The Optimum Diet of Spray-Dried Animal Blood Cells as Protein Source for Adult Screwworms (Diptera: Calliphoridae)[1]

Agustin Sagel, Dennis R. Berkebile,[2,3] John E. Foster and Steven R. Skoda[3]

Department of Entomology, University of Nebraska, Lincoln, NE 68583-0816 USA

J. Entomol. Sci. 37(4): 353-362 (October 2002)

Abstract Spray-dried animal blood cells were evaluated as a protein source for mass rearing adult screwworms, *Cochliomyia hominiorax* Coquerel. Males and females were fed control diets of either a corn syrup carrageenan-gelled diet (Control 1) or corn syrup carrageenan-gelled diet plus 0.05% vitamins (Control 2). Three tests compared these control diets with eight concentrations of protein similarly formulated as the Control 2: 0.16, 0.20 or 0.24% (Test 1), 2, 4 or 6% (Test 2) and 1.25, 2.0 or 2.75% (Test 3). Differences in mean egg production, mortality, fertility and egg hatch were not significant ($P = 0.05$) in Test 1. The higher concentrations of protein in Test 2, concentrations of 2 and 4% protein laid significantly greater number of eggs than those fed the control diets or 6% protein. In Test 3, flies fed protein laid more eggs than did those fed the control diets. A diet of 2% protein from spray-dried-animal blood cells for may be used as an alternative to the diet used for rearing adult screwworms.

Key Words Adult diets, nutrition, screwworm rearing, *Cochliomyia hominivorax*

Screwworms, *Cochliomyia hominivorax* Coquerel, are obligate parasites that affect all warm-blooded animals, including humans. The larvae cause myiasis, a condition where fly larvae develop in the tissues of living animals (Thomas 1993b, Spradbery 1994). The sterile insect technique (Knipling 1955) has been used to eradicate this pest from the United States, Mexico and most of Central America. However, the United States livestock industry could lose about $500 million annually due to death, low milk and meat production, increased veterinary labor and medicine costs if screwworms re-establish in the United States (Wyss and Galvin 1994).

The sterile insect technique involves mass production and release of irradiated sterile screwworm males that overwhelm wild fly populations by mating wild females and rendering their eggs infertile. Research efforts to improve the screwworm rearing techniques (particularly for use in mass rearing) have enhanced success of the program. A number of protein diets have been tested for improvement of screwworm egg production (Brewer 1993, Thomas 1993b, Hammack 1999, Chaudhury et al. 2000), but spray-dried blood (a major ingredient in the screwworm larval diet) has not been tested.

Most adult insects need considerable amounts of carbohydrate, protein and lipid to perform the biological activities necessary for survival and reproduction (Warburg and

[1]Received 10 September 2001; accepted for publication 04 June 2002.
[2]To whom requests for offprints are to be addressed (email: dberkebi@unlnotes.unl.edu).
[3]USDA-ARS, Midwest Livestock Insects Research Unit, Lincoln, NE 68583-0938.

Yuval 1996). The screwworm is generally considered to be autogenous and that larval protein reserves are adequate for the first gonotrophic cycle (Crystal 1966, Mackley and Snow 1982, Thomas 1993a). Crystal (1966) indicated a protein source might be necessary for subsequent egg production.

The fecundity of screwworms was significantly increased by feeding adults egg and milk during the first ovarian cycle (Brewer 1993). Thomas (1993b) observed a 14% increase in the fecundity of screwworms that were fed honey and water supplemented with raw meat. Adult screwworms developed from larvae reared on hosts completed their first ovarian cycle sooner than those from larvae reared on artificial diet (Thomas 1993b). Thus, adequacy of the larval diet probably influences the degree of autogeny expressed in the adults. We rear 15 strains on a gelled carbohydrate diet without a protein supplement. Until recently the mass rearing facility had reared the production strain on a diet of ground meat and honey. The protein source currently being used is spray-dried egg (Chaudhury et al. 2000)

Brody (1939) observed that adult screwworms preferred foods originating from animal sources. Female screwworms are attracted to and feed on diverse protein sources including dung (Mackley and Snow 1982, Hammack 1990) and wound fluids of live animals (Guillot et al. 1977, Thomas and Mangan 1989). Dung alone does not constitute a protein source able to support vitellogenesis in the screwworm (Mackley and Snow 1982), but wounds and carrion may be adequate sources. Parker and Welch (1991a) collected mostly mated, nulliparous female screwworms on rotting liver. They also found that females collected from wounded sheep did not always oviposit but did feed during each visit (Parker and Welch 1991b).

Vitamins also are important nutritional elements of insect diets. Sang (1978) found, when studying *Drosophila,* that if the level of dietary sugar is increased, then the need for thiamine and pantothenate increased due to their primary role as cofactors for glycolytic and energy-producing reactions. In addition, nicotinic acid and folic acid were needed when raising the proportion of dietary protein from 4 to 7%. Even though the presence of B-complex vitamins was not essential for ovarian development in *Phormia regina* Meigen, ovarian maturation was sometimes slightly faster when B-complex vitamins were incorporated in a protein-carbohydrate diet (Rasso and Fraenkel 1954).

The objective of this research was to determine the effect of different levels of spray-dried blood in the adult diet on fecundity, fertility and survival of adult screwworms.

Materials and Methods

The flies used in this study were a strain developed from adult flies collected in Panama in 1995 (P95). It is currently the production strain at the mass production facility. The larvae were reared on an artificial gel media (Taylor et al. 1991); adults were fed a corn syrup-carrageenan diet. At the initiation of this study these flies had been in colonization for about 50 generations.

Spray-dried animal blood cells (AP 301™, American Protein Corporation, Manning, IA) served as the protein source in adult feeding experiments. The minimum content of crude protein of AP 301™ is 92%. The source of carbohydrates was Karo® light corn syrup (CPC Foodsource, Englewoodcliffs, NJ) that contains 30 g of sugars per 30 mL. Carrageenan Type I Commercial Grade (SIGMA, St Louis, MO) was used as a gelling agent to obtain the desired consistency in the food. It is a polymeric

carbohydrate with little nutritive value. Theragran™ Multivitamins (Mead and Johnson, Evansville, IN) supplemented the diet.

Diet preparation. For each 100 mL of the corn syrup carrageenan diet (Control 1), we mixed 250 mg of carrageenan with 50 mL of tap water. The solution was heated to about 80°C, and 50 mL of light corn syrup was added and thoroughly stirred. The diet was dispensed in 237 mL paper ice cream cups. A small amount of clean sawdust was dispersed over the surface to reduce the number of flies sticking in the diet. The diets were refrigerated to activate gelling. A second diet (Control 2) was prepared as described above with the addition of 0.05% (w/v) of multivitamins. The 100 mg caplets of multivitamins were pulverized with a mortar and pestle; 50 mg of the vitamins (Table 1) were mixed with 100 mL of the carrageenan-corn syrup. The mixture was thoroughly stirred before refrigeration.

Three tests were conducted to compare different concentrations of protein, using spray-dried animal blood cells, with the two control diets. Diets containing individual concentrations (w/v) of protein were prepared using the Control 2 diet above. Test 1 compared low levels of protein (0.16, 0.2, and 0.24%), Test 2 compared a broader range of concentrations (2, 4, and 6%) and Test 3 was designed to identify the best level (1.25, 2.0, and 2.75%). To avoid altering the protein and vitamins, the carrageenan was mixed separately with 25 mL of tap water and heated to 80°C. Fifty ml of corn syrup was added and mixed with the heated carrageenan solution. The dried blood and vitamins were mixed with 25 mL of tap water in a separate beaker. This was added and mixed with the cooled corn syrup-carrageenan. The preparation was dispensed into paper ice cream cups and sprinkled with a small amount of sawdust as previously described. Each cup was covered with a plastic lid to avoid dehydration and stored in a refrigerator until used. Each test was replicated 3 times.

Table 1. Vitamin and mineral content in 100 mL of diet

Vitamin	Amount	Mineral	Amount
A	2500 IU	Calcium	20 mg
C	45 mg	Iron	9 mg
D	200 IU	Phosphorous	15.5 mg
E	15 IU	Iodine	75 mcg
K	14 mcg	Magnesium	50 mg
Thiamin	1.5 mg	Zinc	7.5 mg
Riboflavin	1.7 mg	Selenium	10.5 mcg
Niacin	10 mg	Copper	1 mg
B6	1.5 mg	Manganese	1.75 mg
Folate	200 mcg	Chromium	13 mcg
B12	4.5 mcg	Molybdenum	16 mcg
Biotin	15 mcg	Chloride	3.75 mg
Pantothenic Acid	5 mg	Potassium	3.75 mg

Dehydration test. The Control 1, Control 2, and the 1.25, 2.0, and 2.75% protein diets were used to determine rate of dehydration of the diets. One paper ice cream cup for each diet was labeled, weighed, filled with 100 mL of the respective diet and weighed once more to obtain the initial weight of the media. The diet was sprinkled with sawdust, weighed and placed in the refrigerater. One cup of each diet was placed in an environmental growth chamber at 24.5°C and 70% RH for 6 d. The final weights of the contents of the cups were recorded to determine the rate of dehydration. Those data were used to correct the nutrient intake calculations in Test 3. The test was replicated three times.

Nutrient intake. Food intake by adult flies was estimated by weighing the diet at the beginning of Test 3 and subtracting the estimated loss due to dehydration. The amount consumed by each fly was estimated by dividing this by the number of flies at the beginning of the study (225). The data were analyzed as a completely randomized design using PROC GLM (SAS Institute 1988).

Dietary protein impact. For each test, newly-emerged unfed flies were separated by sex. Five stainless-steel (27 cm length × 18 cm width × 23 cm height) gauze-covered cages were setup with 150 females and 75 males. The flies were supplied with one of the diets and tap water. During the 6 d experimental period, the food and water were not replaced. The flies were maintained at 24.5°C and 70% RH and offered oviposition media 6 d after adult emergence. The total weight of eggs from the females of each treatment was recorded. A sample of 100 eggs was placed on moistened filter paper in a Petri dish, incubated at 37°C for ~18 h and egg hatch determined. Fertility was assessed by adding the number of hatched eggs to the number of eggs showing signs of development but not hatching. After oviposition, the numbers of dead males and females were counted in each cage. Fecundity was measured as the egg weight obtained per female that survived to oviposition. Each experiment was replicated three times. An arcsine square root transformation was made on mortality, egg hatch and fertility data before analysis. Data were analyzed as a randomized complete block design using PROC GLM and mean comparisons among the treatments were made using Duncan's Multiple Range Test and orthogonal contrasts (SAS Institute 1988).

Results and Discussion

Test 1. Low levels (0.16, 0.20, and 0.24%) of spray-dried blood diet did not show a significant change ($P > 0.05$) in egg production, egg hatch and adult fly mortality when compared to the control diets (Table 2).

Test 2. Results of Test 1 indicated that higher levels of protein might be required to produce significant changes in egg production, egg hatch or adult mortality. Females fed the 2% dried blood diet produced significantly more eggs than those fed the Control 1 diet ($P < 0.05$) (Table 3). The amount of eggs produced by females fed the 4 and 6% diet were not significantly different from Control 1 ($P > 0.05$). Egg hatch, fertility or mortality was not affected by any of the diets ($P > 0.05$).

Test 3. Since the 2% diet showed a significant increase in egg production over the Control 1 diet in Test 2, a comparison of the two control diets and 1.25, 2.0, and 2.75% dried blood was made in this test. The dehydration rates (mean ± SD) over the 6-day feeding periods were 27.92 ± 2.03, 32.98 ± 3.25, 28.95 ± 2.48, 27.81 ± 1.63, and 26.49 ± 1.32% for Control 1, Control 2, 1.25% protein, 2.0% protein, and 2.75%

Table 2. Effect of low levels of dietary protein on adult screwworm egg production, egg hatch and adult mortality

	Diets*				
			Percentage protein		
Variable	Control 1	Control 2	0.16%	0.20%	0.24%
Egg wt (mg)**	3.36 ± 1.78	2.76 ± 1.32	3.19 ± 1.60	2.99 ± 2.42	3.19 ± 0.96
Egg hatch (%)***	69.75 ± 11.58	71.75 ± 13.15	70.0 ± 7.07	73.25 ± 17.06	75.25 ± 1.70
Mortality (%)***	25.25 ± 16.98	28.25 ± 14.77	26.75 ± 15.13	19.75 ± 8.96	18.0 ± 6.68

* Means (±SD) within rows were not significantly different ($\alpha = 0.05$).
** Egg wt is the average weight of eggs produced per female that survived to oviposition.
*** Actual means are reported. ANOVA and means separations procedure were conducted on arcsine square root-transformed proportions.

Table 3. Effect of three levels of dietary protein on adult screwworm egg production, egg hatch and adult mortality

			Protein level		
	Control 1	Control 2	2%	4%	6%
Egg weight (mg)	1.43 ± 0.65ab	2.58 ± 10.39ac	3.13 ± 0.59c	2.10 ± 0.95abc	1.10 ± 0.79b
Egg hatch (%)***	71.28 ± 7.95a	48.11 ± 42.03a	72.66 ± 10.89a	74.19 ± 16.95a	63.40 ± 15.52a
Fertility (%)***	77.80 ± 4.78a	51.14 ± 44.30a	76.28 ± 9.00a	79.13 ± 18.57a	66.77 ± 14.86a
Mortality (%)***	8.53 ± 1.97a	28.00 ± 17.72ab	28.67 ± 9.88ab	35.33 ± 10.00b	32.00 ± 27.37ab
Female***	6.22 ± 3.36a	25.11 ± 15.53ab	27.56 ± 7.37ab	34.67 ± 9.17b	30.22 ± 29.29ab
Male***	12.00 ± 7.00a	32.33 ± 21.08ab	30.33 ± 13.65ab	36.33 ± 14.01b	34.67 ± 24.58a

* Means (±SD) within rows were not significantly different ($\alpha = 0.05$).
** Egg wt is the average weight of eggs produced per female that survived to oviposition.
*** Actual means are reported ANOVA and means separations procedure were conducted on arcsine square root-transformed proportions.

protein diets, respectively. The nutrient intake did not differ significantly among diets ($F = 1.57$, df = 4, $P > 0.05$) (Table 4).

The mean egg weight was significantly different between treatments ($F = 10.77$, df = 4, $P < 0.05$) as well as between blocks ($F = 5.09$, df = 2, $P < 0.05$). The overall mean egg weight observed was 2.47 mg with a coefficient of variation of about 16.36%. Egg weights from flies fed the Control 1 diet were significantly lower than egg weights from flies fed the other test diets ($P < 0.05$) (Table 4). No statistical difference was observed between Control 2 diet and the 1.25% protein diet ($F = 0.02$, df = 1, $P > 0.05$), but the 2.0 and 2.75% protein diets produced a significantly greater number of eggs than the Control 2 diet ($P < 0.05$). No significant difference was found among the protein diets ($P > 0.05$).

Neither hatching nor fertility varied significantly among the treatments ($P > 0.05$). Fertility ranged from about 71.33% to 80.67% of the eggs obtained from females fed the protein diets (Table 4).

Female and male mortality did not differ among treatments ($P > 0.05$) (Table 4). Female mortality ranged from 7.3% in the group fed 2.75% protein to 10.7% in the group fed Control 2 diet. The minimum (5.0%) and maximum (17.9%) male mortality was recorded in the groups fed Control 1 diet and 2.75% protein diet, respectively. The number of dead males was higher than the number of dead females in all treatments except Control 1 diet.

Our study corroborated results of previous studies. Brewer (1993) used a combination of milk, sucrose and egg to feed adult screwworms and found an increase in fecundity. However, the quantity of eggs obtained in our study was 76.3% higher than that obtained by Brewer (1993), using similar fly population density, approximate temperature, and oviposition timing, but eggs were collected from our females 2 d earlier. The strain used by Brewer had only been colonized for about 17 generations compared to about 50 generations for the strain used in this study. More recently Hammack (1999) and Chaudhury et al. (2000) observed relevant improvement of the screwworm's first gonotrophic cycle using protein in the adult diet. The raw meat used by Hammack (1999) increased the percentage of gravid females by 45% and boosted the number of eggs matured per gravid female by 30%. A diet of spray-dried egg mixed with molasses and honey (Chaudhury et al. 2000) improved the number of gravid females and eggs laid per female. Results from our study are difficult to compare with those of Chaudhury et al. (2000) because they did not standardize the initial populations in test cages.

Protein in insect diets must be soluble in order to be used for ovarian development (Rasso and Fraenkel 1954). The spray-dried blood cells we used were easily soluble in the standard diet. When we incorporated spray-dried egg in a gelled diet, it had poor solubility in water, was highly susceptible to dehydration, and it was less phagostimulating than dried blood.

In Test 3 we determined the best dietary protein level using spray-dried blood to be about 2% for adult screwworms (Table 4). *Phormia regina* (Meigen) was reported to consume 2.8 mg of protein per fly during a 4 h-feeding and that amount was sufficient to induce egg maturation and the production of 218 to 250 eggs per female (Stoffolano et al. 1995a). If we assume that the consumption of all nutrients (protein, vitamin, and carbohydrate) is uniform, the mean amount of protein intake estimated in flies fed *ad libitum* the 2% protein diet during the 6-d experimental period in Test 3 was about 2.89 mg/fly. Protein consumption by *Chrysomya bezziana* Villeneuve decreases rapidly after 3 to 4 d postemergence (Spradbery and Scheweizer 1979). If this is true for

Table 4. Effect of three levels of dietary protein on adult screwworm nutrient intake, reproduction and mortality

Variable	Diets*				
			Percentage protein		
	Control 1	Control 2	1.25%	2.0%	2.75%
Nutrient intake (mg/fly):	137.27 ± 11.72	130.7 ± 5.63	146.84 ± 11.71	144.65 ± 2.46	143.81 ± 8.67
Reproduction:					
Egg wt (mg)**	1.26 ± 0.37c	2.34 ± 0.61b	2.49 ± 0.66ab	3.13 ± 0.72a	3.11 ± 0.04a
Hatching (%)***	75.3 ± 1.53a	76.0 ± 7.8a	69.7 ± 5.68a	77.0 ± 3.6a	69.3 ± 5.69a
Fertility (%)***	78.0 ± 2.0a	78.33 ± 9.07a	71.33 ± 4.72a	80.67 ± 2.08a	71.33 ± 7.64a
Mortality (%):					
Female***	5.33 ± 4.05a	7.11 ± 5.09a	5.33 ± 2.4a	6.44 ± 3.67a	4.89 ± 3.36a
Male***	6.67 ± 4.8a	16.44 ± 4.68ab	15.11 ± 10.69ab	19.11 ± 9.83ab	22.67 ± 13.13b

* Means (±SD) within rows followed by the same letter are not significant different ($\alpha = 0.05$).

** Egg wt is the average weight of eggs produced per female that survived to oviposition.

*** Actual means are reported. ANOVA and means separations procedure were conducted on arcsine square root-transformed proportions.

C. hominivorax, then protein consumption is very similar to that for *P. regina.* The mean number of eggs obtained per female was estimated to be 78 by dividing the mean egg weight of 3.13 mg/fly by an average of 0.04 mg/egg (individual egg weight derived from data in Parker and Welch 1991b) (Table 4).

Protein in the diet of female screwworms serves as a raw material for egg development and may play a role in activating the neuroendocrine cascade that leads to the production of vitellogenin and its uptake by developing follicles (Pappas and Frankel 1977, Stoffolano et al. 1995a). Oosorption of some follicles can occur if the amount of protein provided to flies is less than that physiologically required which can lead to a consequent reduction in the number of eggs produced (Stoffolano et al. 1995a, b, Yin et al. 1993). In *C. bezziana,* resorption occurs early during vitellogenesis during peak protein ingestion (Spradbery and Schweizer 1981). *Phormia regina* had higher levels of juvenile hormone after protein feeding, which increased the rate of vitellogenesis and improved egg production (Yin and Stoffolano 1997). This might explain the improvement obtained by feeding adult screwworms protein during their first gonadotrophic cycle.

Protein has been shown to be important to male reproduction in certain flies. In *P. regina,* protein is required for the development of accessory reproductive glands that regulate mating behavior and the insemination of females (Stoffolano 1974). *Cochliomyia macellaria* (F.) males are less responsive to female pheromones when they are deprived protein (Hammack 1992). The 2% dried blood diet did not markedly affect the ability of male screwworms to inseminate females, and fertility was not significantly different between diets (Tables 3 and 4).

Egg production was increased when the vitamin complex alone was added to the diet in Test 3 but not Tests 1 or 2. The difference observed between the Control 1 diet and Control 2 diet (Table 2) indicates that feeding adult screwworms a diet supplemented with vitamins may result in the production of more eggs. In Test 2, flies fed Control 2 diet (sugar plus vitamins) produced 85.7% more eggs than did flies feeding on Control 1 diet. B-complex vitamins have been shown to increase egg production in *P. regina* (Rasso and Fraenkel 1954). Addition of B-vitamins to *C. hominivorax* protein diet does not improve egg production (Hammack 1999). The addition of potassium ions has been found to be important in egg production in *P. regina* (Pappas and Frankel 1977) and *C. hominivorax* (Hammack 1999). Each 100 mL of spray-dried blood diet contain about 3.73 mg of potassium (Table 1). Although further tests are needed to adapt the use of vitamins and spray-dried blood cells as the protein source for mass rearing the screwworms used in the screwworm eradication program, there may be benefits of more healthy flies that produce more eggs and thus it could be very cost effective.

Use of blood in the diet increased the cost of a 50-mL serving of diet by $0.0052 (from $0.1172 to $0.1224). The increased cost, 4.43%, resulted in 148% more eggs produced by the females when compared with Control 1 diet (Table 3). This might be attributed to the availability of essential amino acids contained in blood, which may enhance metabolic processes including oogenesis in protein fed flies. The introduction of a gelled-proteinaceous adult screwworm diet into the rearing protocol at the mass rearing facility requires more detailed analysis.

Acknowledgments

The authors thank Shripat Kamble and Srinivas Parimi for their helpful comments on an early draft of this manuscript. This work was done in cooperation with the Institute of Agriculture and

Natural Resources, University of Nebraska, Lincoln, NE. This is paper no. 13382, Journal Series, Nebraska Agricultural Research Division. The research was funded in part by Specific Cooperative Agreement No. 58-5440-7-129.

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