The Use of Dried Bovine Hemoglobin and Plasma for Mass Rearing New World Screwworm

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Abstract

The success of the Screwworm Eradication Program is due to continuous mass rearing and dispersal of large numbers of competitive sterile flies in the field. Spray-dried powders of whole bovine blood, chicken egg, and milk substitute constituted the nutritional components of the traditional artificial larval diet used for mass rearing New World Screwworm (NWS), Cochliomyia hominivorax (Coquerel), Diptera: Calliphoridae. However, due to shifting availability and increasing costs of diet ingredients, it is necessary to investigate alternative products for the diet. Recently, spray-dried whole bovine blood became unavailable for purchase in the quantities that the Screwworm Program requires and thus were obliged to purchase bovine blood subproducts. Previous research showed that bovine hemoglobin could be substituted for whole blood with good results in small trials. Here, we report results of NWS larval diets prepared with bovine blood subproducts, hemoglobin and plasma, in 20-liter trays used in mass rearing. Diets were prepared using three separate hemoglobin/plasma ratios. Though all three configurations of hemoglobin and plasma were successful in the larval diet, we found the diets containing 1.5% total plasma, as opposed to 0.5 and 1%, produced heavier larvae and pupae, and resulted in more pupae per unit of diet. Considering cost, we determined that the ideal ratio for the blood portion of the diet for mass rearing is 80% hemoglobin and 20% plasma.

Key words: Cochliomyia hominivorax, screwworm, diet, insect rearing

New World screwworm (NWS), Cochliomyia hominivorax (Coquerel) is a Dipteran parasitic insect pest of warm-blooded vertebrates found throughout most of South America and among five Caribbean countries (Cuba, Dominican Republic, Haiti, Jamaica, and Trinidad and Tobago). Myiasis takes place within the larval life stages of the fly, where eggs laid along open wounds or naval areas of newly born animals. Immediately following eclosion, stage 1 larvae begin eating at the living tissue of the host, forming deep feeding pockets and causing injury, disease, and even death. In turn, this can equate to an economic loss in leather, meat, and milk production, as well as an extreme health risk for populations in endemic areas (Vargas-Teran et al. 2005).

With an original distribution occupying Mexico as well within the southern United States, successful eradication and control of NWS has been achieved over the past 60 years in North and Central America using the sterile insect technique (SIT) originally developed by Knipling and Bushland (Bushland and Hopkins 1953, Bushland et al. 1955, Knipling 1955, Wyss 2000). The United States Department of Agriculture (USDA) Screwworm Program, currently managed by the Panama–United States Commission for the Eradication and Prevention of Screwworm (COPEG), maintains a sterile NWS production facility in Panama. Sterile flies from this facility are dispersed weekly in eastern Panama and into Colombia to prevent re-infestation from South American into NWS-free countries. Although the program is costly, the cost benefit ratio to U.S. producers is estimated to be 1:100, and 1:450 for the national economy (Vargas-Teran et al. 2005). In the case of an outbreak in Texas, for example, the cost to Texan livestock producers alone would climb higher than $560 million, and contribute to a total Texan economic loss of more than $1.4 billion (NPIC 2016).

One of the most critical and costly components of the Screwworm Program is the larval diet. The screwworm larval diet has changed considerably since its development by Melvin and Bushland (1936, 1940) as a mixture of ground beef and citrated bovine blood due to the availability, expense and quantity of ingredients required to maintain a mass rearing colony (Chen 2014), and the development of food processing technology. Over time, the source of nutritional elements of the larval screwworm diet has evolved from use of raw foods such as hamburger and poultry eggs to the use of dry powdered ingredients, with different inert additives used for diet structure...
Prior to the obligated use of spray-dried bovine blood by-products, the latest version of the NWS diet consisted of spray-dried whole bovine blood (SDWB) (APC Inc., Ankeny, IA), spray-dried whole or reduced fat egg and dry powdered milk substitute (Calva Products Inc., Acampo, CA), and cellulose fiber (CF-100, Central Fiber, J. Rettenmaier USA LP, Schoolcraft, MI) for diet structure. The dry ingredients are rehydrated with water, adding formaldehyde for microbial control. Though this diet recipe had functioned well for the NWS program since 2007 (Chen et al. 2014), the active ingredients in the larval diet are constantly subject to change due to availability and changes in food processing technologies.

Recently, market shifts have negatively affected the availability of SDWB for the Screwworm Program. Large producers have adapted to market demand and currently supply hemoglobin and blood plasma as separate commodities, thus necessitating the study of these products for the NWS larval diet.

Here, we present results of studies conducted using multiple bovine blood fractions (hemoglobin and plasma) in the NWS larval insect diet to confirm their use as practical and economical alternative ingredients.

Materials and Methods

Target Values for Larvae and Pupae

The Screwworm Program maintains strict size limits for larvae and pupae. The standard larval diet was designed to meet nutritional needs of the insects to achieve these limits. Since we did not have an adequate supply of whole bovine blood to use as a control, we compared larval and pupal measures to our standard measures listed in Table 1.

Origin of Insects, Bulking Agent, and Dietary Ingredients

This study was conducted using insects from the mass rearing C. hominivorax colony, strain Jamaica 2006 (J-06), at the COPEG screwworm rearing facility in Pacora, Panama. Larval diet ingredients were obtained from the aforementioned companies, with APC providing both the hemoglobin and plasma blood products. The cellulose fiber (CF-100) used as bulking agent was provided by Central Fiber LLC, Wellsville, KS, using the recycled paper product Over Issue News (#9 OIN) as classified by the Institute of Scrap Recycling Industries, Inc. (ISRI, Washington, DC).

Larval Diet Formulation

For all diets, nutritional ingredients comprised 14% of the total diet mixture with a ratio of 5% spray-dried bovine blood products, 5% spray-dried egg, and 4% powdered milk substitute. Cellulose fiber for bulking was added at 5.25% with water at 80.4%. Finally, for microbial control, a 40% formaldehyde solution was added for a final concentration of 0.1% of the total diet. The bovine blood component of the larval diet varied in the three diet variations as follows: 7:3, 8:2, and 9:1, hemoglobin to plasma, respectively. The current diet utilized in the mass rearing of screwworm follows the 8:2 model, and is further referenced as our control.

Rearing Methods

Larvae were reared with some modifications to procedures outlined in Chaudhury and Skoda (2007) and Chaudhury et al. (2015). In total, three replicates were conducted for each diet variation across three consecutive trials. Each larval rearing tray received a total of 20 liters of freshly prepared diet in three feedings of 5, 8, and 7 liters on days 0, 2, and 3, respectively. On the day of incubation (day 0), C. hominivorax eggs were collected from egg masses produced by 6-d-old flies. The incubation diet received 1.45 g of eggs placed on 4 x 4 cm moist paper towels. After 12 h, the paper towel was removed with the egg shells and unhatched eggs to record hatch rate. Temperature and humidity for developing larvae and pupae followed a schedule optimized for each stage of growth as shown in Table 2.

Larvae were allowed to crawl out of their feeding trays and fall into troughs for accumulation. Larvae would then be collected en masse every 4 h forming collection groups in which they are weighed, counted, and placed in sawdust to pupate. Pupae were separated from the sawdust after 24 h, weighed, and placed on perforated trays to continue maturation.

Life History Parameters

Various measurements were made as listed in Table 1. After the initial growth period of 72 h, larvae were placed in a ‘crawl-off’ room where larvae were collected every 4 h after crawling out of the diet.
and into collection troughs. Average larval and pupal weights, as well as the weight of total larvae and pupae produced per treatment, were measured as indicators of insect quality on each diet.

**Statistical Analysis**
A controlled randomized block design was used for rearing larvae in each of the three treatments. Diets were compared using a one-way analysis of variance (ANOVA) with Tukey’s honestly significant difference (HSD) test to compare interactions within the means. This analysis was replicated for larval and pupal count and weight. To achieve this, hemoglobin/plasma ratios were introduced as the continuous response while being blocked by the experiment’s repetitions. All statistical analyses were completed by R statistical software 3.4.3 (R Core Team 2013) (R Foundation for Statistical Computing, Vienna, Austria).

**Results**

**Behavioral Observations**
Feeding across all diets and trials was highly active, forming feeding pockets, or nuclei, of various sizes throughout the media. As well, visual inspection showed reasonably uniform larval development within each diet tray. Upon introduction of new diet layered on top of the old media, larvae moved to the fresh diet and again formed feeding pockets.

**Life History Parameters**
All replicates of all trials within the study showed ≥98% hatch rate. Results of life history measures are shown in Table 3. For all measures, the diet with the greatest fraction of hemoglobin, 9:1, produced the lowest larval and pupal weights (mg, Figs. 1 and 2, respectively). Comparison of weighted means for larval and pupal weights showed no significant differences ($F = 1.419; \text{df} = 2, 6; \text{P} = 0.342,$ and $F = 0.449; \text{df} = 2, 6; \text{P} = 0.667,$ respectively) among hemoglobin and plasma ratios. Weighted means were obtained by directly correlating the average weight of the larvae and pupae collected with the amount recorded during the measurement. This process weakens outliers with low rates of collected larvae, and strengthens those weight averages correlated with a higher collection count, thus developing a more accurate mean weight for statistical analysis. All pupae produced exceeded the expected minimum weight of ≥50 mg.

However, when comparing the mean yields of larvae and pupae, differences between the three diets were more distinct. In mean larval collected throughout all trials (Fig. 3), the control and 7:3 diets performed exceedingly well, causing a significant difference in larval yields ($F = 9.385; \text{df} = 2, 6; \text{P} = 0.031$). This difference, however, did not translate into overall pupation success, as diet showed no impact on pupal yield throughout the trials ($F = 1.669; \text{df} = 2, 6; \text{P} = 0.265$). Furthermore, all larvae collected followed similarly curved accumulation trends, suggesting similar larval fractions collected per collection group (Fig. 4).

Significant statistical differences were noted within all measured factors aside from pupal weight when compared to repetition as the random block. Larval weight, larval count, and overall pupal yield showed significant differences within the 95% CI ($F = 8.085; \text{df} = 2, 6; \text{P} = 0.039,$ and $F = 14.025; \text{df} = 2, 6; \text{P} = 0.016,$ and $F = 9.03; \text{df} = 2, 6; \text{P} = 0.033,$ respectively). A Tukey’s HSD analysis used to compare the means found statistical differences only present when compared to the second repetition.

**Cost Analysis**
A cost analysis of diet was performed for producing pupae (Table 4). When accounting for the blood component alone, the 8:2 and 9:1 ratios have a lower production cost. However, due to the differences in the number of pupae produced, the total annual material cost would be lower in the 7:3 diet when maintaining a 20 million fly per week production quota.

![Average larval weight of stage 3 larvae collected in 4-h intervals after crawling off the diet and into collection troughs.](image)

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**Table 3.** Life history measurements of larvae and pupae by diet media with SEs

| Hemoglobin/plasma ratio | Mean larval wt (mg) | Mean no. larvae collected per replicate | Mean pupal wt (mg) | No. pupae per 21-liter tray |
|-------------------------|---------------------|----------------------------------------|-------------------|---------------------------|
| 9:1                     | 74.1 (1.9)          | 71,598 (1,577)                         | 65.1 (1.5)        | 23,375 (526)              |
| Control (8:2)           | 74.9 (1.9)          | 75,939 (1,612)                         | 66.1 (1.8)        | 25,386 (546)              |
| 7:3                     | 74.6 (1.7)          | 81,001 (1,640)                         | 66.2 (1.5)        | 26,798 (550)              |
Fig. 2. Average pupal weight by collection group. Pupae are weighed in accordance with their crawl-off groupings to ensure specimens are of a similar development stage at time of weighing.

Fig. 3. Average number of larvae collected every 4 h across all trials.

Fig. 4. Mean accumulated larvae collected over separate 4-h collection groups.
Table 4. Cost (USD) of blood component and total nutrient ingredients in NWS larval diet using three hemoglobin:plasma ratios where hemoglobin costs $1.55/kg and plasma $6.00/kg

| Larval diet ratios | 9:1 | 8:2 (control) | 7:3 |
|--------------------|-----|---------------|-----|
| Blood component cost per kg | $2.00 | $2.44 | $2.89 |
| Diet cost per tray (21-liter) | $9.08 | $9.54 | $10.01 |
| Pupae produced per tray | 23,375 | 25,386 | 26,798 |
| Cost per 1 M pupae | $388.24 | $375.89 | $373.52 |
| Annual diet costs at 20 M pupae per week | $404,858.46 | $391,980.41 | $389,509.03 |

Table 5. Multistep process utilized to incorporate rearing changes within the COPEG facility

| Scale | Test phase | Description |
|-------|------------|-------------|
| Small | Initial: 3-liter trays | Determine larval acceptance and effects on larval development. |
| Small | 20-liter | Compare |
| Medium | Half rack | 12 trays used for treatments and control |
| Medium | Full rack | 22 trays used for treatments and control |
| Medium | Three-generation full rack | Full rack, production maintained for three generations to observe stability of life history parameters |
| Large | Half production | Feed half of mass production with ‘new’ diet and compare insect measures to current diet fed to remaining half of production |
| Large | Full production | Changes finally approved at the highest level, and treatments are incorporated into the facility’s SOP. |

Discussion

This study highlights the feasibility for use of combined blood components to replace whole blood in NWS artificial diet media. Further, we include cost estimates for different ratios of plasma and hemoglobin for artificial rearing of C. hominivorax.

Although Duarte et al. (1999) showed liquid bovine blood to be 65% plasma and 35% blood cells giving a hemoglobin:plasma ratio of about 4:6; more plasma than hemoglobin, all tested ratios possessed less plasma for cost efficiency. We found that even with a 9:1 ratio viable screwworm was produced.

Despite the relatively small changes in larval diet, the pupae quality and quantity standard stayed well above the facility’s standard operating procedure (SOP). These results are replicated across all treatments, suggesting flexibility in permissible hemoglobin and plasma ratios based on financial or logistical situations. In the presence of a small budgetary fiscal year, for example, utilizing a diet consisting of cheaper hemoglobin would allow the facility to cut costs temporarily while still producing a quality fly. This is possible, primarily in the fact that though the 9:1 diet performed more poorly when compared to the other treatments, larvae production and quality still exceeded COPEG standards for acceptability. On the other hand, if the need arises, a higher quality insect or a greater yield can be achieved by sacrificing cost efficiency for the beneficial plasma.

Further research should investigate NWS diet interactions upon removal of known key ingredients (hemoglobin, plasma, defatted egg, milk substitute). As well, given the visual upward performance trend in correlation with higher plasma levels, further experimentation should be conducted to determine where this upward trend ends, and if cost efficiency continues to increase throughout the ratio changes.

Currently, facility standards for changing methods or diet are subject to a multistep process involving rearing NWS on small, medium, and large scales before reaching full production (Table 5). This process was designed to slowly scale a tentative dietary change from a small 3-liter scale, to a full rack over three generations to test strain stability involving said differences, and finally completing its process within a full production run.

The significant statistical differences between trials in our research reflect the working conditions within the larger rooms of the mass rearing facility in which they were reared. An increase in repetitions throughout multiple locations within the rearing rooms, as well as greater environmental controls or a smaller rearing area specifically utilized for small-scale research would aid in eliminating such variation in the future. On the other hand, the benefit of utilizing large-scale production areas acts as a validation test when used in conjunction with quantifiable research, as this more accurately replicates the conditions the larvae will experience in the mass rearing process.

The aim of this study was to test, first and foremost, whether these new diet ingredients would be accepted by the larvae. As our results showed, these diet combinations produced acceptable size larvae and pupae. Further experiments are now underway to determine other important life parameters necessary for the success of an SIT program to include, adult emergence, sex ratio, fly longevity, flight ability, fecundity, and fertility of produced eggs, all through several generations. However, good quality pupae are a strong precursor toward the aforementioned properties being generally acceptable and the resulting emergence valuable for SIT, barring the rare cases involving mishandling during the irradiation or field release process.

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Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

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