on the dot sampling*

Behaviour	Experimental phase						SEM	P
	1 (19-27 weeks)			2 (28-35 weeks)				
	Treatment							
	CON	DL	LL	CON	RR	SR		
Foraging	0.28^{bc}	0.22^{c}	0.62^{a}	0.27^{bc}	0.41^{b}	0.32^{bc}	0.047	<0.001
Dust-bathing	0.46	0.19	0.48	0.40	0.29	0.31	0.112	0.394
Feeding	0.35	0.38	0.44	0.39	0.28	0.33	0.063	0.357
Drinking	0.36	0.31	0.50	0.41	0.30	0.30	0.062	0.103
Perching	0.34	0.33	0.51	0.31	0.36	0.32	0.057	0.154
Standing	0.30	0.35	0.52	0.32	0.33	0.35	0.057	0.099
Walking	0.28^{b}	0.34^{ab}	0.55^{a}	0.28^{b}	0.36^{ab}	0.37^{ab}	0.053	0.009
Preening	0.50^{a}	0.21^{ab}	0.47^{ab}	0.47^{a}	0.30^{ab}	0.23^{b}	0.068	0.003
Allo grooming	0.13^{b}	0.10^{b}	0.63^{a}	0.25^{ab}	0.39^{ab}	0.36^{ab}	0.111	0.020
Pecking object	0.28^{b}	0.25^{b}	0.63^{a}	0.27^{b}	0.35^{ab}	0.38^{ab}	0.074	0.010

The overall welfare of the birds was good, and the qualitative behaviour assessment did not suggest there was any effect of treatment on this measure of bird welfare (Figure 1).

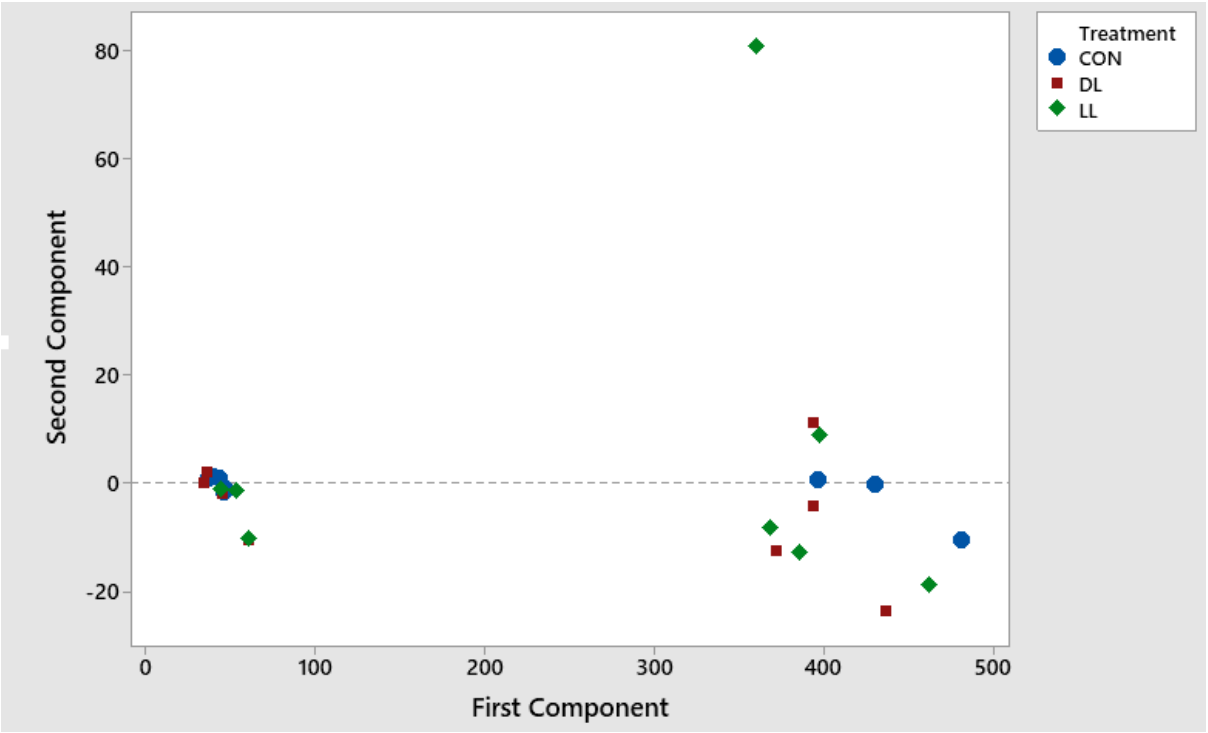

Figure 1 *Principal component analysis of the scores for attitudes assessed by qualitative behavioural assessment.*

The time budgets of the birds (recorded in phase 2) are summarised in Table 3. There was no significant (P>>0.05) interaction between the observation time (relative to feeding) and treatment. The only significant effect that observation time had on behaviour was in the proportion of observations where birds were standing or walking. 0.28 of observations at the time of feeding were accounted for with this behaviour, but this decreased to 0.14 2 h post feeding (P=0.012, SEM 0.028).

As observed by the spot sampling, there was a tendency (P=0.086) for birds offered larvae to spend more time foraging (nearly 45% of their time). CON birds spent significantly more time eating or drinking (P=0.033), which was reflected in their increased feed intake. They also spent more time standing or walking (P=0.030) compared with birds offered larvae; birds that were offered larvae were more likely to be foraging rather than standing, walking or feeding. There was no evidence of any difference between the birds that were fed larvae as a single dose and those that had larvae dropping into their pen at intervals. Foraging is a natural and positive behaviour for hens, and so the tendency for this behaviour to be increased (at the expense of standing or walking with no clear purpose) is a benefit.

Table 3 *The effect of treatment on the proportion of time spent engaged in different activities*

Activity	Treatment (T)			SEM	P		
	CON	RR	SR		T	Time, t	Txt
Foraging	0.197	0.432	0.481	0.0778	0.086	0.370	0.610
Perching	0.166	0.193	0.174	0.0374	0.878	0.534	0.626
Feeding or drinking	0.330	0.171	0.169	0.0369	0.033	0.343	0.998
Standing or walking	0.311	0.166	0.154	0.0340	0.030	0.012	0.172
Dust bathing	0.002	0.039	0.012	0.0223	0.532	0.220	0.532

Antimicrobial resistance (AMR)

In the EU, the median resistance to ampicillin and tetracycline in isolates of *E.* coli taken from poultry was reported to be ‘high’ or ‘very ‘high’ (EFSA, 2021). In this study, the percentage of *E. coli* isolates that demonstrated phenotypic resistance to

ampicillin and tetracycline was 68 and 44% respectively. This was in the absence of any exposure to either antibiotic (certainly during the whole of the experimental period). Once established, it is very difficult to overcome AMR as the plasmids that carry the genes coding for resistance are very persistent, conferring either some other selective advantage on the coliform or encoding a gene that will kill the coliform if it expels the plasmid. The encouraging observation from this study was that, while not statistically significant, there was a tendency (P=0.149) for birds offered BSFL to have coliforms with a lower prevalence of ampicillin resistance (Table 4). If confirmed, the mechanism for this effect is unclear, but worthy of further investigation.

Table 4. *Effect of supplying BSFL on the prevalence of antimicrobial resistance in coliforms isolated from freshly voided excreta (values are least square mean (SEM))*

	Provision of larvae		P
	No	Yes	
E. coli population (log_{10} CFU/g excreta)	5.83 (0.347)	6.31 (0.174)	0.230
% coliforms resistant to:			
Ampicillin	81.5 (10.8)	62.5 (6.44)	0.149
Tetracycline	40.1 (11.7)	44.9 (7.00)	0.730

6. CONCLUSIONS

The provision of small amounts (3 g/bird/d) of BSFL in the form of live larvae encouraged greater foraging activity in laying hens. Hens that were not offered BSFL spent more time preening, which may ultimately have an impact on their feather cover. Providing a larger amount of BSFL (10 g/bird/d) reduced feed intake without affecting egg production, such that feed conversion ratio was improved in birds offered BSFL. There was a tendency for the prevalence of ampicillin resistant coliforms in the excreta of laying hens offered BSFL to be reduced. All these beneficial effects mean that the provision of BSFL to laying hens could have a positive impact on their performance, welfare and environmental impact.

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