Solidifying Agent and Processing of Blood Used for the Larval Diet Affect Screwworm (Diptera: Calliphoridae) Life-History Parameters

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ABSTRACT

Spray-dried whole bovine blood and a sodium polyacrylate polymer gel as a bulking and solidifying agent are among the constituents of the current larval diet for mass rearing screwworm, Cochliomyia hominivorax (Coquerel) (Diptera: Calliphoridae). Locally available, inexpensive dietary materials could reduce rearing cost and address an uncertain commercial supply of spray-dried blood. We compared efficacy of diet prepared from fresh bovine blood after decoagulation with sodium citrate or ethylenediaminetetraacetic acid (EDTA) or after mechanical debrinbrination, with the diet containing spray-dried blood using either gel or cellulose fiber as the bulking and solidifying agent. Several life-history parameters were compared among insects reared on each of the blood and bulking agent diets combination. Diets containing citrated blood yielded the lightest larval and pupal weights and fewest pupae. EDTA-treated blood with the gel also caused reductions. EDTA-treated blood with fiber yielded screwworms that were heavier and more numerous than those from the diet with citrated blood but lighter than those from the control diet using spray-dried blood. A reduction in percentage of adults emerging from pupae occurred from diets with both bulking agents using citrated blood and the diet using EDTA mixed with the gel bulking agent. As a group, the cellulose-fiber diets performed better than the gel diets. Larval diet did not affect adult longevity, weight of the eggs deposited by the females that emerged or subsequent egg hatch. Parameter measurements of insects from both debrinated blood diets were similar to those from the spray-dried blood diets, indicating that fresh, debrinated bovine blood can successfully replace the dry blood in the screwworm rearing medium.

KEY WORDS Cochliomyia hominivorax, insect diet, debrinbrination, anticoagulants

The screwworm, Cochliomyia hominivorax (Coquerel) (Diptera: Calliphoridae) mass rearing facility of the Panama–U.S. Commission for Eradication of Screwworm, is located in Pacora, ~40.234 m (25 miles) east of Panama City, Panama. This facility currently produces ~50 million flies per week, most of which are sterilized and released in the Darien Province of Panama to maintain a biological barrier between Panama and Colombia that prevents reinfestation to Central America, Mexico, and the United States from screwworm-infested areas of South America. The artificial diet currently used for mass rearing screwworm larvae is a semisolid medium consisting of spray-dried whole bovine blood, spray-dried poultry egg product, and a milk substitute, mixed with a bulking and solidifying agent such as sodium polyacrylate polymer gel or cellulose fiber, and water (Chaudhury and Alvarez 1999, Chaudhury and Skoda 2007).

All of the dietary ingredients are imported from the United States and are expensive. To reduce the mass-rearing cost, we continue to investigate the suitability of less expensive locally available materials which can replace costly imported ingredients yet maintain the quality of flies produced, sterilized, and released. Another consideration is the potential unavailability of spray dried whole bovine blood because manufacturing companies are fractionating blood into plasma and cells for higher profit. One of the ingredients considered was the fresh bovine blood obtained from the local abattoirs. The fresh bovine blood requires decoagulation by using chemical or mechanical means to homogeneously mix the blood with other ingredients of the diet to obtain appropriate consistency, texture, and viscosity (Chaudhury and Skoda 2009). Normally, anticoagulants such as ethylenediaminetetraacetic acid (EDTA) and sodium citrate are used to decoagulate blood (Graham 1978, Wisemer-Pedersen 1979, Duarte et al. 1999, Lam et al. 2004). This article reports the effects on development, survival, and female reproduction of screwworms reared on the current standard larval diet by using either polymer gel or cellulose fiber as bulking and solidifying agent, compared with the diet prepared from fresh bovine blood instead of the spray-dried product. In addition, the fresh-blood diets were pre-
pared using sodium citrate, EDTA, or mechanical means of defibrination to determine the effect of de-coagulation on the same set of life-history parameters.

Materials and Methods

Dietary Ingredients and Insects. Fresh bovine blood was collected from a local abattoir free of cost. The blood was collected from the cut neck of the animal in a bucket and then immediately poured into a 1-liter containers for defibrination or into other 500-ml containers with anti coagulant EDTA (Sigma-Aldrich, St. Louis MO; 2.5 ml of 10% solution per 100 ml of blood) or sodium citrate (Sigma-Aldrich; 0.4 g per 100 ml of blood). The blood aliquots collected for defibrination were mechanically stirred using a slow speed overhead stirrer (Daigger, Vernon Hills, IL) before using for the diet. The cellulose fiber (CF-100) was obtained from the Central Fiber Corporation (Wellsville, KS). Sodium polyacrylate gel Aquatain was purchased from the Pioneer Medical Inc. (Lakeland, FL). Spray-dried whole bovine blood and spray-dried poultry eggs were purchased from the California Spray Dry Co. (Stockton, CA), and the dry milk substitute was from the Calva Products Inc. (Acampo, CA). Tests were conducted on the Panama-95 strain of screwworm developed from screwworm flies collected from Panama in 1995.

Preparation of Diets. Diets were prepared by mixing 160 ml of fresh blood subjected to one of the three coagulation methods, 40 g of spray-dried poultry eggs, 40 g of milk substitute, and 60 g of cellulose fiber or 22 g of gelling agent, with water in a slow-speed laboratory blender to make 1 liter of diet. The resulting six test diets were as follows: EDTA-cellulose fiber (EDTA-F), EDTA-gel (EDTA-G), sodium citrate-cellulose fiber (Na Cit.-F), sodium citrate-gel (Na Cit.-G), defibrinated-cellulose fiber (De-fib.-F), and defibrinated-gel (De-fib.-G). The standard rearing medium served as the control diet, which was prepared by mixing spray-dried bovine blood (60 g), spray-dried poultry eggs (40 g), and milk substitute (40 g) with 50 g of cellulose fiber (Control-F) or 18 g of gelling agent (Control-G), followed by sufficient water to produce 1 liter of diet (Chaudhury and Alvarez 1999, Chaudhury and Skoda 2007). Formalin was added to each of the diet formulations at the rate of 1 ml per liter diet to prevent fungal growth. Initial pH of all the test formulations was adjusted to that of the control diet according to the procedures described by Chaudhury and Skoda 2009.

Rearing Methods. Larvae were reared according to the procedures described previously (Chaudhury and Alvarez 1999, Chaudhury and Skoda 2007) with some modifications. Each larval rearing tray received a total of 1.5 liters of diet in three feedings of 0.3, 0.6 and 0.6 liter on day 0, 2 and 3, respectively. Each tray received 100 mg of eggs (≈2,000 eggs) on day 0, placed on a 2-by-2-cm moist paper towel on top of the diet for 24 h to allow the newly-hatched larvae to move into the diets. Egg shells on the paper towel were collected then examined under a dissecting microscope to ensure that normally high hatch rates generally exceeding 90% had occurred. Mature larvae crawled from the diet tray and dropped in sawdust within a larger collection tray placed under each larval rearing tray.

Recording of Life-History Parameters. The weight of a sample of 100 mature larvae from each collection tray was determined and used to calculate an average weight per larva. The sampled larvae were then returned to their respective trays, where all the larvae were allowed to pupate in the sawdust. Five days after pupation, a sample consisting of all pupae within each tray and a subsample of 100 were weighed to estimate the number of pupae obtained per tray. Percent biological yield was calculated by dividing the number of pupae obtained by the expected number (2,000) and multiplying by 100. Percentage of adult emergence per tray was calculated after determining the number of adults that emerged from a randomly selected sample of 100 pupae. To determine adult longevity, newly emerged adult flies were held in cages with honey and water, and their mortality was recorded daily until the death of 50% of the flies. To determine fecundity, 200 pupae from each tray were caged separately for emergence of adults; adults were fed spray-dried egg powder and honey diet (Chaudhury et al. 2000) until they were gravid (7 d old). Females in each cage were then allowed to oviposit for 1 h in a petri dish containing 5 g of ground beef and a few drops of liquid spent larval diet as oviposition stimulant (M.F.C., unpublished data). Eggs from the petri dish were then collected and weighed. To determine fertility, 50 g of eggs from each cage were incubated at 35°C for 20 h in a separate petri dish lined with moist filter paper and each containing 2–3 g of ground beef. Unhatched eggs and empty eggshells from each petri dish were counted for calculation of percentage of egg hatch. Treatments consisting of six experimental diets and two controls as described above were replicated in three consecutive generations with three trays set up for each treatment of each replicate.

Statistics. Data recorded for life-history parameters were analyzed using multivariate analysis of variance of the GLM procedure (SAS Institute 2005). Mean separations for the eight treatments were done using Tukey’s honestly significant difference (HSD) test (SAS Institute 2005). Contrast statements were used to test differences between bulking agents, anticoagulants compared with controls, defibrinated blood compared with controls, sodium citrate compared with EDTA as anticoagulants, and citrate and EDTA with fiber compared with citrate and EDTA with gel bulking agents.

Results

Significant correlation existed between larval weight with pupal weight (0.798) as well as number of pupae with biological yield (0.998): larval weight and biological yield were excluded from multivariate analysis (Table 1). Test statistics from multivariate analysis in SAS GLM were consistent with concurrently reported univariate analysis, allowing the interpreta-
Table 1. Biological parameters (mean ± SD) of screwworms reared on diets using fresh bovine blood treated with the anticoagulants sodium citrate (Na Cit.) and EDTA, mechanically defibrinated (De-fib.) fresh bovine blood or standard spray-dried whole blood (control) and using either cellulose fiber (F) or gel (G) bulking agents.

<table>
<thead>
<tr>
<th>Diet</th>
<th>Larval wt. (mg)</th>
<th>Pupal wt. (mg)</th>
<th>No. Pupae per tray</th>
<th>Biological yield (%)</th>
<th>Adult emergence (%)</th>
<th>Eggs laid (mg)</th>
<th>Egg hatch (%)</th>
<th>Adult longevity (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na Cit.-G</td>
<td>47.6 ± 2.09</td>
<td>32.6 ± 1.57d</td>
<td>984.1 ± 81.8d</td>
<td>49.3 ± 4.06</td>
<td>89.0 ± 2.35d</td>
<td>590.5 ± 116d</td>
<td>88.3 ± 1.5d</td>
<td>15.5 ± 2.1</td>
</tr>
<tr>
<td>Na Cit.-F</td>
<td>50.5 ± 3.04</td>
<td>33.6 ± 2.7d</td>
<td>1106.1 ± 137.5c</td>
<td>55.6 ± 7.04</td>
<td>90.7 ± 3.67cd</td>
<td>604.0 ± 122d</td>
<td>90.3 ± 3.4</td>
<td>16.9 ± 1</td>
</tr>
<tr>
<td>EDTA-F</td>
<td>54.8 ± 1.43d</td>
<td>36.9 ± 1.77c</td>
<td>1485 ± 57.3c</td>
<td>74.2 ± 2.91</td>
<td>950.0 ± 1.58ab</td>
<td>663.2 ± 85d</td>
<td>91.3 ± 2.8</td>
<td>15.0 ± 1.7</td>
</tr>
<tr>
<td>EDTA-G</td>
<td>61.0 ± 2.5</td>
<td>45.3 ± 3.26b</td>
<td>1690.1 ± 91.1a</td>
<td>80.4 ± 4.48</td>
<td>936.4 ± 3.64bc</td>
<td>619.1 ± 139d</td>
<td>90.9 ± 2.3</td>
<td>16.5 ± 1.6</td>
</tr>
<tr>
<td>De-fib.-G</td>
<td>62.2 ± 1.65</td>
<td>46.4 ± 1.43a</td>
<td>1696.7 ± 33.1a</td>
<td>84.8 ± 1.56</td>
<td>97.1 ± 2.42ab</td>
<td>704.0 ± 86d</td>
<td>90.9 ± 3.2</td>
<td>15.9 ± 1.5</td>
</tr>
<tr>
<td>De-fib.-F</td>
<td>64.0 ± 1.78</td>
<td>47.7 ± 1.25ab</td>
<td>1721.4 ± 58.9a</td>
<td>86.1 ± 2.59</td>
<td>98.1 ± 1.83a</td>
<td>714.9 ± 69d</td>
<td>90.6 ± 3.3</td>
<td>17.0 ± 1</td>
</tr>
<tr>
<td>Control-G</td>
<td>61.8 ± 2.26</td>
<td>45.6 ± 1.21ab</td>
<td>1618.6 ± 91.7a</td>
<td>81.0 ± 4.53</td>
<td>95.3 ± 2.5ab</td>
<td>707.2 ± 69d</td>
<td>89.7 ± 2.1</td>
<td>15.9 ± 1.8</td>
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<tr>
<td>Control-F</td>
<td>65.4 ± 2.4</td>
<td>45.5 ± 2.68a</td>
<td>1690.2 ± 55.9a</td>
<td>84.4 ± 2.74</td>
<td>98.1 ± 1.83a</td>
<td>716.0 ± 64d</td>
<td>90.8 ± 2.2</td>
<td>16.6 ± 1.3</td>
</tr>
</tbody>
</table>

F values

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Larval wt. (mg)</th>
<th>Pupal wt. (mg)</th>
<th>No. Pupae per tray</th>
<th>Biological yield (%)</th>
<th>Adult emergence (%)</th>
<th>Eggs laid (mg)</th>
<th>Egg hatch (%)</th>
<th>Adult longevity (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anticoagulants</td>
<td>53.5</td>
<td>37.1B</td>
<td>1237B</td>
<td>65</td>
<td>92B</td>
<td>666.3B</td>
<td>90</td>
<td>16</td>
</tr>
<tr>
<td>Na Citrate</td>
<td>49.1</td>
<td>33.1B</td>
<td>1046B</td>
<td>53</td>
<td>90B</td>
<td>597.3A</td>
<td>90</td>
<td>16</td>
</tr>
<tr>
<td>EDTA</td>
<td>57.9</td>
<td>41.1A</td>
<td>1467A</td>
<td>77</td>
<td>94A</td>
<td>641.3A</td>
<td>90</td>
<td>16</td>
</tr>
<tr>
<td>Defibrinated</td>
<td>63.3</td>
<td>47.0a</td>
<td>1709a</td>
<td>86</td>
<td>97a</td>
<td>709.5a</td>
<td>91</td>
<td>16</td>
</tr>
<tr>
<td>Control</td>
<td>63.5</td>
<td>47.1a</td>
<td>1635a</td>
<td>82</td>
<td>97.8A</td>
<td>711.8A</td>
<td>90</td>
<td>16</td>
</tr>
<tr>
<td>F values</td>
<td>60.2</td>
<td>43.8a</td>
<td>1493a</td>
<td>76</td>
<td>95a</td>
<td>666.3a</td>
<td>91a</td>
<td>17a</td>
</tr>
<tr>
<td>Fiber</td>
<td>56.7</td>
<td>40.4b</td>
<td>1446b</td>
<td>72</td>
<td>94a</td>
<td>663.7a</td>
<td>90a</td>
<td>16b</td>
</tr>
<tr>
<td>Gel</td>
<td>66.9</td>
<td>20.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Considering the blood and bulking agent treatment groups separately and using uppercase, lowercase, and bold letters for separate contrasts performed after obtaining significant F values (P < 0.05), means within parameters and contrasts not followed by the same letter were significantly different from each other.

a Larval and pupal weight as well as number of pupae per tray and biological yield were significantly correlated (0.80 and 0.99, respectively); larval weight and biological yield were not included in multivariate analysis of variance.

b Separate contrasts for comparisons of means by using multivariate analysis of variance do not permit reporting of unique F values.
Discussion

Our results clearly indicated that fresh bovine blood can fully substitute for the spray-dried whole bovine blood in the diet currently used to mass rear screwworm larvae. To our knowledge, this is the first report that describes the rearing of screwworm larvae in an artificial medium containing bovine blood and other solid nutrients, although a crude diet of fresh bovine blood and lean ground meat was initially used in the screwworm rearing program (Melvin and Bushland 1940, Bushland 1960). That early procedure was replaced by simpler and more economical rearing methods (reviewed by Taylor 1992). Further research in support of the screwworm eradication program led to adoption of a gelled, semidefinite, artificial diet for mass rearing larvae (Chaudhury and Alvarez 1999). Chaudhury and Skoda (2007, 2009) subsequently found cellulose fiber superior to gel as a bulking and solidifying agent, a finding confirmed by our current study. Our current results indicated that fresh bovine blood obtained free of cost could replace an unavailable spray-dried blood currently purchased at US$2/kg until more suitable alternatives are found, at an annual savings of US$50,000 to produce 50 million flies per week. Our study also provides data useful for evaluating suitability of alternative commercial sources of bovine blood that may have been treated with anticoagulants or mechanical defibrination before using in the diet.

The significantly lower adult emergences that were observed from the pupae obtained from diets with citrated blood suggest developmental problems in the larval or pupal stage possibly involving synthesis, release, or both of ecdysteroids. The significantly lower egg weights obtained using diets containing the citrate and EDTA anticoagulants was probably related to the lower pupal weights associated with these diets, because pupal and adult body weights tend to be correlated and the latter tends to correlate with egg weights (Spates and Hightower 1970, Hightower et al. 1972). Given these tendencies and the small magnitude of any significant difference between the control and test diets in terms of adult longevity and percentage of egg hatch suggest that the adults surviving from larvae reared on all the diets were all relatively normal in their reproductive physiology and mating behavior.

Fresh bovine blood must be free of clots to mix thoroughly and homogeneously with other dietary ingredients for best effectiveness. Various mechanical means and anticoagulant chemicals, such as EDTA and sodium citrate, are the methods available for deaggregating blood. Results of our tests indicate that citrated bovine blood is an unsuitable substitute for the spray-dried blood used in the current diet to mass rear screwworm larvae. Citrated blood has been successfully used to feed many hematophagous insects and ticks without report of adverse nutritional consequences (Lodha 1961, Schmidt et al. 1966). However, Waladde et al. (1993) reported that a tick, Rhipecephalus appendiculatus Neumann, engorged fully on heparinized blood but only partially on citrated or EDTA-treated blood. In our tests, diet with EDTA-treated blood produced more pupae that were heavier than those obtained from the diet with citrated blood, indicating that EDTA is the better anticoagulant for rearing screwworns. Sodium EDTA is sometimes used as a chelating agent to add trace elements to insect diets in soluble form (Cohen 2004) but proved to be a feeding deterrent (Mittler and Kleinjan 1970). The mechanism by which sodium citrate and EDTA impair the development and survival of screwworns is not clear. Because both anticoagulants are mild chelating agents, they may cause their adverse effects by sequestering metal ions important for larval metabolism. If cellulose fiber is the bulking agent, EDTA would be an acceptable anticoagulant.

Different from the diets using chemically decoagulated blood, diets with defibrinated fresh blood, when averaged across bulking agent, in either bulking agent, produced pupae similar in weight and greater in number than those produced by the control diets, suggesting that fresh bovine blood substituted for the spray-dried product in the screwworn rearing medium should be mechanically defibrinated for best results.

The logistics for the use of fresh bovine blood for mass rearing are debatable. Daily procurement, transportation, storage, defibrination, and other tasks associated with handling of large quantities of fresh blood (although obtained free of cost) will be labor-intensive. However, in the event no spray-dried blood is available, this technology will be useful for mass rearing screwworns.

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